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Microbial Based Biopesticides: Present Status and Future Prospects

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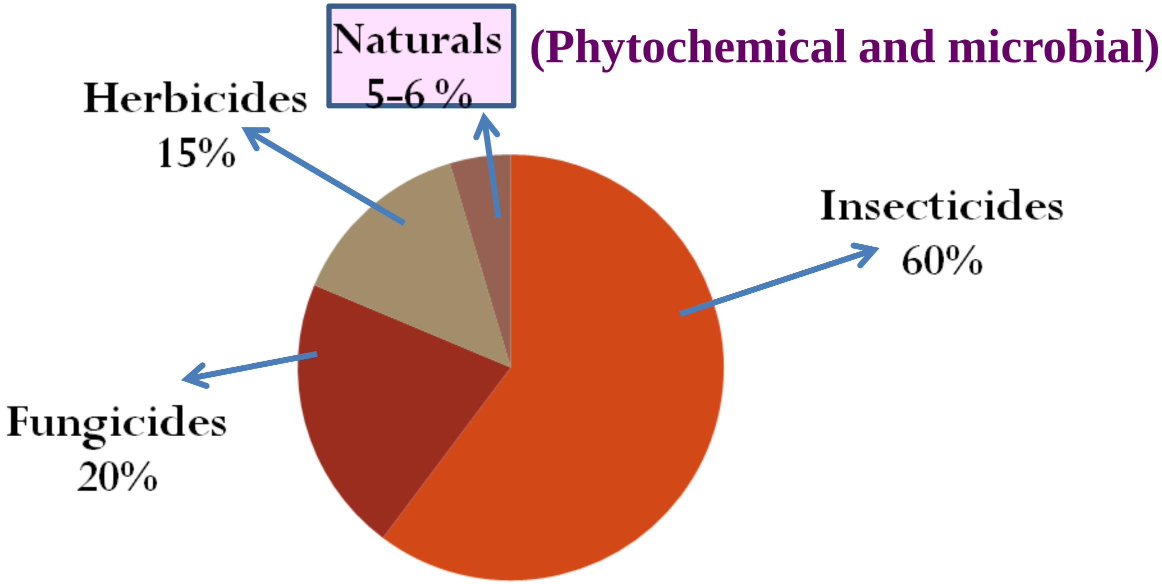
FOOD GRAINS PRODUCTION STATUS

- Agriculture in India accounts for 17.4% of GDP
- Food grain production has increased many fold from around 50 million tons in 1950-51 to 308.65 million tons in 2020-21
- Requires to produce an additional 6.4% million tons annually to meet 2030 target of around 360 million tons
- Need to produce more food per unit of limited resource of land
- **Estimated crop losses (30-40%)**

CROP LOSS ASSESSMENT

1,00,000 10,000 1,000 30,000	**microbial** Pathogens species of **Insects** species of **Nematodes** species of **Weeds**
2540	Vertebrate Pests
Pre-harvest loss **Post-harvest loss**	25-30% 5-10%

Pesticides Use Pattern in India



Total pesticides registered	**299**
Total formulations registered	**746**
Pesticides included in Insecticide schedule	**939**

India uses about 600 g a.i. ha^{-1} as compared to 1-12 kg a.i ha^{-1} in other countries

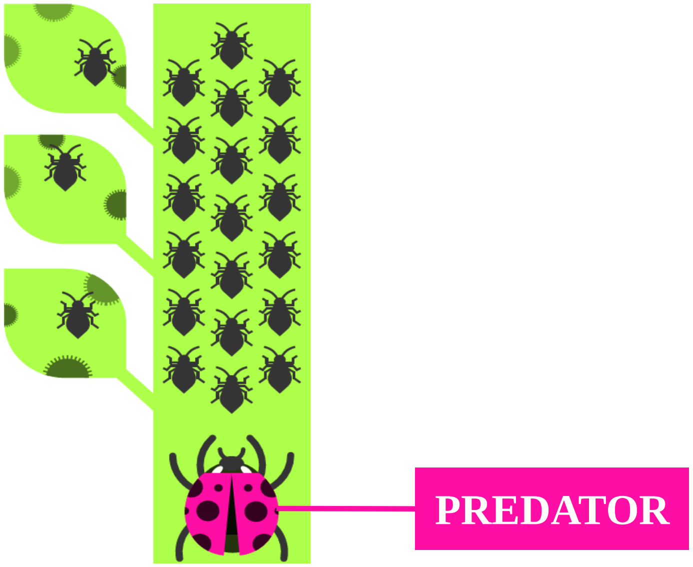
Risks with Conventional Pesticides

- Pesticide poisoning and health hazards
- Pesticide contamination of food commodities
- Endocrine and reproductive defects
- Adverse effects on non-target organisms
- Elimination of natural enemies of pests
- Upsetting the ecological balance
- Environment pollution
- Bioaccumulation/ bio-magnification of residues
- Pest resistance and pest resurgence
- Adverse effect on international trade

CROP PROTECTION PRODUCTS FROM BIORESOURCES

- Biological Control
- <u>Microbial Biopesticides</u>
- Agricultural antibiotics
- Botanical pesticides
  - Safe to environment
  - Safe to non-target organisms
  - Biodegradable
  - Non toxic to non-target organisms

BIOLOGICAL CONTROL



• Biological control involves introducing natural enemies of insect pests which include **predators, parasitoids, pathogens, and competitors**

• However, introducing new species into an ecosystem can have unexpected and damaging consequences

Microbial Biopesticides

(Agricultural Biologicals)

Bacterial biopesticides

Crystalliferous spore formers

Obligate pathogens

Facultative pathogens

Fungal biopesticides

Pyrenomycetes

Hyphomycetes

Zygomycetes

Laboulbeniales

Streptomycetes

Viral biopesticides

Baculoviruses

Nucleopolyhedros

Granuloviruses

Iridoviruses

Parvoviruses

Poxviruses

PolyDNAviruses

Nodaviruses

Picrona like viruses

Tetraviruses

Nematode biopesticides

Steinemema genus

Heterorhabditis genus

Protozoan biopesticides

Nosema genus

Vairimorpha genus

Bioactive secondary metabolites of bacteria, fungi, viruses and EPNs

Microbial control agent	Tradenames of biopesticides	Target pests
Bacteria		
Bacillus thuringiensis subsp. *aizawai*	Agree WG and XenTari DF	Lepidoptera
B. thuringiensis subsp. *israelensis*	Mosquito Beater WSP	Diptera
B. thuringiensis subsp. *kurstaki*	CoStar, DiPel ES, Monterey B.t., and Thuricide	Lepidoptera
B. thuringiensis subsp. *tenebrionis*	Novodor FC	Coleoptera
Paenibacillus popilliae	Milky Spore Powder	Japanese beetle, *Popillia japonica*
Fungi		
Beauveria bassiana	BotaniGard ES, Mycotrol-ESO, Myco-Jaal, and Naturalis-L	One or more pests of Acarina, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, Thysanoptera, and others
Hirsutella thompsonii	ABTEC Hirsutella	
Isaria fumosorosea	NoFly WP and Pfr-97 WDG	
Lecanicillium lecanii	Phule Bugicide	
L. longisporum	Vertalec	
Metarhizium anisopliae	BioCane, Metarril and Ory-X	
M. brunneum	Met52 EC	
Paecilomyces lilacinus	MeloCon WG	Plant-parasitic nematodes
Nematodes		
Heterorhabditis bacteriophora	Nemasys and Terranem	Several orders of soilborne pests
Steinernema carpocapsae	Ecomask and NemAttack	
S. feltiae	Entonem, Fungus Gnat & Rootknot Exterminator, and Scanmask	
H. heliothidis and *S. carpocapsae*	Double-Death	
Viruses		
Granulovirus (GV)		Lepidoptera
Cydia pomonella GV	CYD-X and MADEX HP	
Nucelopolyhedrovirus (NPV)		
Helicoverpa zea NPV	Gemstar LC	
Spodoptera exigua NPV	Spod-X LC	

MICROBIAL INSECTICIDES
(Entomopathogenic Bacteria)

- Microbial insecticides are single cell organisms which are mass produced for use against plant diseases and insect pests damaging agricultural crops

- These are sourced from entomopathogenic bacteria, entomopathogenic fungi, actinomycetes, viruses, protozoa or nematodes

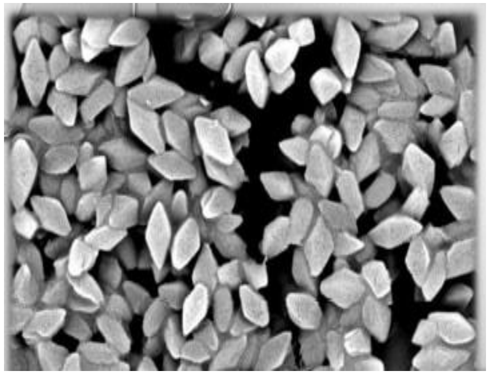
- Some prominent Entomopathogenic Bacteria include:

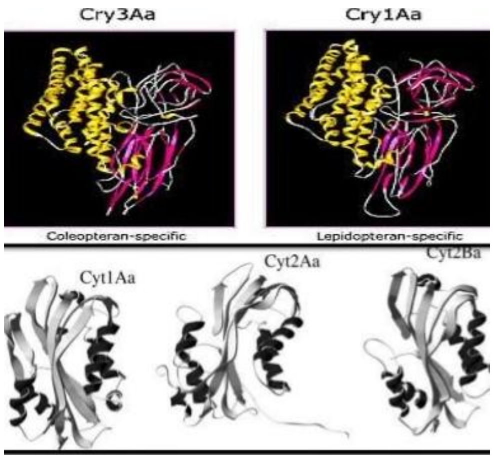
Bacillus species	*Burkholderia rinogensis*	*Chromobacterium substsugae*
Pseudomonas spp.	*Saccharopolyspora spinosa*	*Streptomyces species*
Isaria fumosorosea		

- Most potential Bacillus species are:

B. thuringiensis (Bt)	*B. popilae,*	*B. sphaericus*
Bacillus moritai	*B. licheniformis*	*B. subtilis*
B. pumilus	*Coccobacillus acridorum*	

Bacillus thuringiensis (Bt)





- It is a spore forming gram positive crystal forming soil bacterium

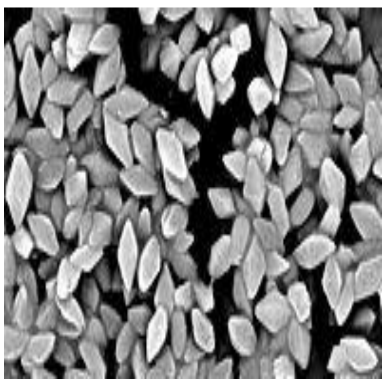
- It produces para-sporal body comprising of crystal and tightly packed protein (δ-endotoxin and exotoxin) or Cry protein) that kills certain insects

- Commercially produced worldwide using fermentation technology and formulated as Dust, WP, EC etc.

- Following *Bt* strains produce insect killing toxins:
  - *Bt* var. *kurstaki* (*Btk*) (Toxic to flies)
  - *Bt* var. *israelensis* (*Bti*) (toxic to flies)
  - *Bt* var. *tenebrionis* (*Btt*) (toxic to beetle larvae)
  - *Bt* var, *san diego* (Btsg) (toxic to beetle larvae)

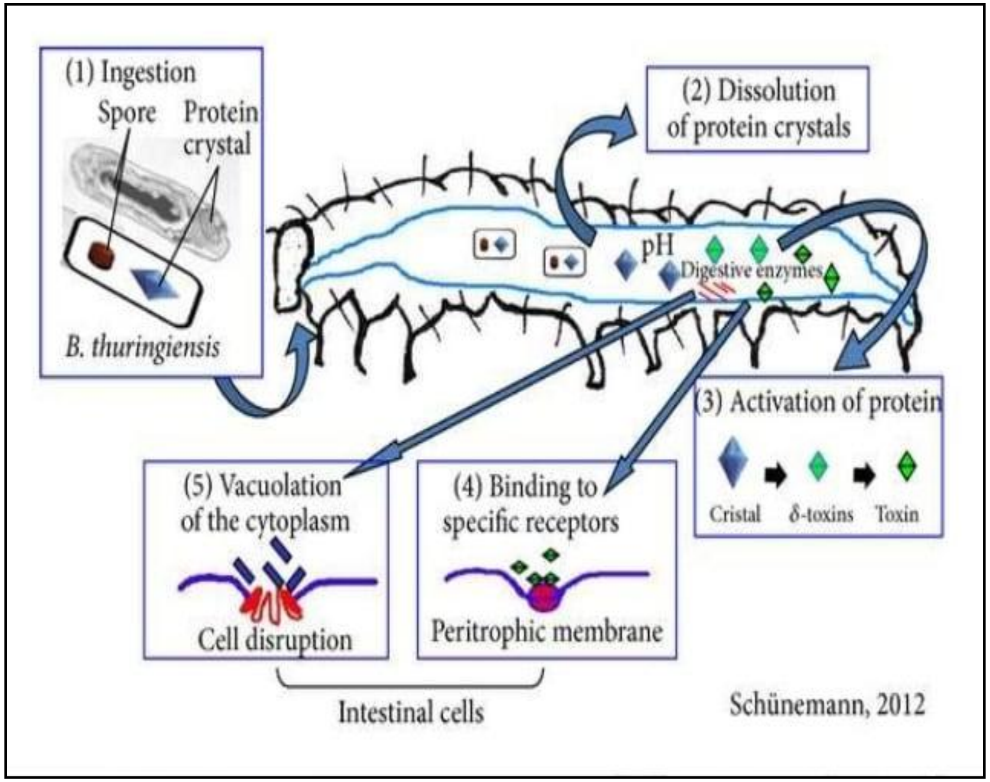
Toxic genes have also been incorporated in crop plants

BT - MODE OF ACTION



- It is highly specific for the control of insect species typically i) Lepidoptera (butterflies/moths), ii) Coleoptera (beetles), and iii) Diptera (flies/mosquitos)
- The Bt is effective when eaten in sufficient quantity by the specific insects with a alkaline gut pH (typically butterflies, moths, beetles, flies and mosquitoes)
- After ingestion, the spores feed on intestinal flora and later burst releasing the protein toxin (Crystalline protein) damaging the gut lining
- Affected insects stop feeding and die from the combined effects of starvation, tissue damage and gastrointestinal infections by other pathogens like bacteria and fungi.
- The crystal is short lived as it breaks down after exposure to sunlight so it is appropriate to spray it in cloudy days.
- Bt is usually formulated with insect attractants to increase the probability that the target insect will ingest the toxin.

Mode of Action



APPLICATION

Bt formulations are sprayed when caterpillars are small

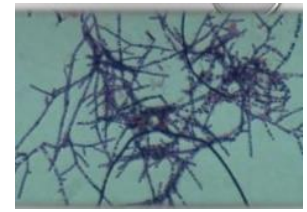
Insect-pest must ingest the bacteria when they are feeding

Spray is done during evenig or during cloudy day but not during rains

COMMERCIAL Bt PRODUCTS

Organism	Trade name	Target pest
Bacillus thuringiensis var. kurstaki	Dipel, Javelin, Thuricide, Worm.Attack, Biovit, Thuricide, Halt, Caterpillar Killer, Condor, Bactospeine, SOK-Bt	Moths
Bacillus thuringiensis var. aizawai	Certan, Agree	Wax moth
Bacillus thuringiensis var. israelensis (Bti)	Vectobac, Teknar, Bactimos, Skeetal, Mosquito Attack	Mosquitoes, black flies
Bacillus popillae and B. lentimorbus	Doom, Japidemic, Grub Attack, Japanese beetle	Japanese beetle
Bacillus thuringiensis var. San diego	M-One	
Bacillus thuringiensis var. Kurstaki plus beta-exotoxin	Javelin	Armyworm and other moths
Bacillus thuringiensis var. tenebrionis	Novodor	Colorado potato beetle
Bacillus thuringiensis var. galleriae	Spicturin	Larvae and moths and forest insects

ACTINOMYCETES



These are large group of Gram-positive bacteria that grow as hyphae like fungi

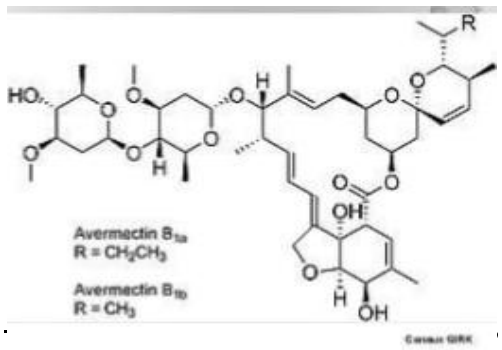
Mortality s due to secretion of bioactive materials which stimulate GABA system or disruption of nicotinic acetylcholine receptors

Some prominent compounds are;

1. Avermectins
2. Milbemectins
3. Spinosads

Avermectins and Milbemectins

- **Avermectin**



Isolated

Major homologues: A1a, A2a, B1a, B2a

Minor analogues A1b, A2b, B1b, and B2b

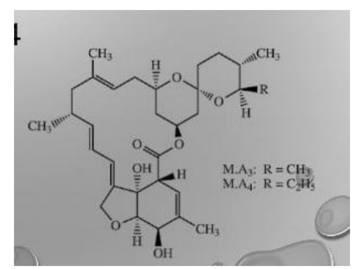
B1b and B2b are effective avamectins

show improved thermal stability and greater water solubility

- **Milbemectin**

Isolated from *S. Hygroscopicum* var. Aureolacromosus

Major analogues: A3 and A4



The commercial products consist of avermectin B_1 which comprises of two components – avermectin B_1a (80-85%) and avermectin B_1b (15-20%)

AVERMECTINS

Exhibit anthelmintic, insecticidal and acaricidal activity against insect pests of crops and ornamental plants.

Used as veterinary medicine for treatment of internal and external parasites and mites of pets and livestock.

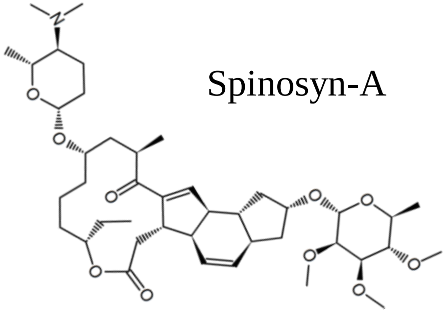
The products are available as
liquid, paste, tablet, chewable, feed premix, pour-on liquid, and as injectables

Semi-synthetic derivatives (ivermectin and emamectin benzoate) used to control many internal and external parasites like lice, hair mites and nematodes.

INSECTICIDAL SPINOSYNS

•Spynosyns are secondary metabolites derived from the aerobic fermentation of the soil actinomycete *Saccharopolyspora spinosa*

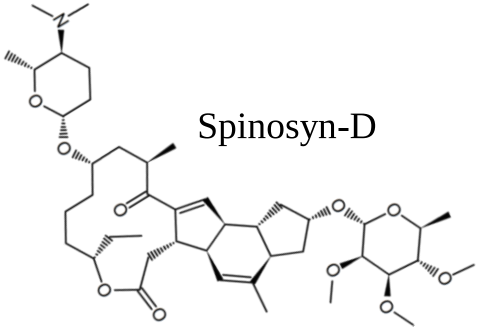
•Spinosad is comprised of two active ingredients namely spinosyn-A and spinosyn D in 85:15 ratio.



Spinosyn-A

•Spinosyn based products exhibit activity at par with some of the synthetic pyrethroid insecticides

•Pose reduced risk to mammals, birds, fish and are effective against a broad range of insect pests in agricultural crops



Spinosyn-D

•Unique mode of action: excitation of the insect nervous system, involuntary muscle contractions, prostration, tremors, cessation of feeding and paralysis

Entomopathogenic fungi (EPF)

(EPF) are capable of infesting insect pests and are thus a potential source of mycoinsecticides. Some potential EPFs include:

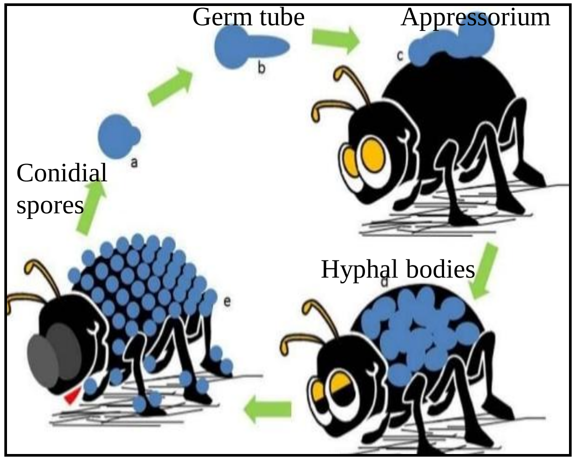
Metarhizium anisopliae,
M. flavoviride,
Beauveria bassiana,
B. brongniari,
Paecilomyces fumoso-roseus
Lagenidium giganteum
Hirsutella thompsoni
Paecelomyces lilacinous
Verticillium species

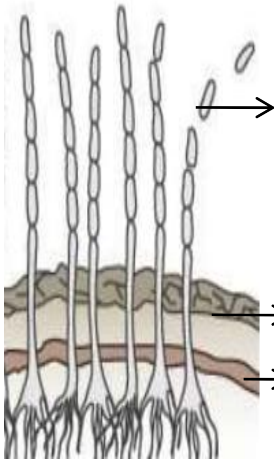
EPFs produce bioactive secondary metbolites like destruxins, beauvericin, cyclosporines, cytochalasins etc.

These are effective against insects pests/termites and act by mycelial penetration of the tissues and toxin production.

The infected insect dies within 3-5 days of hyphal penetration and conidiophore production on outside of the insect's body.

EPF mode of infection





Beauveria bassiana formulation or conidial spores discharged from an infected cadaver produces germ tube and appressorium formed enter into insect body, divide and produces hyphal bodies and invade the host tissue. Fungus emerges from the dead host produces more conidial spores to infect other insects.

EPF toxins like dexstruxines produced by *B. bassiana*, and beauvericin and beauverolides produced by *M. Anisopliae* are responsible for insect mortality

Pre-sowing soil application of *B. bassiana* and *M. Anisopliae* @ 10g/m2 against white grub and cut worm larvae, crickets, and Verticillium lacanii effective against Epilachna beetle

MYCOFUNGICIDES

Following fungi help control plant disease by stimulating plant host defense.

Trichoderma harzianum,
T. virens,
T. Viride
T. polysporum
T. hamatum

Germinating spores produce fungicidal compounds which suppress other soil-borne pathogens by hyperparasitism and exerting antibiosis through release of antifungal metabolites.

TYRICHODERMA - SECONDARY METABOLITES

T. harzianum

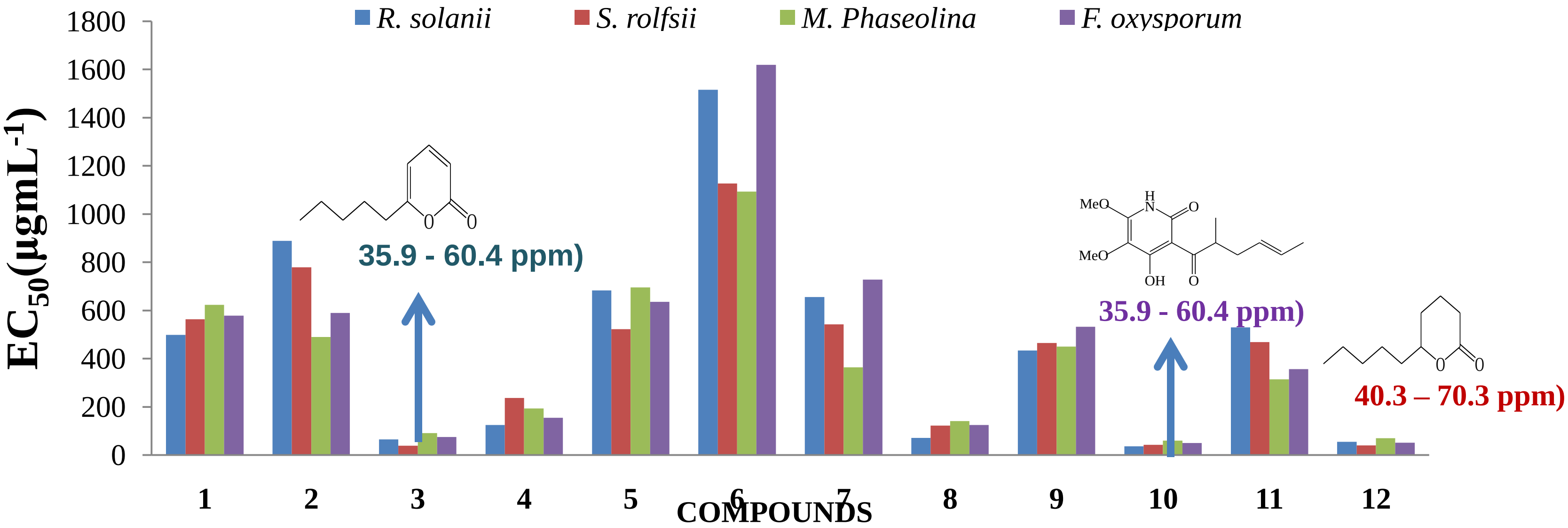
- 6-*n*-pentyl-2*H*-pyran-2-one (6-PAP)
- Harzianic acid
- Harzianopyridone
- Harziphilone
- 2-phenylethanol
- Trichoselin etc.

T. viride

- Viridepyranone,
- Dermadin and virdin
- Gliotoxin
- 6-pentyl-pyran-2-one
- Chrysophanol and emodin
- Trichodermin
- koeningic acid.

These secondary metabolites exhibit significant *in vitro* and *in vivo* control of several phytopathogenic fungi

Isolation, characterization and antifungal activity of *Trichoderma* (T-5) secondary metabolites



Sr. No.	Compounds	Sr. No.	Compounds
1	Palmitic acid	7	1-hydroxy-3-methylanthraquinone
2	1,8-dihydroxy-3-methylanthraquinone	8	δ-decalactone
3	**6-pentyl-α-pyrone**	9	Ergosterol
4	2(5H) furanone	10	**Harzianopyridone**
5	Stigmasterol	11	6-Methyl-1,3,8-trihydroxyanthraquinone
6	β-sitosterol	12	**6-(4-oxopentyl)-2H-pyran-2-one**

- **Oxo-pentylpyrone was most active followed by harzipyridone and pentyl pyrone**
- **Sterols and anthraquinone derivatives were less active.**

Pseudomonas fluorescens

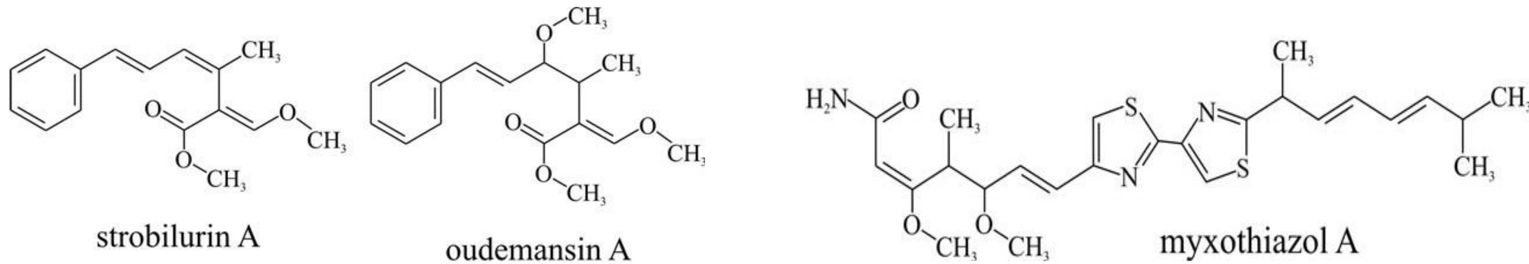
- The bacteria controls root rot and wilts diseases of banana, bean, cotton, groundnut, pigeon pea, soya, and tomato

- Pseudomonas formulations are effective against the rice blast and sheath blight of paddy

- Promote plant growth through the modulation of plant hormonal pathways

- The activity is attributed to bioactive secondary metabolites such as 2,4-diacetylphloroglucinol (DAPG), phenazines, pyrrolnitrin or alkylresorcinol

STROBILURIN FUNGICIDES



•Musilek in 1969 was the first to report the isolation of strobilurin-A From the fungus *Strobilurus tenacellus* and *Oudemansiella mucida* which feeds on decaying plant materials in the soil.

•Since then several strobilurins (B, C D, E, F_1, F_2, G, H, X) and others namely oudemansins and myxothiazoles have been reported.

•Strobilurins exhibit low mammalian and avian toxicity and are active at low application rates.

•Synthetic derivatives like azoxystrobin exhibit greater stability and excellent activity against plant pathogenic fungi including *Ascomycetes* (e.g. powdery mildews), *Basidiomycetes* (e.g. rusts), *Deutoromycetes* (e.g. blast) and *Oomycetes* (e.g. downy mildew).

BACULOVIRUSES

Vaculoviruses are rod-shaped double stranded DNA viruses that can infect and kill a large number of insect pests

NPVs infect immature larvae, sawfliea, mosquitoes, and shrimps

Baculoviruses have limited host range and generally do no allow for insect resistance to develop

Slow killing of target insect pests. To enhance effectiveness, several genes (e.g.Bt toxin, scorpion toxin, wasp toxin, and a neurotoxin like JH esterases can be expressed in NPVs

Larvae infected with GV and NPV usually die within 5-12 days after infection

Commercially produced on the host insects and formulated as liquid and dust formulation

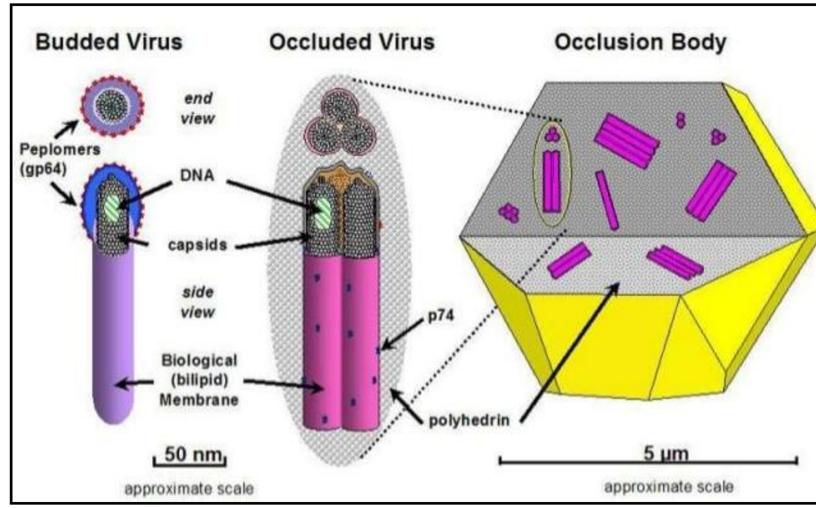
Nucleopolyhedroviruses (NPV)

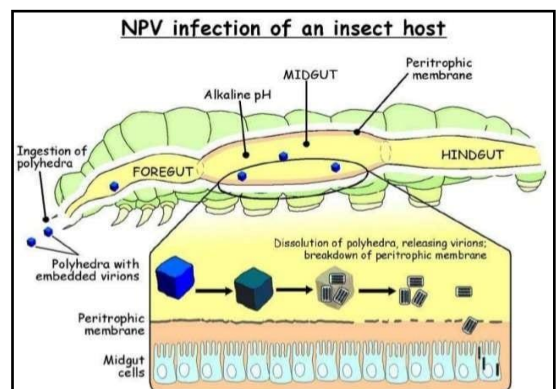
Baculoviruses are rod shaped budded or occluded DNA viruses

These are highly host-specific and pathogenic to lepidopteran (83%), hymnopteran (10%) & dipteran (4%) insect pests

Infection is by ingestion of foods or entry through skin cuticle

NPV causes stress, lethargy, and results in swelling, body discoloration, decomposition (liquificaction) and infected larvae hang from twigs and eventually die





Entomopathogenic nematodes (EPNs)

EPNs are insect-parasitic soil nematodes having high biocontrol potential for managing insect pests of agricultural importance.

- The major EPN species most commonly used in insecticidal preparations are:
  Steinernema carpocapsae, *S. feltiae*, *S. riobravis*, *S. scapterisci*, *S. thermophilum*; and *Heterorhabditis bacteriophora*, and *H. megidis*.

- Each EPN carrying specific symbiotic bacterium (e.g. *Xenorhabdus* and *Photorhabdus*) parasitise and kill a number of insect pests

- EPNs are safe to vertebrates, plants, non -target organisms and the environment

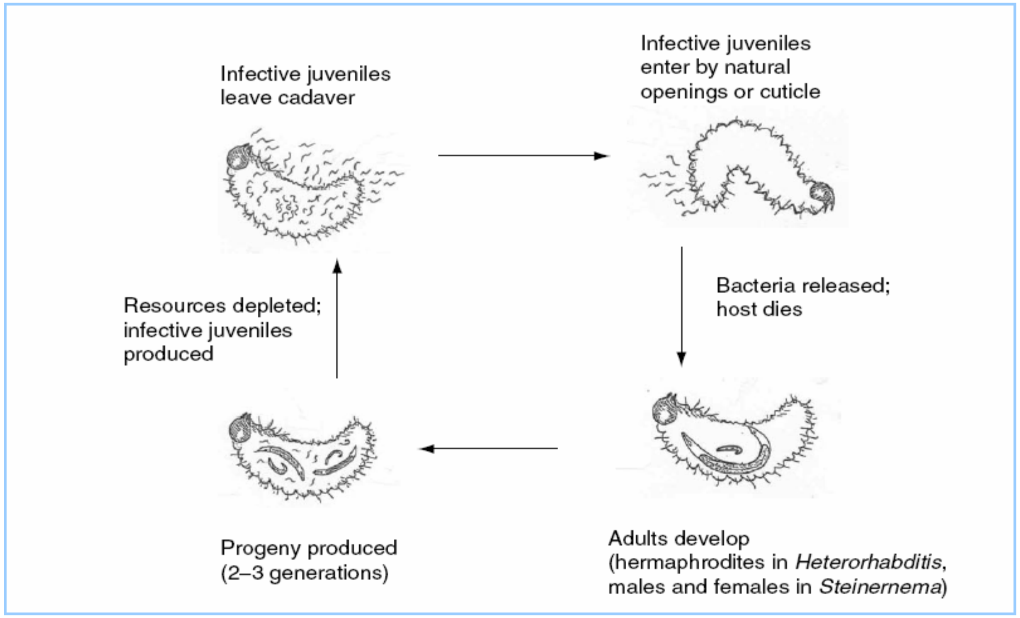
Mode of Infection

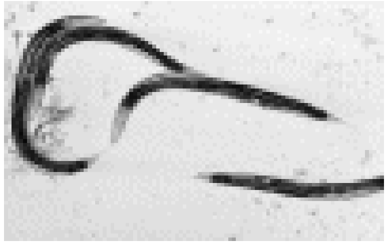
- Infective Juveniles (IJ) of both *Steinernema* and *Heterorhabditis* carries bacteria of genus *Xenorhabdus* and *Photorhabdus*
- IJ enters through the mouth, anus and/or spiracles and make its way to the haemocoel
- Some species may penetrate through intersegmental membranes of the insect cuticle
- In haemocoel, the IJ releases cells of bacterial symbionts from its intestine
- The bacteria proliferate in the nutrient-rich-insect haemolymph and the insect dies within 24-48 hours

EPN-Bacteria -Insect Complex

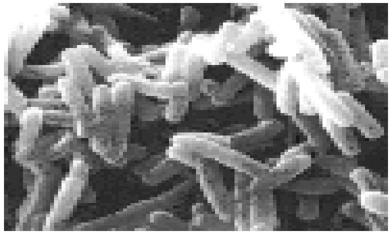
EPNs can be easily cultured, compatible with many pesticides and provide long-term control of a wide range of insect pests.

EPNs enter an insect gut and the infective juveniles release an associated bacteria (*Xenorhabdus or Photorhabdus)* which multiplies rapidly and cause host mortality





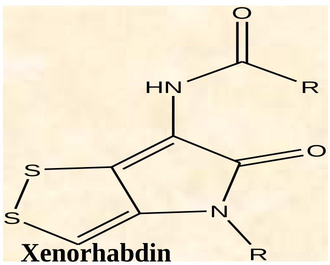
Steinernema

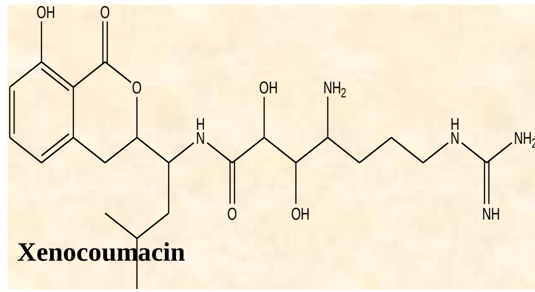


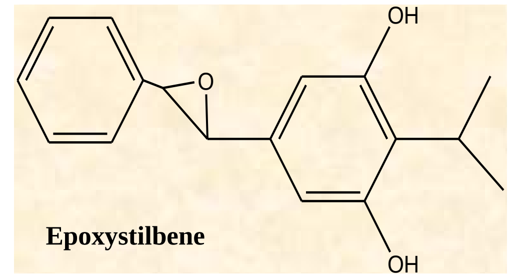
Photorhabdus

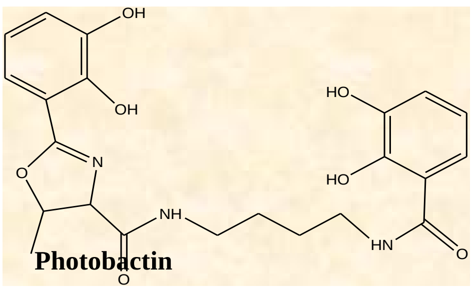
The nematodes provide shelter to the bacteria, which, in return degrade insect tissues and quickly kill the insect host within 1-2 days. Insecticidal activity is due to bioactive secondary metabolites produced by the EPNs

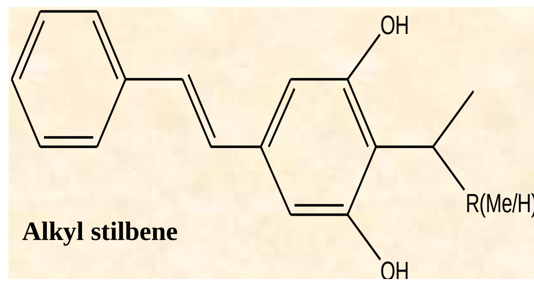
Bioactive secondary metabolites from *Xenorhadus assam* isolate against *Macrophomina phaseolina*

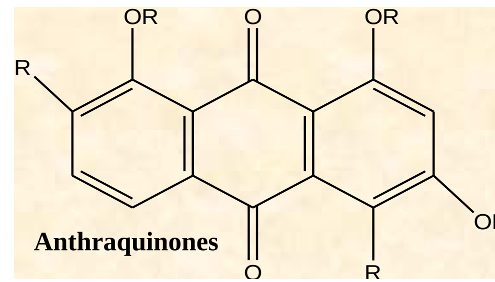

Xenorhabdin

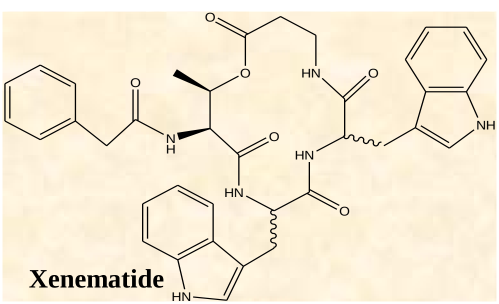

Xenocoumacin

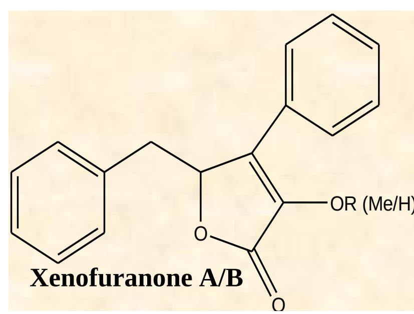

Epoxystilbene

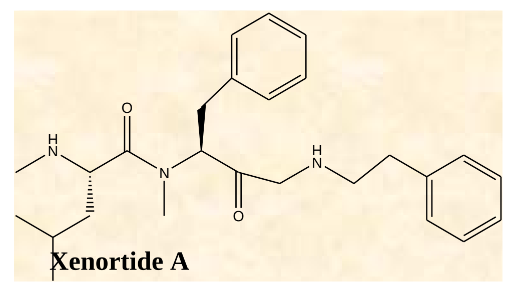

Photobactin


Alkyl stilbene


Anthraquinones


Xenematide


Xenofuranone A/B

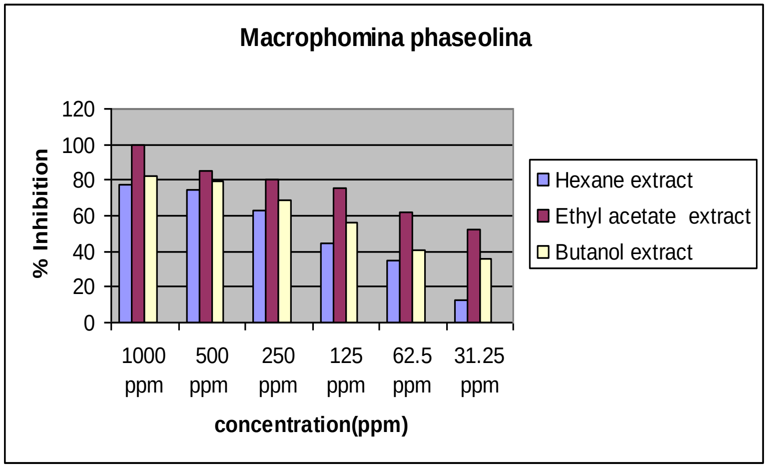

Xenortide A

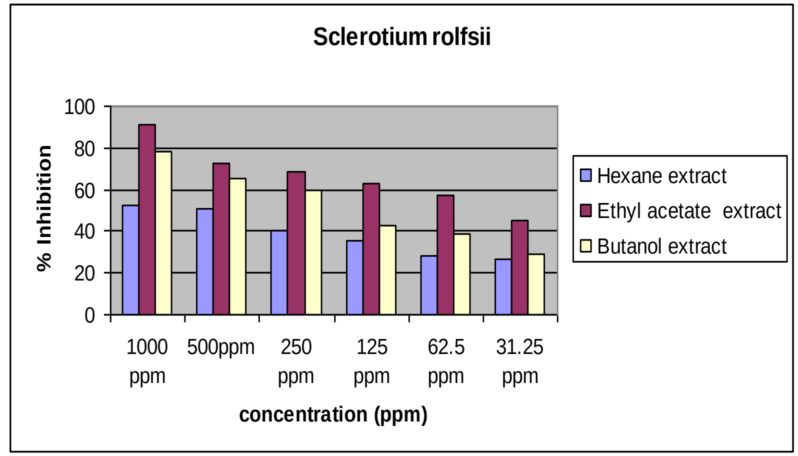
Bacterial metabolites exhibit strong antibacterial and antifungal activity

Antifungal activity (% growth inhibition) of secondary metabolites from *Xenorhadus assam* isolate against *Macrophomina phaseolina*

Organic extracts	Concentration (ppm)						LD_{50} (ppm)
	1000	500	250	125	62.5	31.25	
Hexane extract	72.2	74.4	62.78	44.67	34.4	12.78	179.57
Ethyl acetate extract	100	85.2	80	75.22	61.72	52.6	47.70
Butanol extract	82.2	79.56	68.4	55.8	40.4	35.8	153.097

Antifungal activity of solvent hexane extracts against *Macrophomina phaseolina* and *sclerotium rolfsii*





- Hexane and butanol extracts showed moderate antifungal activity against both the fungi

- Antifungal activity of **<u>Ethyl acetate</u>** extract was found to be the most active against *Macrophomina phaseolina*

- LD_{50} values for ethyl acetate extract was found to be 47and 48.62 ppm for *Macrophomina phaseolina* and *Sclerotium rolfsii*, respectively

EPNs TECHNOLOGY

***Galleria* cadaver based application technology (ICAR-IARI) for *Heterorhabditis indica* is highly promising for management of white grubs and apple root borers in sugarcane crop.**

A heat-tolerant EPN species *S. thermophilum* reported from ICAR-IARI has been licensed to Industry and a gel-based formulation ‘Pusa NemaGel’ is available for commercialization.

Microbial biopesticides Registered in India

S.No		S.No	
1	Ampelomyces quisqualis	11	*Trichoderma harzianum*
2	*Bacillus sphaericus*	12	*Trichoderma viride*
3	*Bacillus subtillus*	13	*Pseudomonas fluoewscens*
4	*Bacillus thuringiensis var. galleriae*	14	NPV of *H. armigera*
5	*Bacillus thuringiensis var. israelensis*	15	NPV of *S. litura*
6	*Bacillus thuringiensis var. kurstaki*	16	Kasugamycin
7	*Beauveria bassiana*	17	Validamucin
8	*Metaehizium anisoplae*	18	Aereofungin
9	Avermectins (Abamectin)	19	Spinosyns
10	Strobilurins		

Due to large biodiversity, huge potential exists for developing novel microbial biopesticides through biochemical, biotechnological, and nanotechnological interventions

Microbial Biopesticides
(CHALLENGES)

Natural crop protectants are biodegradable and exhibit inadequate shelf life, low persistence, slow action, and unpredictable performance under field conditions.

They suffer from drawbacks in term of their inactivation by UV light, sun light, heat, pH, moisture etc.

Bioefficacy and chemical profile of pesticidal plants and microbial agents vary as per the geographic, genetic, climatic, and/or seasonal factors.

Product standardization is a constraint as most of the phytochemical formulations contain multitude of active ingredients.

Microbial Biopesticides
(CHALLENGES)

Due to stringent biodiversity protection laws, many plants are included in the red list and thus their availability is restricted

Like synthetics, excessive and long-term use may result in development of resistance necessitating repeated applications

The high specificity of microbials is disadvantageous particularly when a complex of pest species needs to be controlled.

Since microbial formulations contain living materials, their efficacy is not consistent due to various biotic and abiotic factors

Microbial Biopesticdes
(OPPORTUNITIES)

Solubility, stability and efficacy of natural protectants can be enhanced by nanotechnological interventions

Better plant resources and microbial strains needs to be mined ad optimized culture and extraction technologies developed for better yield and cost-effective production.

New SOPs and analytical protocols for analysis and quality control of technical materials and formulations may be developed for better quality products.

Multidisciplinary R&D approach involving chemists, entomologists, plant pathologists, and engineers will be useful for addressing the emerging problems

धन्यवाद