Biofertilizers and Biopesticides for Sustainable Agriculture

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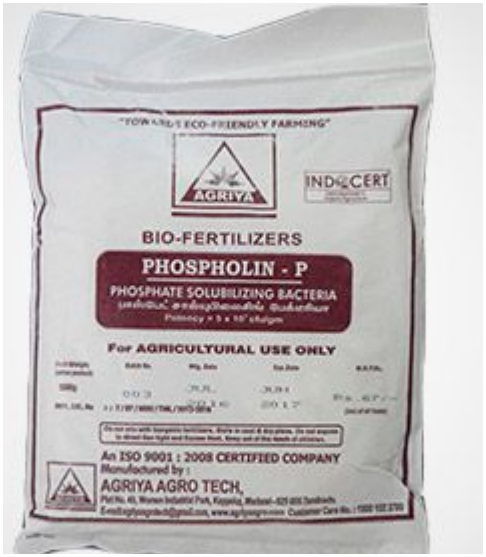
Biofertilizers : Biofertilizers means the product containing carrier based (solid or liquid) living microorganisms which are agriculturally useful in terms of nitrogen fixation, phosphorus solubilization or nutrient mobilization, to increase the productivity of the soil and/or crop.



Following biofertilizers are specified in schedule III of FCO, 1985

- *Rhizobium*
- *Azotobacter*
- *Azospirillum*
- Phosphate solubilizing bacteria
- Mycorrhizae
- Potash Mobilizing bacteria(KMB)
- Zinc solubilizing bacteria(ZSB)
- *Acetobacter*
- NPK consortia

Role of biofertilizers

- Better germination.
- More root proliferation.
- Make the root rhizosphere more lively.
- Makes nutrients available.
- Growth Promoting Substances are produced.
- Improve quality and quantity of produce.
- Improve fertilizer use efficiency.
- More biotic and abiotic stress tolerance.
- Improve soil health.
- Residual Effect.
- Make the system more sustainable
- Replace 25-30% chemical fertilizers
- Increase the crop yields
- Decompose plant residues, and stabilize C:N ratio of soil

Types of biofertilizers

1. Nitrogen fixing microorganism

Symbiotic: *Rhizobium, Bradyrhizobium, Frankia, Azolla*
Associative: *Azospirillum*
Freeliving: *Azotobacter*

2. Phospho-microorganisms

- Phosphate solubilizing bacteria(PSB):
  Bacillus, Pseudomonas

- Phosphate solubilizing fungi(PSF)
  Aspergillus, Penicillium, Schwanionyces occidentalis(Yeast)

- Mobilizing organisms: VAM

3. Potash Solubilizing Bacteria (KSB)

Frateuria aurantia, *Bacillus sp., Pseudomonas sp. etc.,*

4. Zinc Solubilizing Bacteria (ZSB) *Bacillus sp., Pseudomonas sp. etc*

5. Compost inoculants:

- Cellulolytic or lignolytic fungi for rapid decomposition of organic matter or compost preparation

6.Plant growth promoting microorganisms for increasing crop growth

Nitrogen Fixers

- Nitrogen is an essential element for all forms of life and it is the most vital nutrient for plant growth and productivity.
- Nitrogen presents 78 % of the atmosphere, it remains unavailable to the plants.
- No plant species is capable for fixing atmospheric dinitrogen into ammonia and expend it directly for its growth.
- Atmospheric nitrogen is converted into plant utilizable forms by biological nitrogen fixation (BNF) which changes nitrogen to ammonia by nitrogen fixing microorganisms using a complex enzyme system known as nitrogenase

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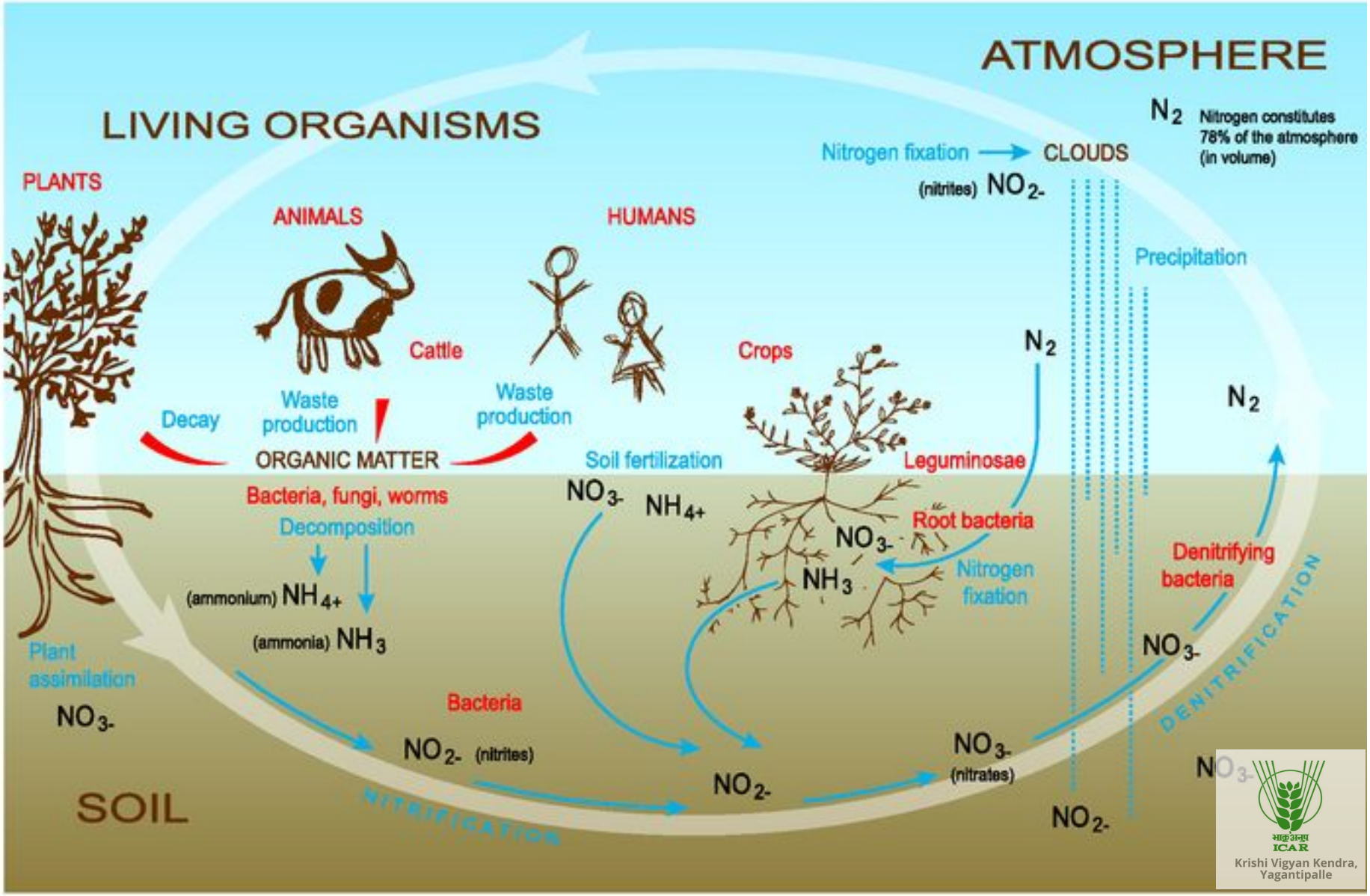
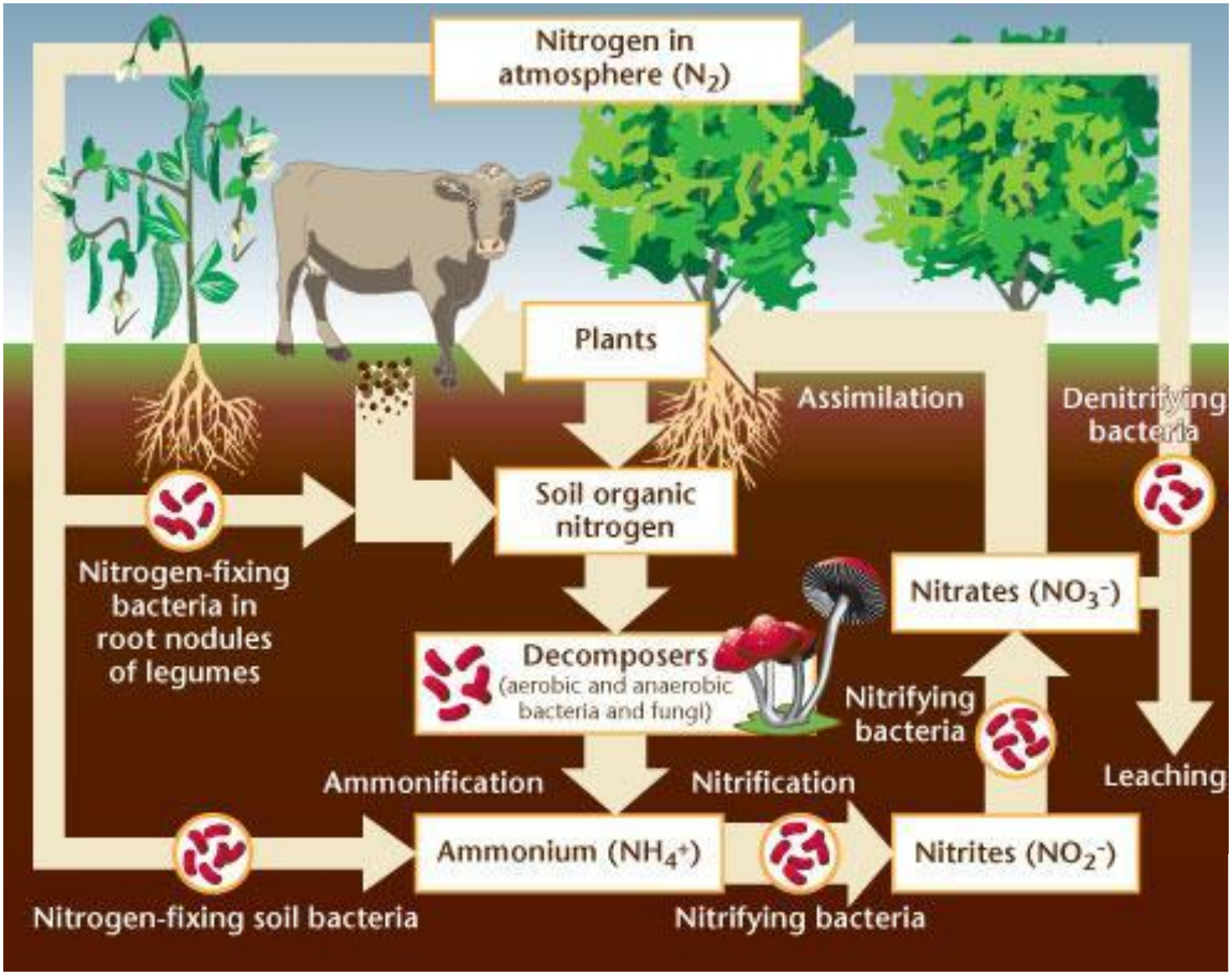
The nitrogen cycle

NITROGEN RESERVOIRS ACTORS PROCESSES





Rhizobia

Rhizobia are a group of small, rod-shaped, aerobic, mostly motile, non-sporulating, gram-negative Nitrogen fixing bacteria.

Many are surrounded by a slimy, thick exopolysaccharide layer that is thought to facilitate attachment to root hairs.

Rhizobia exist in two states as free-living saprophytes in the soil and in a symbiotic relationship with leguminous plants.

Rhizobia produce root nodules in legumes and fix the atmospheric N_2 inside the nodule

Rhizobiu

Recommended doses:

- **Seed Treatment:** 5 – 10 ml/kg seed
- **Seedling dip :** Dip the seedlings in solution containing 5 ml Rhizobium/lt water.
- **Soil application:** 1 lt/kg per acre along with FYM
- **Drip irrigation : 5**00 ml in 100 L of water

Target Crops: All Legume crops

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Azotobacter

- ***Azotobacter*** **is a Free-living, Gram negative aerobic heterotrophic N_2 fixing bacteria form thick-walled cyst and may produce large quantities of capsular slime**

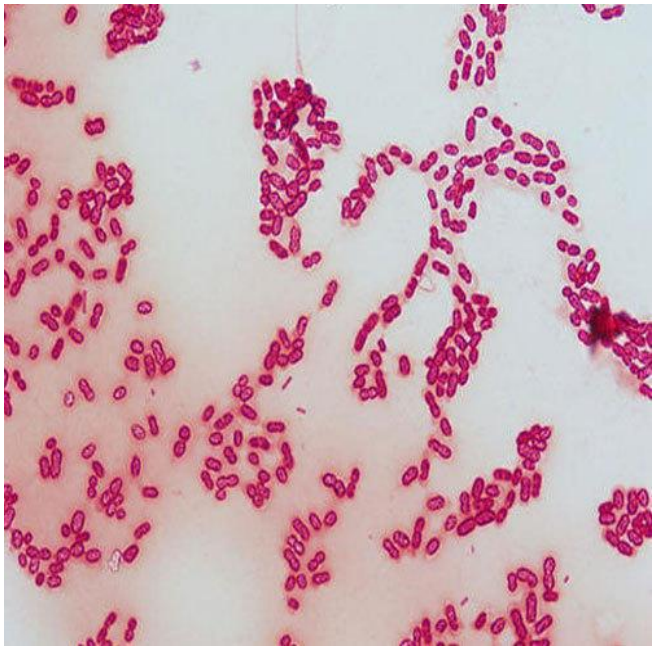
- ***Azotobacter*** **is able to fix atleast 10 mg N per gram of carbohydrate**

- ***Azotobacter chrococcum*** **is the most commonly occurring species**

- **Synthesize growth promoting substances *viz.*, auxins and gibberellins and also to some extent the vitamins.**

- ***Azotobacter*** **also exhibit fungicidal properties against certain species of fungus.**

Azotobacter



Recommended doses:

- **Seed Treatment:** 5 – 10 ml/kg seed
- **Seedling dip :** Dip the seedlings in solution containing 5 ml/lt water.
- **Soil application:** 1 lt/kg per acre along with FYM
- **Drip irrigation : 5**00 ml in 100 L of water

Target Crops:

- Cereal crops Paddy, Cotton, Vegetables, Fruit trees and Ornamental plants.

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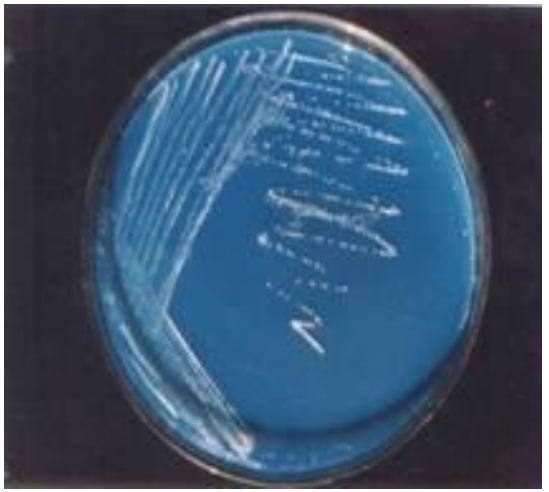
Azospirillum

- These are associative nitrogen fixers
- Fix nitrogen @15-30kg/ha and secrete growth regulating substances



Azospirillum

Recommended doses:

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- **Soil application:** 1 lt/kg per acre along with FYM
- **Drip irrigation : 5**00 ml in 100 L of water

Target Crops:

- Paddy, Cereal crops (Wheat, Maize, Barley, Jowar, Bajra)

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Phosphate Solubilizing Microorganisms (PSM)

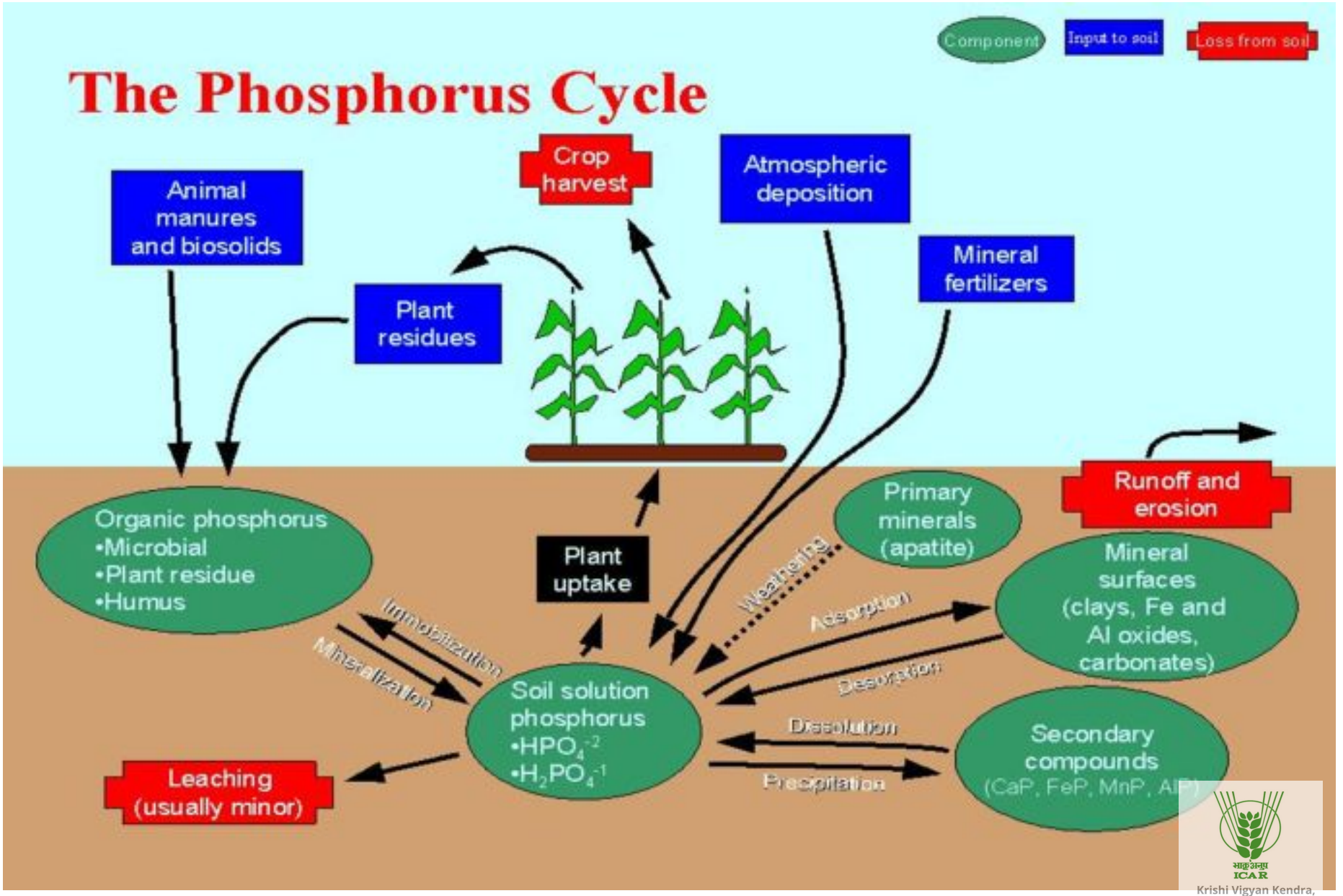
- It plays an important role in virtually all major metabolic processes in plant including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration.

- It is abundantly available in soils in both organic and inorganic forms. Plants are unable to utilized phosphate because 95-99% phosphate present in the insoluble, immobilized, and precipitated form.

- Plants absorb phosphate only in two soluble forms, the monobasic (HPO_4^{2-}) and the diabasic ($H_2PO_4^-$).

- To overcome the P deficiency in soils, there are frequent applications of phosphatic fertilizers in agricultural fields. Plants absorb fewer amounts of applied phosphatic fertilizers and the rest is rapidly converted into insoluble complexes in the soil

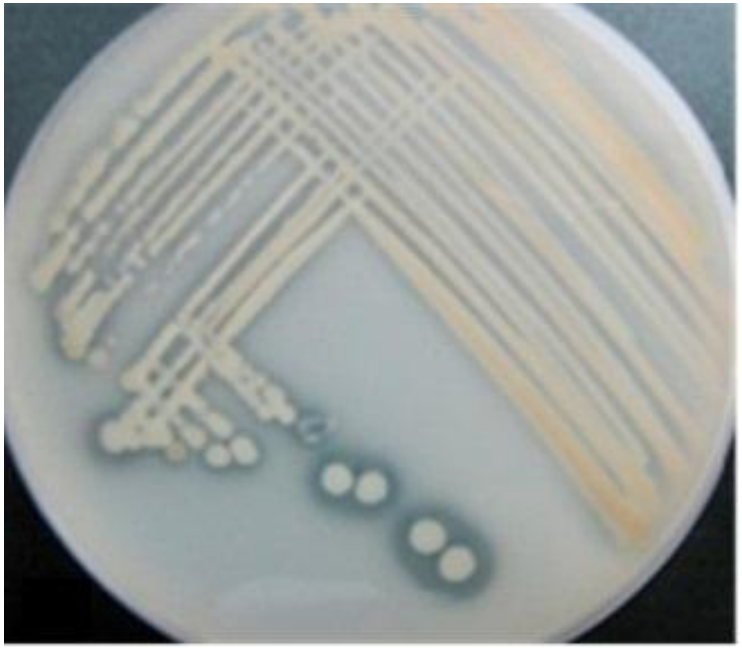
- In this context, organisms coupled with phosphate solubilizing activity, often termed as phosphate solubilizing microorganisms (PSM), may provide the available forms of P to the plants and hence a viable substitute to chemical phosphatic fertilizers.

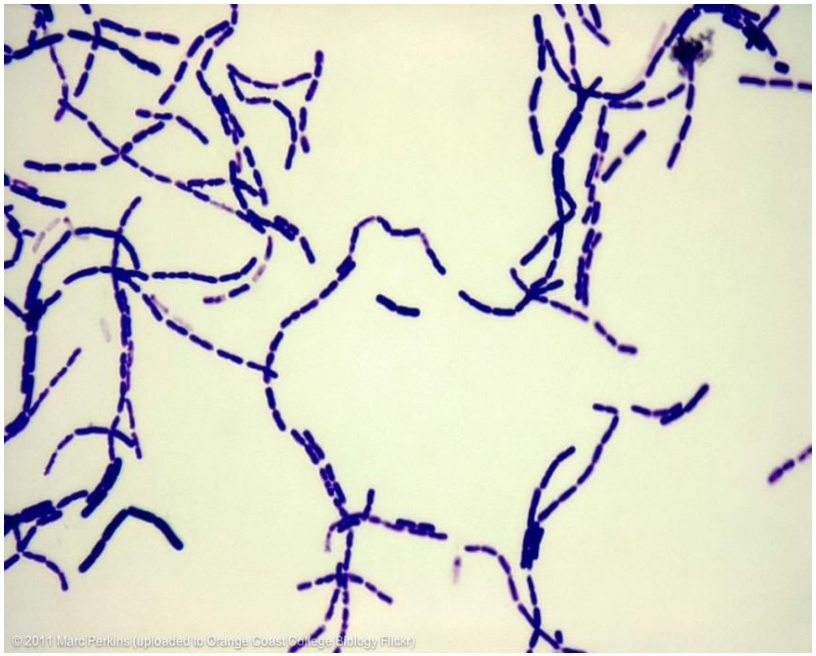


- Solubilization of inorganic ‘P’ by PSMs is due to production of organic acids tartaric, gluconic, ketogluconic, lactic and succinic acid and different phosphatases

- The most efficient PSM belong to genera *Bacillus* and *Pseudomonas* amongst bacteria and *Aspergillus* and *Penicillium* amongst fungi

PSB (Phosphate Solubilizing Bacteria)





Phosphate solubilizers convert insoluble phosphorus into soluble phosphates and make it available to the plants.

Recommended doses:

- **Seed Treatment:** 5 – 10 ml/kg seed
- **Seedling dip :** Dip the seedlings in solution containing 5 ml Rhizobium/lt water.
- **Soil application:** 1 lt/kg per acre along with FYM
- **Drip irrigation : 5**00 ml in 100 L of water

Target Crops: All crops

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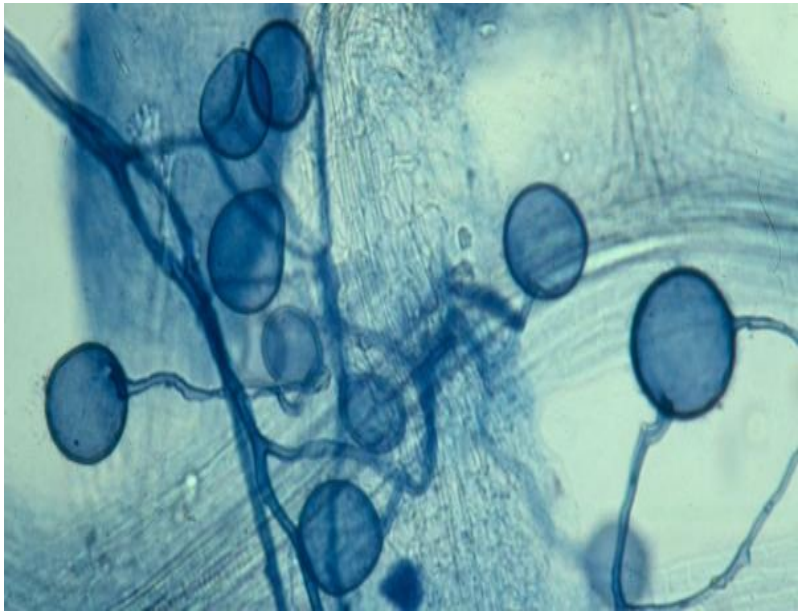
Mycorrhiza



Mycorrhiza have symbiotic associations with roots of majority plants.

Increase the absorption of phosphorus (P), zinc (Zn), manganese (Mn) and copper (Cu) and water.

Application dose : 3-4 kg/ acre

Target crops:

- Useful for all crops

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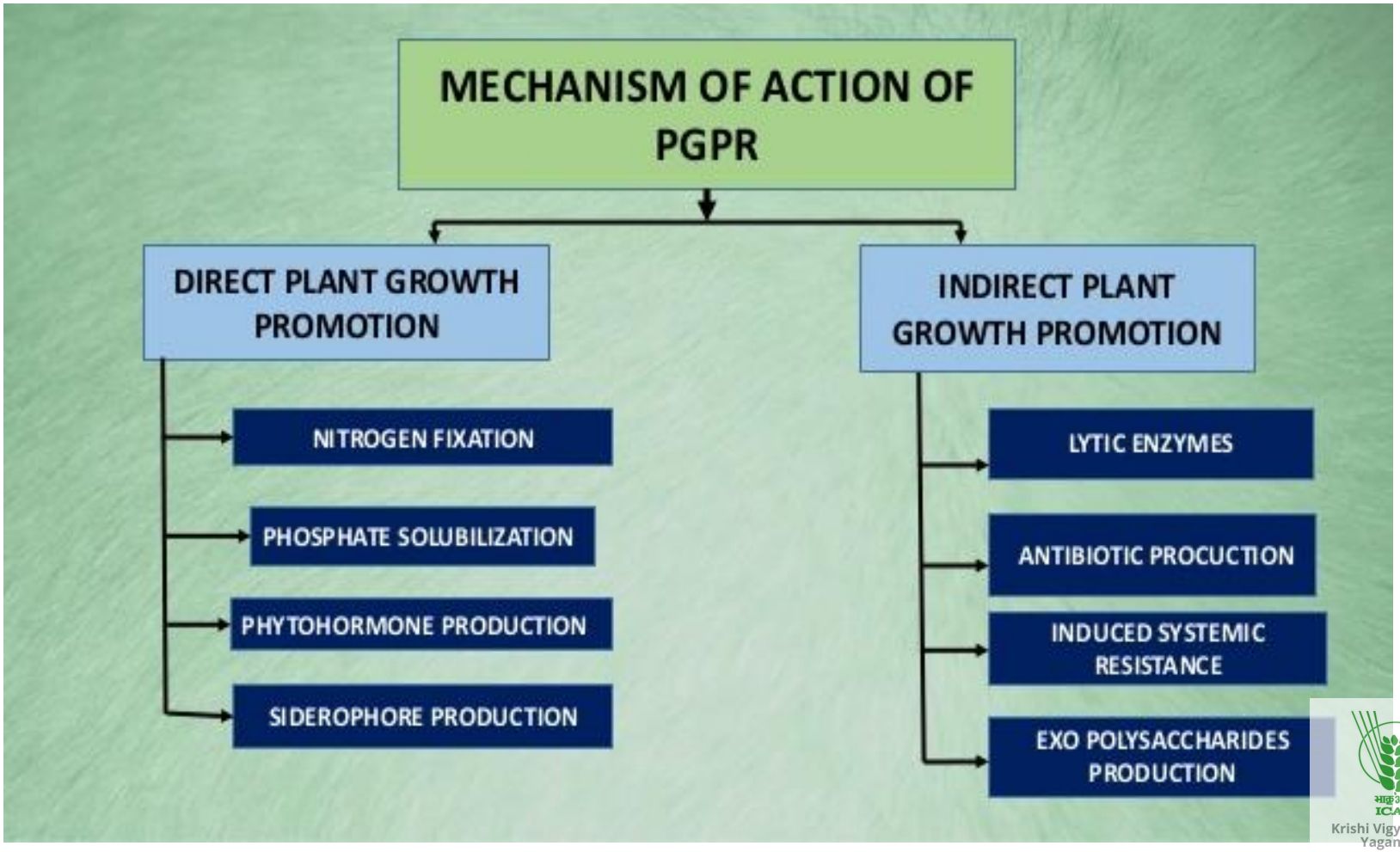
Plant Growth Promoting Rhizobacteria



Compost inoculants

Methodology of Mass Multiplication by farmers :

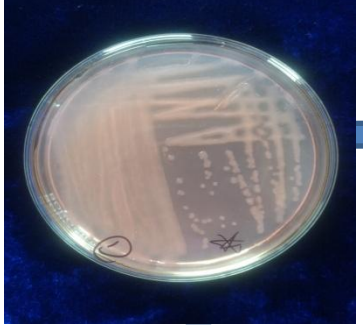
Mix 2 kg jaggery in 200 litre of water in a container and stir well

Open the bottle and pour the contents into the solution and stir well every day

Cover container with a paper/cardboard

The waste decomposer gets ready after 5 days

Liquid Biofertilizers Production



Purification

Supply to farmers

Serial dilution

Mother culture

Fermentation for Large scale production

Packing

Rhizosphere soil/Nodules

Broth culture

Multiplication of Biofertilizers on shaker

Quality analysis

On-Farm Production of *Rhizobium*

1. Mother culture of *Rhizobium*

2. Prepare jaggery solution (10 g jaggery & 1g of common salt/L

3 Transfer jaggery solution into glass bottles Close the bottle mouth with cotton plug

4 Sterilize the jaggery solution in pressure cooker for 40 min and cool at room temperature

5. Inoculate sterilized jaggery solution with starter culture

6. Incubate bottles at room temperature for 5-7 days

7. Shake bottles 3-4 times everyday

8. *Rhizobium* culture ready to use

9. Liquid *Rhizobium* culture for seed treatment (@200ml/ acre seed)

DG NIPHM with Visitors

Approximate cost for on farm production unit of biofertilizers

S. No.	Item	Size/unit	Approxi.cost/ unit (Rs.)	Remarks
1.	LPG cylinder 14.5 Kg (extra)	1	2000	
2	Pressure cooker- 20 L	1	6000	
3	Gas stove -2 burner	1	5000	
4	Inoculation chamber	45 x 45 x 40 cm	3000	
5	Sterilisable vials- 5 ml	500	2500	
6	Miscellaneous (bottles, Spirit lamp Jaggery, Cotton, etc.)	-	6500	
	Total		25000	

Quality of Biofertilizer

The quality of biofertilizer demands the presence of the right type of bacteria in active form and in the desired numbers.

Quality is one of the most important factors influencing the success and acceptance of the product by farmers

Quality controls maintained by

- Fermentation process
- Carrier preparation
- Mixing of carrier and bacterial broth
- Packing and storage

It is important that greater R&D efforts to be focused on *identify more suitable strains* to develop better production technology and quality control methods

Government of India is promoting bio-fertilizers through various schemes

- National Mission on Sustainable Agriculture (NMSA)
- Paramparagat Krishi Vikas Yojana (PKVY)
- Rashtriya Krishi Vikas Yojana (RKVY)
- National Mission on Oilseeds and Oil Palm (NMOOP)
- National Food Security Mission (NFSM)
- Indian Council of Agricultural Sciences (ICAR)

BIO PESTICIDES

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BIO PESTICIDES

A Biopesticide is defined as "a form of pesticide based on micro-organisms or natural products".

According to the US Environmental Protection Agency (EPA), Biopesticides "**include naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs".**

- ***http://www.epa.gov/opp00001/biopesticides/***

Bio-pesticides belong to three categories:

1. Living organisms (i.e. natural enemies), which include invertebrates (e.g. predatory insects), nematodes and micro-organisms;
2. Naturally occurring substances which include plant extracts and semiochemicals e.g. insect pheromones);
3. Genetically modified plants that express introduced genes that confer protection against pests or diseases (so called plant incorporated products).

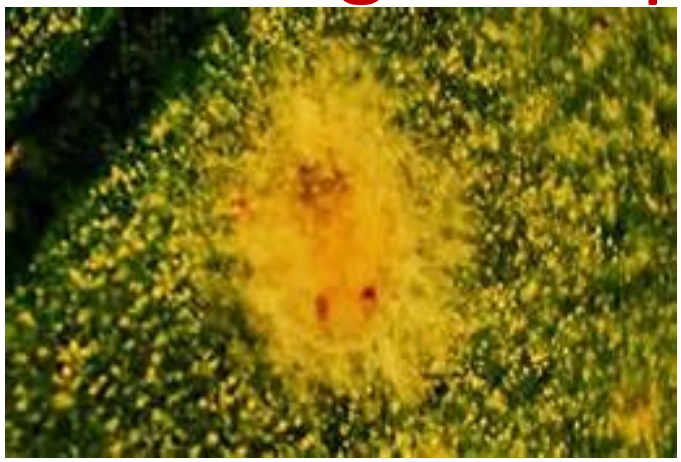
Types of biopesticides

- Microbial pesticides
- Plant-incorporated-protectants (PIPs)
- Biochemical pesticides
- Botanical pesticides
- Biotic agents (parasitoids and predators)

Microbial Pesticides

- Microbial pesticides are composed of microscopic living organisms (viruses, bacteria, fungi, protozoa, or nematodes) or toxin produced by these organisms
- Applied as conventional insecticidal sprays, dusts, or granules.
- Their greatest strength is their specificity as most are essentially nontoxic and non pathogenic to animals and humans.
- Microbial pesticides includes insecticides, fungicides, herbicides and growth regulators of microbial origin.

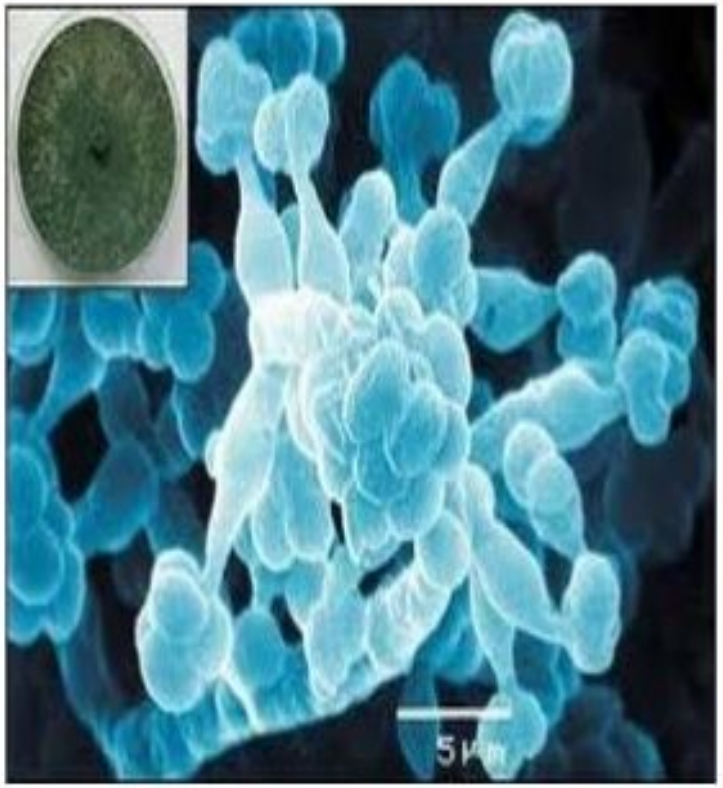
Working examples of biocontrol



- Several insect-pathogenic fungi are used as microbial control agents, including *Beauveria*, *Metarhizium*, and *Paecilomyces*.
- These are most often used against foliar insect pests in greenhouses or other locations where humidity is relatively high.

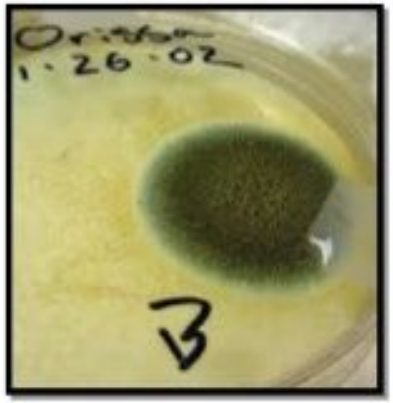
Trichoderma is….

- Very effective biological agent
- Free living
- Highly proliferating
- Non- pollutive
- Easily accessible
- Non phytotoxic
- Systemic ephemeral
- Readily biodegradable
- Cost effective
- Synergistic effect
- Longer shelf life
- Greater compatibility

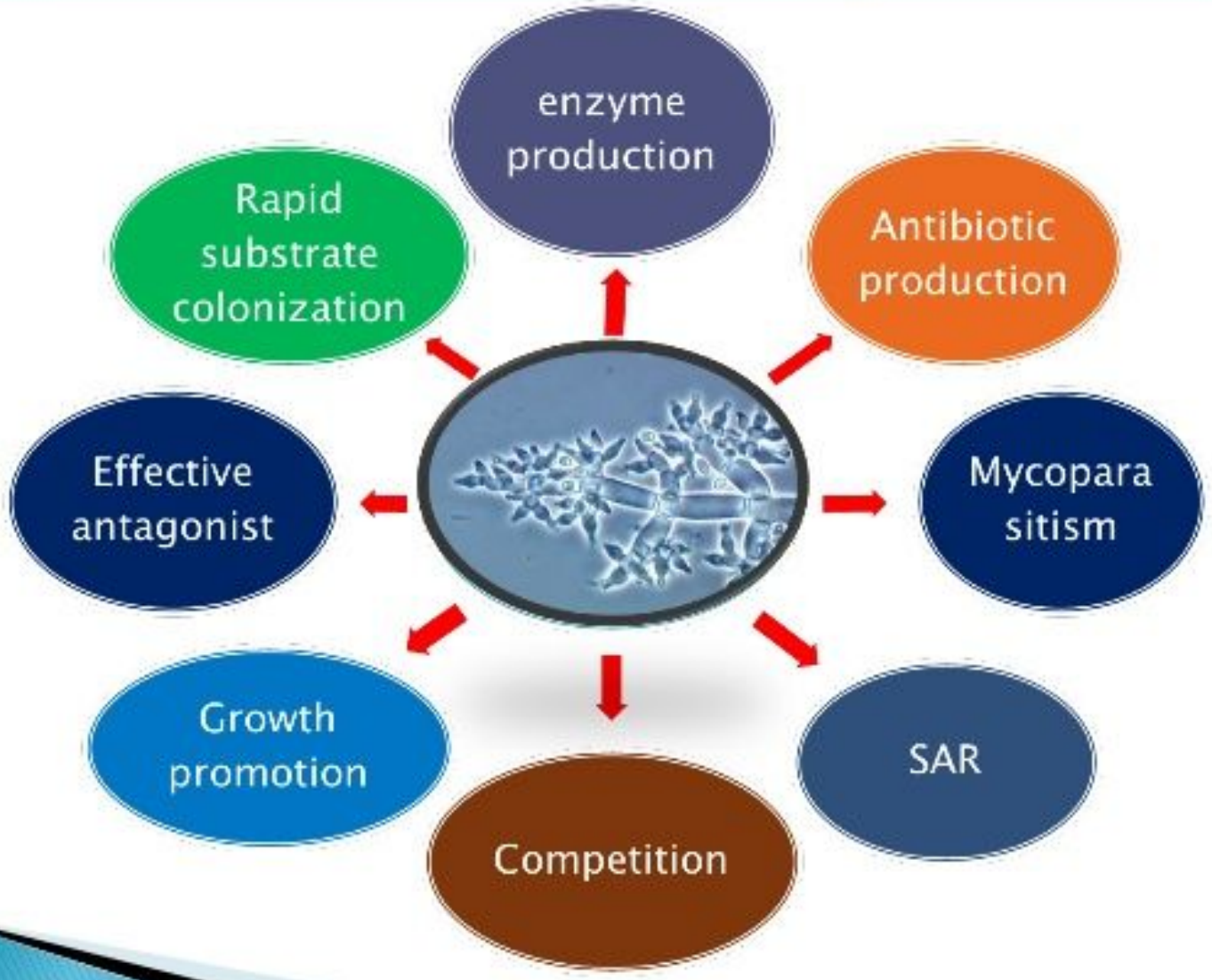


General Characters of *Trichoderma spp.*

- Cultures are fast growing at **25-30° C**
- Conidia forming within on week in compact or loose tufts in **shades of green** or **yellow or less frequently white**
- Yellow pigment may be secreted into the agar, specially on PDA
- A characteristic **sweet or 'coconut' odour** is produced by some species



Potential bio control activities exhibited by Trichoderma

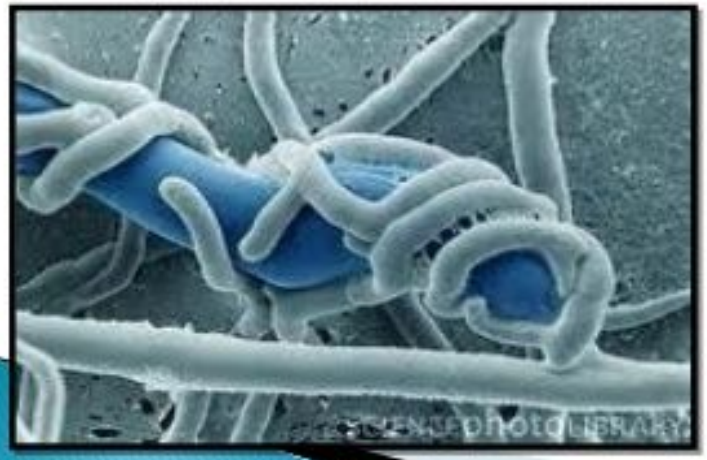


Kamala and Indira, 2012, Manipur

Mycoparasitism

- Antagonist fungi parasitize other pathogenic fungi
- Hyphae of *Trichoderma* either grow along the host hyphae or coil around it

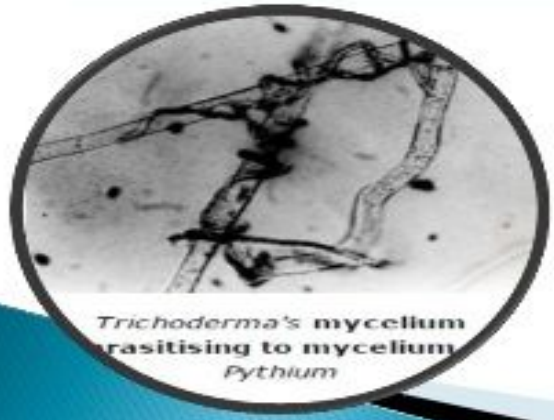
E.g. : ***T. harzianum*** **and** ***T. hamatum*** **were mycoparasite of both** ***Scelerotium rolfsii*** **and** ***R. solani***



Interaction –

- Coiling of hyphae around the pathogen,
- Vacuolization,
- Penetration by haustoria and
- lysis (Omero *et al.*, 1999).

- Recognize and attach to the pathogenic fungus and excrete extra-cellular lytic enzymes like β-1,3-glucanase, chitinase, proteases and lipase (Schlick *et al.*, 1994).



Application methods

1.Seed treatment

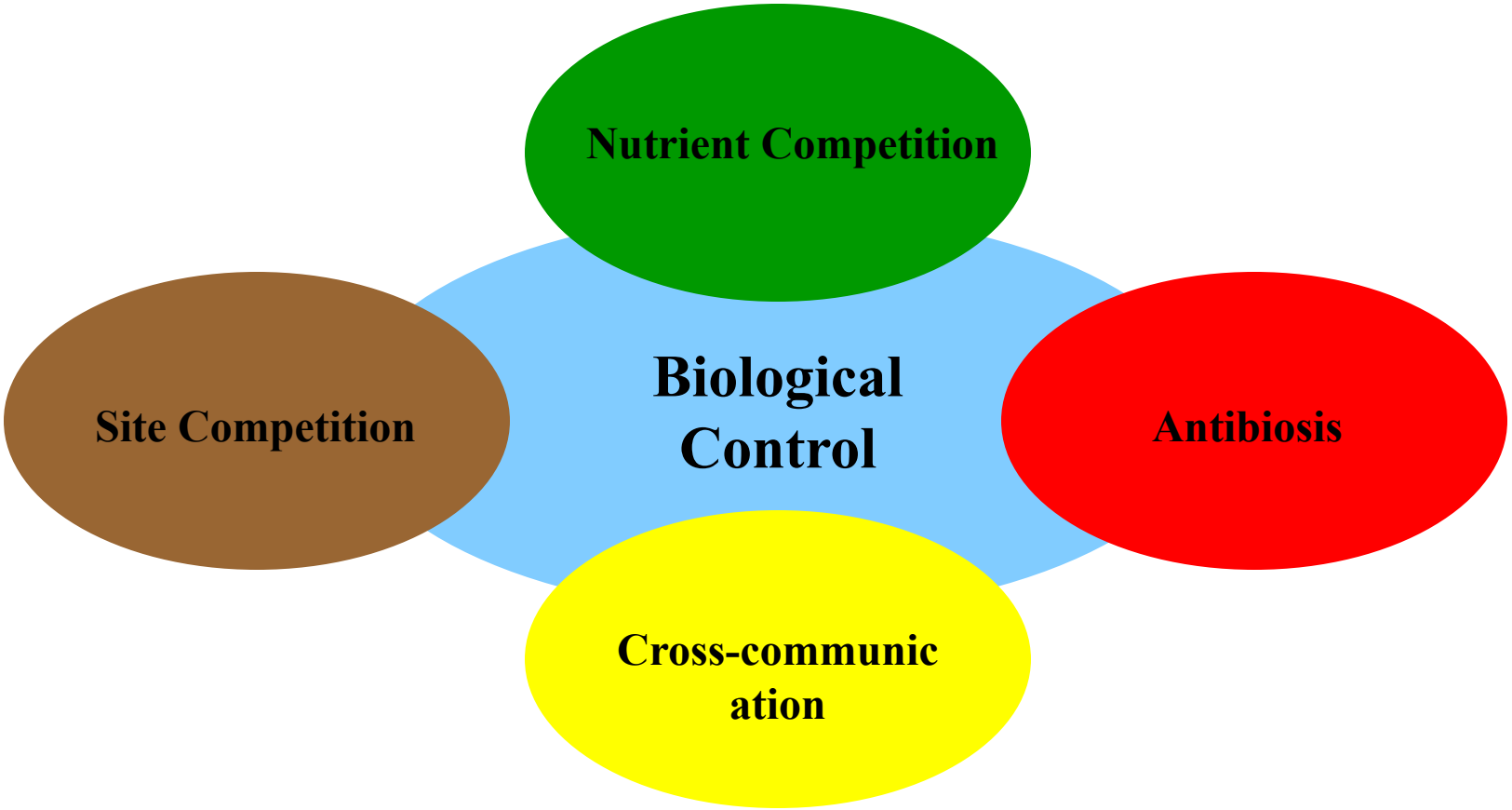
- **Dose**: @5 g /kg of seed
- **Method**: Make a paste or slurry adding 5 g in 10-20 ml of water . Pour 1kg of seed on to the paste or slurry and mixed properly to coat the seeds uniformly .Shade dry the coated seeds for 20-30 minutes before sowing

2.Tuber/Rhizome/Cutting treatment:

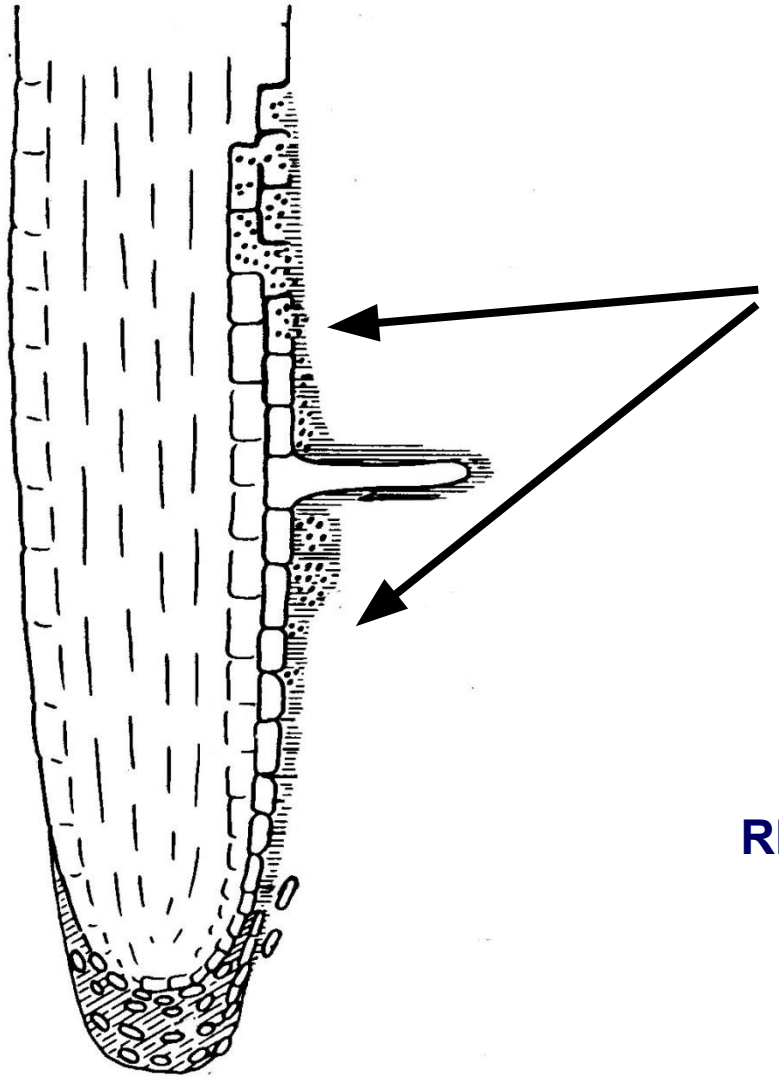
- **Dose**: @ 10 g /litre of water
- **Method**:Dip the tuber / rhizome/cuttings in the suspension prepare @ 10 g /litre of water. Shade dry for 15 minutes before planting

(Medhi,2009)

How does Biological Control by Pseudomonads work?



The Rhizosphere



The zone of soil influenced by the plant root

Plants can exude *ca*. 70% of fixed carbon through their roots

Rhizosphere is a dynamic environment

Entomopathogenic *Fungi*

Metarhizium anisopliae

Beauveria bassiana infection of Clover Worm

Beauveria bassiana infection of worm within woody substrate

Entomopathogenic fungi -in Insect Control

The Pests which are difficult to control by Pesticides can be controlled by Biopesticides

→ Beauveria infected Helicoverpa

→ Paecilomyces infected tea mites

→ Metarhizium infected rice bugs

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Lecanicillum lecanii (*Verticillum lecanii*) infects Sucking pests

Whitefly

Greenscale

Aphids

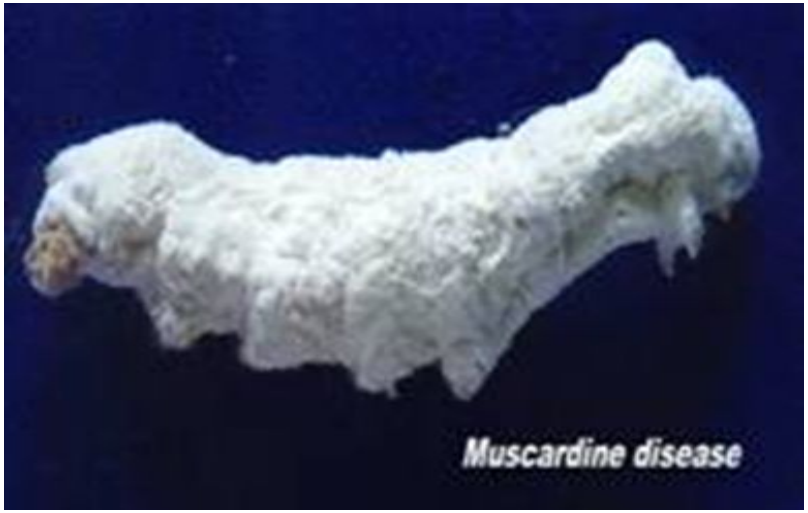
Mealybugs

Lecanicillum lecanii

Beauveria bassiana infects Sucking and Caterpillars pests

Lygus

Spider



Silk worm

Hairy caterpillar

Beauveria bassiana

coffee-borer beetle

Thrips

Armyworm larva

White fly

Metarhizium Anisopliae infects Weevils, Beetles and Grasshopper

Weevils

Grasshopper

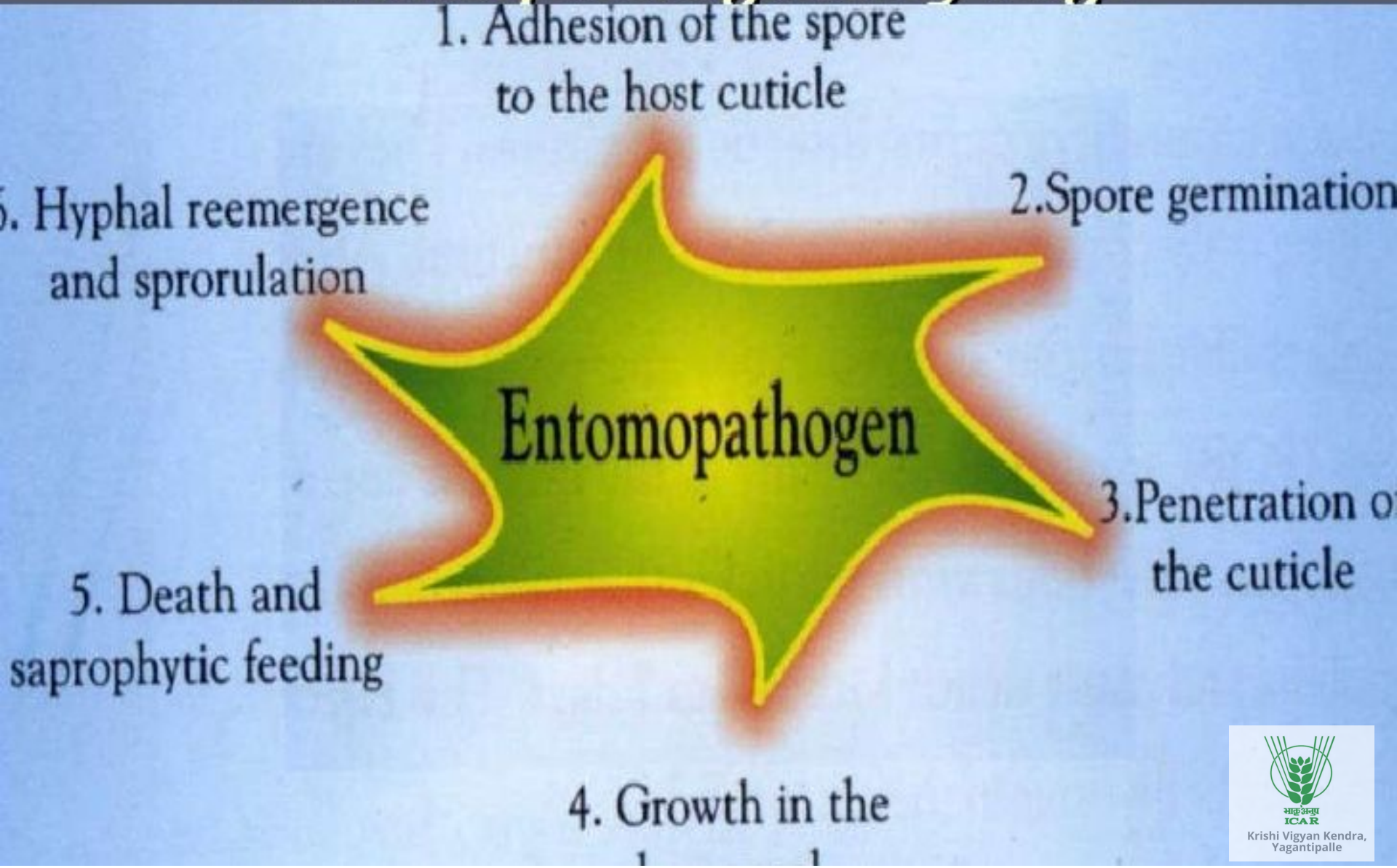
Metarhizium Anisopliae

Beetles

Table: 7 Selected metabolites of important Entomopathogenic fungi

Pathogen	Metabolite
Metarhizium anisopliae	Destruxins (>27 types), cytochalasin
Beauveria bassiana	Bassianin, beauvericin, bassianolide, tenellin
Beauveria brogniartii	Oosporein
Paecilomyces fumosoroseus	Beauvericin, beauverolies, pyridine-2,6-dicarboxylic acid
Verticillium lecanii	Dipcolonic acid, hydroxycarboxylic acid, vertilecannins, bassianolide
Tolypocladium spp.	Cyclosporin, efrapeptins (5 types)
Hirustella thompsonii	Hirsutellin A, hirsutellin B, phomalatone

Mode of action of Entomopathogenic fungi



Botanicals

Chrysanthemum sp.

Annona squamosa

Tagetes erecta

Ipomoea fistulosa

Ricinus communis

Allium cepa

- Pesticides derived from plants
- Generally act in one of two ways:
  - contact poison
  - stomach poison
- About 2,50,000 plant species evaluated
- 2121 useful in pest management
  - 1005 exhibited insecticidal activity
  - 384 antifeedants
  - 297 repellents
  - 27 attractants
  - 31 growth inhibiting properties

Purohit and Vyas, 2004

MODE OF ACTION OF BOTANICALS

- Ovipositional deterrent
- affects the egg laying and egg hatching
- may be due to strong odour of product or presence of substance which causes malfunctioning of ovariole in females. eg. *Pongamia pinnata*, *Annona squamosa*
- Ovicidal
- kill eggs and disrupts embryonic development so prevents hatching of eggs. Eg. *Annona squamosa, Parthenium* sp.
- Attractants eg.
- Repellents eg. Fennel (*Foeniculum vulgare*), *Eucalyptus globulus, Moringa oleifera, Allium cepa, Mentha*
- Feeding deterrents/ Antifeedants
- Gustatory substances which causes the pest to stop feeding and starve to death or causes cessation of feeding. Eg. *Melia azedarach,* Neem, Garlic, Datura

Contd....

- Antigonadal agents
- Vapours of oil of *Acorus calamus* reported to inhibit the development of ovaries of a no. of stored grain pests
- In male insects it showed sperm malformation and agglutination
- Insect growth regulators eg. *Lantana* sp., *Pongamia pinnata*
- Physiological effects
- slow necrosis of mid gut epithelial cells
- reduction in size and no. of cells
- Malformation of circular and longitudinal muscles or welling of organelles when taken as stomach poison
- Neurotoxin

Table: 13 Important plants with pesticidal activity

Common name	Scientific name	Plant parts used	Active principle
Custard apple	*Annona squamosa*	Leaves and bark	Annonin, squamocin
Pongam	*Pongamia glabra*	Leaves, fruits, seeds, oil roots and flowers	Pongamol, Pongapin, pongone, karanjanin
Eucalyptus	*Eucalyptus globulus*	Leaf and oil	Camphene, limonene, linalool, α- terpienol
Moringa	*Moringa olefifera*	Leaves, flowers	Moringyne
Clerodenderon	*Clerodenderon indicum*	Leaves	Trans- decalin, clerodin
Ipomoea	*Ipomoea fistulosa*	Leaves, flowers and whole plant	Ipomomin, isoergin, ergine, ipalbdinium
Murraya	*Murraya koenigii*	Leaves and bark	Murraxonin, murrayanone
Jatropha	*Jatropha curcas*	Leaves, seed, seed cake, oil	Jatrophin, curcusone, jatrophol

Common name	Scientific name	Plant parts used	Active principle
Datura	Datura strmonium	Leaves, fruits, dried seeds, roots	Atropine, hyoscyamine
Tobacco	*Nicotiana tabacum*	Leaves, whole plant	Nicotine, nornicotine, anabasine
Sweet flag	*Acorus calamus*	Rhizomes	Calamol, α asarone, β asarone
Lantana	*Lantana camera*	Leaves, whole plant	Lantonolic acid, lantic acid
Indian *Aloe*	*Aloe vera*	Leaves, rhizomes	Aloesin, aloin
Calotopis	*Calotropis gigantea*	Leaves, roots	Calatropin, calatoxin
Mint	*Mentha spicata*	Leaves, flowers, whole plant, oil	Menthole, limonene, menthone
Ocimum	*Ocimum sanctum, O. basilicium*	Leaves, stems, whole plants, oil	Juvocimene- I, II, ocimin
Onion	*Allium cepa*	Bulb	Oleic acid, cepocycle-D, α and β tocopherols

Common name	Scientific name	Plant parts used	Active principle
Garlic	*Allium sativum*	Leaves, flowers, whole plant, bulbs	Allicin, diallyl sulphide
Chilli	*Capsicum annum*	Leaves and fruits	Capsacin
Marigold	*Tegetes erecta*	Leaves, flowers, roots	Mycene, tagetone. allopatulein
Lemon grass	*Cymbopogon marginatus*	Leaves and roots	Cymbopogon, Cymbopogonal
Turmeric	*Curcuma longa*	Rhizomes	Curcumol, curcumin
Ginger	*Zingiber officinali*	Rhizomes	Gingerols, arcurcumene
Khejiri	*Prosopis julifora*	Leaves and seeds	Juliprosopine, prosopidione, juliflorinine
Ardusa	*Ailanthus excelsa*	Leaves	Ailanthone
Castor	*Ricinus communis*	Leaves and oil	Ricin, ricinnie

Source: Dodia *et al.* (2008)

Use of Bioinoculants is the key For Sustaining Agriculture

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THANK YOU

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