Committee Recommendations on Registration of Biopesticides

Introduction

Management of pests will continue to play a key role in sustainable production and productivity in agriculture. Synthetic pesticides play a major role in pest management. However, their adverse effects on the user, consumer and the environment draw major concern. On the other hand, biopesticides are highly selective to target pests, easily degradable and safe to non-target and beneficial organisms. They are amenable to bio-intensive pest management and ideally suited for organic niche products including export-oriented commodities. They can also be used in IPM programmes for increased efficacy, higher yield and lowering chemical usage. They can also be used effectively in management of pesticide resistance and generate less harmful pesticide residues. It is now widely recognized that biopesticides can be successfully used in modern agriculture replacing the synthetic pesticides. As a result, there is huge scope for growth of the bio-pesticide market globally. An annual increase of about 15% with revenue of 6.6 billion US dollars is anticipated in 2020.

Global situation

Bio-pesticides have successfully been used in a number of countries in the sustainable management of agricultural pests. USA, India, Thailand and a number of other countries have already made significant progress in promoting the local registration, production and use of bio-pesticides. Indigenous microorganisms, plant extracts and natural enemies have been successfully developed as plant protectants for local farmers. Local research institutes, regulating agencies, extension services, small and medium scale companies and NGOs have played an active role in developing and promoting new, safe crop protection techniques using their natural resources. They have amended their pesticide regulation Acts to facilitate registration of local bio pesticides. The flexible and enabling regulatory environment has been a contributing factor in facilitating these developments. Allowing minimal toxicity testing, provision of waivers and the acceptance of published generic data especially for in use botanicals and microbials minimizing expensive data requirements. As a result, in India, a large number of small industries are involved in the production of bio-pesticides utilizing local resources. India has formulated separate regulations to cater to different types of bio-pesticides under section 9(3b) and 9(3) of their insecticide Act, 1968. These amendments have helped greatly in these developments. For example, different regulations exist for different botanicals (Neem, Pongomia etc.) and microbial pesticides (fungi, bacteria and virus). Similarly, EPA, European Union and many other countries have already amended their regulations to encourage the use of bio-pesticides.

The relevance to local conditions

Pest and disease control in Sri Lanka depends mainly on the use of synthetic pesticides. Due to the increasing trend of the occurrence of non-communicable diseases such as cancer and kidney failure, which are suspected to be correlated to pesticide and fertilizer usage in agriculture, the public demand for safer food is increasing. Further, safer products are needed to produce agricultural commodities for the export markets. Under these circumstances, the government has

launched a three year national programme, namely *Wasa Visa nathi Ratak*, to eliminate the use of synthetic pesticides and synthetic fertilizers in agriculture towards the end of year 2018. Therefore, it has become the foremost responsibility of researchers, regulating authorities and industrialists to ensure the availability of suitable local biopesticides for undisruptive agriculture production.

Due to the tropical humid climate, Sri Lanka is rich in biodiversity and provides ample resources for the production of bio-pesticides. Our fore fathers have efficiently used biological resources for medicinal and agricultural purposes and therefore, we have rich traditional knowledge on their sustainable utilization. Utilizing these opportunities, local researchers and small scale industries have developed biopesticides in the previous decade. However, many were not able to register their products, as they were unable to provide the extensive data/details required by the authorities. The current pesticide regulation Act No 33 of 1980 and its amendments focus mainly on registration with regards to import of synthetic pesticides. The Act does not provide the necessary registration guidelines for bio-pesticides. Further, the locally available laboratory facilities are insufficient to generate the required data required by the current Act. Therefore local manufacturers have to obtain these reports from foreign laboratories at a cost of over Rs. 5 million. This situation has hampered the development of the local biopesticide industry which has now become a national priority.

Committee Recommendations

Except for the introduction of a few bio-control agents, Sri Lanka depends on synthetic pesticides for the control of pests and diseases. The demand for pesticide residue free foods has increased in Sri Lanka during the last decade, especially due to public awareness after the emergence of non-communicable diseases such as CKDu and cancer to significant levels and also due to the increasing demand for safe food in the local and export markets as encouraged by the government. The high biological diversity prevailing in the country provides enough natural resources for local development and production of bio-pestcides not only for local use but also for export purpose.

Under these circumstances, formulation of a favorable national policy encouraging local researchers and industries to develop bio-pesticides from locally available resources has become an urgent national requirement and priority. **As the current pesticide regulation Act No 33 of 1980 does not provide guidelines for the registration of bio-pesticides, the committee strongly recommends that the current pesticide Act be amended to include guidelines/regulations for this purpose. This would enable the local biopesticde industry to cater to recent requirements.**

The proposed guidelines need to comply with plant protection and other relevant Acts in place in Sri Lanka and also strictly limited to locally produced biopesticides.

The following factors should be considered.

1. Acceptance of published data where appropriate.
   e.g. adoption of available toxicity data for active ingredients/technical material/formulations of bio-pesticides.
2. Waivers for registration dossiers where adequate data are already available (e.g. neem)
3. Adoption of a fast system for registration of bio-pesticides where active ingredient/ microbe/ technical material is already in use and safety is generally accepted.
4. Formulation of appropriate requirements to register different active ingredients including microorganisms.
5. Establishment of a fully equipped laboratory within a 3 year period or upgrade existing laboratories in order to provide data requirements for the registration process.
6. Granting of a provisional registration, effective for three years for the locally manufactured bio-pesticides with commonly used active ingredients/technical materials which have proven bio-efficacy of the formulation tested by the relevant Institute accepted by ROP. This provision should be granted only for products manufactured locally, where the production process is accessible to ROP for inspection and monitoring. However, the manufacturer should be able to submit the full dossier fulfilling the data requirement specified in the proposed guideline to obtain the full registration.
7. Exempting macrobial bio-control agents and pheromones without synthetic active ingredients/technical material from the amendments of the Act.
8. Exempting traditionally used bio-control preparations/practices which have no commercial interest from the Act.

Definition: Bio-pesticides are types of pesticides derived from such materials as animals, plants, bacteria and certain minerals (US-EPA). Bio-pesticides fall into three major categories.

1. Living organisms: These include invertebrates, natural enemies (parasitoids, predatory insects, nematodes) and micro-organisms. They generally pose little or no risk to man and the environment.
   e.g . Microbial pesticides can be used to control different kinds of pests and most of them are specific for the target pests (strains of *Bacillus thuringiensis* produce insecticidal proteins, which bind to a larval gut receptor and specifically kills one or a few related species of insect larvae of different insect orders).

Naturally occurring substances: These include plant extracts, which pose adverse effects on pests (e.g. analogues of insect hormones, toxicants, antifeedants, repellants) and semiochemicals, which influence the physiology and behavior of the target organism (e.g insect pheromones and allelochemicals). They are expected to pose little or no risk to man and the environments, e.g Neem extracts.

2. Plant –incorporated protectants (PIPs): Genetically modified plants that express introduced genes that confer protection against pests or diseases. **Currently PIPs are not allow to be used in Sri Lanka.**

The suggested Registration Requirements of Locally Produced Botanical Pesticides

Registration requirements should be vary with the active ingredient or ingredients in the formulation. Therefore, registration requirements and guidelines for products containing different active ingredients should describe separately. Further, the quantitative and qualitative composition of active substances in botanical products may vary due to number of factors (geographical area and climatic conditions). As a result, AI concentration of the formulation may vary from batch to batch. Therefore for botanical formulations, the minimum concentration/range of the AI (s) should be considered.

1. Registration requirements of Neem and other Botanical Pesticides

A.	**GENERAL**		
	Description	Other botanicals	Neem
	Name of the Local Manufacturer	R	R
	Address and contact details	R	R
	Company Registration details	R	R
B	PRODUCT DETAILS		
1	**CHEM ISTRY**		
	Description	Other botanicals	Neem
1.1	Name and the Part of the Plant(s) to be used for extraction of the active ingredients /	R	R

	Technical material.		
1.2	(a) Chemical identity and quantity of the active ingreadient/s.	R The minimum quantity of the AI should be worked out by standard bioassay by the competent Authority	R Azadirachtin content in the formulated products based on seed extracts should contain not less than 1500 ppm and neem oil based formulated product it should be not less than 300 ppm
1.3	Complete chemical composition of the formulation should be submitted (the kind, name and percentage of all ingredients) The analytical test report obtained from a reputed national/international laboratory accepted by ROP should be submitted.	R	R
1.4.	Complete identity and physicochemical characteristics of various ingredients' used in the formulation should be submitted	R	R
1.5.	Shelf-life claim for the product	R	R
2	**BIO-EFFICACY OF THE FORMULATED PRODUCT**(Should be obtained from the relevant Institute recommended by ROP)		
	Description	Other botanicals	Neem
2.1	Bio-effectiveness	R	R
2.2	Phytotoxicity	R	R
2.3	Purpose of manufacture	R	R
2.4	Intended use (crops/pests)	R	R

	Products recommended for household purpose should get permission from the Ministry of Health.		
2.5	Direction concerning dosage	R	R
2.6	Application frequency	R	R
2.7	Time of application	R	R
2.8	Waiting period	R	R
2.9	Application equipment	R	R
2.10	Information regarding registration status of the AI in other countries, if any	R	NR
2.11	Information regarding traditional use (medicine, Pest control)	R	NR

3	**TOXICITY OF THE FORMUYLATED PRODUCT : (Should be obtained from local/foreign laboratory accepted by the ROP)**		
	Description	Other botanicals	Neem
3.1	Acute oral rat and mice	R	R
3.2	Acute dermal	R	R
3.4	Eye Irritation	R	R
3.5	Neuro-behavioral toxicity, reproductive toxicity, carcinogenicity, mutagenicity and teratogenicity	R	NR
C	PACKAGING AND LABELLING		
	Description	Other Botanicals	Neem

1.	Labels and Leaflets as per Sri Lankan regulations	R	R
2	Type of packaging	R	R
3	Manner of Labeling (Specimen in label accordance with the section 8 of the Act in duplicate should be submitted)	R	R
4	Specification for primary package (Specimen sample should be submitted)	R	R
5	Specification for secondary package	R	R
6	Specification for transport package	R	R
7	Instructions for storage and use	R	R
8	Information regarding disposal of used package	R	R

R- Required NR- Not required.

THE SUGGESTED DATA REQUIREMENTS FOR REGISTRATION OF ENTOMOPATHOGENIC FUNGI AS MICROBIAL PESTICIDES

I STANDARD OF FORMULATIONS:

1. Colony Forming Unit (CFU) count on selective medium should be minimum of $1x\ 10^8$ / ml or g for entomopathogenic fungi.

1. Contaminants:

2.1 Biological contaminants:

2.1.1 Pathogenic contaminants such as gram negative bacteria *Salmonella*, *Shigella, Vibrio* and such other microbials should not be present.

2.1.2 Other microbial contaminants should not exceed 1×10^4 / ml or per g of formulation.

2.2 Chemical/botanical pesticide contaminants should not be present.

2. Stability of CFU counts at 30 °C and 65% RH

11 REGISTRATION REQUIREMENTS:

A. BIOLOGICAL CHARACTERISTICS AND CHEMISTRY

Product should full fill the following requirements

No.	Requirements	
1.	Systematic name (Genus, species and strain)	R
2.	Common name, if any	R
3.	Source of origin as per Annexure-1.1	R
4.	Natural occurrence of the organism and morphological description	R
5.	Composition of the product	R
5.1	CFU/g of the product	R
5.2	Percent content of the biocontrol organism in the formulation & nature of biomass.	R
5.3	Percentage of carrier/filler, wetting/ dispending agent, stabilizers/ emulsifiers, contaminants/ impurities etc.	R
5.4	Moisture content	R
6.	Specification of the product as per Annexure-I	R
7.	Manufacturing process including type of fermentation and biological end products: The microbial cultures are multiplied by liquid solid fermentation. Information pertaining to use of entire mycelial mats with spores separated must be provided in terms of biomass.	R
8.	Test method:	

8.1 8.2	Pathogencity test on insect pest as per Appendix-I Bioassay procedure for *Plutella xylostella,* are given in Appendix-II	R R
9.	Qualitative analysis	R
9.1	CFU on selective medium	R
9.2 9.2.1. 9.2.2. 9.2.3.	Contaminants: Pathogenic contaminants such as *Salmonella, Shigella, Vibrio* and such other microbials Other microbial contaminants Chemical and botanical pesticide contaminants	 R R R
9.3. 9.3.1.	Shelf life claims (Not less than 6 months): Data on storage stability as per shelf life claims as detailed in **Note-2**	R R
10.	A sample for verification (100 g)	R

B. BIOEFFICACY:

11. 11.1	Field tests: Data on bioeffectiveness from Institute certified by ROP/SL	R••
11.2	Data on non-target organism: One season/one year on effect on product against natural parasites/ predators	R
12.	Laboratory tests: The product should be tested at laboratory certified by ROP/SL	R

C. TOXICITY*:

13. 13.1 13.2 13.3 13.4 13.5	For mother culture Single dose oral (rat and mouse) Single dose pulmonary Single dose dermal Single dose intra-peritoneal Human safety records.	 R R R R R
14 14.1 14.2 14.3 14.4 14.5 14.6	For formulation Data on mother culture as in (13) above Single dose oral (rat & mouse) Single dose pulmonary Primary skin irritation Primary eye irritation Human safety records	 R R R R R R
15.	<u>For formulated product to be directly manufactured:</u> (Mammalian toxicity testing of formulations)	

15.1	Single dose oral (rat & mouse) Toxicity/Infectivity/Pathogenicity	R
15.2	Single dose pulmonary Toxicity/Infectivity/Pathogenicity (Intratracheal preferred)	R
15.3	Single dose dermal Infectivity	R
15.4	Single dose intraperitoneal (Infectivity)	R
15.5	Primary skin irritation	R
15.6	Primary eye irritation	R
15.7	Human safety records (Effect/Lack of effects)	R
16.	**Environmental safety testing*: Core information requirements** (For formulation only)	
16.1	**Non-target vertebrates**	R
16.1.1	Mammals[a]	R
16.1.2	Bird (two species)[b]	R
16.1.3	Fresh water fish[c]	
16.2	**Non-target invertebrates**	R
16.2.1	Terrestrial invertibrates[d]	R
16.2.2	Soil invertebrates[e]	

* Formulation free from other contaminant microorganisms, toxicity data and environmental safety data can be exempted only for microorganisms in use (e.g. *Metarhizium* strains)

D. Packaging & Labelling

Formulation:

17.	Manufacturing process/process of formulation	
17.1	Raw material	R
17.2	Plant and Machinery	R
17.3	Unit Process operation/Unit process	R
17.4	Out-put (Finished product and generation of waste)	R
18.	Packaging:	
18.1	Classification-solid, liquid or other types of product.	R
18.2	Unit pack size – In metric system	R
18.3	Specification – Details of primary, secondary and transport pack	R
18.4	Compatibility of primary pack with the product (Glass bottles are	R

	not recommended).	
19.	Labels and leaflets: As per pesticide regulations- indicating the common name, composition, antidote/storage, statements etc	R

Notes:

1. Applicants are required to submit an undertaking that strain is indigenous, naturally occurring, not exotic in origin, and not genetically modified as per Annexure 1.1.

2. Additional data of two months for six months claim and additional data of three months for one year shelf-life claim at two different agro climatic locations at ambient temperature along with meteorological data should be submitted.

3. Considering the fact that many small entrepreneurs are engaged in the business of cultivation of entomopathogenic fungi the following simplifications have been considered.

   3.1 If the same microbial strain is used for making formulations by different entrepreneurs then the information submitted once on the said strain will be sufficient. All entrepreneurs need not generate relevant data.

   3.2 If the same microbial strain, same method and same adjutants, stabilizers etc. are used for making the given formulation, data once submitted for validitating these claims will be sufficient for subsequent registrants, as substantiated by the relevant supportive documents.

4. The packaging material should also be ensured to be free from contamination during handling, storage and transportation and is as per prescribed standards, as the case may be.

Abbreviations:

R = Required

R** = Two seasons/years data on bioeffectiveness from a minimum of two agro-climatic conditions

16.1

a =Information on infection and pathogenicity in mammals will be available from mammalian safety testing.

b =Information on infection and pathogenicity: suggested test: single-dose, oral test. suggested test species: pigeon and chicken.

c = Information on infection and pathogenicity: suggested test species: *Tilapia mossambica* or other appropriate spp.

d = Information on mortality effects. It is recommended that information be obtained for honey bee and silk worm (*Bombyx mori*).

e = Information on mortality effects. It is recommended that test species include an earthworm (*Lumbricus terrestris*) or other appropriate macro invertebrates of ecological significance.

Annexure-I

STANDARDS for ENTOMOPATHOGENIC FUNGI DRAFT SPECIFICATIONS

1. Form and appearance
2. pH
3. Composition
   - 3.1 CFU/g of the product
   - 3.2 Percent content of the biocontrol organism in the formulation & nature of biomass.
   - 3.3 Percentage of carrier/filler, wetting/ dispending agent, stabilizers/ emulsifiers, contaminants/ impurities etc.
   - 3.4 Moisture content
4. CFU counts: Minimum $1x10^8$ CFU/ml or gm. (Stability at 30 °C and 65% RH).
5. Contaminants:
   - 5.1 Biological Contaminants:
     - 5.1.1 Pathogenic Contaminants: such as gram negative bacteria *Salmonella, Shigella, Vibrio* etc.: **absent**
     - 5.1.2 Other contaminants should not exceed $1x10^4$/ml or g
   - 5.2 Chemical/botanical pesticides contaminants: **absent.**
6. Method of analysis:
   - 6.1 FU counts by serial dilution and examination under regular compound research microscope with bright field optics.
   - 6.2 Plating for contaminants on specific media
   - 6.3 Entomopathogenic capability on target insects by bioassay.
7. An undertaking should be submitted that strain is indigenous, naturally occurring, not exotic in origin and not genetically modified as per Annexure-1.1.

Laboratory bioassay procedures for screening fungal pathogens on *Spodoptera litura* and *Helicoverpa armigera*

Insect pathogens:

Beauveria bassiana, Metarhizium anisopliae, Nomuraea rileyi

Preparation of Fungal inoculum for bioassays:
The fungus is grown on SDAY/SMAY medium for 10 days in slants and aqueous spore suspensions of various concentrations are prepared using sterile water. The spore count is estimated by Haemocytometer. (10^4 - 10^{10} spores/ml). Tween-80 is added @ 0.01% to get uniform spore suspension.

Rearing insects:
H.armigera, S.litura - Artificial diet (Semi-synthetic diet) or natural host

Stage of insect for bioassay
H.armigera, S.litura - II instar larvae to be used for bioassay protocols for lepidopteron pests

Method of inoculation

S. litura
1. Cut castor or cabbage leaf discs of 3.0 cm diameter, rinse in sterile distilled water and place each leaf disc in a sterile Petri plate and allow it to air dry in a laminar flow system
2. Apply ten micro liters of the spore suspension of each concentration on the leaf disc and spread it uniformly on the leaf surface and allow to it air dry in a laminar flow system. Treat the other side of the disc similarly.
3. Release ten numbers of second instar larvae of *S. litura* on the leaf surface and incubate the discs in an incubator at 25 ^{0}C and 90% RH
4. After 24 hours, shift the larvae to the polypots containing the semi-synthetic diet or cabbage disk and incubate in an incubator at 25 ^{0}C and 90% RH
5. After 5 days of incubation, record the mortality of the larvae for each concentration tested
6. Calculate Lc_{50} using a software package
Standard for LC_{50}: Not more than 2.00×10^5 spores/ml (3.0×10^3 spores/mm^2)

H. amigera:
Instead of castor leaves, cabbage, soybean leaves can be used for ***H. amigera*** and the procedure is the same as above.

Standard for LC_{50}: Not more than 4.00×10^6 spores/ml (6.0×10^3 spores/mm^2)

Appendix-II

Bioassay procedure for *Plutella xylostella*

Various concentrations of *Beauveria bassiana* formulation ranging from 6×10^8 to 2×10^{10} are to be screened to assess the mortality.

Fresh undamaged radish or cabbage leaves free from pesticide application are to be collected and washed thoroughly in sterile distilled water and air-dried. Individual leaves are dipped in respective concentrations for 30 seconds. After complete drying of leaves ten late 2^{nd} instar larvae of *Plutella xylostella* are released per treatment. A water dipped radish, cabbage leaf is maintained simultaneously as control.

To prevent desiccation of leaves, the petiole is covered with a moist cotton swab. Each treated leaf is placed in a plastic container of dimension 12.5 x 10 cm containing moist filter paper, Whatman No.41 to provide humidity.

Each treatment has to be replicated thrice. Fresh radish or cabbage leaves should be provided as feed at 24 hours interval. This set up has to be maintained at 25 + 1 ^{0}C and 70-80% RH for 7 days. Observations on larval mortality are to be made at 3, 5 and 7 days after treatment.

Standard for LC_{50} = Not more than 3×10^9 cfu/g

Annexure-1.1

UNDERTAKING BY MANUFACTURERS OF MICROBIAL PESTICIDES

I,----------,aged-------years, s/o----------, R/o----
----------and----------of M/s.----------
----------Registered Office at----------
----------do hereby undertake as follows:

(a) That the product----------based on----------, Strain----------, manufactured by M/s.----------and /or imported by M/s.....does not contain any genetically modified organism (GMO) .

(b) That I/We shall abide by the provisions contained in the International Plant Protection Convention with regard to the import of this product.

(c) That I/We shall abide by the provisions in context of International Standards for Phyto-Sanitary Measures-Code of Conduct for the import and release of exotic biological control agents of the International Plant Protection Convention (IPPC), FAO, Rome.

(d) That I/We shall provide the samples of our----------product as and when desired by the competent authorities of Government of SL for verification.

(e) That I/We further undertake that in the event of the above product having proved otherwise by any competent authority and resulting in environmental damage, I/We shall inform the ROP/SL, the relevant authorities for Manufacturing Licensing, Pollution Control and of appropriate District/Province/National Level and shall comply with the directions/decisions from them.

(f) That my/our above undertaking is true, and no portion is false and I have concealed nothing relevant to the above matter.

Signature:
Name----------
Designation----------
Seal of the Company--------

Date----------

Place----------

THE SUGGESTED DATA REQUIREMENTS FOR REGISTRATION OF ENTOMOPATHOGENIC BACTERIA AS MICROBIAL PESTICIDES

1. BIOLOGICAL CHARACTERSTICS AND CHEMISTRY

Following requirements should be fulfilled by products

No.	Parameter	Technical	Formulations
1.1	Common name of Entomotoxic bacteria	R	R
1.2	Systematic name: (Genus, species, serotype and strain)*	R	R
1.2.1	Insecticidal toxins classification	R#	R#
1. 3	Physical specification	R	R
1.3.1	Form and appearance	R	R
1.3.2	Moisture content	R	R
1.3.3	pH, particle size, suspensibility, miscibility	R	R
1.4	Detailed composition	R	R
1.4.1	Endotoxins content – through acceptable methods)	R	R
1.4.2	Exotoxins content – to be ruled out through stranded bioefficacy test (e.g.	R	R
1.4.3	House fly bioassay)		
1.4.4	Adjuvants	R	R
1.4.5	Human pathogens (culture method)	R	R
1.4.6.	Other microorganisms (not more than 10^4 / g)	R	R
	Present or absent of chemical and botanical pesticide contaminants	R	R
1.5	Natural occurrence of the organism	R	R
1.6	Identification – morphology, biochemistry, serology or molecular method	R	R
1.6.1	Potency of product by bioassay method (LC_{50} on target larvae and potency against a reference using artificial diet or leaf disc method or in the water for mosquito larvae as detailed in **Appendix-II**).	R	R
1.7	Shelf life/stability		
1.7.1	Shelf life claim (not less than 6 months)	R	R
1.7.2	Shelf life data in support of shelf life claim as detailed in **Note-2**	R	R
1.8	A sample for test (100 g)	R	R

2. BIOEFFICACY:

Sl. No.	Parameter	Technical	Formulations
2.1	**Laboratory Test**: LC_{50} / LD_{50} values for each insect species under laboratory conditions should be generated at respective institutes recommended by ROP.	R	R
2.2 2.2.1	**Field Test**: Data on bioeffectiveness and plant pathogenicity generated at Research Institute recommended by ROP (Two seasons).	R**	R**
2.2.2	Data on non-target organisms : One season / one year data on the effect of the product on natural predators / parasites (only for new organisms).	R	R

2. TOXICITY:

	Parameter	Technical	Formulations
3.1	**Single exposure studies**	R	R
3.1.1	Oral toxicity	R	R
3.1.2	Dermal toxicity	R	R
3.1.3	Inhalation toxicity	R	R
3.1.4	Skin and eye irritation	R	R
3.1.4	Mucous membrane irritation	R	R
3.1.6	Allergy / sensitization / immuno supression	R	R
3.2	**Eco-toxicity**		
3.2.1	Toxicity to birds[a]	NR	R
3.2.2	Toxicity to fish[b]	NR	R
3.2.3	Toxicity to honeybees	NR	R
3.2.4	Toxicity to silkworm	NR	R

* Formulation free from other contaminant microorganisms, toxicity data and environmental safety data can be exempted only for microorganisms in use (e.g. beneficial strains of Bt)

5. PACKAGING & LABELLING

	Parameter	Technical	Formulations
4.1	Packaging requirements as pesticide regulations	R	R
4.1.2	New Packaging system approved by global standards	R	R
4.2	**Manner of Packing**		
4.2.1	Specification of primary packing	R	R
4.2.2	Specification of secondary packing	R	R
4.2.3	Specification of transport packing	R	R
4.2.4	Detailed information for completely filled transport packing containing quantity of primary and secondary packing	R	R
4.3	**Manner of labelling**		
4.3.1	Specification of primary packing	R	R
4.3.2	Specification of secondary packing	R	R
4.3.3	Specification of transport packing	R	R
4.4	**Container Content Compatibility**		
	If BIS methodology is not available, then as per protocols being approved by ROP	R	R
4.5	**Labels and leaflets**		
4.5.1	3 copies of from each pack size	R	R

Notes:

1. Applicants are required to submit an undertaking that strain is indigenous, naturally occurring, not exotic in origin, and not genetically modified as per **Annexure 1.1.**

2. Additional two months data for six months self life claim / three months additional data for one year shelf-life claim at two different agro climatic locations at ambient temperature along with meteorological data should be submitted.

3. The packaging material should also be ensured to be free from contamination from handling, storage and transportation and is as per prescribed standards,

as the case may be.

4. The percentage of ingredient relative to total material is required to be stated and may vary from 2-7 per cent, the balance being inert ingredients. In addition, the labels will have to contain a measurement of toxin protein as percent protein, referring to the Lepidopteran-active toxin(s) present in the crystal.

5. Bt products should be labeled with biopotency and (or) toxin content.

6. The presently used Bt var. kurstaki standard is HD-1-S-1980 and its potency was calculated at 16,000 IUs per milligram of powder (Beegle et al. 1986. Standardization of HD-1-S-1980: US Standard for Lepidopterous-active *Bacillus thuringiensis*. Bulletin Ent. Soc. America 32: 44-45.).

7. Defined potency and toxin concentration – Bioassay would require the use of an insect species. Normally manufacturers could select *Trichoplusia ni* / *Helicoverpa armigera* for Lepidopteran specific Bt formulations. *Spodoptera* Units (SPU), *Leptinotarsa* Units (LTUs) or International Toxin Units (ITUs) are to be used for denoting a specific insect.

8. No test for beta exotoxin is required for *Bacillus sphaericus*, because this species is not known to produce exotoxins.

9. The biopotency of products based on *B. thuringiensis* subsp. *israelensis* (*Bti*)

Is compared against a reference strain(e.g. IPS82, 1884 using early fourth-

Instar larvae of Aedes aegypti (strain Bora Bora). The toxicity of IPS82 has

an arbitrarily assigned toxicity of 15,000 ITU/mg powder.

10. The bioefficacy of products based on *B. sphaericus* (*Bsh*) is determined against a reference standard (e.g. SPH88, strain 2362) using early fourth-instar larvae of Culex pipiens pipiens (strain Montpellier). The toxicity of SPH88 has an arbitrarily assigned toxicity of 1,700 ITU/mg of the powder (Guidelines for laboratory and field testing of mosquito larvicides, WHO 2005 pp 45).

11. The use of alternative bacterial reference powders and / or strains must be approached cautiously. Such alternatives must be the subject of careful cross-calibraation against the reference powders and should be conducted by recognized laboratories. The alternative powders/strains and the cross-calibration data which support them, should be made available to anyone who wishes to use, or check, the test with the alternative powders/strains.

12. Water content should not exceed 5 %, to preclude premature degradation of the product.

Abbreviations:

R = Required
NR = Not Required

1.2.1 **R#** If H-Serotype is not known, it is mandatory to provide the details of Cry toxin to confirm that it is *Bacillus thuringiensis*.

2.2.1 R** = Two seasons/years data on bioeffectiveness from minimum two agro-climatic conditions

3.2 **a** =Information on infection and pathogenicity: suggested test: single-dose, oral test. suggested test species: pigeon and chicken.

b= Information on infection and pathogenicity: suggested test species: *Tilapia mossambica* or other appropriate species.

Annexure-I

STANDARDS for ENTOMOTOXIC BACTERIA TECHNICAL /FORMULATION DRAFT SPECIFICATIONS

No.	Details
1. SCOPE	
1.1	This standard prescribes the requirements and the method of sampling and test for Entomotoxic bacteria technical and formulation. The product is a biopesticide active against target insects. The product is not for human consumption.
2. REQUIREMENTS	
2.1	Common name: i.e., *Bacillus thuringiensis or B. sphaericus etc.*
2.2	Systematic name (Genus, species, serotype, strain and Cry-toxin* along with cry gene)
2.3	Physical specification 2.3.1 Form and appearance 2.3.2 Moisture content 2.3.3 pH
2.4	Composition 2.4.1 Delta endotoxin content (Minimum 2.0%) – estimation as per Appendix-V 2.4.2 Adjuvants 2.4.3 Beta Exotoin content – Negative through housefly bioassay test as per Appendix-IV 2.4.4 Human pathogens (gram negative bacateria Salmonella, shigella & vibrio etc) - Absent 2.4.5 Other microorganisms (not more than 10^4 / g) 2.4.6 Chemical/botanical pesticide contamination – Absent
2.5	Natural occurrence of the organism
	2.5.1 Its relationship of the organisms 2.5.2 History (exotic or indigenous strain) 2.5.3 The isolate should not be genetically modified organism (GMO).
3. SAMPLING	
3.1	Representative samples of the material shall be drawn in accordance with regulations
4. TESTS	
4.1	An appropriate test procedure and criteria used for identification, such as morphology, biochemistry and / or serology / immunology 4.1.1 Morphology description, particle size 4.1.2 Immunology assays: ELISA test or any other sensitive standard immunology test. 4.1.3 Method of analysis

	4.1.4 Level of beta exotoxins to be identified if expressed by Housefly bioassay method. 4.1.5 Potency of product by bioassay method (**Appendix-II**) 4.1.5.1 Bioassay method a) LC_{50} or LD_{50} on target larvae and potency against a reference using artificial diet or leaf disc method or in the water for mosquito larvae (Appendix-I) b) Housefly Bioassay method for Beta-exotoxin content (Appendix-IV) c) Determination of toxin content by ELISA / Dot Blot Assay Method (Appendix-V) 4.1.5.2 A technique for the separation and purification of the crystals (Appendix III) is to be used by the manufacturer and the antisera to be raised using solublized toxin. Toxin content (3.5 %) to be standardized in the formulation using this antisera (ELISA /Dot blot assay).

2.2 Crytoxin* If H-Serotype is not known, it is mandatory to provide the details of Cry toxin to confirm that it is *Bacillus thuringiensis.*

Appendix I

Bioassay Method

Diet incorporation

The following protocol is used for diet incorporation of oral toxicants to test their toxicity on target insects. The example presented here is to bioassay Cry I Ac on *H. Armigera* (First instar larva of other test insects are used for similar bioassay).

1. Pipette out 3 ml of the solution into a 40 ml plastic cup.
2. Pour lukewarm diet, approx 60^0 C, into the cup to a total volume of 30 ml. Place the lid and shake the cup vigorously for a minute to mix properly.
3. Pour the diet to 0.5 cm height, into wells of a 24-cell insect- rearing tray. Allow the diet to cool in laminar airflow under UV lamps for I h to surface sterilize the diet.
4. If concentration of the toxicant in the stock solution was 2 µg/ml, the final concentration in the diet would now be 0.2µg/ml diet. Thus the final concentration of toxin in diet was diluted 10-fold.
5. Release first instars into the diet rearing trays at the rate of one per well. Cover the diet tray with semi-permeable wrap and close the lid.
6. It is recommended that the lid be tightly secured to the tray with rubber bands, to prevent the larvae from escaping. Because the diet is unsuitable, larvae try constantly to escape from the diet rearing trays.
7. Keep controls with larvae released on untreated diet, for all the experiments.
8. The unused rearing trays with diet can be stored in a refrigerator for a week.
9. Change the diet for the larvae every two or three days.
10. Record mortality observations at 8 hourly intervals until the end of seven days, for median lethal time LT_{50} calculations. LT_{50} is the time at which 50 % of the test population is killed with the specific dose tested. A simple linear regression equation can be worked out to calculate the LT_{50}
11. Otherwise, record mortality at alternate days until the end of seven days, for median lethal concentralion LC_{50} calculations. LC_{50} is the concentration that kills half the test population.
12. Record weights of surviving larvae at the end of seven days, for median effective concentration EC_{50} and LC_{50} is the concentration that prevents half the test population from reaching 50% of the weight attained by control larvae. For example, if the average weight of larvae on the control diet (without toxin) was 80 mg, EC_{50} represents the

concentration at which 50% of the test population is unable to gain a weight more than 40 mg. LC_{50} is the concentration that inhibits half the test population from reaching the third instar.

Diet incorporation for filter paper bioassays

1. For bioassays with bollworms, 10 ml toxin incorporated diet is poured over a16 sq cm piece of filter paper. The filter papers layered with diet are cooled and cut into smaller squares of 2 x 2 cm, and 10 first instar larvae are released in small plastic cups 3 x 3 cm (d x h) cups containing a square. Change the strips every alternate day.

2. Record mortality observations until the seventh day.

Surface coating of semi-synthetic diet

I. Prepare the diet and pour it into the trays or the rearing plastic cups. Generally 10 µl of the toxin can be used to coal 1 sq cm surface area. Gently swirl the diet surface to ensure uniform and complete spread of the solution over the diet surface.

2. Allow the surface to dry in a laminar airflow under UV light for 2-3 hours to surface sterlize.

1. Release one first instar *H. armigera* larva per well. Always maintain proper controls with untreated diet.

4. Change the diet on alternate days and record mortality until the seventh day. Then, weight of surviving larvae should be recorded on the final day of the bioassay.

The method has the advantage of obtaining constantly reliable results because the toxin is unlikely to be affected by either improper mixing or heat as can occur in the diet-incorporation method. Moreover, less amount of the toxin is required for the assay, compared to the diet-incorporation method.

Calculation of results:

The potency of the sample (International Units – IUs)

$$\text{IU/mg sample} = \frac{\text{LC50 Standard}}{\text{LC50 Sample}} \times \text{IU/mg Standard}$$

(IU/mg Standard, i.e., HD-1-S-1980 is 16,000 IUs/mg; each registrant should prepare a "self reference" and should deposit it with the Registering Authority. Each self reference will be expressed as IU/mg using International standard)

Appendix-II

Dot Blot assay of Bacillus thuringiencis (B.t.) toxin protein as alternate of Bioassay.

1) B.t. grown till sporulation in shake flask or in fermenter vessel and let the cells lyse and release spore/crystals into the medium

2) Cells are harvested by centrifugation at 10k for 15 mins.

3) Wash the pellet with 1M NaCl to remove the Bt. associated seine/metallo proteases and washd twice with sterile distilled water.

4) Pellet resuspended in 50MM NaOH to solublize the toxin protein for 2 hours at R.T. with slow shaking and centrifuged again at 10K for 15 Mins.

5) Supernatent was adjusted to pH 8.0 with Tris HCL pH 8.8

6) Protein contents estimated by Lowry's protocol.

7) Two fold serial dillutions of test protein were made in PBS and known amount at protein applied on NCP using S&S or Biorad Dot Blot manifold apparatus and applying water vaccum for 30 mins.

8) NCP was carefully removed from Dot Blot set and soaked in excess of 3% Skim milk in PBS for blocking the remaining acetic sites on NCP for 2-3 hours at R.T/O/N at 4°C.

9) Wash the NCP with excess PBS with 0.01% Tween 20, 3-4 times and then finally with PBS

10) Polyclonal antiserum raised against total crystal protein was suitably diluted in PBS and added to the 'seal a meal' containing NCP and incubated for 1-2 hours with shaking.

11) Remove the NCP from the bag and was several times (as mentioned in step.No.9)

12) Antirabbit antibodies conjugated with HRPO/alkaline Phosphate was diluted as per the suppliers instruction and incubated NCP (as in step 10)

13) Was as in step 11

14) For HRPO:

    a) Diaminobenzeden (4mg/10ml PBS)/4-Chloro-1-Napthol (4mg/10ml 20% Alcohol) were dissolved and 10 ml of 30% of H2O2 per 10 ul substrate soluion

was added and colour reaction developed in dark for 5-10 mins (DAB gives brick red colour. 40N gives blue colour).

b) For alkaline Phosphatase:

Alkaline Phosphatase Buffer:

1M Tris pH 8.8	- 10 ml/
4M NaCI	- 2.5 ml/ make up to 100 ml
1M Mgc12	- 0.5 ml/

For 10ml of above buffer add NBT-66 ul and BCIP-33 ul and developed and colour reaction

15. Stop the reaction by removing the substrate and washing with PBS.
16. Keep on filter paper and dry.

DIFFERENT PROTEIN CONCENTRATION

10ug 5ug 2.5ug 1.25ug 512.5ng 256.25ng 128ng 64ng 32ng 16ng 8ng 4ng

Determination if cell dry weight

Take a known volume of Bacterial culture spin down at 4R for min.
Wash the pellet in minimal distilled water
Transfer to a pre weighed container
Incubate at 80 ^{0}C for 16-18 hours till become dry and weight becomes constant.

Appendix-III

PURIFICATION OF CRYSTALS BY GELATIN METHOD

Centrifuge the sporulated material and wash pallet twice with 1M Nacl. Add 200ml. of 0.5% Gelatin, stir and remove all froth completely. Dilute with sterile water and centrifuge. Take debris and stir with 20ml. of 1.5M sucrose. Further add 50 ml of 1.5M sucrose, stir and centrifuge at 3000 RPM for 2 hours. Remove supernatant and purified crystals are harvested.

Appendix-IV

Beta-Exotoxin determination by House Fly Bioassay Method

Fly Assay Diet Condition		**Laboratory**	
Agar	16g	Temp	25°C + 2°C
Milk powder	100g	R.H.	70%
Yeast	100g	Test insect	-2 days old Hot fly larvae
Methyl Paraben	2.1g		
Water	1000ml	No. of Replications	2

PROCEDURE

i) 1 g sample thoroughly mixed with 9 ml. of sterile saline. This solution is heat treated at 65 °C (Water bath) for 45 minutes and incubate at rotary shaker for 2 hrs. at room temp.

ii) Then centrifuge this sample at 12,000 RPM for 10 minutes.

iii) This suspension is serially diluted (1:10) to 10^{-6} dilutions

iv) Liquid diet 200g. for each replicate is placed in trays/beakers.

v) 5ml of heat treated culture supernatant (10^{-6}) is poured on diet. Let it solidify at room temp. For control, use 5ml of sterile water

vi) 2 days old House fly larvae (50) in each replicate i.e. two replicate each for sample and control and cover with wire mesh/clot.

vii) Incubate the trays at 25 °C + 2 °C till emergence.

viii) After 24 hours, just put 5 g wheat bran in each tray on the top (on 8^{th} to 10^{th} day). On adult emergence freeze the trays for 2 hours to count the adults and % mortality may be calculated as :-

% Mortality = (100 – Number of Normal Adults).

Appendix – V

Quantification of Bt endotoxin using ELISA Technique

The Cry1Ac Bt-Quant is an ELISA kit, which facilitates a precise quantification of Cry1Ab or Cry1Ac, present in Bt based biopesticides. The kit is simple, cost effective and very reliable. It takes about 2 hrs for completion of one set of ELISA assay. Each ELISA plate can be used for 96 samples (including four wells for standards and two for blank). Depending on the capabilities of a laboratory hundreds of samples can be processed in a single day. ELISA plate reader is a requirement for use of the kit.

Materials

1. **96 well ELISA plate** coated with antibody (Store refrigerated).
2. **Calibrated standards**
3. **Substrate** (Store refrigerated).
4. **PBST** (10x) Dilute it before use.
5. **IgG-conjugate** (Store refrigerated).
6. **Stop Solution** (ready to use)

Procedure

1. Grind the sample into a fine solution with 0.5 ml sample extraction buffer
2. Centrifuge the sample at 10,000 rpm (optional)
3. Prepare serial dilutions of the standards provided, for a range between 0.01 to 0.5 ppm.
4. Pipette out 50 µl of antibody-conjugate into each well.
5. Add 50 µl (microliter) of the sample into each well of the ELISA plate.
6. Pipette 50 µl of each of the standard solutions into the wells of a particular column. Pipette out buffer only in one or two wells of the ELISA plate to maintain blanks.
7. Incubate for 1 hour at room temperature, preferably in a humid chamber.
8. Wash the plate with wash buffer (PBST) 3 times and empty wells.
9. Add 50 µl substrate to each well. Incubate for 20-30 minutes. Blue color develops in positive samples.
10. Add 40 µl stop solution to each of the wells. Positive samples turn yellow
11. Read absorbance at 450 nm.

Calculations for ELISA to quantify Cry1Ac

Example

Weight of sample = 68 mg
Buffer quantity = 500 µl
Crush the sample thoroughly
50 µl was pipetted into each well

Standards

1 ppm (1 µg Cry1Ac per 1000 µl)
0.2 ppm (200 ng Cry1Ac per 1000 µl)
0.04 ppm (40 ng Cry1Ac per 1000 µl)
0.008 ppm (8 ng Cry1Ac per 1000 µl)

Standards	Cry1Ac quantity		
	1 ml (1000 µl)	**100 µl**	**50 µl**
1 ppm	1000 ng	100 ng	50 ng
0.2 ppm	200 ng	20 ng	10 ng
0.04 ppm	40 ng	4 ng	2 ng
0.008 ppm	8 ng	0.8 ng	0.4 ng

50 µl was pipetted into each well, hence each well contains the following amount of Cry1Ac

Results

	Standards				
	Cry1Ac ng/well	**O.D**			
	50	1.6			
	10	0.44			
	2	0.22			
	0.4	0.17			
	0	0.11			
Steps			**1**	**2**	**3**
Samples	**Sample weight in mg**	**O.D**	**ng/well**	**ng/sample**	**ng/gm**
1	68	0.67	18.1	180.7	2657
2	72	0.12	-0.8	-8.3	0
3	54	0.98	28.7	287.2	5319
4	48	1.12	33.5	335.3	6986
5	77	0.88	25.3	252.9	3284
6	82	0.76	21.2	211.6	2581
7	59	0.65	17.4	173.8	2946
8	49	0.11	-1.2	-11.8	0
9	55	0.74	20.5	204.7	3723
10	62	0.82	23.2	232.2	3746

To construct the standard curve to obtain regression equation.

Regression equation with standard

$y = bx + a$

$y = 0.0291x + 0.1442$

y represents O.D (optical density or absorbance)

x represents amount of Cry1Ac

b represents slope

a represents constant

Step 1. Use formula to derive $x = (O.D-a)/b$ to get ng/well

Step 2. Multiply the value of ng/well with 10 (because $1/10^{th}$ of the sample was pipetted into each well)

Step 3. Calculate ng/gm using the following formula

= (ng/sample x 1000)/weight of sample

Annexure-1.1

UNDERTAKING BY MANUFACTURERS OF MICROBIAL PESTICIDES

I,----------,aged-------years, s/o----------, R/o----
----------and----------of M/s.----------
----------Registered Office at----------
----------do hereby undertake as follows:

(a) That the product----------based on----------, Strain----------, manufactured by M/s.----------and /or imported by M/s.....does not contain any genetically modified organism (GMO) .

(b) That I/We shall abide by the provisions contained in the International Plant Protection Convention with regard to the import of this product.

(c) That I/We shall abide by the provisions in context of International Standards for Phyto-Sanitary Measures-Code of Conduct for the import and release of exotic biological control agents of the International Plant Protection Convention (IPPC), FAO, Rome.

(d) That I/We shall provide the samples of our----------product as and when desired by the competent authorities of Government of SL for verification.

(e) That I/We further undertake that in the event of the above product having proved otherwise by any competent authority and resulting in environmental damage, I/We shall inform the ROP, the relevant authorities for Manufacturing Licensing, Pollution Control and of appropriate District/Province/National Level and shall comply with the directions/decisions from them.

(f) That my/our above undertaking is true, and no portion is false and I have concealed nothing relevant to the above matter.

Signature:
Name----------
Designation----------
Seal of the Company--------

Date: ----------

Place:----------

THE SUGGESTED DATA REQUIREMENTS FOR REGISTRATION OF ANTAGONISTIC FUNGI AS MICROBIAL PESTICIDES

I STANDARD OF FORMULATIONS:

1. Colony Forming Unit (CFU) count on selective medium should be minimum of 2x 10^6 /ml or g for *Trichoderma* spp. Minimum CFU may vary for other antagonistic fungi.
2. **Contaminants:**
   - 2.1 Biological contaminants:
   - 2.1.1 Pathogenic contaminants such as bacteria *Salmonella*, *Shigella*, *Vibrio* and other such microbials should not be present.
   - 2.1.2 Other microbial contaminants should not exceed 1 x 10^4 / ml or per g of formulation.
   - 2.2 Chemical / botanical pesticide contaminants should not be present
3. Stability of CFU counts at 30 °C and 65% RH

II. REGISTRATION REQUIREMENTS:

A. BIOLOGICAL CHARACTERISTICS AND CHEMISTRY

The following requirement should be fulfilled

No.	Requirements	
1.	Systematic name (Genus and species)	R
1.1	Strain name	R
2	Common name, if any	R
3.	Source of origin as Annexure-1.1	R
4.	Habitat and morphological description	R
5.	Composition of the product	R
5.1	CFU/g of the product	R
5.2	Percent content of the Biocontrol organism in the formulation & nature of biomass.	R
5.3	Percentage of carrier/filler, wetting/ dispending agent, stabilizers/ emulsifiers, contaminants/ impurities etc.	R
5.4	Moisture content	R
6.	Specification of the product as per Annexure-I	R
7.	Manufacturing process including type of fermentation and biological end products: The microbial cultures are multiplied by liquid solid fermentation. Information pertaining to use of entire mycelial mats with spores separated must be provided in terms of biomass.	R

8. 8.1 8.2	Test Methods: Dual culture to attain at least 50% reduction in target organism. Bioassay: based on disease severity and root colonization as detailed in Appendix-I	 R R
9.	Qualitative analysis	R
9.1	CFU on selective medium	R
9.2 9.2.1. 9.2.2. 9.2.3.	Contaminants: Pathogenic contaminants such as *Salmonella, Shigella, Vibrio* and such other microbials Other microbial contaminants Chemical and botanical pesticide contaminants	 R R R
9.3.	Moisture content	R
9.4. 9.4.1.	Shelf life claims : Not less than 6 months Data on storage stability as per shelf life claims as detailed in **Note-2**	R R
10.	A sample for verification (100 g)	R

B.BIOEFFICACY:

11.	**Field studies**: Data from DOA, RRI, CRI, TRI or other research institutes certified by ROP/DOA, Sri Lanka	R••
12.	**Laboratory studies**: The product should be tested at a laboratory under DOA, RRI, CRI, TRI or other research Institute certified by ROP/DOA, Sri Lanka	R

C. TOXICITY *:

13. 13.1 13.2 13.3 13.4 13.5	**For mother culture** Single dose oral (rat and mouse) Single dose pulmonary Single dose dermal Single dose intraperitoneal Human safety records.	 R R R R R
14 14.1 14.2 14.3 14.4 14.5 14.6	**For formulation** Data on mother culture as in (13) above Single dose oral (rat & mouse) Single dose pulmonary Primary skin irritation Primary eye irritation Human safety records	 R R R R R R

15.	**For formulated product to be directly manufactured:*** (Mammalian toxicity testing of formulations)	
15.1	Single dose oral (rat & mouse) Toxicity/Infectivity/Pathogenicity	R
15.2	Single dose pulmonary Toxicity/Infectivity/Pathogenicity (Intratracheal preferred)	R
15.3	Single dose dermal Infectivity	R
15.4	Single dose intraperitoneal (Infectivity)	R
15.5	Primary skin irritation	R
15.6	Primary eye irritation	R
15.7	Human safety records (Effect/Lack of effects)	R
16.	**Environmental safety testing: Core information requirements*** (For formulation only)	
16.1	**Non-target vertebrates**	
16.1.1	Mammals[a]	R
16.1.2	Bird (two species)[b]	R
16.1.3	Fresh water fish[c]	R
16.2	**Non-target invertebrates**	
16.2.1	Soil invertebrates[d]	R

* Formulation free from other contaminant microorganisms, toxicity data and environmental safety data can be exempted only for microorganisms in use (e.g. beneficial strains of *Trichoderma* spp.)

D. Packaging & Labelling

Formulation:

17.	**Manufacturing process/process of formulation**	
17.1	Raw material	R
17.2	Production and Machinery	R
17.3	Unit Process operation/Unit process	R
17.4	Out-put (Finished product and generation of waste)	R
18.	**Packaging:**	
18.1	Classification-solid, liquid or other types of product.	R

18.2 18.3 18.4	Unit pack size – In metric system Specification – Details of primary, secondary and transport pack Compatibility of primary pack with the product (Glass bottles are not recommended).	R R R
19.	**Labels and leaflets**: As regulation of SL pesticide Act, indicating the common name, composition, antidote, storage, statements etc	R

Notes: 1. Applicants are required to submit an undertaking that strain is indigenous, naturally occurring, not exotic in origin, and not genetically modified as per Annexure 1.1.

2. Additional data of two months for six months shelf-life claim and additional data of three months for one year shelf-life claim at two different agro climatic locations at ambient temperature along with meteorological data should be submitted.

3. Considering the fact that many small entrepreneurs are engaged in the business of cultivation of antagonistic fungi the following simplifications have been considered.

3.1 If the same microbial strain is used for making formulations by different entrepreneurs then the information submitted once on the said strain will be sufficient. All entrepreneurs need not generate relevant data.

3.2 If same microbial strain, same method and same adjutants, stabilizers etc. are used for making the given formulation, data once submitted for validitating these claims will be sufficient for subsequent registrants, as substantiated by the relevant supportive documents.

4. The packaging material should also be ensured to be free from contamination during handling, storage and transportation and is as per prescribed standards, as the case may be.

Abbreviations:

R = Required

R** = Two seasons/years data on bioeffectiveness from minimum of two agro-climatic conditions

16.1

a =Information on infection and pathogencity in mammals will be available from mammalian safety testing.

b =Information on infection and pathogenicity: suggested test: single-dose, oral test. suggested test species: pigeon and chicken.

c = Information on infection and pathogenicity: suggested test species: *Tilapia mossambica* or other appropriate spp.

d =Information on mortality effects. It is recommended that test species include an earthworm (*Lumbricus terrestris*) or other appropriate macro invertebrates of ecological significance.

Annexure-I

STANDARDS for ANTAGONISTIC FUNGI
DRAFT SPECIFICATIONS

1. Form and appearance
2. pH
3. Composition
   - 3.1 CFU/g of the product
   - 3.2 Percent content of the Biocontrol organism in the formulation & nature of biomass.
   - 3.3 Percentage of carrier/filler, wetting/ dispending agent, stabilizers/ emulsifiers, contaminants/ impurities etc.
   - 3.4 Moisture content
4. CFU counts: *Trichoderma* $2x10^6$ CFU/ml or gm. (Stability at 30 °C and 65% RH).
5. Contaminants:
   - 5.1 Biological Contaminants:
   - 5.2 Pathogenic Contaminants: such as gram negative bacteria *Salmonella, Shigella, Vibrio* etc.: **absent**
   - 5.3 Other contaminants should not exceed $1x10^4$/ml or g
   - 5.4 Chemical/ botanical pesticides contaminants: **absent.**
6. Method of analysis:
   - 6.1 CFU counts by serial dilution and examination under regular compound research microscope with bright field optics.
   - 6.2 Plating for contaminants on specific media
   - 6.3 Antagonistic mycolytic capability on target organism by bioassay on plants (Laboratory test).
   - 6.4 Bioassay procedure based on disease severity and root colonization as detailed in Appendix-I
7. An undertaking should be submitted that the strain is indigenous, naturally occurring, not exotic in origin and not genetically modified as per Annexure-1.1.

Appendix-I

Bioassay for plant disease antagonists based on disease severity and root colonization.

The target pathogen to be tested against has to be grown in Sand maize medium. The Sand maize medium is prepared by adding sand 90 g, maize 10 g. and water 10 ml in a glass bottle of 300 ml capacity and then autoclaved twice. Then 5 mycelial discs of the test pathogen are transferred into the bottle and left for incubation for 15 days. Once the culture has grown well, the Sand maize medium is mixed along with the fungal growth and 1 g of this preparation is used as the inoculum after adjusting the cfu to 1 x 10/g by addition of sand.

The plastic cups (5-6 cm diameter) filled with soil and compost (3:1) have to be used. In each cup the filling should be done upto ¾th level. The pathogen inoculum is mixed with sand and has to be applied up to a depth of 2 cm in the plastic cups.

The bioefficacy of the bioagent should be tested by both seed treatment and soil application. For seed treatment, the recommended dose of the formulation has to be used (5 to 10 g.). For soil application, the bioagent is added at the rate of 1g of formulation (minimum cfu should be the $2x10^6$). The germination percentage, disease intensity and seedling vigour are to be recorded.

Another set of plastic cups filled with sterile soil and sterile compost has to be used to confirm whether the bioefficacy was due to the isolate of the bioagent tested or due to the native isolates of the bioagent present in the soil.

The keys for grading the efficacy mentioned below should be used. However, for registration purpose, bioagents that are Highly Efficient, Efficient or Moderately Efficient in the plastic cup test under glass house conditions (in the presence of pathogen) can be allowed i.e. germination percentage of 70% or above, disease incidence of 30% or less can be considered for registration.

Disease Grading Key

Disease incidence (%)	Description	Rating of bioefficacy of bioagents
0	Germination>90%, no seed rotting, seedling healthy, root and shoot portions well developed.	Highly Efficient (HE)
1-15	Germination 80-90%, infection on main as well as lateral roots, seedlings are well developed.	Efficient (E)
16-30	Germination 70-80%, development of roots restricted and growth is less compared to Score 1. Infection occurred on roots. Shoot portions developed but growth retarded compared to Score 1.	Moderately Efficient (ME)
31-45	Germination 60-70%, length of roots and shoots short compared to Score 1. Germination of seeds inhibited. 50% of root area infected. Shoot portions also showed infection.	Moderately Inefficient (MI)
46-60	Seed germination 50-60%. Development of roots and shoots greatly retarded. Shoot portions also showed infection.	Efficient (I)
Above 60	Less than 50% germination and seed rotting.	Highly Inefficient (HI)

For the root colonization assay, the rhizosphere region of the plants tested above has to be collected and the soil adhering to the root surface has to be removed by gently tapping the roots. The root bits have to be cut into 1 cm bits and randomly 25 bits should be selected for each treatment. They have to be plated on (TSM) and the percentage of root bits colonized has to be recorded. This has to be performed in sterile soil and non sterile soil. One control treatment without the biocontrol agent being tested, should be kept for both the sterile and non-sterile soil to rule out of the possibility of interference of native micro flora in the bioefficacy assay.

Annexure-1.1

UNDERTAKING BY MANUFACTURERS OF MICROBIAL PESTICIDES

I,----------,aged-------years, s/o----------, R/o----
----------and----------of M/s.----------
----------Registered Office at----------
----------do hereby undertake as follows:

(a) That the product----------based on----------, Strain----------, manufactured by M/s.----------and /or imported by M/s.....does not contain any genetically modified organism (GMO) .

(b) That I/We shall abide by the provisions contained in the International Plant Protection Convention with regard to the import of this product.

(c) That I/We shall abide by the provisions in context of International Standards for Phyto-Sanitary Measures-Code of Conduct for the import and release of exotic biological control agents of the International Plant Protection Convention (IPPC), FAO, Rome.

(d) That I/We shall provide the samples of our----------product as and when desired by the competent authorities of Government of SL for verification.

(e) That I/We further undertake that in the event of the above product having proved otherwise by any competent authority and resulting in environmental damage, I/We shall inform the ROP/ SL, the relevant authorities for Manufacturing Licensing, Pollution Control and of appropriate District/Province/National Level and shall comply with the directions/decisions from them.

(f) That my/our above undertaking is true, and no portion is false and I have concealed nothing relevant to the above matter.

Signature:
Name----------
Designation----------
Seal of the Company--------

Date ----------

Place- ----------

THE SUGGESTED DATA REQUIREMENTS FOR REGISTRATION OF ANTAGONISTIC BACTERIA AS MICROBIAL PESTICIDES

I STANDARD OF FORMULATIONS:

1. Colony Forming Unit (CFU) count on selective medium should be a minimum of 1×10^8 / ml or g for Antagonistic bacteria.
2. **Contaminants:**
   - 2.1 Biological contaminants:
   - 2.1.1 Pathogenic contaminants such as gram negative bacteria *Salmonella*, Shigella, *Vibrio* and such other microbials should not be present.
   - 2.1.2 Other microbial contaminants should not exceed 1×10^4 /ml or per g of formulation.
   - a. Chemical/botanical pesticide contaminants should not be present.
3. Stability of CFU counts at 30 °C and 65% RH

11 REGISTRATION REQUIREMENTS:

The following requirements should be fulfilled

A. BIOLOGICAL CHARACTERISTICS AND CHEMISTRY

Sl. No.	Requirements	
1.	Systematic name (Genus, species and strain)	R
2.	Common name, if any	R
3.	Source of origin as per Annexure-1.1	R
4.	Natural occurrence of the organism and morphological description	R
5.	Composition of the product	R
5.1	Percent content of the biocontrol organism in the formulation & nature of biomass.	R
5.2	CFU/g or ml of the product.	R
5.3	Percentage of other components: carrier/filler, wetting/dispersing agent, stabilizers/emulsifiers, contaminants/impurities etc.	R
5.4	Moisture content	R
6.	Specification of the product as per Annexure-I	R
7.	Manufacturing process including type of fermentation and biological end products: The microbial cultures are multiplied by liquid solid fermentation. Information pertaining to use of entire mats with spores separated must be provided in terms of biomass.	R
8.	**Test method**:	
8.1	Dual culture for antagonistic bacteria to attain at least 35%	R

8.2	reduction in target organism Bioassay: based on disease severity and root colonization as detailed in **Appendix-I**	R
9.	**Qualitative analysis**	R
9.1	CFU on selective medium	R
9.2	**Contaminants**:	
9.2.1.	Pathogenic contaminants such as *Salmonella, Shigella, Vibrio* and such other microbials	R
9.2.2.	Other microbial contaminants	R
9.2.3.	Chemical and botanical pesticide contaminants	R
9.3.	Shelf life claims (Not less than 6 months):	R
9.3.1.	Data on storage stability as per shelf life claims as detailed in **Note-2**	R
10.	A sample for verification (100 g)	R

B.BIOEFFICACY:

11. 11.1	**Field tests**: Data from respective Institute certified by Director Research or Head of the Institute	R**
11.2	Data on non-target organism: One season/one year on effect on product against natural parasites/ predators	R
12.	**Laboratory tests**: The product should be tested at respective laboratory	R

C. TOXICITY*:

13.	**For mother culture**	
13.1	Single dose oral (rat and mouse)	R
13.2	Single dose pulmonary	R
13.3	Single dose dermal	R
13.4	Single dose intra-peritoneal	R
13.5	Human safety records.	R
14	**For formulation**	
14.1	Data on mother culture as in (13) above	R
14.2	Single dose oral (rat & mouse)	R
14.3	Single dose pulmonary	R
14.4	Primary skin irritation	R
14.5	Primary eye irritation	R
14.6	Human safety records	R
15.	**<u>For formulated product to be directly manufactured:</u>** (Mammalian toxicity testing of formulations)	
15.1	Single dose oral (rat & mouse) Toxicity/Infectivity/Pathogenicity	R

15.2	Single dose pulmonary Toxicity/Infectivity/Pathogenicity (Intra-tracheal preferred)	R
15.3	Single dose dermal infectivity	R
15.4	Single dose intra-peritoneal (Infectivity)	R
15.5	Primary skin irritation	R
15.6	Primary eye irritation	R
15.7	Human safety records (Effect/Lack of effects)	R
16.	**Environmental safety testing*: Core information requirements** (For formulation only)	
16.1	**Non-target vertebrates**	
16.1.1	Mammals[a]	R
16.1.2	Birds (two species)[b]	R
16.1.3	Fresh water fish[c]	R
16.2	**Non-target invertebrates**	
16.2.1	Terrestrial invertebrates[d]	R
16.2.2	Soil invertebrates[e]	R

* Formulation free from other contaminant microorganisms, toxicity data and environmental safety data can be exempted only microorganisms in use.

D. Packaging & Labelling

17.	**Manufacturing process/process of formulation**	
17.1	Raw material	R
17.2	Production and Machinery	R
17.3	Unit Process operation/Unit process	R
17.4	Out-put (Finished product and generation of waste)	R
18.	**Packaging:**	
18.1	Classification-solid, liquid or other types of product.	R
18.2	Unit pack size – In metric system	R
18.3	Specification – Details of primary, secondary and transport pack	R
18.4	Compatibility of primary pack with the product (Glass bottles are not recommended).	NR
19.	**Labels and leaflets**: As per pesticide regulations indicating the common name, composition, antidote, storage, statements etc	R

Notes:

1. Applicants are required to submit an undertaking that strain is indigenous, naturally occurring, not exotic in origin, and not genetically modified as per Annexure 1.1.
2. Additional data of two months for six months shelf-life claim and additional data of three months for one year shelf-life claim at two different agro climatic locations at ambient temperature along with meteorological data should be submitted.
3. Considering the fact that many small entrepreneurs are engaged in the business of cultivation of antagonistic bacteria the following simplifications have been considered.

3.1 If the same microbial strain is used for making formulation by different entrepreneurs then the information submitted once on the said strain will be sufficient. All entrepreneurs need not generate relevant data.

3.2 If the same microbial strain, same method and same adjutants, stabilizers etc. are used for making the given formulation, data once submitted for validating these claims will be sufficient for subsequent registrants, as substantiated by the relevant supportive documents.

4. The packaging material should also be ensured to be free from contamination during handling, storage and transportation and is as per prescribed standards, as the case may be.

Abbreviations:

R = Required

R** = Two seasons/years data on bioeffectiveness from minimum two agro-climatic conditions

16.1

a = Information on infection and pathogenicity in mammals will be available from mammalian safety testing.

b = Information on infection and pathogenicity: suggested test: single-dose, oral test. suggested test species: pigeon and chicken.

c = Information on infection and pathogenicity: suggested test species: *Tilapia mossambica* or other appropriate spp.

d = Information on mortality effects. It is recommended that information be obtained for honey bee and silk worm (*Bombyx mori*).

e = Information on mortality effects. It is recommended that test species include an earthworm (*Lumbricus terrestris*) or other appropriate macro invertebrates of ecological significance.

Annexure-I

STANDARDS ANTAGONISTIC BACTERIA DRAFT SPECIFICATIONS

1. Form and appearance
2. pH
3. Composition

3.1 Percent content of the biocontrol organism in the formulation & nature of biomass

3.2 CFU/g or ml of the product.

3.3 Percentage of other components: carrier /filler, wetting/ dispersing agent, stabilizers /emulsifiers, contaminants/impurities etc.

3.4 Moisture content

4. CFU counts: Minimum 1 x 10^8 CFU/ml or g. (Stability at 30 °C and 65% RH).

5. Contaminants:

5.1 Biological Contaminants:

5.1.1 Pathogenic Contaminants: such as gram negative bacteria *Salmonella, Shigella, Vibrio* etc.: **absent**

5.1.2 Other contaminants should not exceed $1x10^4$/ml or g

5.2 Chemical/botanical pesticides contaminants: **absent.**

6. Method of analysis:

6.1 CFU counts on specific medium.

6.2 Plating for contaminants on specific media

6.3 Antagonistic capability on target organism by bioassay.

6.4 Bioassay procedure based on diseased severity and root colonization as detailed in Appendix-I

7. An undertaking should be submitted that the strain is indigenous, naturally occurring, not exotic in origin and not genetically modified as per Annexure-1.1

APPENDIX- I

Bioefficacy assay for plant disease antagonists based on disease severity and root colonization:

The pathogen to be tested has to be grown in sand maize medium. The sand-maize medium is prepared by adding sand 90 g, maize 10 g and water 10 ml in a saline or any glass bottle of 300 ml capacity and then autoclaved twice. Then 5 mycelial discs of the test pathogen are transferred into the bottle and left for incubation for 15 days. Once the culture has grown well, the sand maize medium is mixed along with the fungal growth and 1 g from this preparation is used as the inoculum after adjusting the cfu to 1 x 10/g by addition of sand.

The plastic cups (5-6 cm diameter) filled with soil and compost (3:1) have to be used. In each cup the filling should be done up to $3/4^{th}$ level. The pathogen inoculum is mixed with sand and has to be applied up to a depth of 2 cm in the plastic cups.

The bioefficacy of the bioagent should be tested by both seed treatment and soil application. For seed treatment, the recommended dose of the formulation has to be used (5 to 10 g). For soil application, the bioagent is added at the rate of 1g of formulation (minimum cfu should be the 2×10^6). The germination percentage, disease intensity and seedling vigour are to be recorded.

Another set of plastic cups filled with sterile soil and sterile compost has to be used to confirm whether the bioefficacy was due to the isolate of the bioagent tested or due to the native isolates of the bioagent present in the soil.

The keys for grading the efficiency mentioned below should be used here. However, for registration purpose, bioagents that are Highly Efficient, Efficient or Moderately Efficient in the plastic cup test under glass house condition (in the presence of pathogen) can be allowed i.e. germination percentage of 70% or above, disease incidence of 30% or less can be considered for registration.

Disease Grading Key

Disease incidence (%)	Description	Rating of bioefficacy of bioagents
0	Germination>90%, no seed rotting, seedling healthy, root and shoot portions well developed	Highly Efficient (HE)
1-15	Germination 80-90%, infection on main as well as lateral roots, seedlings are well developed	Efficient (E)
16-30	Germination 70-80%, development of roots restricted and growth is less compared to Score I. Infection occurred on roots. Shoot portions developed but growth retarded compared to Score I.	Moderately Efficient (ME)
31-45	Germination 60-70%, length of roots and shoots short compared to Score I. Germination of seeds inhibited. 50% of root area infected. Shoot portions also showed infection	Moderately Inefficient (MI)

46-60	Seed germination 50-60%. Development of roots and shoots greatly retarded. Shoot portions also showed infection.	Inefficient (I)
Above 60	Less than 50% germination and seed rotting	Highly Inefficient (HI)

For the root colonization assay, the rhizosphere region of the plants tested above has to be collected and the soil adhering to the root surface has to be removed by gently tapping the roots. The root bits have to be cut into 1 cm bits and randomly 25 bits should be selected for each treatment. They have to be plated on TSM and the percentage of root bits colonized has to be recorded. This has to be performed in sterile soil and non sterile soil. One control treatment without the biocontrol agent being tested should be kept for both the sterile and non sterile soil, to rule of the possibility of interference of native microflora in the bioefficacy assay.
For the bacterial antagonists, the above bioassay procedure has to be followed where only the % root colonization will be considered and other parameters are not required. The % root colonization required is 80%.

Annexure-1.1

UNDERTAKING BY MANUFACTURERS OF MICROBIAL PESTICIDES

I,----------,aged-------years, s/o----------, R/o----
----------and----------of M/s.----------
----------Registered Office at----------
----------do hereby undertake as follows:

(a) That the product----------based on----------, Strain----------, manufactured by M/s.----------and /or imported by M/s.....does not contain any genetically modified organism (GMO) .

(b) That I/We shall abide by the provisions contained in the International Plant Protection Convention with regard to the import of this product.

(c) That I/We shall abide by the provisions in context of International Standards for Phyto-sanitary Measures-Code of Conduct for the import and release of exotic biological control agents of the International Plant Protection Convention (IPPC), FAO, Rome.

(d) That I/We shall provide the samples of our----------product as and when desired by the competent authorities of Government of SL for verification.

(e) That I/We further undertake that in the event of the above product having proved otherwise by any competent authority and resulting in environmental damage, I/We shall inform the ROP/SL, the relevant authorities for Manufacturing Licensing, Pollution Control and of appropriate District/Province/National Level and shall comply with the directions/decisions from them.

(f) That my/our above undertaking is true, and no portion is false and I have concealed nothing relevant to the above matter.

Date----------

Place----------

Signature:
Name----------
Designation----------
Seal of the Company--------

THE SUGGESTED DATA REQUIREMNNTS FOR REGISTRATION OF BACULOVIRUSES - NUCLEAR POLYHEDROSIS VIRUS (NPV) & GRANULOSIS VIRUS (GV) AS BIOPESTICIDES

I. STANDARD OF FORMULATIOS:

1. Viral unit: NPVs $1x10^9$ POB /ml or g. minimum and GVs: $5x\ 10^9$ capsules /ml or g. minimum

2. Contaminants

2.1 Biological contaminants:

2.1.1 Pathogenic contaminants (*Salmonella, Shigella, Vibrio* etc.)

2.1.2 Other microbial contaminant

2.2 Chemical and botanical pesticides contaminants should not be present.

II. REGISTRATION REQUIREMENTS

Following requirements should be fulfilled

A. **BIOLOGICAL CHARACTERISTICS AND CHEMISTRY**

Sl. No.	Parameters	
1.	Identification of Virus	R
1.1	Systematic name (Genus and species)	R
	Strain name	R
2	Common name, if any	R
3.	Source of origin as Annexure-1.1	R
4.	Specification of the product as per Annexure-I.	R

5.	Composition of the product	R
5.1	Viral Unit: POB/Capsule count per ml/g of the product	R
5.2	Percent content of the bio-control organism in the formulation and nature of biomass	R
5.3	Percent of carrier/filler, wetting/dispersing agent, stabilizers/ emulsifiers, containments/ impurities etc.	R
5.4	Moisture content	R
6.	Manufacturing process	R
7.	Test procedure and criteria used for identification by DNA test (Restriction enzymes analysis test).	R
8.	**Method of analysis**	
8.1	Viral unit: NPVs $1x10^9$ POB/ml or g. minimum GVs: $5x10^9$ capsules /ml or g. minimum (For NPV/GV, POB/Capsule Count will be taken with Haemocyto meter as detailed in Appendix-I)	R
8.2	**Biological assays** for determining the LC_{50} / LD_{50} of the formulation	R
9.	**Contaminants:**	R
9.1	Pathogenic contaminants (*Salmonella, Shigella, Vibrio* etc.)	R
9.2	Other microbial contaminants	R
9.3	Chemicals and botanical pesticide contaminants	R
10.	Shelf life claim: Not less than 6 months	R

10.1	Data on storage stability as detailed in Note 2.	R
11.	A sample for verification (100 ml or g)	R

B. BIOEFFICACY:

12.	**Field studies** :data from respective institute certified by Director Research or Head of the Institute	R**
13.	**Laboratory studies** data on LC_{50} values for each target insect species should be generated at a respective laboratory.	R

C. TOXICITY:

14.	**For mother culture**	
14.1	Single Dose Oral (rat and mouse)	R
14.2	Single dose pulmonary	R
14.3	Single dose intravenous	R
14.4	Cell culture	R
14.5	Human safety records.	R
15	**For formulation**	
15.1	Data on mother culture as in 14 above	R
15.2	Single Dose Oral (Rat & Mouse)	R
15.3	Single dose pulmonary	R

15.4	Primary skin irritation	R
15.5	Primary eye irritation	R
15.6	Human safety records	R
16	**For formulated product to be directly manufactured:**	
	(Mammalian toxicity testing of formulations)	
16.1	Single Dose Oral (Rat & Mouse)	
	Toxicity/Infectivity/Pathogenicity	R
16.2	Single dose pulmonary	
	Toxicity/Infectivity/Pathogenicity	R
	(Intra-tracheal preferred)	
16.3	Single dose intravenous	
	Toxicity/Infectivity/Pathogenicity	R
16.4	Human safety records (Effect/Lack of effects)	R
16.5	Primary skin irritation	R
16.6	Primary eye irritation	R
16.6	Cell culture	R
17.	**Environmental safety testing: Core Information requirements**	
	(For formulation only)	
	Non-target Vertebrates	

	Mammals[a]	
17.1	Birds(two species)[b]	NR
17.1.1	Fresh water fish[c]	NR
17.1.2	**Non-target invertebrates**	NR
17.1.3	Terrestrial Invertebrates[d]	
	Soil invertebrates[e]	NR
17.2		NR

D. **Processing,Packaging & Labelling**

Formulation:

18.	**Manufacturing process/process of formulation**	
18.1	Raw material	R
18.2	Plant and Machinery	R
18.3	Unit Process operation/Unit process	R
18.4	Out-put (Finished product and generation of waste)	R
19	Packaging:	
19.1	Classification-solid, liquid or other types of product.	R
19.2	Unit pack size – In metric system	R
19.3	Specification – Details of primary, secondary and transport pack	R
19.4	Compatibility of primary pack with the product	NR

20.	**Labels and leaflets** As per Insecticides Rules, 1971 indicating the common name, composition, antidote, storage, statements etc	R

Notes:

1.Applicants are required to submit an undertaking that strain is indigenous, naturally occurring, not exotic in origin, and not genetically modified as per Annexure 1.1.

2. Additional data of two months for six months shelf life claim and additional data on three months for one year shelf-life claim at two different agro climate locations at ambient temperature along with meteorological data should be submitted.

3. Considering the fact that many small entrepreneurs are engaged in the business of cultivation of NPV/GV, the following mentioned simplification has been suggested.

3.1 If same mother culture is used for making formulation by different entrepreneurs then the information submitted once on mother culture will be sufficient. All entrepreneurs need not to submit data.

3.2 If same mother culture, same method and same adjutants, stabilizers etc. are used for making formulation, then data once submitted will be sufficient for subsequent registrants.

4. The packaging material should also be ensured free from contamination from handling, storage and transportation.

Abbreviations:

R = Required

NR = Not Required

R** = Two seasons/years data on bioeffectiveness from minimum two agro climatic conditions

17.1

a=Information on infection and pathogencity in mammals will be available from mammalian safety testing.

b=Information on infection and pathogenicity, suggested test: single-dose, oral test. Suggested test species: pigeon and chicken.

c=Information on infection and pathogencity. Suggested test species;

*Tilapia mossambica*or other appropriate spp.

d=Information on morality effects. It is recommended that information be obtained for honey bee and Bombyxmori (silk worm).

e=Information on morality effects. It is recommended that test species include an earthworm (*Lumbricusterrestris*) or other appropriate macro invertebrates of ecological significance.

BACULORIVUS

SPECIFICATIONS

Form and composition of the product

1. Viral Unit: POB/Capsule count pr ml/g of the product

2. Percent content of the bio-control organism in the formulation and nature of biomass

3. Percent of carrier/filler, wetting/dispersing agent, Moisture content, pH

4. Viral Unit:

NPVs (*Helicoverpa&Spodeptera*) - $1x10^9$ POB/ml or gm (minimum)

(POB –Polyhedral Occlusion Body)

GV (Chilo, Plutella&Acheae) - $5x10^9$ Capsules/ml or g. (minimum).

5. **Contaminants**:

5.1 **Biological contaminants**:

5.1.1 **Pathogenic contaminants**: Pathogenic contaminants such as gram negative bacteria *Salmonella, Shigella, Vibrio* etc. should be absent

5.1.2. **Other microbial contaminants:** Other microbial contaminants should not exceed $1x10^4$ /ml or g

5.2 Chemical/botanical pesticides contaminants should be absent.

6.Identification of Baculovirus by DNA test (Restriction enzyme analysis test).

7.An undertaking should be submitted that the strain is indigenous, naturally occurring and not exotic and not genetically modified as per Annexure-1.1

8. **Method of analysis:**

Viral Unit:

NPVs (Helicoverpa and Spodeptera) =$1x10^9$ POB/ml or gm. minimum

GVs = $5x10^9$ Capsules/ml or gm. minimum.

8.1 In case of NPVs/, POB/Capsule count should be taken with Haemocytometer using shallow depth counting chamber as detailed in Appendix – I

8.2 Biological assay for determining the LC_{50} or LD_{50} of the formulation:
8.2.1 Bioassay for NPV by the Diet Surface Contamination

Method as detailed in Appendix-II OR

8.2.2 Bioassay for GV against *Chiloinfuscatellus* as detailed in Appendix-III OR

8.2.3 Bioassay for GV against *Plutellaxylostella*as detailed inAppendix-IV.

8.2.4 Bioassay for GV against Acheaejanta as detailed in Appendix-V.

8.3 Plating for contaminants on specified media.

Appendix-I

COUNTING OF NPV/GV (POB/CAPSULE) USING IMPROVED NEUBAUER HAEMOCYTOMETER COUNTING CHAMBER.

A haemocytometer is used for estimating of NPVs/GVs in a unit volume of the product. The Improved Neubauer Haemocytometer comprised a thick glass slide with a shallow depression in the central section divided into two halves (figure-1). Each side, the base of the depression has a fine ruled grid of squares (figure-2) which is visible under a microscope. The dimensions of this grid are defined. Place a standard cover slip placed over the depression and a one half halves of the slide chamber using a micro pipette. The particles require 2-5 minutes to sediment to the chamber floor.

Either dark field or a phase contrast microscope is used to identify and count polyhedral occlusion bodies (POB) or capsule. With the counting chamber under the microscope, the number of Polyhedra/capsule in a given number of grid squares can be counted. Each count consists of a tally of the number of polyhedra completely contained within a big square plus the number of touching the top and left sides. Polyhedra touching the bottom and right sides are not counted. Since both the depth of the chamber and the grid dimensions are known. It is then a

straight forward calculation to determine the number of polygedra /capsule per ml of test suspension.

Number of NPV (POB) per ml/gm $= \frac{D \times X}{N \times K}$

Where:

D = Dilution factor

X = Total number of polyhedra counted

N = Number of squares counted

K = Volume above one small square in $cm^3 = (2.5 \times 10^{-7} cm^3)$

Area of each small square is 1/400 mm^2 = 0.0025 mm^2. Depth of chamber is o.1mm. Volume of liquid above a single small square is 0.0025 mm^2 x 0.1mm= 0.00025 mm^3. To covert to cm^3 multiply by 1/1000 to get a volume of 2.5 x 10^{-7} cm^3 above 1 small square. Hence, $K=2.5 \times 10^{-7} cm^3$

Worked example:

Suppose in a sample diluted by a factor of 1000 we count 535 polyhedra in 160 small squares then:

D = 1000

X = 535

N = 160

K = 2.5 x 10^{-7} cm^3

$$\text{Thus, POB count} = \frac{1000 \times 535}{160 \times 2.5 \times 10^{-7}} = 1.34 \times 10^{10} \text{ POB/ml of test sample}$$

Note: (i) Usually, this procedure is repeated 3 times and an average taken to get a more accurate estimate.

(ii) Same procedure will beused for GV also for counting the number of capsule per unit volume of the product.

Annexure-1.1

UNDERTAKING BY MANUFACTURERS OF MICROBIAL PESTICIDES

I,----------,aged-------years, s/o----------, R/o----
----------and----------of M/s.----------
----------Registered Office at----------
----------do hereby undertake as follows:

(a) That the product----------based on----------, Strain----------, manufactured by M/s.----------and /or imported by M/s.....does not contains any genetically modified organism (GMO) .

(b) That I/We shall abide by the provisions contained in the International Plant Protection Convention with regard to the import of this product.

(c) That I/We shall abide by the provisions in context of International Standards for Phyto-Sanitary Measures-Code of Conduct for the import and release of exotic biological control agents of the International Plant Protection Convention (IPPC), FAO, Rome.

(d) That I/We shall provide the samples of our----------product as and when desired by the competent authorities of Government of India for verification.

(e) That I/We further undertake that in the event of the above product having proved otherwise by any competent authority and resulting in environmental damage, I/We shall inform the Central Insecticides Board and Registration Committee, the relevant authorities for Manufacturing Licensing, Pollution Control and of appropriate District/State/National Level and shall comply with the directions/decisions from them.

(f) That my/our above undertaking is true, and no portion is false and I have concealed nothing relevant to the above matter.

Signature:

Date_____ Name----------

Designation----------

Place:----------

Seal of the Company--------

3. SOURCE

(Details pertaining to this section should be furnished in the spaces given against the respective Items)

Item 3 .1 ☐ Item 3.2 ☐ Item 3.3 ☐

	Manufacturer	Formulator	Supplier
Company Name			
Contact Name			
Contact Title			
Address			
City			
Postal Code			
T/Phone Number			
Fax Number			
E-mail Address			

3.4 ☐ Declaration of the manufacturer of the technical material is attached: YES / NO

3.5 ☐ Declaration of the formulator of the pesticide is attached: YES / NO

FORM-1

Application
For Registration of Biopesticides

Control of Pesticides Act, No.33 of 1980

Sri Lanka

Please refer the Guideline before compilation of the application.

1. GENERAL

(Details pertaining to this section should be furnished in the spaces given against the respective Items)

1.1 ☐ Name of the registrant:

1.2 ☐ Name and designation of the authorised signatory:

1.3 ☐ Address:

1.3.1 ☐ Office:

1.3.2 ☐ Warehouse/ Factory:

1.4 ☐ T/Phone No: ☐ Fax No: ☐ E-mail:

2. PRODUCT DETAILS

(Details pertaining to this section should be furnished in the spaces given against the respective Items)

2.1 ☐ Trade name:

2.2 ☐ Use category: INSECTICIDE/FUNGICIDE/WEEDICIDE/OTHER (delete the sections not ☐ relevant)

2.2.1 ☐ If other, please specify:

2.3 Intended user/market

2.4 Use classification: AGRICULTURAL/DOMESTIC/INDUSTRIAL/PUBLIC HEALTH/ VERINARY/WAREHOUSE/FORMULATION PURPOSES//OTHER (delete the sections not ☐ relevant)

2.4.1 If other, please specify:

2.5 ☐ Common name(s) of active ingredient(s):

2.6 ☐ Strength of formulation (Range or minimum concentration):

2.7 ☐ Type of formulation:

2.8 ☐ Type of containers:

2.9 Size of containers:

2.10 Registration in other countries, if available:

2.10.1. ☐ Other countries where registered (copies of registration certificate should be authenticated as per the Guideline):

2.11 Do you already hold a valid registration for the proposed formulation or applied for registration from an alternate source. YES/NO

If "YES": Application No: Registration No:

7. ADDITIONAL SUBMISSIONS

7.1 ☐ Specimen labels prepared in accordance with the Section 8 of the Act, in duplicate

7.2 ☐ Specimen containers

7.3 ☐ Specimen samples of

7.3.1 ☐ Formulation (from the source declared above) (100 g or 100 ml)

7.3.1.1 ☐ Aerosols (2X100 ml or more)

7.3.1.2 ☐ Mosquito Coils (5X10 pieces)

7.3.1.3 ☐ Mosquito Mats (3X30 pieces)

7.2.1.4 ☐ Others (Refer to Pesticide Registration Authority)

7.4 ☐ Non refundable payment a sum of Rs.6500.00 as filing fee (Cheques must be drawn in favour of the Director General of Agriculture. Money-orders must be payable at the Post Office- Peradeniya.)

I hereby solemnly declare and affirm that all information given by me is true and correct.

.....

Signature, name & designation

360 × 7500 / 1000 = 2700 / 2 = 1350.00

Date:

Notes:

1.Applications will not be accepted if sections 1,2,3 and 7 are incomplete. Authority may waive details of certain technical data requirements of the Sections 4,5 and 6 or accept applications lack any of those details for evaluation on the basis that it may not increase any potential adverse effect on human health and the environment.

2.The decision of registration of the pesticide is based on the submission of document along with the registration application on the assumption that they are final and accurate declarations for that purpose. Therefore, administrative or other legal actions would not be reviewed on the grounds that those declarations contained incorrect information by an oversight or typing mistake unless the reports pertaining to such claims are verified at the expense of the applicant through independent sources decided by the Registration Authority. However, the Registrant may propose amendments or alterations to their submissions if they find the claims need to be updated based on subsequent field observations, published reports with proof or proprietary data by the patent holder.

3.Upon acceptance of the application for registration, a sum of Rs.4,000.00 (A cheque drawn in favour of the Director General of Agriculture or a Money-order payable at Post Office- Peradeniya shall be submitted as registration fee.)

Office use only

Application No:
Registration No:
Application accepted/returned:
Reasons for the return:

Date received:
Date Registered:

- ☐ Incomplete application. (items: .....)
- ☐ Technical documents are not numbered
- ☐ Relevant references are not indicated in the applicatio
- ☐ Technical documents are not in duplicate

4. ACTIVE INGREDIENT

(Details pertaining to this section should be furnished in separate documents. Indicate respective number(s) of the annexed documents against relevant Items below. Please see section 'D' in the Guideline)

4.1 ☐ Summary of chemical and physical properties

4.2 ☐ Data on environmental behavior (not required for biopesticides in use)

4.3 ☐ Bio-toxicological effects (not required for biopesticides in use

4.4 ☐ Source(s) of reference

5. FORMULATION (Product)*

(Details pertaining to this section should be furnished in separate documents. Indicate respective number(s) of the annexed documents against relevant Items below. Please see section 'D' in the Guideline)

6.1 ☐ Summary of chemical and physical properties

6.2 ☐ Specific detailed composition

6.3 ☐ Method of analysis

6.4 ☐ Shelf-life

6.5 ☐ Toxicological data

6.6 ☐ Summary of bio-efficacy data

- Declaration of the applicant that the product does not contain any other pesticide active ingredients (AIs) other than AI(s) mentioned above as an adulterant.