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ENVIRONMENTAL IMPACT OF MICROBIAL PESTICIDESP.S. VIMALA DEVI^{1*}, G.V. RANGA RAO², S. GOPALAKRISHNAN² AND G. SIVAKUMAR³**ABSTRACT**

Biological control constitutes an important component of integrated pest management (IPM). However, the non-availability of efficient biocontrol agents is one of the major constraints in adopting IPM practices. Microbial control, which makes use of naturally occurring microbes to control insect pests, pathogens, and weeds, is less harmful to nontarget organisms and the environment than the chemical pesticides. Microbials are promising alternatives to chemical pesticides and have opened up new vistas in insect pest management to aid promotion of safe, eco-friendly pest management. The use of microbial pesticides in pest management is quite limited because of lack of appropriate formulations and the availability of quality products to the farmers. Since 2006, the registration of the microbial pesticides for commercial purposes has been made mandatory in India. It warrants information on toxicological results against mammals and eco-toxicity data on nontargets such as fishes, birds, earthworms, honeybees, and silkworm. The data is to be generated with technical product and the formulation of every strain intended for commercialization. It is also mandatory to generate data on the safety of the formulation to natural enemies along with data on the bioefficacy and phytotoxicity to the crop. Fourteen primary microbial pesticide products and their formulations have been registered in India by 2009. There are 478 products of the 14 microbial pesticides registered in India. There are 184 products for the management of plant pathogens

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belonging to Trichoderma viride, T. harzianum and Pseudomonas fluorescens. Microbial pesticides registered for the management of insect pests include 18 products belonging to Bacillus thuringiensis var. kurstaki, 62 to Beauveria bassiana, 51 to Verticillium lecanii, 13 to Metarhizium anisopliae, 18 to nuclear polyhedrosis virus (NPV) of Helicoverpa armigera, and 3 to NPV of Spodoptera litura. There is a paradigm shift in the use of biopesticides for use under the IPM. Large-scale field application of microbial pesticides for pest management can help generate tangible information on their environmental effects for use in the future.

INTRODUCTION

Insect pest management in agriculture is important to safeguard crop yield and increase productivity. In India, on an average 33% of crop loss occurs due to insect pests, and has been estimated to be Rs. 200 billion annually. Regardless of the adverse effects of chemical pesticides on the environment, health and socio-economic conditions of the community, farmers resort to self-defeating practices such as increasing the dosage or frequency of pesticide application to minimize the crop loss. In addition, Rs.1,000 crores worth of agricultural exports is rejected every year due to the presence of unacceptable levels of pesticide residues. This warrants reduced dependence on pesticides by exploring the use of safer alternatives for pest management. This has led to search for eco-friendly pest management strategies with emphasis on bio-intensive integrated management. The National Agricultural Policy 2001 has laid special emphasis on the integrated pest management (IPM), particularly with emphasis on the use of "bio-agents in order to minimize the indiscriminate and injudicious use of chemical pesticides". However, the non-availability of good quality biotic agents at the farm level on time is one of the major constraints faced in adopting the IPM practices.

The need for sustainable and eco-friendly pest management practices is strongly felt with the increasing awareness of the harmful effects of the synthetic insecticides on the nontarget organisms, humans and the environment. Microbial pesticides are considered promising alternatives to chemical pesticides, and have opened up new vistas in insect pest management to aid in the promotion of safe, eco-friendly pest management. Due to biodegradable nature, they do not leave any residues on crops, and do not contaminate the aquatic systems. Microbial control includes all aspects of utilization of microorganisms or their by-products for the control of insect pests and plant diseases. Microbial agents are relatively host specific and do not interfere with other biotic systems. The use of microbial pesticides in pest management has been limited to the generation of information on the efficacy in micro-plots at research farms, but the use in farmers' fields has been quite limited. Large-scale use of microbial pesticides for pest management will provide tangible information on their environmental impact, but such an effort is yet to gain momentum. Hence, it is too early

to make a statement on the impact of microbial pesticides on the environment.

Among the different microbial agents developed and tested for pest management, bacteria, fungi, and baculoviruses are quite promising for pest control. Bacteria and fungi are gaining importance due to their amenability for mass multiplication on artificial media. Microbial insecticides currently used in India for controlling economically important pests affecting agricultural and horticultural crops include *Bacillus thuringiensis* var. *kurstaki* (Btk), nuclear polyhedrosis virus (es) (NPV) of *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fab.), the entomopathogenic/nematicidal fungi, *Beauveria bassiana* (Balsamo) Vuillemin, *Verticillium lecanii* (Zimm.) Viegas, *Paecilomyces lilacinus* Thom. and *Metarhizium anisopliae* (Met.) Sorokin. Antagonistic fungi and bacteria found promising for plant disease management include *Trichoderma viride* Pers., *T. harzianum* Rifai, and *Pseudomonas fluorescens* Migula. The development of microbial pesticides for effective pest control in the context of sustainable agriculture will be a major challenge. A truly integrated approach to address the present day plant protection issues is to obtain maximum benefit. Because of the low adverse environmental impact and high specificity of the microbial agents, they should be an ideal component of IPM in the future pest management programs.

IMPACT OF MICROBIAL PESTICIDES ON THE ENVIRONMENT

The rationale for the development and deployment of microbial insecticides for pest management is their environmental safety, specificity, and biodegradability. Some pathogens selected for commercial development, such as viruses and bacteria, may infect only a single or small number of closely related insect species. Others, such as fungi and nematodes, may affect a fairly wide range of insects and related arthropods. However, the commercially available microbial pathogens are target specific and have not been shown to infect vertebrates or plants. The biodegradable nature of the microbial pesticides does not leave any harmful residues in the environment, and does not enter the food chain.

Fate of *Bt* in the environment has been well documented. The *Bt* spores are released into the soil from the decomposing dead insects after they have been killed by it. It is rapidly inactivated in soils with a pH below 5.1. Microbial pesticides such as *Bt* are classified as immobile because they do not move or leach with the groundwater. Because of rapid breakdown and low toxicity, they do not adversely impact the aquatic systems. Safety of the *Bt* toxins in terms of toxicity and allergenicity towards mammals and other nontarget organisms is well documented. Lack of receptors that bind to *Bt* toxins and rapid degradation of *Bt* toxins in human digestive system make them innocuous to human beings. *Bt*-sprays are safe to nontarget organisms such as soil microorganisms (protozoa and fungi), Collembola,

Mollusca, Crustacea, Arachnida, aquatic insects, predators, parasitoids, honeybees, earthworms, salamanders, bird, and mammals.

Spores of entomopathogenic fungi do not withstand high temperatures and cannot persist on the foliage for long. However, infected cadavers that drop to the soil sporulate under congenial microclimatic conditions and overwinter in the soil. A meager percentage of these conidia survive through the summer and express in the subsequent rainy season after the pest population builds up. Baculoviruses, among the insect viruses, are regarded as safe and selective bio-insecticides, and are restricted to invertebrates. They have been used worldwide against many insect pests, mainly Lepidoptera. Their application as microbial pesticides, however, has not met their potential to control pests in crops, forests, and pastures, with the exception of NPV of the soybean caterpillar, *Anticarsia gemmatalis* (Hub.), which is used on approximately one million ha annually in Brazil. Problems that have limited the use of baculoviruses include narrow host range, slow killing speed, technical and economical difficulties for *in vitro* commercial production, timing of application based on host population monitoring, and variability in their efficacy in the field under diverse climatic conditions. Epizootics of baculovirus diseases are frequent in Lepidoptera and sawflies with very high larval mortality, resulting in a substantial reduction in insect population. Baculoviruses survive for a long period in the soil. Long-term benefits can be achieved through the use of NPV since most of the dead larvae remain on the plant with their integument ruptured, resulting in the release of NPV laden hemolymph that persists in the soil, resulting in the epizootic spread of the disease to the next crop. Reservoirs of baculoviruses in the soil have long-term importance, and initiate epizootics when insect populations resurge following a phase of low density.

IMPACT OF MICROBIAL PESTICIDES ON THE NATURAL ENEMIES

Research pertaining to the development of microbial pesticides in India has focused on the identification of virulent isolates for effective management of the target pests. Information pertaining to their effects on natural enemies, nontarget pests, and the environment is quite scanty. Research on the microbial pesticides over the past decade has focused on generation of information pertaining to their safety to the natural enemies, persistence in the environment, phytotoxicity, etc. (Table 1), in addition to generating information on the bioefficacy. Field trials at Vishakapatnam in Andhra Pradesh (India) for the management of brinjal spotted beetle, *Henosepilachna vigintioctopunctata* Fab. employing *B. bassiana* showed that the fungus persisted in the soil for 30 days after application (Padmaja and Kaur 1998). *Nomuraea rileyi* (Farlow) Samson was found to be safe to the larval parasitoid, *Microplitis maculipennis* Szep. and the honey bee, *Apis cerana indica* Fab., (Mulimani and Kulkarni 2004). Field trials with spinosad for the management of major insect pests in rice ecosystem have shown no significant effects on the spider population that predominates predatory

Table 1: Interactions of microbial pesticides with natural enemies of crop pests.

Microbial pesticide	Natural enemy/ beneficial insect	Field/lab	Fate/effect	Reference
<i>Beauveria bassiana</i>	-	Field trials against spotted beetle on brinjal	Conidia persisted in soil for 30 days after application	Padmaja and Kaur (1998)
	<i>Trichogramma chilonis</i> Predatory spiders	Lab studies Field trials on sunflower	Safer for adult emergence No reduction in predator population and no phytotoxic effects	Vimala Devi and Hari (2009) Vimala Devi and Hari (2009)
<i>Nomuraea rileyi</i>	<i>Apis cerana indica</i> <i>Microplitis maculipennis</i> <i>Cotesia</i> spp. <i>Apanteles</i> spp.	Lab studies	Safer to honeybees, <i>Microplitis</i> , <i>Cotesia</i> and <i>Apanteles</i>	Mulimani and Kulkarni (2004)
Spinosad	Predatory spiders	Field studies against <i>Spodoptera litura</i> on groundnut and castor Field trials against major insect pests on rice Field trials against <i>Helicoverpa armigera</i> on pigeonpea Lab studies Lab studies	No reduction in predatory spider population	Vimala Devi et al. (2002) Karthikeyan et al. (2008)
<i>Bacillus thuringiensis</i>	<i>T. chilonis</i> <i>T. chilonis</i> <i>Microplitis maculipennis</i>	Field trials on castor	Poor adult emergence Safe Safe, no phytotoxic effects	Mittal and Ujagir (2005) Boomathi et al. (2005) Boomathi et al. (2005) Vimala devi and Sudhakar (2006)

Table 1: (Contd...)

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Microbial pesticide	Natural enemy/ beneficial insect	Field/Lab.	Fate /effect	Reference
NPV of <i>Helicoverpa armigera</i>	<i>T. chilonis</i> , <i>Chrysoperla carnea</i> , honeybee and <i>Bombyx mori</i>	Lab studies	Safe	Jeyarami et al. (2008), Boomathi et al. (2005)
	<i>Campoplex chloridiae</i>	Field trials against <i>H. armigera</i> on chickpea	Low reduction of parasitoid in comparison to endosulfan	Ranga Rao et al. (2008)
	Aerial and soil inhabiting natural enemies	Field trial against <i>H. armigera</i> on chickpea	Low reduction in aerial and soil dwelling natural enemies.	
Combination of <i>Metarhizium anisopliae</i> , <i>Bacillus subtilis</i> , <i>B. pumilus</i> and <i>Serratia marcescens</i>	Earthworm	In the soil	10-fold higher population over farmer's practices	Rupela (2008)

fauna in the rice ecosystem (Karthikeyan et al. 2008) and on pigeonpea (Mittal and Ujagir 2005). However, Boomathi et al. (2005) reported deleterious effects of spinosad on the egg parasitoid, *Trichogramma chilonis* Ishii, resulting in poor adult emergence (14%). The use of HaNPV @ 3×10^{12} POBs ha⁻¹ and Spicturin (commercial *Bt* formulation) @ 2.0 L ha⁻¹ have been found to be safe to the egg parasitoid. In laboratory studies, a UV-selected strain of HaNPV has been found to be safer to *T. chilonis*, *Chrysoperla carnea* (Stephen), honeybee, and *Bombyx mori* L. (Jeyarani et al. 2008).

Considerable data have been generated on the safety of formulations of *Btk* and the entomofungal pathogens *B. bassiana* and *N. rileyi* to natural enemies and on their phytotoxicity to the target crops (Vimala Devi et al. 2002). *Nomuraea rileyi* is safe to the larval parasitoids, *Cotesia* spp. and *Apanteles* spp. when applied for the control of *S. litura* on groundnut and castor. Incidence of the larval parasitoid, *Microplitis maculipennis* Szep. was observed only in castor-fields sprayed with DOR *Bt*-1 formulation for the management of castor semilooper, *Achaea janata* (Linn.), but the insect pest was absent in the quinalphos sprayed plots (Vimala Devi and Sudhakar 2006). *Beauveria bassiana* formulated as a 30% suspension concentrate (SC) was found to be safer to the egg parasitoid, *T. chilonis* under laboratory conditions, and to spiders in field trials for the management of *H. armigera* on sunflower (Vimala Devi, Unpublished). No phytotoxic effects were recorded with DOR *Bt*-1 formulation on castor, and the *B. bassiana* SC formulation on sunflower.

Low reduction (3%) of *H. armigera* parasitoid, *Camptolitis chloridaeae* Uchida and other natural enemies was recorded in the HaNPV sprayed plots as compared to 60% reduction in the endosulfan treated plots in chickpea. HaNPV (@ 250 LE ha⁻¹) application on chickpea resulted in a reduction of aerial and soil inhabiting natural enemies by 15 and 22%, respectively, over the control plots, while the reduction in the endosulfan sprayed plots was 52.4 and 63.1%, respectively (Ranga Rao et al. 2008). There was a 3-fold difference in the numbers of beneficial insects in plots sprayed with biopesticides as compared to those treated with the synthetic chemicals. Studies with low cost input based IPM involving *M. anisopliae*, *Bacillus subtilis* Ferdinand Cohn, *B. pumilus* Meyer, and Gottheil and *Serratia marscescens* Bizio resulted in 7 to 10 fold increase in the population of earthworms in the biopesticide treated plots over the plots maintained under normal agronomic practices (Rupela 2008). Little is known about the effects of biocontrol inoculants on the nontarget fungi in the rhizosphere. Studies carried out with *P. fluorescens* (CHARO-Rif), which produces the antimicrobial polyketides 2, 4-diacetylphlorogelucinol (Phl) and pyoluteorin (Plt), and protects cucumber from several fungal pathogens. Strain CHARO-Rif (pME3424), which over produces Phl and Plt displays improved biocontrol efficacy as compared to CHAO-Rif (Sivakumar, NBAII, Unpublished).

REGISTRATION OF MICROBIAL PESTICIDES

Plant protection against pathogens, pests, and weeds has been progressively re-oriented from a remedial approach to a rational use of pesticides in which consumer health and environmental conservation prevail over any other consideration. Microbial pesticides have been introduced for crop protection, and a new generation of microbial pesticides is being promoted for pest management. The development of microbial pesticides requires several steps to be addressed right from its isolation in pure culture to bioefficacy assays performed *in vitro*, *ex vivo*, *in vivo*, or in pilot trials under field conditions. For commercial delivery of a microbial pesticide, the biocontrol agent must be produced at an industrial scale (fermentation), preserved for storage, and formulated by means of biocompatible additives to improve its survival and application and stability of the final product. Because of the unique nature of biocontrol agents, some data requirements are different from those necessary for registration of chemical pesticides, but the general principle that the product should demonstrate effectiveness and should not be hazardous to users, consumers of treated foods, or to the environment including natural enemies and beneficial organisms, still applies.

As of October 2009, altogether 14 primary microbial pesticide products and their formulations were registered in India. Around 150 companies are involved in the production of microbial pesticides. Estimates indicate that 478 products of 14 microbial pesticides have been registered in India. The microbial pesticides registered for plant diseases include 184 products belonging to *T. viride*, 19 to *T. harzianum*, and 93 to *P. fluorescens*. Microbial pesticides registered for the management of insect pests include 18 products belonging to *B. thuringiensis* var. *kurstaki*, 62 to *B. bassiana*, 51 to *V. lecanii*, 13 to *M. anisopliae*, 18 to NPV of *H. armigera*, and 3 to NPV of *S. litura* (Table 2). The data requirements for microbial pesticides are designed to provide information on basic hazards due to the exposure for a microorganism with totally unknown properties. In actual practice, present microbial pest control agents are well identified, which enables the regulatory authorities to predict their properties and behavior (Table 3). This is particularly true in the categories of biocontrol agents related to human health and plant pathogenicity. Clinical medicine and agricultural science have identified many microorganisms associated with many diseases. If the microbial pesticide under consideration is taxonomically similar to a clinically or agriculturally-significant microorganism, its properties and effects should be examined in greater details than suggested by the tests generally required under the registration guidelines.

In India, the import, manufacture, sale, transport, distribution, and the use of pesticides is regulated under the Insecticide Act 1968, and the rules framed there under. Accordingly, the Department of Agriculture and Cooperation, Government of India has notified the inclusion of microbial pesticides in the schedule of the Act by Gazette notification number G.S.R.

Table 2: Microbial pesticides registered in India as of October 2009.

No	Microbial pesticide	Formulations	No. of products
Bacteria			
1	<i>Bacillus thuringiensis</i> var. <i>israelensis</i>	5.0% WP, 5.0% AS	6
2	<i>Bacillus thuringiensis</i> var. <i>kurstaki</i>	0.5%, 5.0% & 7.5% WP	18
3	<i>Pseudomonas fluorescens</i>	0.5%, 1.0% WP	93
4	<i>Bacillus subtilis</i>	1.5% AS	2
Fungi			
5	<i>Ampelomyces quisqualis</i> Ces.	2.0% WP	1
6	<i>Beauveria bassiana</i>	1.0%, 1.15% or 2.15% WP, 10.0% SC	62
7	<i>Metarhizium anisopliae</i>	1.0%, 1.5% WP	13
8	<i>Paecilomyces lilacinus</i>	1.0%	7
9	<i>Trichoderma harzianum</i>	0.5%, 1.0%, 2.0% WP	19
10	<i>Trichoderma viride</i>	1.0% WP	184
11	<i>Verticillium chlamydosporium</i> Godd.	1.0% WP	2
12	<i>Verticillium lecanii</i>	1.15%	51
Virus			
13	NPV of <i>Helicoverpa armigera</i>	0.43%, 0.5%, 0.64%, 2.0%	18
14	NPV of <i>Spodoptera litura</i>	0.5%, 2.0%	3

Source: CIB and RC website, Oct 2009.

224(E), dated 26.03.1999, and G.S.R. 69 (E) dated 05.11.2001. To ensure an early availability of the microbial pesticides to the farming community, the Central Insecticides Board has simplified the registration procedure, and allowed the commercialization during the provisional registration period, unlike the chemical pesticides. Registration of microbial pesticides for commercial purposes has been made mandatory in India since 2006. It warrants generation of toxicological data against mammals as well as ecotoxicity data on nontargets such as fishes, birds, earthworms, honeybees, and silkworm. The data is to be generated with technical formulation of every strain intended for commercialization. It is also mandatory to generate data on safety of the formulation to natural enemies along with data on bioefficacy and phytotoxicity to the crop. The guidelines were reviewed two times in 2004 and 2008. Based on the feedback from the industry and the scientific community, the Registration Committee has revised the existing guidelines to ensure quality of the microbial pesticides coupled with the simplification of the aspects contributing to promoting their commercial production and use in the IPM. The revised guidelines have become effective since 1 January 2010. The information can be accessed from the official website of the Central Insecticides Board: <http://www.cibrc.nic.in>.

CONCLUSIONS

The regulatory framework for microbial pesticides is aimed at ensuring availability of good quality biopesticides for pest management. The system ensures manufacture and supply of good quality microbial pesticides as long

Table 3: Information requirements for the registration of microbial pesticides.

1.	Identity of the product	Active agent	<ul style="list-style-type: none"> • Physical and chemical properties • Common name. Systematic name and strain for bacteria, protozoa, fungi, etc. • Natural occurrence of the organism, its relationships to other species, and history • Manufacturing process • Appropriate test procedures and criteria used for identification, such as morphology, biochemistry, and/or serology • Composition of unintentional ingredients, their nature and identity, and content of extraneous organisms • Methods of analysis • Physical and chemical properties • Quantity of active agent • Name and type of formulation • Nature and quantity of diluent • Identity of non-active ingredients such as UV protectors, water retaining agents, etc. • Stability of product, effect of temperature and storage conditions on biological activity • Methods of analysis • Shelf-life
		Finished product	
2.	Biological properties	Information on the biological agent (active agent)	<ul style="list-style-type: none"> • Natural occurrence • Target pest species and the pathogenicity or antagonism to that pest • Infective dose/level, transmissibility and mode of action • Types of crops or premises to be protected • Manner, rate and frequency of application • Oral toxicity/pathogenicity • Dermal toxicity/pathogenicity • Inhalation toxicity/pathogenicity • Primary skin irritation • Mucous membrane irritation • Allergy/sensitization/immunosuppression • Primary eye irritation
3.	Toxicology data	*Primary toxicology data (single exposure studies)	

Table 3: (Contd...)

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	**Eco-toxicity	<ul style="list-style-type: none"> • Toxicity to fish • Toxicity to honey bees • Toxicity to earthworm • Toxicity to silkworm • Compatibility of primary pack with the product
4.	Container content compatibility	
5.	Labeling	<ul style="list-style-type: none"> • Common name • Composition • Antidote • Statements
6.	Processing and packing	<ul style="list-style-type: none"> • Raw material • Plant and machinery • Unit process operation/unit process • Out-put (finished product and generation of waste) • Classification – solid, liquid or other types of product. • Unit pack size – in metric system • Specification – details of primary, secondary and transport pack
	Labels and leaflets as per Insecticides Rules 1971	
	Manufacturing process/process of formulation	
	Packaging	

* It should be established that the active agent is not a known pathogen of man or other mammals and that the preparation does not contain such pathogens as contaminants or mutants as determined by acceptable tests.

**It is important to assess ecological risks due to microbial pesticides. In order to do this, their degree of species specificity and adverse effects on non-target species must be given careful consideration.

as they comply with the guidelines. Unhygienic production facilities can result in the contamination of products with human pathogens such as *Escherichia coli* Migula, *Salmonella* spp., *Shigella* spp., *Vibrio* spp., etc., as well as with other microbial contaminants. The adoption of stringent in-house quality control measures by producers of microbial pesticides is the key to avoiding inundation of the environment with microbial contaminants. There is a paradigm shift in the use of bio-intensive IPM for pest management, which will gain momentum with more effective and registered microbial pesticides becoming available commercially to the farming community. Biopesticides are more environment friendly because of their target specificity, short half-life, and biodegradability. Systematic in-depth studies, however, are essential to determine their impact on the environment.

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