MICROBIAL BIOTECHNOLOGY

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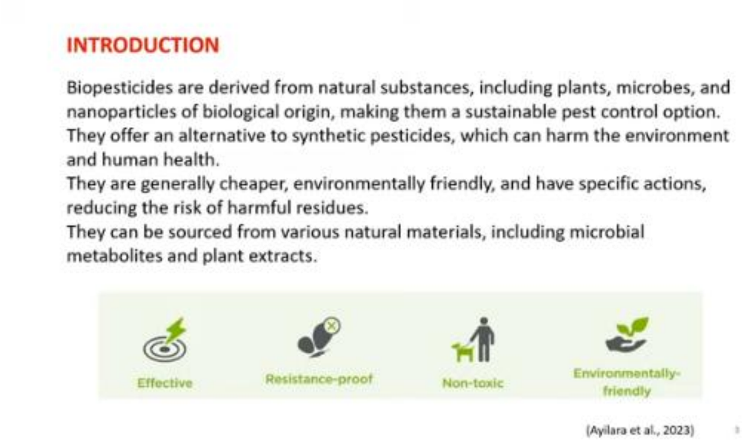
Lecture-25
Lec 25: Biopesticides and Integrated Pest Management

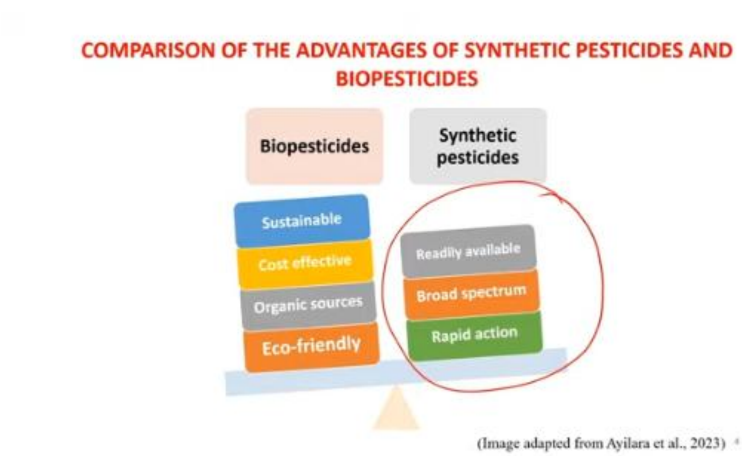
Hello friends, welcome to my course on microbial biotechnology. We are in module 7. Today, we will be discussing biopesticides and integrated pest management. A few concepts we will revisit here have already been discussed in earlier lectures. So, we have three sections here.

We will discuss these sections in detail as we come across them. First, we will have an introduction and comparison of the advantages of synthetic pesticides and biopesticides, then classification of biopesticides, natural enemies, plant-incorporated protectants, biochemical metabolites, botanicals, secondary metabolites, and the mode of action of semiochemicals. Biopesticides are derived from natural substances, including plants, microbes, and nanoparticles of biological origin, making them a sustainable pest control option. They offer an alternative to synthetic pesticides, which can harm the environment and human health. They are generally cheaper, environmentally friendly, and have specific actions, reducing the risk of harmful residues.

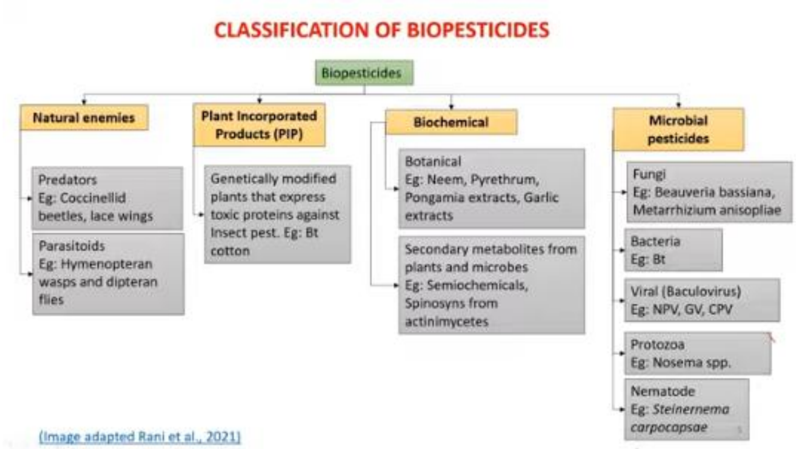
They can be sourced from various natural materials, including microbial metabolites and plant extracts. For the purpose of integrated pest management, we will discuss plant extracts briefly, but our focus will mostly be on the role of microbes. Comparing the advantages of synthetic pesticides and biopesticides, we see that biopesticides have more advantages because they are sustainable and cost-effective. We can obtain them from renewable sources, and they are eco-friendly. However, synthetic pesticides are readily available.



They are broad-spectrum and very rapid in their action. But from the point of view of environmental health and human health, the advantages of biopesticides outweigh those of synthetic pesticides. So, we can divide biopesticides into different classes, like natural enemies, predators, and parasitoids, which we discussed already in the earlier class. Then we have plant-incorporated products, PIPs. These are genetically modified plants that express toxic proteins against insects, like Bt cotton.



Then you have biochemicals and botanicals, mostly derived from plants. Then we also have biochemicals like secondary metabolites from plants as well as microbes, semiochemicals, spinosyns from actinomycetes, etc. Then we have microbial pesticides, where we use the whole microorganism like fungi, bacteria, and then viruses, which of course are not microorganisms, and then protozoa and also nematodes. We will be discussing all these and, to some extent, a little bit about the PIPs. So, natural enemies—we can just have a small discussion.



These are organisms that naturally control pest populations. They play a crucial role in maintaining ecological balance by keeping the pest population. Predators are organisms that hunt and consume pest organisms during their lifetime. They are essential in biopesticide strategies for controlling various agricultural pests. Then we have parasitoids.

These are organisms that lay their eggs on or in a host insect. The developing larvae feed on the host, eventually killing it. Parasitoids are highly specific, making them effective in targeted pest control. Then we have plant-incorporated protectants, which are pesticidal substances produced by plants as a result of genetic engineering. These substances are intended to protect the plants from pests by incorporating specific genes into the plant's genome.

PIPs are created by transferring specific genes from other organisms, like bacteria, into the plant. These genes enable the plant to produce pesticidal substances, typically proteins that target and kill specific pests. So, what is the mechanism of action of these PIPs? For example, in Bt crops, the Bacillus thuringiensis gene encodes a protein that, when ingested by certain insect larvae, binds to receptors in the insect's gut, causing cell lysis and death. The target of these kinds of toxins is very specific.

PLANT INCORPORATED PROTECTANTS

Plant-Incorporated Protectants (PIPs) are pesticidal substances produced by plants as a result of genetic engineering. These substances are intended to protect the plants from pests by incorporating specific genes into the plant's genome.
PIPs are created by transferring specific genes from other organisms (e.g., bacteria) into the plant. These genes enable the plant to produce a pesticidal substance, typically a protein, that targets and kills specific pests.

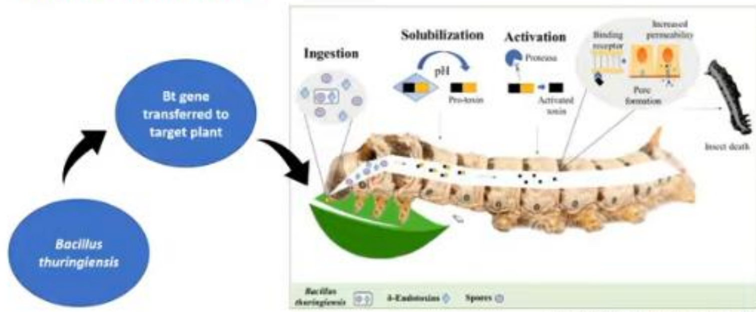
This ensures that only particular pests are affected, minimizing harm to non-target organisms, including beneficial insects. A common example is the incorporation of genes from the bacterium Bacillus thuringiensis into crops like cotton, corn, and soybean, which allows these plants to produce a protein toxic to specific insect pests. So, this is the bacteria from which the Bt gene is transferred to a target plant. When these insect larvae eat the leaf of this transgenic plant, the Bt protein is ingested and is first released as a protoxin. As it moves down the gut, it becomes activated due to the action of enzymes. The released peptides bind to receptors in the gut, increasing permeability due to pore formation, leading to fluid imbalance. The insect feels overfed and stops feeding, eventually starving to death.

PLANT INCORPORATED PROTECTANTS

Action Mechanism
Bt Crops: The *Bacillus thuringiensis* (Bt) gene encodes for a protein that, when ingested by certain insect larvae, binds to receptors in the insect's gut, causing cell lysis and death.
Targeted Pest Control: The specificity of PIPs ensures that only particular pests are affected, minimizing harm to non-target organisms, including beneficial insects.

A common **example** is the incorporation of genes from the bacterium *Bacillus thuringiensis* (Bt) into crops like corn, cotton, and soybeans, allowing these plants to produce a protein that is toxic to specific insect pests.

MODE OF ACTION OF PIPS



(Image adapted from Fernández-Chapa et al., 2019)

So, biochemical pesticides, on the other hand, are naturally occurring substances or synthetic analogs that control pests through non-toxic mechanisms. Unlike conventional pesticides, which typically kill pests directly, biochemical biopesticides affect the behavior, growth, or development of pests, making them an eco-friendly alternative in pest management. For example, we have botanicals like neem from Azadirachta indica, and then we have garlic extract, and so on. We will not spend much time on these botanicals because our focus is mostly on secondary metabolites produced by microorganisms like Bacillus thuringiensis, for example, semiochemicals,

spinosyns by actinomycetes, and strobilurin by wild mushrooms, etc. Botanical extracts are plant-derived substances used as biopesticides to manage pests, including insects, fungi, bacteria, and weeds. These extracts are typically made by isolating and concentrating the bioactive compounds from plants with pesticide properties. Botanical extracts are valued in sustainable agriculture for their natural origin, biodegradability, and reduced environmental impact compared to synthetic pesticides. And these are some of the famous examples, like azadirachtin, which is obtained from

BIOCHEMICAL (METABOLITES)

Biochemical biopesticides are naturally occurring substances or synthetic analogs that control pests through non-toxic mechanisms. Unlike conventional pesticides, which typically kill pests directly, biochemical biopesticides affect the behavior, growth, or development of pests, making them an eco-friendly alternative in pest management.

- **Botanicals** (Eg: Neem (*Azadirachta indica*), Pyrethrum (*Chrysanthemum cinerariifolium*), Garlic Extract (*Allium sativum*), Capsaicin (*Capsicum* spp.), Quassia (*Quassia amara*), Sabadilla (*Schoenocaulon officinale*)
- **Secondary metabolites** (Semiochemicals, *Bacillus thuringiensis* (Bt), Spinosyns (actinomycetes), strobilurin (wild mushroom *Strobilurus tenacellus*) etc.)

BIOCHEMICAL: BOTANICALS

Botanical extracts are plant-derived substances used as biopesticides to manage pests, including insects, fungi, bacteria, and weeds. These extracts are typically made by isolating and concentrating the bioactive compounds from plants with pesticide properties. Botanical extracts are valued in sustainable agriculture for their natural origin, biodegradability, and reduced environmental impact compared to synthetic pesticides.

neem, particularly the neem seeds. Then there is pyrethrum from the chrysanthemum flower. We have these bioactive compounds, the pyrethrins. These target mosquitoes, flies, moths, and various other insects. They particularly affect the nervous system of the insects, leading to paralysis and death.

SOME BOTANICALS

Neem (*Azadirachta indica*)

Active Compound: Azadirachtin

Uses: Insecticide, antifeedant, growth regulator.

Pests Targeted: Aphids, whiteflies, beetles, caterpillars, and other insects.

Mode of Action: Disrupts insect growth and reproduction, repels pests, and inhibits feeding.

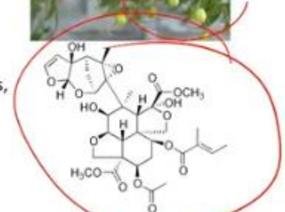


Image: Neem, Azadirachtin structure
Source: Edgar181, Public domain, via Wikimedia Commons

Pyrethrum (*Chrysanthemum cinerariifolium*):

Active Compounds: Pyrethrins

Uses: Insecticide.

Pests Targeted: Mosquitoes, flies, moths, and various insects.

Mode of Action: Affects the nervous system of insects, leading to paralysis and death.

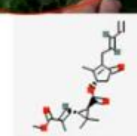
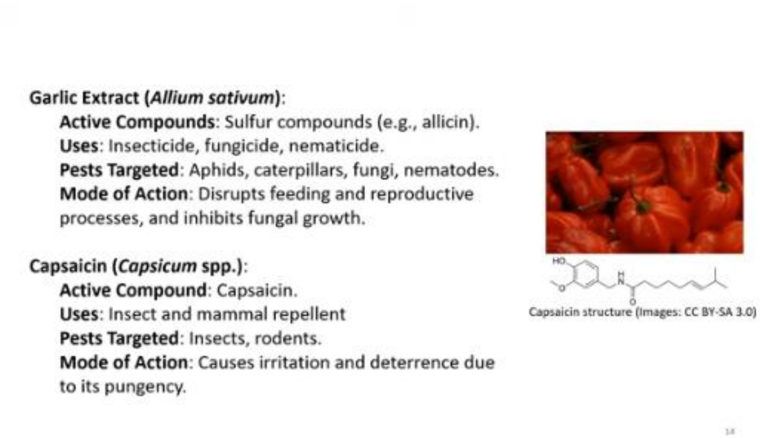


Image: Pyrethrum, Pyrethrins structure
Source: CC BY SA 3.0 https://pubchem.ncbi.nlm.nih.gov/compound/Pyrethrin.

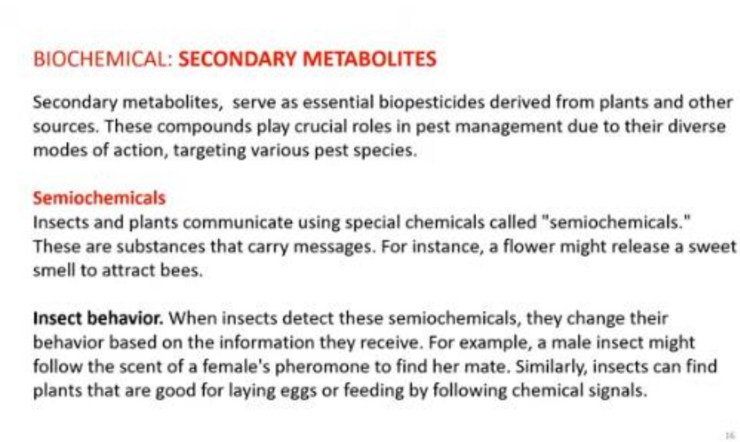
And then we have garlic extract, which has sulfur compounds like allicin, and these are fungicidal and nematicidal. And then it can target feeds, caterpillars, fungi, and nematodes. They basically disrupt feeding and reproductive processes and inhibit fungal growth. Then we have capsaicin, which is obtained from chili. It is used as an insect and mammal repellent and targets insects and rodents.

It causes irritation and deterrence due to its pungency. Then we have Quassia, which produces quassinoids, and they are used as insecticides against aphids, caterpillars, and beetles. They disrupt insect feeding and digestion. Another class of biochemicals are the secondary metabolites, which serve as essential biopesticides derived from plants and other sources.



These compounds play a crucial role in pest management due to their diverse modes of action targeting various species. Semiochemicals: Insects and plants communicate using special chemicals called semiochemicals. These are substances that carry messages. For instance, a flower might release a sweet smell to attract bees. When insects detect these semiochemicals, they change their behavior based on the information they receive.

For example, a male insect might follow the scent of a female pheromone to find his mate. Similarly, insects can find plants that are good for laying eggs or feeding by following chemical signals. The mode of action of semiochemicals occurs in different ways. It may disrupt the mating cycle where female sex pheromones are used by insects to communicate, for example, in vineyards, synthetic grapevine moth pheromones can be sprayed to confuse male grapevine moths, preventing them from finding females to mate with.


BIOCHEMICAL: **SECONDARY METABOLITES**

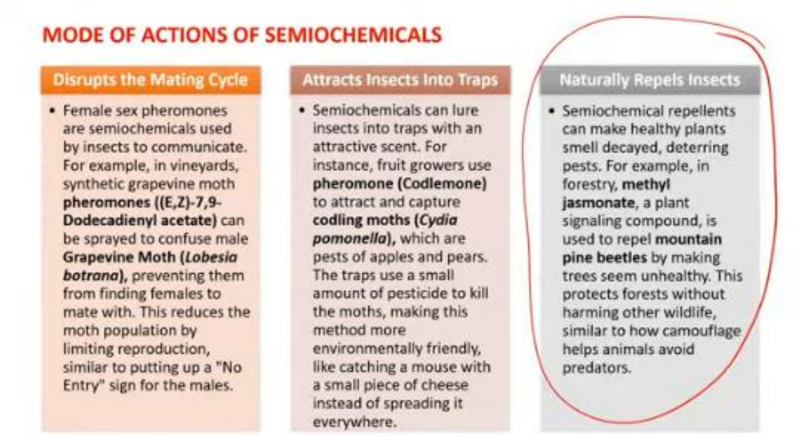
Secondary metabolites, serve as essential biopesticides derived from plants and other sources. These compounds play crucial roles in pest management due to their diverse modes of action, targeting various pest species.

Semiochemicals
Insects and plants communicate using special chemicals called "semiochemicals." These are substances that carry messages. For instance, a flower might release a sweet smell to attract bees.

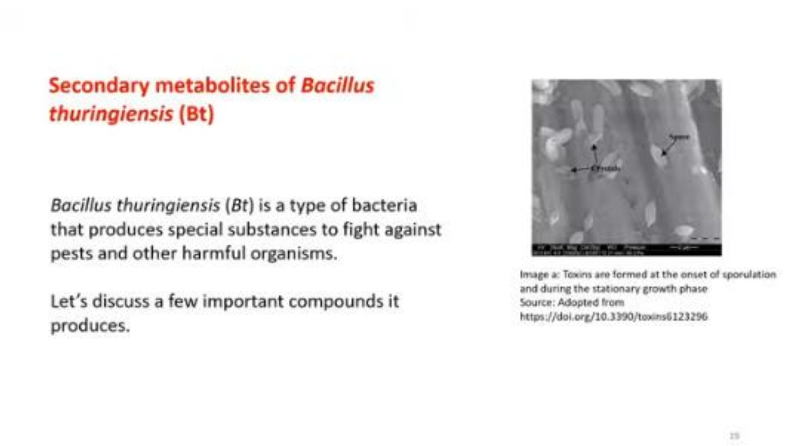
Insect behavior. When insects detect these semiochemicals, they change their behavior based on the information they receive. For example, a male insect might follow the scent of a female's pheromone to find her mate. Similarly, insects can find plants that are good for laying eggs or feeding by following chemical signals.

This reduces the moth population by limiting reproduction, similar to putting up a no-entry sign for the males. Secondly, it may attract insects into traps where semiochemicals are used to lure insects with an attractive scent. For instance, fruit growers use pheromones to attract and capture codling moths, which are pests of apples and pears. The trap uses a small amount of pesticide to kill the moths, making these methods more environmentally friendly, like catching a mouse with a small piece of cheese instead of spreading poison everywhere. Thirdly, they naturally repel insects.

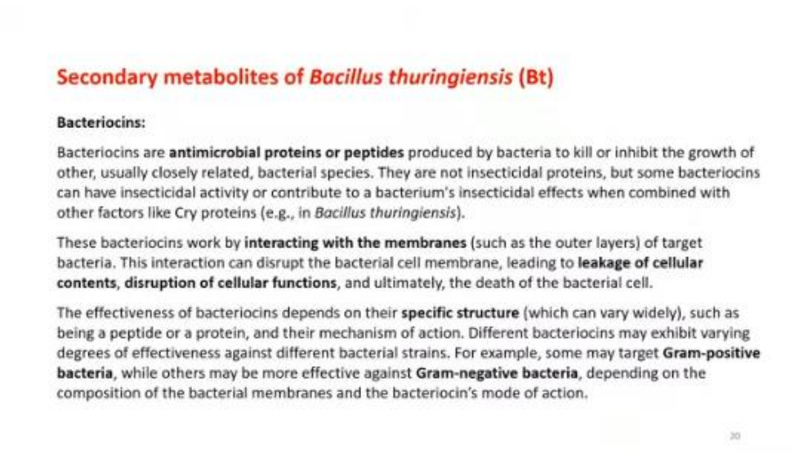
Semiochemical repellents can make healthy plants smell decayed, deterring pests. For example, in forestry, methyl jasmonate, a plant signaling compound, is used to repel mountain pine beetles by making trees seem unhealthy. These protect trees without harming other wildlife, similar to how camouflage helps animals avoid predators. Let us now discuss the secondary metabolites of Bacillus thuringiensis which is a type of bacteria that produces special substances to fight against pests and other harmful organisms.



This is apart from the Cry protein or endotoxin that we discussed earlier in this lecture. Basically, they produce certain secondary metabolites, for example, bacteriocins. These are antimicrobial proteins or peptides produced by bacteria to kill or inhibit the growth of other, usually closely related, bacterial species. They are not insecticidal proteins, but some bacteriocins can have insecticidal activity or contribute to a bacterium's insecticidal effect when combined with other factors like Cry proteins. These bacteriocins work by interacting with membranes, such as the outer layers of target bacteria.



These interactions can disrupt the bacterial cell membrane, leading to leakage of cellular content, disruption of cellular functions, and ultimately, death of the bacterial cell. The effectiveness of bacteriocins depends on their specific structure, which can vary widely, such as being a peptide or a protein, and their mechanism of action. Different bacteriocins may exhibit varying degrees of effectiveness against different bacterial strains. For example, some may target Gram-positive bacteria, while others may be more effective against Gram-negative bacteria, depending on the composition of the bacterial membranes and the bacteriocin's mode of action. Bacillus thuringiensis produces several bacteriocins, which are antimicrobial peptides that help the bacterium compete with other microorganisms.



Some of the notable ones are Thuricin. This was discovered in 1989 and is one of the first bacteriocins identified from Bacillus thuringiensis. Thuricin exhibits antimicrobial activity against various Gram-positive bacteria. Then there is Tochicin, which was discovered in 1997. This is an antibiotic bacteriocin with antimicrobial properties and is produced by certain Bacillus thuringiensis strains.

Then there is which was identified in 2000, basically a cycling antimicrobial peptide with activity against a broad range of gram-positive bacteria. Then there is Thuricin 7, discovered in 2001, which is a more potent variant of Thuricin, having broader antimicrobial spectrum or activity and is particularly effective against gram-positive bacteria. Then there is another variant Thuricin S identified in 2007 which has unique antimicrobial properties that differentiate it from the other forms of Thuricin. Then there are others like Thuricin 439A and 439B identified in 2003. They are closely related bacteriocins which demonstrate activity against gram-positive bacteria.

Then entomocin, which was discovered in 2003, has been reported to have insecticidal properties. Then there is bacthuricin F4, discovered in 2005, which is an antimicrobial peptide with activity against the gram-positive bacteria. Then there is thuricin CD discovered in 2010, which is a potent bacteriocin with strong antimicrobial effects. So, we see that bacillus thuringiensis offers so many different kinds of bacteriocins, and some of these are effective against gram-positive bacteria, and some are also having insecticidal properties. Let us discuss about secondary metabolites from actinomycetes like spinosynes.

Bacillus thuringiensis produces several bacteriocins, which are antimicrobial peptides that help the bacterium compete with other microorganisms. Notable bacteriocins identified from *B. thuringiensis* include:

- **Thuricin** (Discovered in 1989) : One of the first bacteriocins identified from *B. thuringiensis*, **Thuricin** exhibits antimicrobial activity against various Gram-positive bacteria.
- **Tochicin** (Discovered in 1997): **Tochicin** is a lantibiotic bacteriocin with antimicrobial properties, also produced by certain *B. thuringiensis* strains.
- **Kurstakin** (Identified in 2000): A cyclic antimicrobial peptide with activity against a broad range of Gram + bacteria.
- **Thuricin 7** (Discovered in 2001): A more potent variant of **Thuricin**, has a broader antimicrobial spectrum and is particularly effective against Gram + pathogens.
- **Thuricin S** (Identified in 2007): Another variant of **Thuricin**, has unique antimicrobial properties that differentiate it from the other thuricin forms.
- **Thuricin 439A and 439B** (Identified in 2003): Closely related bacteriocins, demonstrate activity against Gram + bacteria.
- **Entomocin** (Found in 2003): Has insecticidal properties.
- **Bacthuricin F4** (Discovered in 2005): Another antimicrobial peptide with activity against Gram + bacteria.
- **Thuricin CD** (Found in 2010): Is a potent bacteriocin with strong antimicrobial effects.

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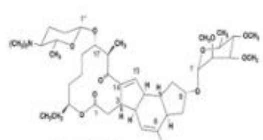
These are a noble class of highly potent natural insecticides discovered in the culture of the soil dwelling actinomycete. Saccharopolyspora spinosa, this bacterium produces spinosyn A and spinosyn D. which are the active insecticidal components in the commercial product known as Spinosad. The name Spinosad, we can see here in this image, one Spinosyn A and Spinosyn D is derived by the combination of Spinosyn A and Spinosyn D. The first one is approximately 85% and the second one is around 15%. So, how does spinosad which contains 85% spinosyn A and spinosyn D kill the target insect?

(James & Mary, n.d.)

Secondary metabolites Spinosyns (actinomycetes)

Spinosyns are a novel class of highly potent natural insecticides discovered in a culture of the soil-dwelling actinomycete *Saccharopolyspora spinosa*. This bacterium produces spinosyn A and spinosyn D, which are the active insecticidal components in the commercial product known as spinosad. The name "spinosad" (**Image a**) is derived from a combination of "spinosyn A" and "spinosyn D" (approximately 85% A and 15% D).



(1) Spinosyn A (R = H)
(2) Spinosyn D (R = CH_3)

Image a: Structures of spinosyn A (1) and spinosyn D (2). Source: (Kirst, 2010)

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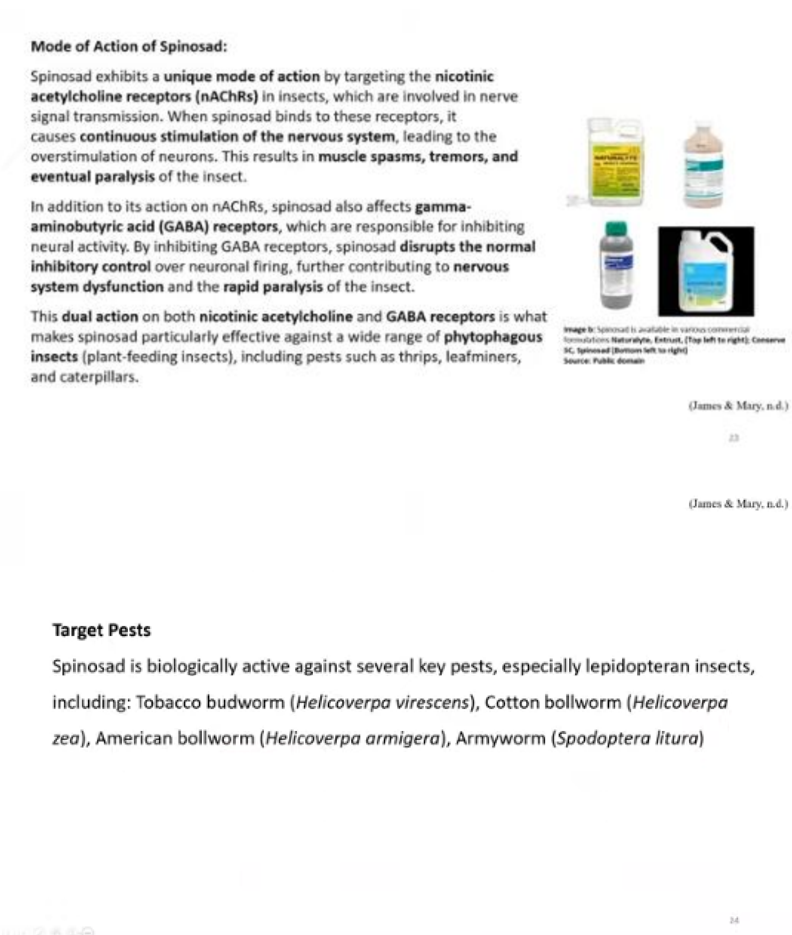
They exhibit a unique mode of action by targeting the nicotinic acetylcholine receptors in insects. These receptors are involved in nerve signal transmission. When spinosad binds to these receptors, it causes continuous stimulation of the nervous system, leading to the overstimulation of neurons. This results in muscle spasms, tremors, and eventually paralysis of the insect. In addition to its action on the nicotinic acetylcholine receptors, spinosad also affects gamma-aminobutyric acid receptors, or GABA receptors, which are responsible for inhibiting neural activity.

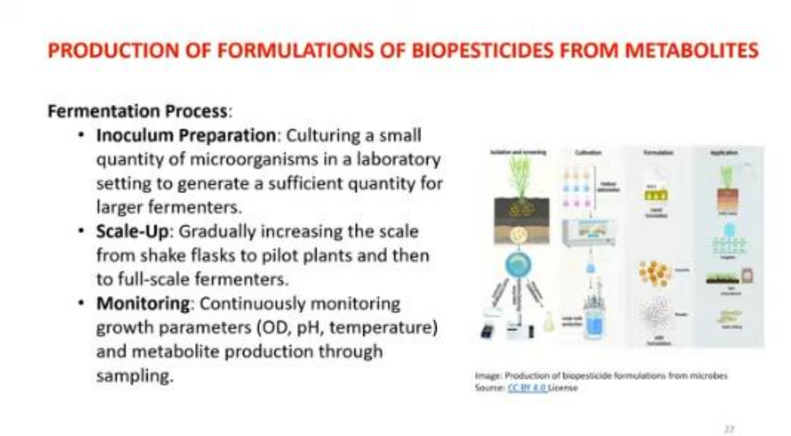
By inhibiting GABA receptors, spinosad disrupts the normal inhibitory control over neuronal firing, further contributing to nervous system dysfunction and the rapid paralysis of the insect. The dual action on both the nicotinic acetylcholine receptors and GABA receptors makes spinosad particularly effective against a wide range of phytophagous insects. This includes pests such as thrips, leafminers, and caterpillars. Spinosad is biologically active against several key pests, especially lepidopteran insects, including tobacco budworm, cotton bollworm, American bollworm, and armyworm (Spodoptera litura). Secondary metabolites are produced from wild mushrooms like Strobilurus tenacellus, which produces strobilurin.



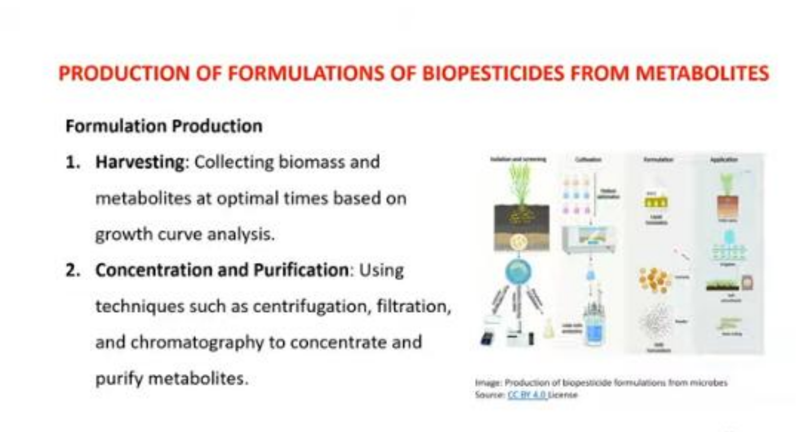
These strobilurins represent a new generation of fungicides derived from this particular wild mushroom. Particularly, strobilurin A and B are the first such compounds isolated from the wild mushroom Strobilurus. How do these strobilurins act? They inhibit mitochondrial respiration in fungi, both higher and lower fungi, disrupting energy production. They exhibit translaminar movement, moving across the leaf blade to protect both surfaces, even if only one side is treated.

Some, like azoxystrobin, also move systematically within the plant, offering comprehensive protection. So, commercially, these fungicides are now available in various formulations. So, some of these are Azoxystrobin, Quadris, Abound, Amister, and Heritage, as well as Triflooxystrobin, Flint, Stratego, and Compass. These are effective against various plant diseases. For example, studies on pearl millet downy mildew showed that azoxystrobin provides up to 91% protection, which is quite high. Now, let us discuss the production of biopesticide formulations from metabolites.

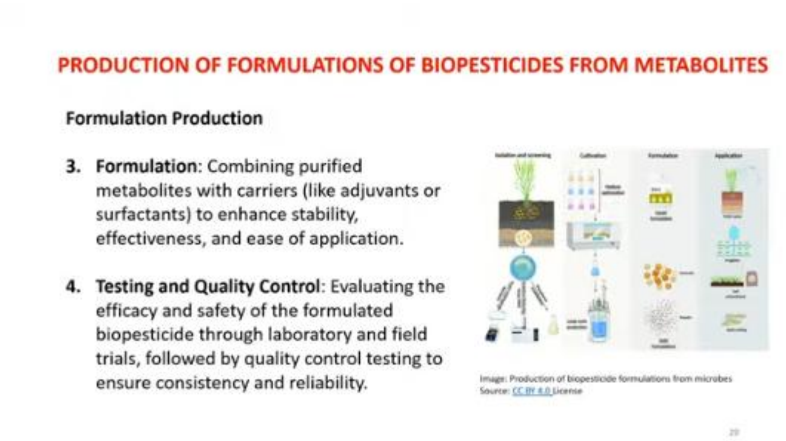
Mostly, we depend on fermentation, where we start with inoculum preparation. We culture a small quantity of microorganisms in a laboratory setting to generate a sufficient quantity for large fermenters. So, we gradually scale up, increasing the size from shake flasks to pilot plants and then to full-scale fermenters. Continuous monitoring of growth parameters, such as optical density, pH, temperature, and metabolic production through sampling, is very important to understand the growth dynamics. So, next comes the formulation of this production.



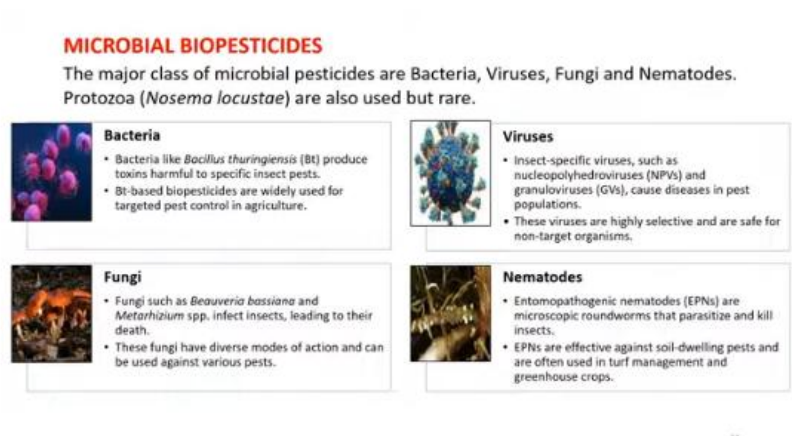
We may start with isolation and screening from natural sources, followed by characterization, flask cultivation, lab-scale cultivation, and finally, industrial-scale large-scale production. The next important stage is the formulation stage. Once we harvest the biomass or the microorganisms and the metabolites at the optimal time based on growth curve analysis, we proceed with concentration and purification using techniques such as centrifugation, filtration, and chromatography to concentrate and purify the metabolites. We then formulate granules, powder, or liquid or solid formulations. We combine purified metabolites with carriers like adjuvants or surfactants to ensure stability, effectiveness, and ease of application.



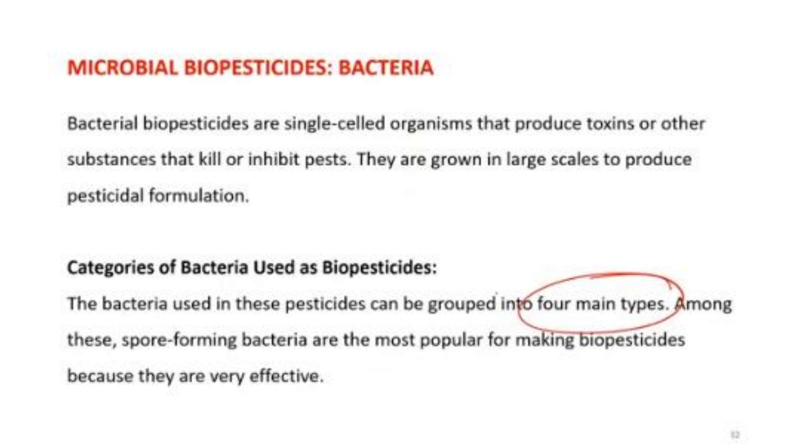
We also conduct testing and quality control, evaluating the efficacy and safety of the formulated biopesticide in laboratory and field trials, followed by quality control testing to ensure consistency and reliability. Then, of course, we proceed with various kinds of applications, which can be foliar or soil applications, and field trials are very important, as is constant monitoring throughout this entire process. Now, let us move to Section 3, where we will discuss various microbial biopesticides in which microbes are directly used as biopesticides. Here, we mostly used metabolites as biopesticides. For example, we can use bacteria like Bacillus thuringiensis, which produces toxins harmful to specific insects.

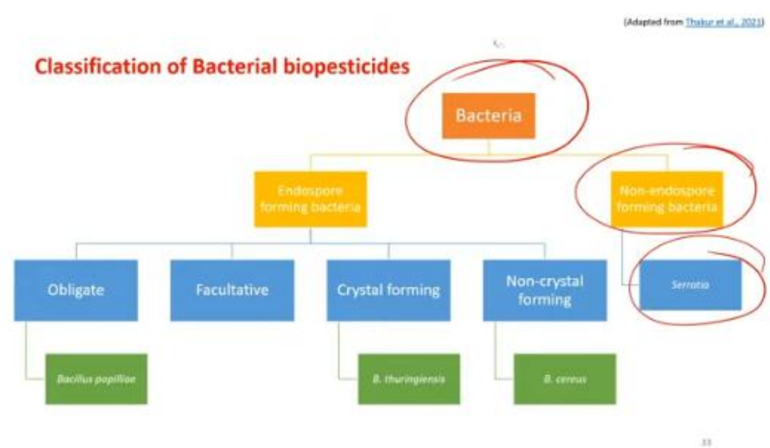


Alternatively, we may use fungi such as Beauveria bassiana, which infect insects, leading to their death. We may also use insect-specific viruses, such as nucleopolyhedroviruses. Another option is entomopathogenic nematodes, which are microscopic roundworms that parasitize and kill insects. So, let us begin with bacteria. Bacterial biopesticides are single-celled organisms that produce toxins or other substances to kill pests.



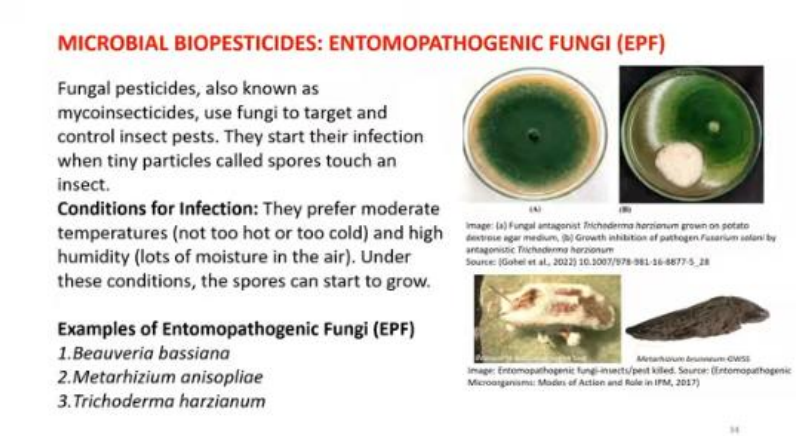
They are grown on a large scale to produce pesticidal formulations. There are different categories of bacteria used as pesticides, mainly four types. Among these, spore-forming bacteria are the most popular for making biopesticides because they are highly effective. Bacterial biopesticides can be classified into endospore-forming bacteria, such as Bacillus, and non-endospore-forming bacteria, such as Serratia. Some endospore-forming bacteria are obligate, like Bacillus popilliae, while others are facultative. Some are crystal-forming, like Bacillus thuringiensis, and others are non-crystal-forming, like Bacillus cereus.

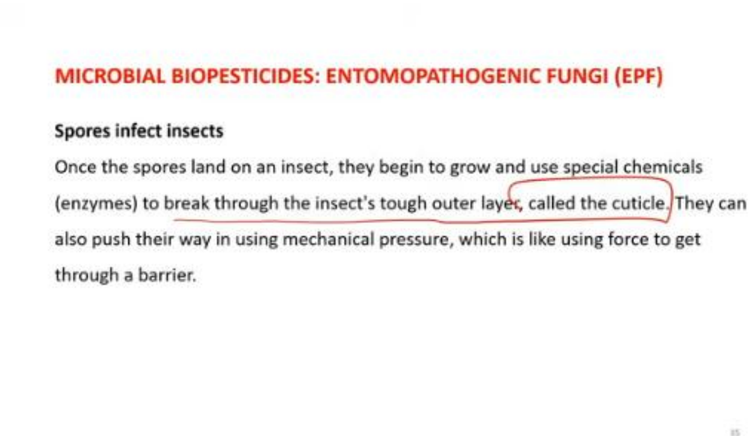




So, then we have these entomopathogenic fungi, which are used as biopesticides. These are known as fungal pesticides or mycoinsecticides, which use fungi to target and control insect pests. They start their infection when tiny particles called spores touch an insect. The conditions for infection are moderate temperatures—not too hot or too cold—and high humidity. Under these conditions, the spores can start to grow.

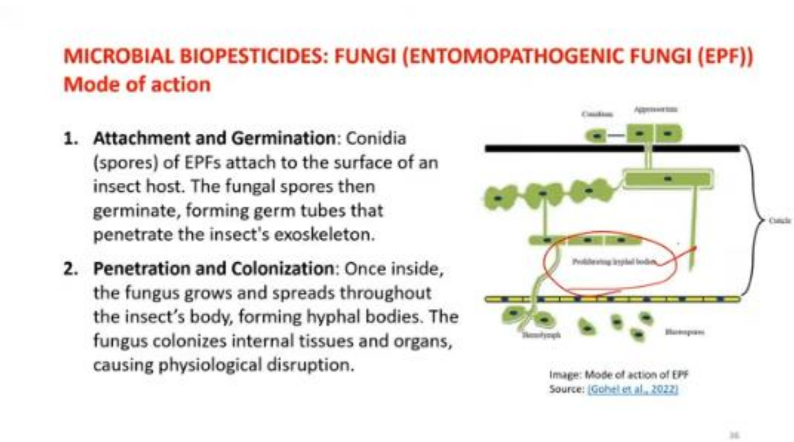
Some examples of entomopathogenic fungi are Beauveria bassiana, Metarhizium anisopliae, and Trichoderma harzianum. Then we have certain spores that infect insects, which are produced by the entomopathogenic fungi. Once the spores land on an insect, they begin to grow and use special chemicals, particularly enzymes, to break through the insect's tough outer layer, the cuticle. They can also push their way in using mechanical pressure, which is like using force to get through a barrier. So, the first step is attachment and germination.



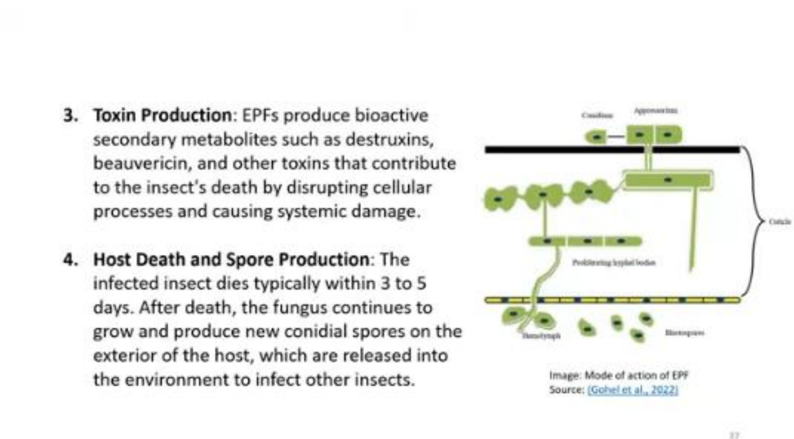


Conidia or spores of entomopathogenic fungi attach to the surface of an insect host. The fungal spores then germinate, forming germ tubes that penetrate the insect's exoskeleton. Then there is penetration. So, this is the cuticle you can see over here. So, here is the haemolymph, and then these are the proliferating hyphal bodies—the conidium, which forms the appressorium, and then it slowly penetrates the cuticle.

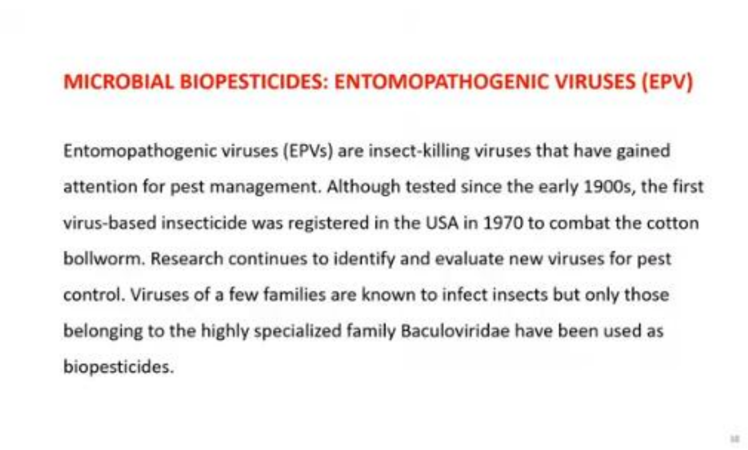
Once inside, the fungus grows and spreads throughout the insect's body, forming hyphal bodies. The fungus colonizes internal tissues and organs, causing physiological disruption. It also produces toxins. These entomopathogenic fungi produce bioactive secondary metabolites, such as destruxin, beauvericin, and other toxins, which contribute to the insect's death by disrupting cellular processes and causing systemic damage. Finally, there is host death and spore production.



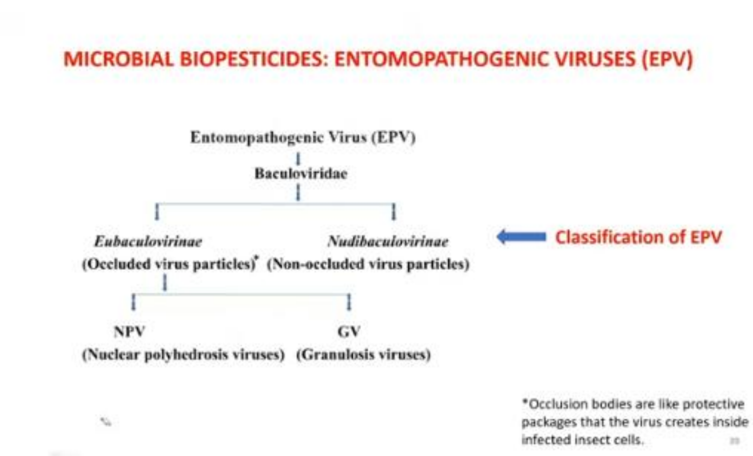
The infected insect typically dies within three to five days. The fungus continues to grow and produces new conidial spores on the exterior of the host, which are released into the environment to infect other insects. Let us now discuss entomopathogenic viruses, or EPVs. They kill insects and have gained attention for pest management. Although tested since the early 1900s, the first virus-based insecticide was registered in the USA in 1970 to combat the cotton bollworm.



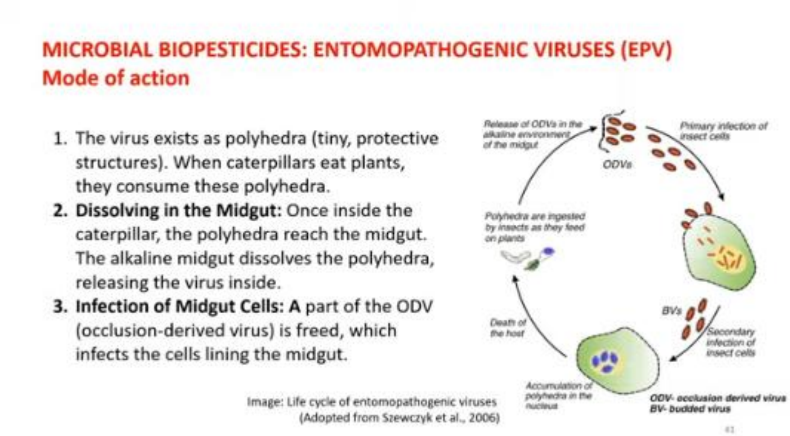
Research continues to identify and evaluate new viruses for pest control. Viruses from a few families are known to infect insects, but only those belonging to the highly specialized family Baculoviridae have been used as biopesticides. So, we have two types of Baculoviridae under the entomopathogenic viruses. One is the Eubaculovirinae, and another is the Nudibaculovirinae. Eu has occluded virus particles, and Nudi has non-occluded virus particles.



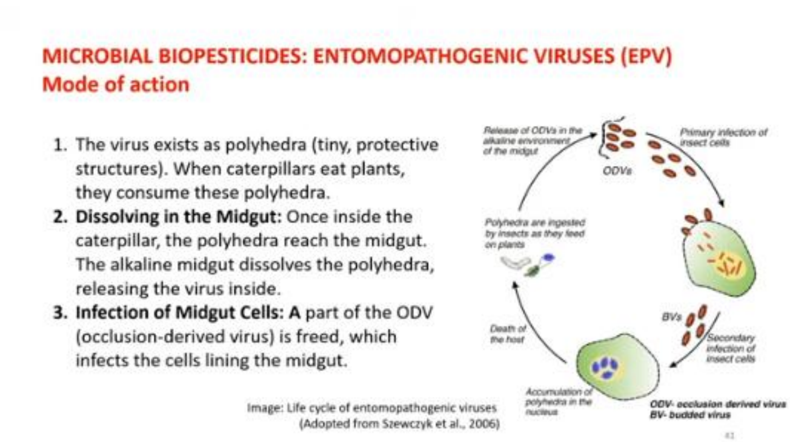
The examples of Eu Baculovirinae contains nuclear polyhedrosis virus NPV and granulosis viruses. So, some of the examples of EPV and their target pests. So, we have these SeNPV that is targeted against beet armyworm and we have HzNPV which targets heliothis or helicoverpa. Then CpNPV, codling moth, AgNPV, velvetbean caterpillar.



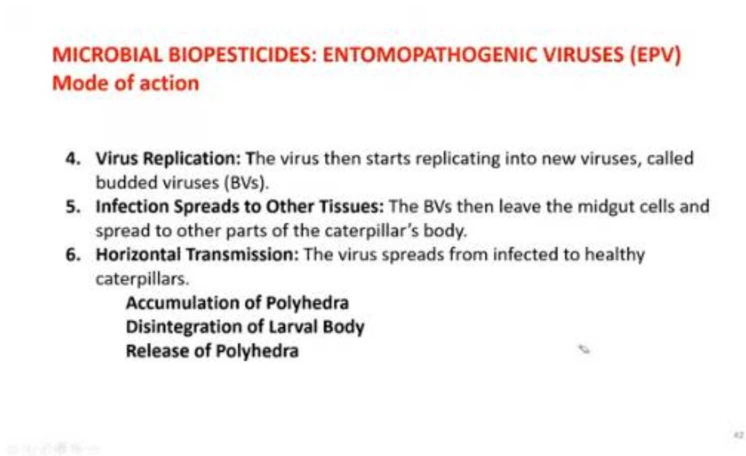
Then AcNPV, autographa californica and SlNPV which targets spodoptera litura. So, what is the mode of action of these entomopathogenic viruses? The virus exists as polyhedra or tiny protective structures when caterpillar eat plant they consume these polyhedra. The polyhedra are ingested by the insects as they feed on plants. Once inside the caterpillar the polyhedra reach the



The alkaline midgut dissolves the polyhedra releasing the virus inside. So, this is released in the alkaline environment of the midgut and primary infection of the insect cell will follow. A part of the ODV, occlusion-derived virus, is freed, which infects the cell lining, the midgut. And this will also further cause secondary infection and accumulation of the polyhedra and finally the death of the host. So, the virus will start replicating into new viruses called budded viruses and infection will spread to other tissues.

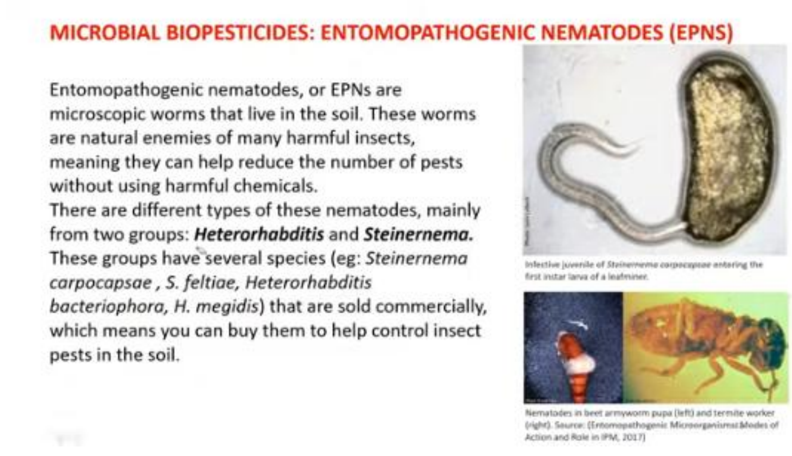


It will leave the midgut cells and spread to other parts of the caterpillar's body. There may also be horizontal transmission. The virus spreads from infected to healthy caterpillars due to the accumulation of polyhedra, disintegration of the larval body, and the release of the polyhedra. Let us now discuss another important microbial biopesticide where we use entomopathogenic nematodes. These are microscopic worms that live in the soil.



These worms are natural enemies of many harmful insects. Meaning, they can help reduce the number of pests without using harmful chemicals. There are different types of these nematodes, mainly from two groups: Heterorhabditis and Steinernema. These groups have several species. S. feltiae, Heterorhabditis bacteriophora, and H. megidis.

These are also available commercially, so we can source them from suppliers to control insect pests in the soil. One important aspect is the safety of these entomopathogenic nematodes. They are safe for other living organisms. They do not harm animals like pets or humans, plants, or other beneficial organisms in the environment. This makes them an eco-friendly option for pest control, which is very important for sustainable farming.



MICROBIAL BIOPESTICIDES: ENTOMOPATHOGENIC NEMATODES (EPNS)

Safety of EPNs:

- One of the best things about EPNs is that they are safe for other living things. They do not harm animals (like pets or humans), plants, or other beneficial organisms in the environment.
- This makes them an eco-friendly option for pest control, which is very important for sustainable farming.

So, what is the mode of action of these entomopathogenic nematodes? The young infective stages of these nematodes are very active. They search for insect hosts to enter. They can enter insects through natural openings in the insect's body, such as the mouth, spiracles, anus, or intersegmental membranes. Once nematodes enter the insect, they release symbiotic bacteria, which they carry with them.

MICROBIAL BIOPESTICIDES: ENTOMOPATHOGENIC NEMATODES (EPNS)
Mode of Action

Once the nematodes enter the insect, they release bacteria (symbiotic bacteria) they carry with them. The bacteria cause infection in the insect, leading to bacterial septicemia, which is when the bacteria spread throughout the body and can cause death within 1-2 days. This is a very fast process!

Bacteria Carried by Nematodes:

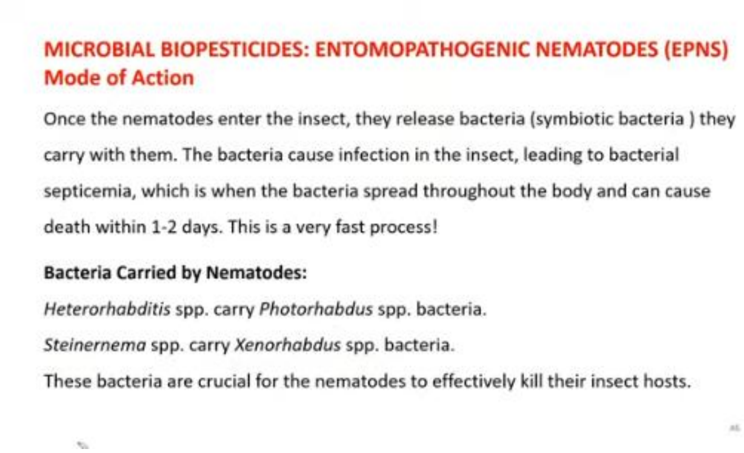
Heterorhabditis spp. carry *Photorhabdus* spp. bacteria.

Steinernema spp. carry *Xenorhabdus* spp. bacteria.

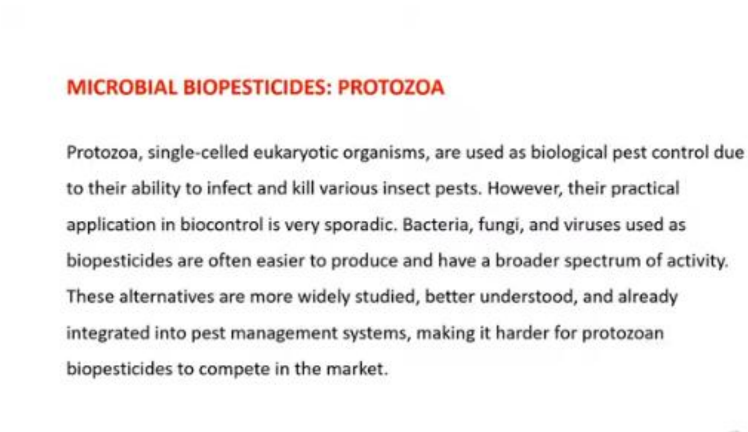
These bacteria are crucial for the nematodes to effectively kill their insect hosts.

The bacteria cause infection in the insect, leading to bacterial septicemia, where the bacteria spread throughout the body and can cause death within one to two days. This is a very fast process. So, the infective juveniles enter through natural openings or cuticles. The bacteria are released. Then, the adult nematodes develop, produce progeny for two to three generations, and when resources are depleted, they are released from the cadaver.

What are the bacteria carried by these nematodes? For example, Heterorhabditis carries Photorhabdus species. Steinernema carries Xenorhabdus bacteria. These are crucial for the nematodes to effectively kill their insect hosts. Let us now discuss another important microbial biopesticide: protozoa, which are single-celled eukaryotic organisms used as biological pest control agents due to their ability to infect and kill various insect pests.

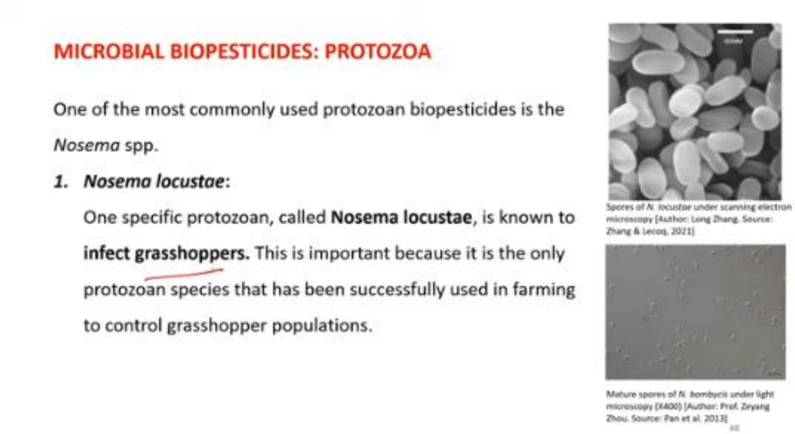


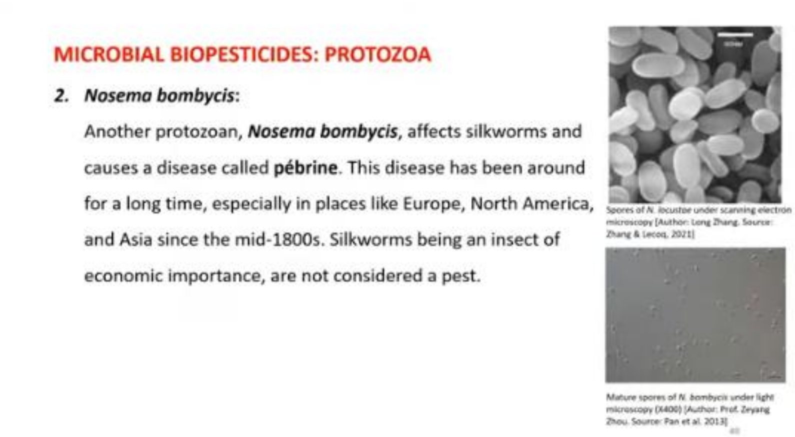
However, the practical application in biocontrol is very sporadic. Bacteria, fungi, and viruses used as biopesticides are often easier to produce and have a broader spectrum of activity. These alternatives are more widely studied, better understood, and already integrated into placement in the systems, making it harder for protozoan biopesticides to compete in the market. One of the most commonly used protozoan biopesticides, in spite of these challenges, is the Nosema species. For example, we have Nosema locustae, which is known to infect grasshoppers.



This is important because it is the only protozoan species that has been successfully used in farming to control grasshopper populations. Then we have Nosema bombycis, which affects silkworms and causes a disease called pebrine. This disease has been around for a long time, especially in places like Europe, North America, and Asia since the mid-1800s.

Silkworms, being insects of economic importance, are not considered pests, and in fact, efforts are made to control Nosema bombycis infections. How do protozoans infect insects?





Protozoans can produce tiny particles called spores. They are like seeds that can spread the infection. When an insect eats these spores, they enter the insect's body and start to grow in a part of the digestive system called the midgut. Once inside, the spores begin to develop and break out of their protective covering. Then they invade the insect's cells, causing significant damage to the insect's organs and tissues.

MICROBIAL BIOPESTICIDES: PROTOZOA
How Do Protozoans Infect Insects?

- Protozoans can produce tiny particles called **spores**. These spores are like seeds that can spread the infection. When an insect eats these spores, they enter the insect's body and start to grow in a part of the digestive system called the **midgut**.
- **Infection Process:** Once inside, the spores begin to develop and break out of their protective covering. They then invade the insect's cells, causing a lot of damage to the insect's organs and tissues.

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After causing damage, the infected tissues can produce more spores. These new spores can then be expelled from the infected insect and can be eaten by other healthy insects, spreading the disease further. In nature, some insects that eat other insects are called predators. And certain types of wasps, also known as parasitoids, can help spread the protozoan disease. They act like carriers, moving the spores from one insect to another, which helps the disease spread even more.

MICROBIAL BIOPESTICIDES: PROTOZOA
How Do Protozoans Infect Insects?

- **Reproduction of Protozoans:** After causing damage, the infected tissues can produce more spores. These new spores can then be expelled from the infected insect and can be eaten by other healthy insects, spreading the disease further.
- **Role of Other Animals:** In nature, some insects that eat other insects, called **predators**, and certain types of wasps, known as **parasitoids**, can help spread these protozoan diseases. They act like carriers, moving the spores from one insect to another, which helps the disease to spread even more.

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Let us now discuss the biopesticides from microalgae. Some types of microalgae produce biologically active compounds. These compounds can fight off harmful bacteria and fungi, which are types of pathogens that make plants sick. Because of these compounds, they can be used as biopesticides to protect crops. Microalgae biomass can be used instead of chemical pesticides.

MICROBIAL BIOPESTICIDES: PROTOZOA
How Do Protozoans Infect Insects?

- **Reproduction of Protozoans:** After causing damage, the infected tissues can produce more spores. These new spores can then be expelled from the infected insect and can be eaten by other healthy insects, spreading the disease further.
- **Role of Other Animals:** In nature, some insects that eat other insects, called **predators**, and certain types of wasps, known as **parasitoids**, can help spread these protozoan diseases. They act like carriers, moving the spores from one insect to another, which helps the disease to spread even more.

So, what are the different types of biopesticides from microalgae that have shown the ability to act as biopesticides? Some of these are the Nostoc species, which is a type of filamentous cyanobacterium. It is a long, thin, thread-shaped microorganism or microalgae. It can help protect plants from certain diseases. Then we have Anabaena variabilis.

This is being explored as a possible bio-fertilizer and bio-pesticide for growing rice sustainably, helping to improve crop yields without the negative effects of chemicals. Then we have Chlamydopodium fusiforme, which is a single-celled green algae that can also help fight off plant pathogens. In spite of all the good things we have discussed, there are certain limitations associated with biopesticides. For example, we have challenges with storage.

BIOPESTICIDES FROM MICROALGAE

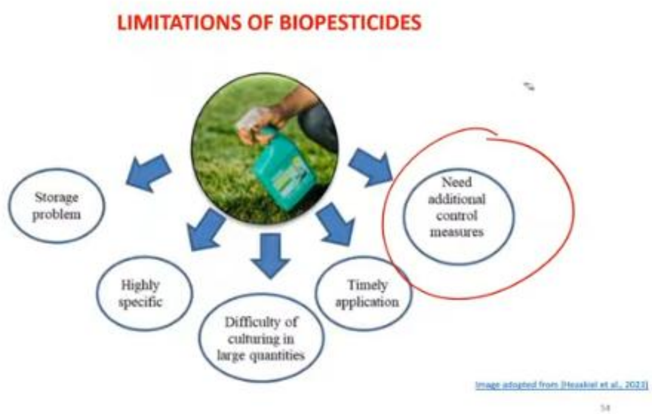
Microalgae with Biopesticide Activity:
Tpes of microalgae that have shown the ability to act as biopesticides:

- ***Nostoc spp.*:** This is a type of filamentous cyanobacterium, which means it looks like a long, thin thread. It can help protect plants from certain diseases.
- ***Anabaena variabilis*:** This microorganism is being studied as a possible biofertilizer and biopesticide for growing rice sustainably, helping to improve crop yields without the negative effects of chemicals.
- ***Chlamydopodium fusiforme*:** This is a single-celled green algae that can also help fight off plant pathogens.

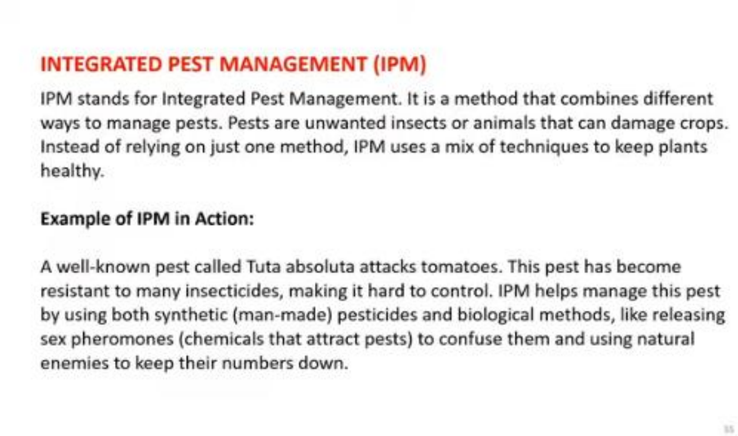
They may become unviable, particularly the living organisms used as biopesticides, which require specialized storage. Then another problem is their high specificity. Not all of them have broad-spectrum activity. This limits their effectiveness.

But when it comes to single-species infection in an agricultural field, they are actually very beneficial. And then there is always the challenge of culturing in large quantities or scaling up. Timely application may sometimes also be a challenge. We may not be able to obtain sufficient amounts due to the time required for large-scale production. Or even the supply chain may sometimes not be very logistically sound.

And it requires many additional control measures. For example, if we are using live microbes like fungi, bacteria, etc. as biopesticides, and if the field is already contaminated with broad-spectrum pesticides, which are harmful to these organisms, the application of biopesticides may fail. So, we need a total organic system where these biopesticides can be highly effective.



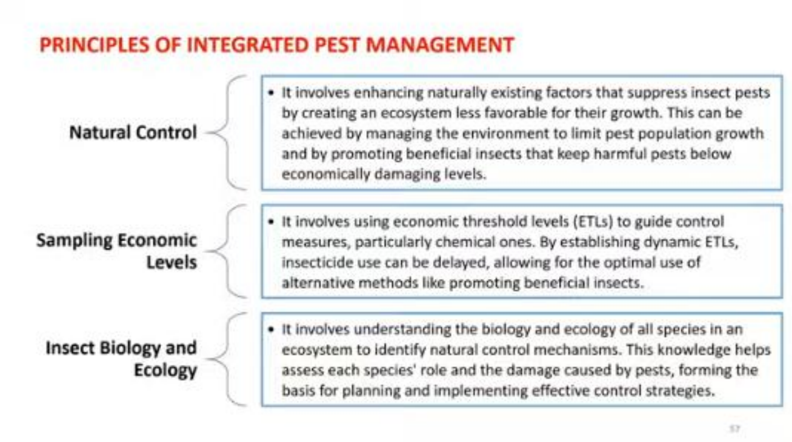
So, let us once again revisit the concept of pest management that combines different ways to manage pests, which are unwanted insects, animals, or microorganisms that can damage crops. Instead of relying on just one method, IPM uses a mix of techniques to keep plants healthy. So, we use all the different kinds of pest control methods available in IPM. So, what are the benefits of IPM?



Number one, it provides environmental protection. It's highly profitable. It also results in societal benefits and reduces risks. However, there are challenges in adopting IPM because not many farmers use it due to reasons like lack of awareness or user preference. using synthetic pesticides or due to different reasons, maybe marketing being one of them, and then technology inaccessibility. Many times, IPM is mostly like a package, and it needs to be taken to the field by extension workers. Certain farmers may not have access to that kind of technological support.

The policy and culture impact the adoption of IPM in a particular region or locality. So, what are the different principles of integrated pest management? It relies on various principles like natural control, sampling economic levels, insect biology, and ecology. Natural control involves enhancing naturally existing factors that suppress insect pests by creating an ecosystem less favorable for their growth. This can be achieved by managing the environment to limit pest population growth and by promoting beneficial insects that help keep harmful pests below economically damaging levels.

In the sampling of economic levels, we determine economic threshold levels to guide control measures, particularly chemical ones. By establishing dynamic economic threshold levels, insecticide use can be delayed, allowing for the optimal use of alternative methods like promoting beneficial insects. Insect biology and ecology involve understanding the biology and ecology of all species in an ecosystem to identify natural control mechanisms. This knowledge helps assess each species' role and the damage caused by pests, forming the basis for planning and implementing effective control strategies. So, this figure provides a general step in IPM program development.



This is taken from the European Union draft guidance document for IPM published in 2008. So, the first step starts with prevention and separation, principle number one. Principle number two: constant monitoring. Number three: decision-making based on scientific studies like sampling economic levels and also understanding the biology and ecology of all species in an ecosystem. So, principle one involves the combination of tactics into preventive strategies.

Principle two monitoring involves observation, forecasting, and diagnostics, and principle three involves the use of thresholds and multiple criteria. Then we have principles 4 to 7, which mostly deal with intervention. Non-chemical methods are the most preferred. Then using agents which have the least side effects. Then reduced pesticide use and anti-resistance strategies.

Biopesticides may also develop some kind of resistance in the long run. So, that also has to be taken into account. Finally, the principle of evaluation involves the assessment of the entire process and the adoption of new standards. These principles are to be followed in a sequential manner for efficient integrated pest management. With this, we come to the end of this lecture. Thank you for your patient hearing. Thank you.

GENERAL STEPS OF IPM PROGRAM DEVELOPMENT

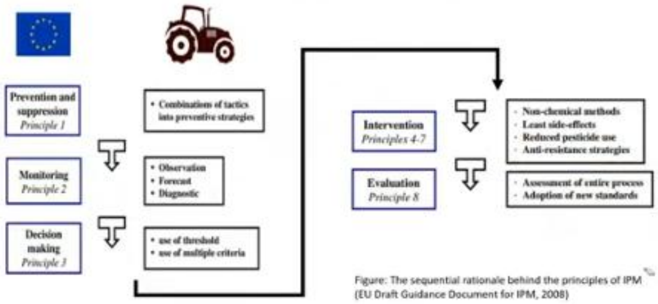


Figure: The sequential rationale behind the principles of IPM
(EU Draft Guidance Document for IPM, 2008)

Barzman et al., 2015
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