Biological Pesticide Technologies and Applications for Agriculture in ASEAN

Ministry of Science
and Technology

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1. INTRODUCTION

The world's food security is increasingly becoming an alarming issue, as the global population is expected to reach 9.1 billion in 2050, with an increase additional food demand for up to 60%. In 2015, the Food and Agriculture Organization of the United Nations (FAO) has shown an estimated number of undernourished people globally at 795 million people, most of them are living in developing countries including Asia (FAO, IFAD&WFP, 2015). Yet, resources needed to produce food, such as land and water, are becoming increasingly scarce and under potential threats from the ever-growing impacts of climate change and economic uncertainties. As part of Asia, our ASEAN region is no stranger to these challenges.

ASEAN is one of the most productive agriculture baskets in the world. In 2012 for instance the region produced 129 million tons of rice, 40 million tons of corn, 171 million tons of sugarcane, 1.44 million tons of soybean, and 70.34 million tons of cassava. Rice production this year is forecast to increase by 3% to 132.87 million tons (ASEAN, 2020). It is important to note that an effective business model for an agriculture enterprise should always include profitability of the crop yield and the expected quality of marketable produce. To that end, chemicals have been widely used in agriculture to fertilize soils and plants or to kill pests and pathogens that are limiting factors to optimum productivity. In recent decades however, an awareness of the impacts of pesticide use on the environment and human health have resulted in efforts to reduce reliance on chemical controls. With the increasing of public concern towards both health and the environment, biological pesticide technology and application for agriculture has been promoted by Governments in countries all around the world including ASEAN countries which is a part of their strategy increase levels of food safety and reduce contamination of the environment (ASEAN, 2016).

This document summarizes biopesticide applications from the AMS experts who participated the ASEAN Forum on Biological Pesticide

Technologies and Applications for Agriculture on 19-20 November 2019, Vientiane, Lao PDR and other open sources and publications.

2. TYPES OF BIOPESTICIDES

There are a number of definitions given by various organizations on biopesticide that all of them include reference to natural or biological origin of the active ingredient. According to Lengal & Muthoml (2018), biopesticides are products and by-products of naturally occurring substances such as insects, nematodes, microorganism, plants as well as semiochemicals. Biopesticides are categorized as biofungicides, bioinsecticides and bioherbicides in general. According to Dhakal & Singh (2018), biopesticides can divide into three main groups such as microbial pesticides, biochemical pesticides and plant-incorporated-protectants. Plants and microorganism are considered as major sources of biopesticides because of the high components of bioactive compounds and antimicrobial agents. Microbial biopesticides consists of microorganism (e.g. bacteria, fungi, viruses, viroids or protozoa) or their products (metabolites, e.g. protein toxins) as the active substance. Entomopathogenic nematodes are sometimes classed as microbial pesticides. Microbials include also insects' natural enemies (e.g. parasitoids such as *Trichogramma* wasps or predators such as coccinellid beetles) and entomopathogenic nematodes (though the latter are often considered as microbials). Botanicals are active substances based on material of plant origin (roots, seeds, foliage etc.) that during production, extraction or processing are not intentionally altered. Botanicals are extremely mixed array of substances ranging from simple plant powders or unprocessed and processed plant extract.

In terms of semiochemicals, they are substances that produce a behavioral or physiological response in another organism. The best known group of semiochemicals are the pheromones produced by members of the insect order Lepidoptera, including moths and butterflies. They are familiar as monitoring lures, but they are also used as plant protection products for insect pest management.

In many ASEAN countries, a biopesticide needs to be authorized as a plant protection product before it can be used in crop protection. The regulatory process is the same as for conventional chemical pesticides, but there is some adaptation of the data requirements.

Before a biopesticide can be deployed successfully by farmers in the field, several steps must be taken:

- Pest management needs must be understood.
- Registered products containing biopesticide active substances that are effective against the target pests have to be identified.
- Biopesticides have to be available, either commercially or through local production.
- Farmers and the field technicians who advise them have to know how to use the biopesticides properly so that they can achieve good results.

3. ADVANTAGES AND DISAVANTAGES OF BIOPESTICIDES

Biopesticides is one of the effective methods to control pets and diseases (Gasic & Tanovic, 2013). They provide significant roles to reduce environmental pollution, maintain ecological balances, reduce pesticide residual effect, conserve of biological resources, reduce human and animal health hazards and generating rural employment. There are several advantages in order to apply biopesticides in agriculture production. However, some disadvantages associated with the usage are also highlighted as illustrated in Table 1.

Table 1: Advantages and Disadvantages of Biopesticides

Advantages of biopesticides	Disadvantages of biopesticides
▪ Usually inherently less toxic than conventional pesticides (Cao et al., 2010) ▪ Lower potential for residues ▪ Can be cheaper than chemical pesticides (if locally produced) ▪ Often effective in small quantities and decompose quickly ▪ May be more effective in the long term ▪ Less prone to development of resistance in target ▪ Generally, more specific, only affecting target organism and closely related organisms (Alam, 2000) ▪ Potential for long-term self-dissemination/ maintenance (classical biocontrol) ▪ Lower non-target effects (Alam, 2000) ▪ Use in IPM system can maintain yield while reducing use of conventional pesticides (Birch et al., 2011) ▪ Often less onerous registration requirements	▪ Slow speed of action (not always) ▪ High specificity (not always) ▪ Can be more expensive commercial products ▪ Variable efficacy due to biotic and abiotic factors ▪ Require careful storage and handling

4. DECISION FOR SELECTING BIOPESTICIDES

Biopesticides can potentially be incorporated into Integrated Pest Management strategies to address the needs. However, not all biopesticides will be appropriate, so certain key points must be taken into consideration when selecting biopesticides for use. To help assess whether or not a particular biopesticide would be an appropriate choice, a decision matrix is provided in Table 2. This decision matrix can be applied to all crop protection agents (CPAs), not just biopesticides. It covers many of the points that regulators consider when deciding whether or not to register a CPA (FAO, 2017).

Table 2: Decision Matrix for Selecting Biopesticides

Biopesticides: identify of what sort of biopesticide to be applied **Target pests:** identify of what sort of pests to be controlled		
Is the biopesticide effective against the target pests?	Is evidence available that is effective against the target problem? Is the control measure known to work reliably under normal farm condition?	- If yes, then proceed to the next point - If no, then rejected
Is the biopesticide safe?	Are the risks posed by the biopesticide to human health and the environment acceptable?	- If yes, then proceed to the next point - If no 1) Biopesticide that meet any of the HHP criteria are considered to pose an unacceptable hazard and should be rejected. 2) Where there are other serious human health (e.g. endocrine disruption) or environmental hazards (e.g. bioaccumulation, aquatic toxicity) mitigation measures should be put in place to reduce risk. For example, the biopesticide should only be adopted for use if appropriate personal protection equipment (PPE) is available.
Is the biopesticide sustainable?	Does evidence indicate that the biopesticide will not affect agronomic sustainability? Is the risk of the development of pest resistance low? Does it pose low risk to pollinators, natural enemies and other beneficial organisms?	- If yes, then proceed to the next point - If no 1) Assess whether mitigation measures can be put in place to reduce risk. 2) If not, the biopesticide should be rejected.

Is the biopesticide practical?	Given the local circumstances, is the biopesticide practical for farmers to use? Is its use realistic given farmers' time and labor constraints? Are appropriate application equipment and storage facilities available? Is the biopesticide compatible with other crop protection measures applied in the production system? Is the biopesticide appropriate for use by small-scale farmers (or is it only effective when used for area-wide management)?	- If yes, then proceed to the next point. - If no 1) Assess whether the practicalities can be overcome, e.g. by adjusting the production system. 2) If the impracticalities cannot be overcome the biopesticide should be rejected.
Is the biopesticide locally available?	Is the biopesticide registered for use in the country? Can the biopesticide be sourced locally?	- If yes, then proceed to the next point. - If no 1) For particularly compelling biopesticides with strong evidence of efficacy, consider exploring the possibility of registration. 2) For biopesticides that are registered for use but not locally available, liaise with manufacturers or consider establishing production facilities.
Is the biopesticide economic?	Will the revenue be greater than the cost associated with the biopesticide?	- If yes, then there are no impediments to using the biopesticide, suggesting that it will be appropriate to incorporate into an IPM strategy. - If no, the biopesticide should be rejected.

5. COMMONLY AVAILABLE BIOPESTICIDES

There are many biopesticide products that have been adopted and used globally nowadays as well as in AMS countries. They provide a significant benefit for green and sustainable agriculture. In Table 3, some effective biopesticides that are commonly available in the markets and used by farmers in ASEAN countries to control pests and diseases. In order to use the biopesticides however need to be supported by scientific research and supervise by biopesticide practitioners or experts before their application.

Table 3: List of Commonly Available Biopesticides

List of Commonly Available Biopesticides				
Biopesticide		**Active substance**	**Target**	**Crops (Field of use)**
Biofungicide				
Micro-organism	Fungal	*Trichoderma asperellum (spp)*	Soil-borne pathogens, *Fusarium oxyporum*	Wide range of edible crops, herbs and ornamentals
	Bacterial	*Bacillus subtilis*	*Botrytis cinerea*	All edible and non-edible crops
Botanical	Botanical	Cinnamon *(Cinnamomum osmophloeum)*	*Verticillium fungicola, Sclerotinia homeocarpa, Fusarium moniliforme* var. *Subglutinans* (Copping, 2004)	Wide range of crops and fruit trees
Bioinsecticide				
Micro-organism	Fungal	*Metarhizium anisopliae* var. *anisopliae*	Vine weevil (Otiorhychussulcatus) and wide range of insect larvae	Soft fruit and ornamentals and wide range of vegetables and herbs
		Beauveria bassiana	Many significant pests including caterpillars, beetles, mites, whiteflies, aphids and thrips	All edible and non-edible crops

	Bacterial	*Bacillus thuringiensis* var.	Larvae (Lepidoptera), Caterpillars	Various edible crops
	Baculovirus	Nucleopoly-hedrosis virus (NPV)	Caterpillars and larvae such as *Helicoverpa armigera*, *Helicoverpa zea*, *Spodoptera exigua*, *Spodoptera littoralis*, *Heliothis virescens*	All edible and non-edible crops
	Entomo-pathogenic nematodes	*Heterorhabditis* spp., *Steinernema* spp.	Many insect pests of soil dwelling stages of their lifecycle, including white grubs (*Phyllophaga* spp.), cutworms (*Agrotis* spp.) and armyworms (*Spodoptera* spp.)	Wide range of edible crops, herbs and ornamentals
Botanical	Botanical	Neem (*Azadirachta indica*) extract	Wide range of pests such as aphids, whiteflies, thrips and flea beetles	All edible and non-edible crops
		Tinospora rumphii	Green leafhopper and brown plant hopper, diamond back moth, Asian corn borer (Leonardo, 1983)	Wide range of fruit trees, edible crops, herb and ornamentals
		Tagetes erecta (Marigold)	Diamond back moth, Green peach aphids (Morallo-Rejesus and Eroles, 1978; Morallo-Rejesus and Decena, 1982)	Wide range of crops
Semioche-mical	Pheromones	Synthetic blends of pheromones	Wide range of field pests such as *Helicoverpa armigera*, *Heliothis virescens*, *Phthorimaea operculella*, *Spodoptera exigua*,	Wide range of fruit trees, edible crops, herb and ornamentals

			Spodoptera frugiperda, *Spodoptera litura* and *Tuta absoluta* as well as stored product pests such as cigarette beetle, tobacco moth and warehouse moth	
Bionematicides				
Micro-organism	Bacterial	*Bacillus firmus*	Nematodes, primarily *Meloidogyne* spp. but also other nematodes such as *Trichodorus* spp., *Ditylenchus* spp.	Wide range of edible crops, herbs and ornamentals
Botanical	Plant extract	Garlic extract	Free-living nematodes	Wide range of edible crops, herbs and ornamentals
		Tagetes erecta (Marigold)	It is most effective against the nematode species (*Pratylenchus penetrans*)	Wide range of edible crops, herbs and ornamentals
Natural enemies				
Insect	Predatory insects	*Trichogramma* wasps	*Chilo infuscatellus*, *Helicoverpa armigera*, Corn army worm, *Helicoverpa armigera*, *Chilo suppressalis*, *Plutella xylostella*	Wide range of crops and vegetables

5.1 Characteristics and applications of some common biopesticides

Biopesticides have been studied and applied in agricultural for decades. However, some common items are essential to take into account especially characteristics and the application information of the biopesticides (Keith Holmes et al., 2019). Some of them are as following:

5.1.1 *Trichoderma* for management of soil-borne pathogens in crops

- Active substance: *Trichoderma* spp.
- Substance group: Microbial – Fungus
- Target pests: Many significant soil-borne pathogens of crop and horticultural plants including *Pythium* spp., *Phytophthora* spp., *Thielaviopsis basicola*, *Rhizoctonia solani*, *Fusarium* spp., *Verticillium* spp.
- Application site and/or stage: Seedbed
- Special considerations for use: If applying to external seedbeds, apply during late evening to avoid high temperatures during initial establishment. Can be incorporated into growth substrate or applied as seed treatment. As with all biological agents wear appropriate PPE, as indicated on product label.
- Overview of the regulatory status: *Trichoderma* spp. are registered worldwide. Check the latest list of nationally registered pesticides to make sure that it is allowed for use in crops. *Trichoderma* are fungi that are known to be present in most soils globally. They are also found within host plant tissues as endophytes (beneficial symbionts). *Trichoderma* species have been extensively researched as biological control agents of fungal pathogens, and have been shown to control most fungal pathogens studied, although the particular species or strain and its efficacy may vary for any given fungal pathogen.

The mechanisms employed by *Trichoderma* species include:

- Mycoparasitism
- Antibiosis
- Competition for nutrients or space
- Induced resistance
- Improvement of nutrient utilization. Overall increased plant health may improve crop vigor, yields and quality, especially under stressful conditions.

Trichoderma spp. have been studied extensively as biocontrol agents in soil-based systems and for aerial application, and are known to as act as opportunistic plant symbionts. *Trichoderma* may demonstrate rhizosphere competence; the ability to colonize the area, usually less than 1 cm, around the plant roots. It has also been demonstrated that *Trichoderma* species colonize the root tissues, where they can induce local or systemic resistance within the host plant. *Trichoderma* species are known to enhance plant health by improving root growth, removing potential plant pathogens from the rhizosphere, and also enabling nutrient uptake by plants. In addition, *Trichoderma*'s innate resistance to agricultural chemicals such as copper and sulphur means it may be used in conjunction with these. The use of *Trichoderma* in nurseries and glasshouse systems is well established and it provides important protection to the early stages of plant production. For example, in plant nurseries it can provide protection to the seed/seedling from damping-off pathogens to enhance production. In addition, those strains of *Trichoderma* with rhizosphere competence or endophytic ability can in principle remain with the host plant when transplanted to the field and provide further direct protection of the root zone, increased stress tolerance (e.g. drought) and aboveground protection against pathogens through induced resistance. *Trichoderma* can be incorporated into growth substrates directly as a solid substrate/powder/granule or as a solution diluted in water or oil (depending on the hydrophobicity of the spores). It can be applied using a knapsack sprayer to the substrate surface. Application rates can vary (see product label for application rate). *Trichoderma* products should be stored in a cool, dry location and used within the recommended time-frame (check product label for details of storage conditions and expiry date).

5.1.2 *Bacillus subtilis* for management of plant pathogens

- Active substance: *Bacillus subtilis*
- Substance group: Microbial – Bacteria
- Target pests: Oomycetes, Fungi

- Application site and/or stage: For plant disease control, these include foliar application and products applied to the root zone, compost, or seed.
- Special considerations for use: While *B. subtilis* is not a known human pathogen or disease-causing agent, it does produce the enzyme subtilis in, which has been reported to cause dermal allergic or hypersensitivity reactions in individuals repeatedly exposed to this enzyme in industrial settings. As with all biological agents wear appropriate Personal Protection Equipment (PPE), as indicated on product label.
- Overview of the regulatory status: *Bacillus subtilis* is registered for use in crop and horticultural plants in many countries. *Bacillus subtilis* is a ubiquitous, naturally occurring, saprophytic bacterium that commonly occurs in soil, water, air and decomposing plant material. Under most conditions, however, it is not biologically active and is present only in spore form. Different strains of *B. subtilis* can be used as biological control agents in different situations. There are two general categories of *B. subtilis* strains: those that are applied to the foliage of a plant and those that are applied to the soil or transplant mix when sowing or transplanting.

Bacillus subtilis **bacteria act through:**

- Antibiosis: *Bacillus subtilis* produces natural antibiotics that enable it to out-compete other microorganisms either by killing them or by reducing their growth rate.
- Competition for sites and/or nutrients.
- Induction of systemic acquired resistance (SAR).
- Improvement of nutrient utilization; this leads to overall increased plant health, which may improve crop vigor, yields and quality, especially under stressful conditions.

When applying *B. subtilis* as a spray follow the general principles indicated below:

- Adjust the application rate and/or spray intervals of *B. subtilis* according to the product label instructions.
- Ensure that product use conforms to resistance management strategies, which may include rotating and/or tank mixing with other products with different modes of action.
- Use the product in sufficient water to achieve thorough coverage (see label instructions).
- Maintain agitation during mixing and application to ensure uniform product suspension.
- Add a surfactant, known to be safe for crops, to the spray tank to improve penetration and coverage of above-ground portions of the plant.
- Use an appropriate application rate and/or spray interval (refer to product label).
- Conduct a spray compatibility test if a mixture with other pesticides, surfactants, or fertilizers is planned.
- Do not exceed label dosage rates.
- Maintain a spray solution with a pH between 4.5 and 8.
- Heavy rainfall or irrigation shortly after application may result in the need to re-apply.
- Products should be stored in a cool, dry location and used within recommended time-frame (check product label for details of storage conditions and expiry date).

5.1.3 *Bacillus firmus* for nematode management

- Active substance: *Bacillus firmus*
- Substance group: Microbial – Bacteria
- Target pests: Nematodes, primarily *Meloidogyne* spp. but also other nematodes such as *Trichodorus* spp., *Ditylenchus* spp.
- Application site and/or stage: Depending on the product, *B. firmus* may be applied as a seed treatment, in the seedbed or in the field.

- Special considerations for use: Apply during late evening to reduce temperature effects on its establishment. As with all biological agents wear appropriate PPE, as indicated on product label.
- Overview of the regulatory status: *Bacillus firmus* is registered for nematode management in some countries where crop and horticultural plants is grown.

Check the registered pesticide list to confirm that it is registered and allowed for use in crops. *Bacillus firmus* is a naturally occurring soil bacterium that associates with the root systems of plants and possesses nematicidal activity. *B. firmus* colonizes the egg sacs of root knot nematodes, subsequently destroying the nematode eggs. Thus, it serves to suppress nematode populations, providing early season protection against nematodes. Treatment with *B. firmus* results in improved plant vigor for more uniform, higher yielding plants. Studies in crops such as tomato have demonstrated that it can achieve control levels equivalent to those of chemical controls and can keep nematode populations below economic threshold levels. In areas with high nematode infestation additional control measures may be needed. There is no evidence that *B. firmus* poses a risk to human health, and it is non-harmful to non-target organisms and the environment. Commercialized products containing *B. firmus* are applied as a seed treatment or directly to the soil in the greenhouse or field. For products that are used as seed treatments, apply *B. firmus* as a water-based slurry. When used in the field, it is generally recommended that the product is applied several days prior to planting to treat the soil by spraying or by drip irrigation in furrows. It can also be applied for a second time mid-season for additional control.

Bacillus firmus is compatible with many seed treatments and soil fungicides. It is not compatible for use at the same time as soil sterilants, but it can be used after the application of soil sterilants for improved control. *Bacillus firmus* is thermo-tolerant, so it can be used in conjunction with soil solarization. For more information on how to use products containing *B. firmus*, refer to the product's label. Products containing *B. firmus* should be stored in a sealed container under cool, dry conditions (check product label for details of storage conditions and expiry date).

5.1.4 *Bacillus pumilus* for management of downy mildew, powdery mildew and other diseases of crops

- Active substance: *Bacillus pumilus*
- Substance group: Microbial – Bacteria
- Target pests: For use against a wide range of fungal diseases, including blue mould Training Guide for Field Technicians and Farmers.
- Application site and/or stage: Foliar application
- Special considerations for use: Apply in the evening or early in the morning. Do not apply in full sun. As with all biological agents wear appropriate PPE, as indicated on the product label.
- Overview of the regulatory status: *Bacillus pumilus* is registered for use in tobacco and crops in many countries.

Bacillus pumilus is a common bacterium that occurs in soils and water worldwide. It is a broad-spectrum preventive biopesticide used for the control or suppression of many important diseases of tobacco and crops, including *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp., *Alternaria* spp., *Aspergillus* spp. and blue mould (*Peronospora hyoscyami*). It acts as a fungicide by forming a physical barrier between the plant surface and the fungal spores, inhibiting fungal development on the plant surface and then colonizing the fungal spores. It also stimulates treated plants' immune systems. It promotes root growth as well, which helps in the development of vigorous root systems and uniform plants. It is not harmful to human health or the environment. Multiple strains of *B. pumilus* have been commercialized and are allowed for use in tobacco and crops. Peat-based products containing *B. pumilus* are available, which can be used as a growing medium for tobacco seedlings for transplants. This can be used in floating seedbed systems. Other products containing

B. pumilus can be applied with normal spray equipment for ground application or chemigation. It can be applied to the soil for management of many diseases and also to the plant surface for management of blue mould.

Bacillus pumilus is most effective when used as part of a preventive disease management program. It is compatible with many fungicides, bactericides, insecticides and adjuvants. It can be applied on its own, tank mixed with other CPAs or used in rotation with other fungicides for improved management. Using *B. pumilus* with a surfactant can improve penetration and coverage of above-ground portions of the tobacco plants. When conditions are conducive to heavy disease pressure, *B. pumilus* should be used in a rotational program with other registered fungicides. Likewise, when the weather conditions are expected to be conducive to disease development, if the field has a history of disease problems, or if minimum/low-till programs are in place, use the higher application rates suggested. *Bacillus pumilus* can be applied up to and including the day of harvest. It should be stored in a dry place at 25°C.

5.1.5 *Beauveria bassiana* for managing insect pests

- Active substance: *Beauveria bassiana*
- Substance group: Microbial – Entomopathogenic fungus
- Target pests: Many significant pests of crops and horticulture plants, including caterpillars, beetles, mites, whiteflies, aphids and thrips
- Application site and/or stage: Field
- Special considerations for use: Apply in the evening or early in the morning. Do not apply in full sun. As with all biological agents wear appropriate PPE, as indicated on product label.
- Overview of the regulatory status: *Beauveria bassiana* is one of the most widely registered biopesticides for use in crop and horticultural plants globally.

Beauveria bassiana is one of the biopesticides most commonly used worldwide to control arthropod species. It occurs naturally in the soil throughout the world and is registered for use in many countries. There are many potential advantages to using *B.bassiana*; for example, residues are not an issue, and it can be applied up to the day of harvest. Depending on the circumstances in which it is used, the cost and labor requirements for *B.bassiana* may be similar to those of other CPAs. It is a reduced-risk CPA that poses little hazard to human health or the environment. When *B. bassiana* is applied regularly in combination with other cultural controls, farmers are often able to replace or reduce their use of other CPAs. Since *B. bassiana* is a biopesticide containing living spores, its use differs in some ways from that of other chemical CPAs. If not used correctly, it may be ineffective.

Beauveria bassiana is an entomopathogenic fungus, and has a broad host range. It attacks many significant pests of crops, including caterpillars, beetles, mites, whiteflies, aphids and thrips. *Beauveria bassiana* can be applied to the soil or as a foliar application. When an insect host comes into contact with *B. bassiana* spores, the spores stick to the insect's skin (cuticle), allowing the fungus to infect and kill the insect. *Beauveria* products should be stored in a cool, dry location and used within the recommended time-frame (check product label for details of storage conditions and expiry date).

Procedures:

Beauveria will be most effective if it is applied when pests are detected at onset of infestation. Therefore, early scouting and detection followed by application of *Beauveria* when insect number are low will result in the most effective control and the key points regarding the mode of application and application timing:

- Wear appropriate PPE as described on the label when applying and mixing.

- Always check the product label for dosage and target pests. Water volume depends on spray equipment, crop canopy and target pest.
- Apply in the evening or early in the morning. Do not apply in full sun.
- Shake the container well before mixing to ensure that spores are suspended.
- To mix, fill spray tank with half the desired amount of water and agitate to mix. Shake the product, slowly add the desired quantity to the spray tank and then add the remaining amount of water.
- Once mixed, *Beauveria* will only be viable for a limited time, so the mixture should be applied as soon as possible. Do not mix more product than is needed for that day.
- Continue agitation during spraying.
- Apply as recommended on the product label.
- *Beauveria* may take some days to infect and kill pests. When immediate control is required in order to avoid crop losses, pair it with an appropriate insecticide that is compatible for use with *Beauveria*.
- Many fungicides are not compatible with *Beauveria*. Do not tank mix *Beauveria* with fungicides unless compatibility can be assured. Wait at least 48 h before applying fungicides to areas that have been treated with *Beauveria*.
- Avoid applying to areas where honey bees are active.
- There is no limit on the number of applications or the total amount of product that can be applied in one season.
- There is no pre-harvest interval for *Beauveria*. It can be applied up to the day of harvest.

Since *Beauveria* is a living organism, storage conditions are important. Make note of the following points:

- Always store the product in a cool, dry place out of direct sunlight (between 40°F/4°C and 80°F/27°C). Avoid storing or transporting at high temperatures as this may harm the live spores of *Beauveria*.

- Close containers tightly after use in order to avoid contamination and loss of efficacy. Whenever possible, once a container has been opened, use all of its contents.
- Do not store or let *Beauveria* preparations stand for more than 4h.

5.1.6 Using *Metarhizium* to manage grasshoppers and other insect pests

- Active substance: *Metarhizium* spp.
- Substance group: Microbial – Fungus
- Target pests: Many significant pests of crops and fruit trees including *Oryctes rhinoceros* L., caterpillars, beetles, mites, whiteflies, aphids and thrips
- Application site and/or stage: Field
- Special considerations for use: Apply in the evening or early in the morning. Do not apply in full sun as it is UV sensitive. As with all biological agents wear appropriate PPE, as indicated on product label.
- Overview of the regulatory status: *Metarhizium* spp. are registered for use in crops and horticultural plants in many countries.

In Africa in particular it has been used successfully to replace many highly hazardous pesticides for the control of locusts and other swarming grasshoppers. *Metarhizium* is an entomopathogenic fungus, i.e. a type of fungus that parasitizes insects. It can kill or disable the target insect host. *Metarhizium* spp. are found in soil or as endophytes (beneficial plant symbionts). The spores or mycelium can attach to the surface of the insects. They can penetrate the host insect's cuticle and use enzymes to penetrate the insect. Once inside the host insect body cavity *Metarhizium* can multiply. Once the insect is dead, the fungus colonizes the insect and then sporulates. These spores are then disseminated and the process begins again. The application of these entomopathogens to control insect pests in the field is well established and many commercial products are available.

The products are composed of dry spores which can be mixed with agricultural adjuvants or sprayed in oil formulations. *Metarhizium* products should be stored in a cool, dry location and used within the recommended time-frame (check product label for details of storage conditions and expiry date).

5.1.7 *Bacillus thuringiensis* for managing caterpillars and larvae (Lepidoptera) in crops

- Active substance: Bt: *Bacillus thuringiensis* subsp. *Aizawi* or *B. thuringiensis* subsp. *kurstaki*
- Substance group: Microbial -- Bacteria
- Target pests: Caterpillars and larvae such as *Helicoverpa armigera*, *Helicoverpa zea*, *Spodoptera exigua*, *Spodoptera littoralis*, *Heliothis virescens*
- Application site and/or stage: Field
- Special considerations for use: As with all biological agents appropriate PPE should be worn, as indicated on product label.
- Overview of the regulatory status: *Bt* is one of the most widely registered biopesticides for use in crops and horticultural plants globally.

Bacillus thuringiensis (*Bt*) is one of the most commonly registered biopesticides for use in crops and horticultural plants around the world. *Bt* is a bacterium naturally found in soils worldwide and infects many insect pests, including beetles, mosquitoes, blackflies, caterpillars and moths. The proteins produced by the bacteria are toxic to insects and their action is very specific; each type of *Bt* strain targets a specific group of insects. *B. thuringiensis* subsp. *Aizawi* and *B. thuringiensis* subsp. *kurstaki* infect lepidopteran larvae such as *Helicoverpa armigera*, *Helicoverpa zea*, *Spodoptera exigua*, *Spodoptera littoralis*, *Heliothis virescens*. For this reason, the type of *Bt* to be used must be selected carefully to match and control the target insect pest. To be effective, the bacterium must be eaten by caterpillars and reach the gut of the insect before it will take effect. When small caterpillars and larvae start feeding on crops leaves sprayed with *Bt*, the bacteria reach the gut where the toxins are activated and start to break down the gut. The infected larva dies of infection and starvation.

As *Bt* strains are highly specific to insect groups, *Bt* sprays usually do not harm beneficial insects. Other benefits of using *Bt* include no residue issues, and it is safe for use in the environment, with no known effects on wildlife. As some insect pests have developed resistance due to intensive spraying over many years, a pesticide resistance management strategy is highly recommended.

How to use:

- Apply when eggs are due to hatch or when caterpillars or larvae are small (1st and 2nd in star larvae). These are the most susceptible instars, because the larvae feed on open leaf surfaces that are accessible to sprays. Once the larvae have bored into the stem, they are protected from sprays.
- Early scouting and detection followed by application of *Bt* products when caterpillar and larvae numbers are low will result in the most effective control.
- Wear appropriate PPE as described on the label when applying and mixing.
- Check the product label for dosage and target pests. Water volume depends on spray equipment, crop canopy and target pest.
- *Bt* breaks down under the UV light of the sun, therefore apply in the evening or early in the morning. Do not apply in full sun.
- Do not apply *Bt* product through any type of irrigation system.
- To mix, fill spray or mixing tank three quarters full with the desired amount of water and start agitation. Slowly pour the desired quantity of product into water with the agitator

running, and add the remaining amount of water. Agitate as necessary to maintain suspension.

- Continue agitation during spraying.
- Additional adjuvants, spreaders or stickers approved for crops may be added to improve product performance, especially under heavy dew or rainy conditions.
- Use diluted sprays as soon as possible and within 48 hours. Do not mix more product than is needed for that day.
- For adequate insect control, thorough and uniform crop coverage is required. Ensure good spray coverage on the top and bottom of the foliage.
- To assure efficacy of the product, larvae must be actively feeding on treated, exposed plant surfaces.
- There is no pre-harvest interval for *Bt*. It can be applied up to and on the day of harvest.
- Re-entry interval (REI) is 4 hours before entering treated areas.
- Repeat applications as necessary under a pest management program that includes close scouting, usually at 3 to 14days intervals depending on plant growth rate, moth egg-laying activity and rainfall after treatment.
- In the case of a heavy infestation, where quick action is required to avoid crop losses, pair with an appropriate contact insecticide compatible for use with *Bt. Bt* products can be mixed with commonly used insecticides or fungicides, which are generally not deleterious to the *Bt* product if the mix is used promptly. Before mixing in the spray tank, it is recommended to test physical compatibility by mixing all components in a small container in proportionate quantities. *Bt* is toxic to aquatic invertebrates and highly toxic to honey bees exposed to direct treatment. Do not apply directly to water or while bees are actively visiting the treatment area.
- *Bt* should be stored in its original container in a cool, dry place (under 90°F/32°C), inaccessible to children and away from heat and direct sunlight.

- Protect from freezing.

5.1.8 Nucleopolyhedrosis virus for managing caterpillars and larvae (Lepidoptera) in crops

- Active substance: Nucleopolyhedrosis virus (NPV) of *Angrapha falcifera*, *Autographa californica*, *Helicoverpa armigera*, *H. zea*, *Spodoptera exigua*, *S. littoralis*
- Substance group: Microbial – Baculovirus
- Target pests: Caterpillars and larvae such as *Helicoverpa armigera*, *Helicoverpa zea*, *Spodoptera exigua*, *Spodoptera littoralis*, *Heliothis virescens*
- Application site and/or stage: Field
- Special considerations for use: As with all biological agents wear appropriate PPE, as indicated on product label. Do not expose to direct sunlight.
- Overview of the regulatory status: NPV is registered for use in crops and horticultural plants in many countries globally.

Nucleopolyhedrosis virus (NPV) is highly specific, safe and environmentally friendly, making it ideally suited for inclusion in an integrated pest management (IPM) approach in cropping systems. NPV particles are called polyhedral inclusion bodies (PIBs) and must be eaten by the larvae for infection to occur. Once the PIB is ingested, the virus infects the epithelial cells of the mid gut. Ingestion of a single PIB is usually sufficient to kill the insect. NPV belongs to a group of insect diseases called baculoviruses that infect and kill the larvae of moths and sawflies. NPV can kill young larvae within 4 days of ingestion, and older larvae within 5 to 7 days, depending on dose and temperature. NPV is self-propagating in the field. The infection of one caterpillar/larva will result in the death of a whole population. This reduces the number of chemical sprays generally required in the field.

NPV will not affect non-target organisms.

NPV is usually formulated as the lyophilized powder of dead insect larvae/ caterpillars or a suspension of the PIB.

NPV can be applied in the field in the same way as a standard CPA.

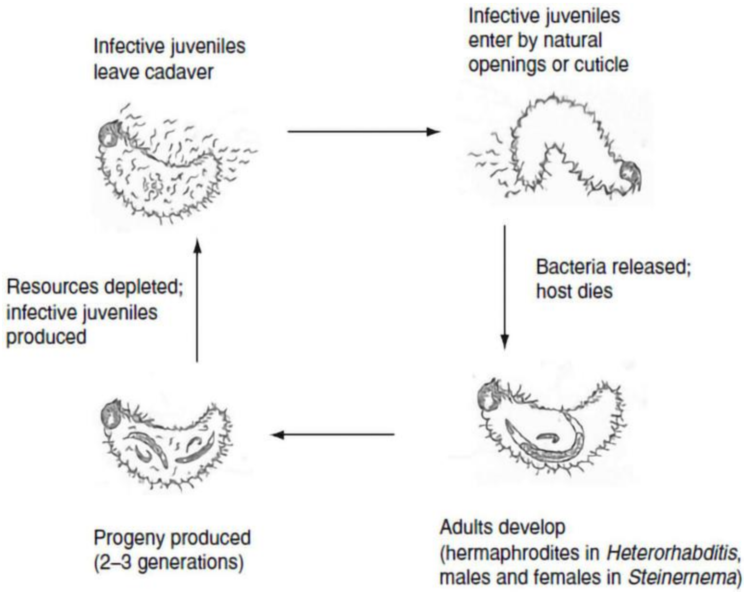
NPV should be stored in a cool dry location Powder formulation may be stored frozen but not liquid formulations.

5.1.9 Entomopathogenic nematodes for managing insect pests in tobacco

- Active substance: *Heterorhabditis* spp., *Steinernema* spp.
- Substance group: Microbial – nematodes
- Target pests: Entomopathogenic nematodes are effective against many insect pests of crops and horticultural plants with soil dwelling stages of their lifecycle, including white grubs (*Phyllophaga* spp.), cutworms (*Agrotis* spp.) and armyworms (*Spodoptera* spp.)
- Application site and/or stage: Field
- Special considerations for use: Apply in the evening or early in the morning. Do not apply in full sun.
- Overview of the regulatory status: Most countries do not require the registration of native species of entomopathogenic nematodes.

Entomopathogenic nematodes (EPNs) are a group of nematodes that parasitize and kill many different types of insects that come into contact with the soil, such as armyworms, cutworms, white grubs, etc. The EPNs most commonly used for the biological control of insects are found within the genera *Steinernema* and *Heterorhabditis*. Rapid death by EPNs reflects the pathogenicity of their bacterial symbionts. In the case of *Steinernema* and *Heterorhabditis* these bacteria are from the *Xenorhabdus* and *Photorhabdus* genera, respectively. A simplified illustration of the infection and subsequent death of an insect host can be seen in Figure 1 below. EPNs employ different strategies to find host larvae; some species actively search for the host insect larvae while others wait and use an ambush strategy. EPNs can be supplied as formulated products in clay or another carrier, or on moist sponge.

Figure 1: Simplified illustration of the infection and subsequent death of an insect host



Simplified life cycle of entomopathogenic nematodes (Griffin *et al.*, 2005).

Procedure:

As the EPNs are living organisms and that the right environmental conditions are important for the survival and success of EPN.

- **Moisture:** Sufficient soil moisture is required for the nematodes' efficacy and survival. EPNs use the water channels like roads to reach their hosts. Therefore, the soil needs to be moist below the level of the grubs. Keeping the soil wet for 2–3 days after an application can help to ensure EPN efficacy.

- **Texture:** Different soil textures have varying capacities for oxygen, which is an important factor in the nematodes' survival. Different soil types can influence the efficacy of the nematodes; the lowest survival for EPNs is recorded in clay soil (compared with sand, sandy loam and clay loam). This lower survival rate is probably due to the lower oxygen levels present in the small pores of clay soils. Note: if the soil is saturated from heavy rains or lack of drainage, the nematodes could die from lack of oxygen.
- **Temperature:** The effect of temperature on survival varies with nematode species and strains. Soil temperature determines the activity and efficacy of EPNs. If it is too cold, they are inactive and will not actively seek their hosts. Conversely, if the soil is too warm, they will use up their energy source too quickly.
- **Check viability of the product:**
  - Check product label for expiry date.
  - Confirm product has been transported and stored appropriately before arriving in the field, e.g. in a cool box with icepacks, kept refrigerated.
  - Check product for visual contamination by fungi.
  - Check odor of product; an earthy smell is healthy.
  - If possible, conduct a laboratory viability test as indicated in the activity above.

5.1.10 Pheromones for monitoring insect pests in crops and fruit trees

- Active substance: Pheromone blend appropriate for the target pests.
- Substance group: Semiochemicals.
- Target pests: Pheromones have been commercialized for a wide range of field pests such as *Helicoverpa armigera*, *Heliothis virescens*, *Phthorimaea operculella*, *Spodoptera*

exigua, *Spodoptera frugiperda*, *Spodoptera litura* and *Tuta absoluta* as well as stored product pests such as cigarette beetle, tobacco moth and warehouse moth.

- Application site and/or stage: Seedbed, field, storage.
- Special considerations for use: Apply in the evening or early in the morning. Do not apply in full sun.
- Overview of the regulatory status: Pheromones have been registered in many countries globally, and in some countries, e.g. the USA, they are exempted from the registration requirement.

Pheromones are chemicals produced by animals to attract a mate. For many species of insects, females emit pheromones to attract males. Pheromones tend to be highly specific, so only males of the same species are attracted to the pheromones that the females emit. Synthetic blends of pheromones are available commercially in many countries globally, e.g. Brazil, South Africa and the USA.

Pheromones are used for monitoring insect populations, helping farmers to decide when to apply CPAs, and, for some insect species, can be used for control, through mass trapping or by applying pheromones in the field to confuse the males so that they are unable to locate females. However, mass trapping or mating disruption is generally more likely to be effective if used over a large area. In areas with many small farms, this would be difficult to organize.

Pheromones can be used in the seedbed, field or warehouse to monitor populations of armyworm, tobacco moth, click beetle and cigarette beetle. Pheromones are low risk, and present no known hazards to humans or the environment. Because they are species-specific and tend to be released in small quantities, no adverse impacts on non-target organisms are expected. The frequency with which the trap should be checked, and the threshold for action should be applied, will depend on the pest in question and the local conditions.

Guidelines for use:

- Use 10 pheromone traps/ha (or double that if infestation persists).
- Replace the lures every 2 months.
- Lures should be stored below 5°C.
- Use a delta or funnel trap, depending on the pest:
  - To mount the delta trap, attach the side flaps to the lower fittings. The glue refill is placed on the inside of the trap with the glue uppermost. Place the pheromone septum on top of the glue in the center of the refill.
  - The funnel trap is already mounted and the septum should be trapped at the top.
- Check the trap every week and count the insects captured.

5.1.11 *Trichogramma* spp. for managing lepidopteran pests

- Active substance: *Trichogramma* spp., e.g. *T. pretiosum* or *T. evanescens*
- Substance group: Macroorganism – Parasitoid wasp
- Target pests: Caterpillars and larvae such as *Helicoverpa* spp., *Heliothis virescens*, *Ephestia elutella* (in storage)
- Application site and/or stage: Field/Storage
- Special considerations for use: Do not use on small, isolated fields
- Overview of the regulatory status: With a few notable exceptions (e.g. Brazil), most countries do not require native *Trichogramma* spp. to be registered, but be careful when considering the use of exotic *Trichogramma* spp.

Trichogramma is one of the most widely used biological control agents. Despite its tiny size — 1 mm or less — *Trichogramma* is an efficient natural enemy of lepidopteran pests of many crops and horticultural plants such as budworm and tobacco moth. *Trichogramma* are released as parasitized eggs attached to a piece of card known as a Tricho-card. Adult *Trichogramma* females emerging from these Tricho-cards search for pest eggs into which they can lay their eggs. This will kill the larvae before they hatch, thus preventing them from doing any damage. The release of *Trichogramma* can have many advantages. For example, residues are not an issue, and Tricho-cards can be applied up to the day of harvest. Also, applying Tricho-cards poses no hazard to human health or the environment. Applying *Trichogramma* in combination with other cultural controls means that farmers are often able to replace or reduce their use of conventional CPAs.

Procedure:

It is well known that *Trichogramma* wasps are very small but also very effective, killing the moth eggs before they can hatch and cause any damage. In order to be able to use *Trichogramma* wasps for the management of lepidopteran pests in the fields:

- Place the Tricho-cards into the field shortly before the *Trichogramma* wasps emerge from the eggs on the card (based on information from the provider).
- Make sure that the printed side is facing up as this will protect the *Trichogramma* on the back side from rain and direct sunlight.
- Avoid release during hot times of the day and during or before heavy rain.
- Put out 100 Tricho-cards for each hectare of field. The release points should be about 10 m apart. Each Tricho-card harbors about 1000 *Trichogramma* wasps, resulting in the release of 100,000 wasps per hectare.
- The *Trichogramma* wasps emerging from the cards could be killed by any insecticides applied to the crop. Thus, DO NOT spray pesticides, particularly insecticides, either 1 week before or after the release of *Trichogramma*!
- With *Trichogramma* releases, insecticide applications are often made unnecessarily. If there happens to be serious pest occurrence, consult a local plant protection agency for advice.

- *Trichogramma*, and many other beneficial insects, can benefit from nectar sources around the treated crop. It would therefore be an advantage to preserve and/or grow plants providing flowers and nectar, for example, sesame or soybean plants.
- The efficacy of *Trichogramma* is very much improved if they are released into a larger area, e.g. if farmers from a village agree to combine their efforts. If they are only released in a single field, some *Trichogramma* might leave that field, thereby reducing their effectiveness.

5.1.12 Neem-based products for managing whiteflies, aphids, thrips and caterpillars in crops and fruit trees

- Active substance: Azadirachtin (and other limonoids/ terpenoids)
- Substance group: Plant extract/Botanical
- Target pests: Insects
- Application site and/or stage: Soil or foliar application, field
- Special considerations for use: Ensure appropriate PPE is worn, as indicated on the product label.
- Overview of the regulatory status: Registered for use in crops and horticultural plants in many countries.

Neem extracts can control numerous insect pests, including several important ones found on crops such as whiteflies, aphids, thrips, caterpillars and leafhoppers. Neem products have also been shown to be effective against some pests that have developed resistance and are difficult to control by most synthetic insecticides (Lokanadhan *et al.*, 2012).

Most commercial neem products are oil-based, emulsifiable concentrates containing azadirachtin at various concentrations. Azadirachtin disrupts or inhibits the normal development of eggs, larvae or pupae by preventing normal hormone releases triggering growth and maturation. It also acts as a repellant, feeding and ovipositional deterrent, and mating disruptor (Ghewande et al., 1993).

Neem products are easy to use and relatively safe in comparison with conventional synthetic pesticides. In particular, neem is generally of low toxicity to humans, and does not persist in the environment.

Neem can be applied as a foliar spray application (see product label for application rates and timing). It has a slow mode of action, and its effects are usually not immediately visible. Some neem extracts (e.g. oil extracts) may be phytotoxic. Therefore, it is important to test the extract on plants before full-scale spraying in the field.

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