EFFICACY OF CERTAIN BIOCIDES IN THE MANAGEMENT OF INSECT IN DISTRICT BANKURA WEST BENGAL

Nandini Singha Deb

Research Scholar, Sri Satya Sai University of Technology and Medical Sciences, (India)

ABSTRACT

A biocidal product (or biocide) can be a pesticide or an antimicrobial containing or generating an active substance(s) that is used to prevent or control various types of harmful or unwanted organisms. Such products include disinfectants, preservatives, insect repellents, rodenticides and insecticides. Biocides control the intended target organism by a chemical or biological action. Biocides can be used by professionals and members of the public in a wide spectrum of use areas that are classified into product-types. To make sure the use of biocidal products do not have unacceptable risks for people, non-target animals and the environment, they are regulated to control their marketing, sale and use in order to minimise any risk. Biocidal products can be distributed and used in bankura, West Bengal if they have been notified to or authorised by the Competent Authority (The Department of Agriculture, Food and the Marine).

I INTRODUCTION

The straggle between man and insects began long before the dawn of civilization, has continued to the present time, and will continue as long as the human race endures. In the recent years entomologists have emphasized the importance of economic threshold as they apply to manage the insects of economica importance. The development of economic threshold require bio mathematical and economic expertise in pest management. It is difficult to determine the economic threshold of most pests on most crops. To do so it requires an ability to predict the probable consequences of continuing increase population if management tactics are not exerted in relation to injury level. The control of insect pests is a goal which challenges every fibre of man's ingenuity and intelligence. For this purpose man has been involved in devising newer methods, tools and techniques. However, every time insects counter ways to blunt every weapon invented by man to exterminate them.

More recent is the discovery of Betaproteobacteria species that show broad-spectrum insecticidal properties. This group includes specific strains of Burkholderia spp. and Chromobacterium spp. Lastly, certain Actinobacteria species have gained high scientific and commercial interest in relation to the production of a variety of metabolites acting as potent insecticides. This is the case for Streptomyces and Saccharopolyspora species.

II INSECT PATHOGENIC BACTERIA

2.1 Bacillaceae

Bacillus thuringiensis

Bacillus thuringiensis (Bt) is the most studied entomopathogenic species and some of its crystal producing strains have certainly represented the main active substances used for the microbial pest management during the last decades. The pathogenic action of this bacterium normally occurs after ingestion of spores and crystalline inclusions containing insecticidal δ-endotoxins that specifically interact with receptors in the insect midgut epithelial cells. It is largely demonstrated that these toxins, mostly represented by Cry proteins, after being solubilized and activated in the insect midgut, act through a pore-forming mechanism determining the disruption of natural cell membrane permeability, with consequent cell lysis followed by gut paralysis and death.

Most commercially available formulations are based on spore-crystal mixtures with effectiveness against different pest species. B. thuringiensis subsp. kurstaki (Btk) is generally used against young Lepidopteran larvae and includes different strains with significant commercial interest like HD-1, SA-11, SA-12, PB 54, ABTS-351 and EG2348. Strains of B. thuringiensis subsp. aizawai (Bta) (i.e., ABTS-1857) are also used against armyworms and diamondback moth larvae. Besides, strains belonging to the subsp. israelensis (Bti) and tenebrionis (Btt) have been employed for the management of mosquitoes and simulids, and against coleoptera, respectively. Continuous research activities have led to the isolation of many strains and to the discovery and characterization of various Bt insecticidal toxins produced in different bacterial stages (i.e., Cyt, VIP). The insecticidal potential of bacterial strains closely related to B. thuringiensis has also been demonstrated.

Additionally, the integration of cry genes into genetically modified plants has been successfully implemented to confer resistance to specific crop pests.

Lysinibacillus sphaericus

Entomopathogenic strains belonging to the L. sphaericus (formerly Bacillus sphaericus) species group are featured by the production of spherical endospores closely associated with parasporal crystals containing an equimolar ratio of binary protein toxins (BinA and BinB). The insecticidal mode of action includes damages to the microvillar epithelial cells in the midgut comparable to the ones known for B. thuringiensis. In addition, vegetative cells of certain strains produce mosquitocidal toxins (Mtx proteins).

The main targets of commercial formulations based on L. sphaericus strains are mosquitoes, blackflies and non-biting midges.

Paenibacillus spp.

The genus Paenibacillus includes different species showing pathogenicity against insects like the causative agent of the honeybee disease American Foulbrood (AFB), P. larvaesubsp. larvae.

On the other hand, the spore-formers P. popilliae (Dutky) Pettersson et al. and P. lentimorbus (Dutky) Pettersson et al. are the causal agents of milky disease in phytophagous coleopteran larvae. The production of parasporal inclusions within the sporangial cells has been observed in P. popilliae, even if they are not directly responsible for the insecticidal action. However, homology between a 80 kDa parasporal protein this species produces and Bt Cry toxins has been demonstrated. After spores are ingested by the host, they germinate in the midgut. The following pathogenicity seems to be in relation to the septicemia caused by vegetative cells.

Brevibacillus laterosporus

Brevibacillus (former Bacillus) laterosporus is a pathogen of invertebrates and a broad spectrum antimicrobial species. During sporulation it produces a typical canoe-shaped parasporal body (CSPB) firmly associated with the spore coat, which gives this species a unique morphological feature. The insecticidal action of different B. laterosporus strains has been reported against insects in different orders, including Coleoptera, Lepidoptera and Diptera, and against mollusks, nematodes, phytopathogenic bacteria and fungi. In relation to its antifungal and antibacterial properties, due to the production of antibiotics, it has also found use in medicine.

The whole genome of B. laterosporus has recently been published, which reveals the potential to produce different toxins. Certain strains showing toxicity against the corn rootworms (Diabrotrica spp.) and other coleopteran larvae, produce insecticidal secreted proteins (ISPs) that act as binary toxins in the insect midgut and have high homology with B. thuringiensis vegetative insecticidal proteins (VIPs).

Specific strains toxic to mosquitoes produce parasporal inclusion bodies reminiscent of those produced by B. thuringiensis. These bodies contain proteins and their implication in the mosquitocidal action has been reported. Spores of a strain lacking parasporal crystals are highly toxic to the house fly Musca domestica L., and the mode of action implies histopathological changes in the midgut with disruption of the microvillar epithelium.

2.2 Clostridiaceae

Clostridium bifermentans

A strain of *C.* bifermentans (Weinberg and Séguin) Bergey *et al.* serovar malaysia (C.b.m.), isolated in Malaysia, shows high toxicity against mosquitoes and black flies. During sporulation, this bacterium produces three major proteins involved in the insecticidal action. These include the mosquitocidal protein Cbm71 showing homology to *B.* thuringiensis delta endotoxins.

2.3 Gammaproteobacteria

Photorhabdus spp. and Xenorhabdus spp.

The entomopathogenic members of the genera *Photorhabdus* and *Xenorhabdus* are represented by endosymbionts of insecticidal nematodes. The first are typically associated with entomopathogenic nematodes in the genus *Heterorhabditis*, while the second to *Steinernema* species. The pathogenic action usually involves the release of symbiotic bacteria in the insect hemocoel once the nematodes have actively entered the insect body. Here the bacteria proliferate producing various antimicrobial compounds to contrast the growth of other microorganisms. They also release different enzymes that contribute to the degradation processes in the hemocoel, thus creating an ideal environment for the development of the nematode population. A variety of bacterial virulence factors are involved in the interaction with the susceptible host.

Different *Photorhabdus* and *Xenorhabdus* species producing an insecticidal toxins complex (Tc) have high potential for pest management. Generally, the Tcs are high-molecular weight and multi-subunit proteins that include three components, A, B and C, orally active against different insects. While the mode of action is not completely understood, all these components are normally needed to achieve full toxicity.

Another example of insecticidal proteins produced by these bacterial species is represented by the *Photorhabdus* insect related (Pir) proteins, produced by *P. luminescens* (Thomas and Poinar), that show similarity to *B. thuringiensis* delta-endotoxins and have been proposed to be mimics of the juvenile hormone esterases (JHEs) interfering with insect development regulation.

In addition to insecticidal toxins and various metabolites, these bacterial endosymbionts have evolved different mechanisms to face the insect immune response. For instance, it has been shown that certain *Photorhabdus* species exploit lipopolysaccharide modifications to resist the action of insect antimicrobial peptides (AMPs), while *X. nematophila* (Poinar and Thomas) interferes with the expression mechanisms of host AMPs.

In addition to *P. luminescens* and *X. nematophila*, most studied species include *P. asymbiotica* Fischer-Le Saux *et al.*, *P.temperata* Fischer-Le Saux *et al.*, *X. beddingii* (Akhurst) Akhurst and Boemare, *X. bovienii*(Akhurst) Akhurst and Boemare, *X. japonica* Nishimura, and *X. poinarii* (Akhurst) Akhurst and Boemare. Besides, new species are continuously being identified and characterized. So far, the commercial use of these bacterial endosymbionts is related to the employment of the nematode species with which they are associated.

Serratia spp.

The association of *Serratia* spp. with insects or with entomopathogenic nematodes is well documented. Different species in this genus produce a variety of virulence factors. Common is the production of toxin complexes analogous to those produced by *Xenorhabdus* spp. and *Photorhabdus* spp. *S. entomophila* Grimont *et al.*, a pathogen of the grass grub, *Costelytra zealandica*(White) (Coleoptera: Scarabaeidae), produces Sep proteins (SepA, SepB, SepC), a group of insecticidal toxins showing similarities to the insecticidal toxins of *P. luminescens*.

On the other hand, the recent genome sequencing of *S. nematodiphila* Zhang *et al.* also highlighted other pathogenic factors of *Serratia* species. Among these was a variety of secreted extracellular enzymes such as proteases, lipases, and chitinases.

It has recently been demonstrated that the pathogenicity of *S. marcescens* Bizio is increased by the action of a serralysin metalloprotease it secretes and that this bacterium is able to suppress cellular immunity by decreasing the adhesive properties of immunosurveillance cells of the insect host.

Yersinia entomophaga

Isolated from the New Zealand grass grub, *C. zealandica*, *Y. entomophaga* is a non-spore-forming entomopathogenic bacterium characterized by the production of an insecticidal toxin complex (Yen-Tc) showing similarity to those produced by *Photorabdus* spp. These complexes include three Yen protein families, A, B and C, and two chitinases (Chi1 and Chi2).

The broad insecticidal range of these toxins and their post-ingestion histo pathological action in the insect midgut epithelium have been reported. Promising are the studies conducted in field conditions with insecticidal formulations containing *Y. entomophaga* against the pasture pest porina (*Wiseana* spp. larvae).

Pseudomonas entomophila

P. entomophila is a ubiquitous bacterium that orally infects larvae of insects in different orders determining extensive gut cell damages. Host-pathogen interactions have been studied in experiments with *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), which highlighted a specific post-ingestion immune response.

Recent complete genome sequencing of *P. entomophila*revealed a specific secretion system and the associated toxins probably responsible for its entompathogenic properties.

III INTEGRATED PEST MANAGEMENT

Insect pathogenic bacteria and their derived products represent the active substances of various “biopesticides”. There is a range of definitions for this term, but it essentially includes mass reared living organisms (natural insect predators and parasitoids), nematodes and micro-organisms (bacteria, fungi, microsporidia, virus), natural compounds from plant extracts, and semiochemicals (e.g., insect pheromones). Besides, in countries where their use is permitted, biopesticides comprise genetically modified plants that express genes conferring resistance against pests or diseases (plant incorporated products).

Biological control agents, like bacterial entomopathogens, are generally recognized as lower risk substances than conventional chemical pesticides, and various benefits are associated with their use. For instance, their mode of action is normally more complex than conventional chemicals, targeting a diversity of action sites, which makes the development of resistant pests less likely. Although entomopathogenic bacteria can be used as stand-alone products for pest management in organic farming, their use in rotation or combination with chemicals is strongly encouraged to achieve full efficacy and eco-sustainability. Many studies have highlighted compatibility and synergistic effects of entomopathogenic bacteria and chemical substances. Among the other advantages of including biopesticides in pest management programs are their safety for workers, the reduction of residues on crop and the flexibility on harvests, due to minimal or no pre-harvest interval.

The effectiveness of entomopathogenic bacteria is often associated with a proper application in the field. For instance, in the case of products that act by ingestion (*i.e.*, *Bt* based products), timing is critical to ensure that the bacterial toxins remain stable in the environment until they are ingested by their target insect stage. Another aspect is to ensure a proper coverage of substrates (e.g., foliage) frequented or eaten by insects. This has led to the development of special processing and formulation of bacteria-based bioinsecticides, with the aim of maximizing shelf-life, improving dispersion and adhesion, reducing spray drift and above all enhancing efficacy. A variety of adjuvants and additives for microbial formulations have been developed by the industry. These include dispersants, surfactants, wetters, spreaders, drift control agents, pH buffers, antifoam agents, carriers, phagostimulants and attractants. Depending on the application target and on the adverse environment conditions, a choice of solid and liquid formulations is available. The first include dusts, granules, briquettes and wettable powders (WPs), while liquid suspensions may consist of suspension concentrates (SCs), emulsions and encapsulations. Advanced technologies aiming at increasing residual effects comprise microencapsulations and microgranules.

The variety of entomopathogenic bacteria based active substances authorized for commercialization and of their relative commercial products is significantly increasing worldwide. Consequently, their market is expanding into new segments, thus broadening their use for pest management, traditionally relegated to niche contexts (forest, public health, protected crops).

Besides optimizing efficacy, modern pest management strategies tend to minimize the impact on the environment and on non-target organisms. This is also in line with the need to meet current regulations on the maximum residue levels (MRLs) for synthetic pesticides. The management of pest counter-adaptation (resistance) to pesticidal products is another concern in the agro-ecosystem. The integration of bio-based pesticides in pest management programs in many cases represents an important resource to face these challenges. During the last years, the number of microbial products available has grown significantly and many efforts are dedicated to increasing the awareness and to fostering the adoption of biopesticides in integrated pest management programs. This includes the implementation of worldwide industrial understandings like the Biopesticide Industry Alliance (BPIA) and the International Biocontrol Manufacturers' Association (IBMA). According to this trend, a database of available biopesticides is maintained within the context of the biopesticide program of the Interregional Research Project Number 4 (IR-4) at Rutgers University (U.S.).

Many biopesticides have recently gained interest within the legislative framework of most important regions like USA and Europe, fostering the use of low risk active substances in agriculture. In the United States, for the pre-market approval (registration) of any pesticide, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) requires a specific evaluation by the Environment Protection Agency (EPA). Since 1994, to facilitate the registration of biopesticides a Biopesticides and Pollution Prevention Division was established in the Office of Pesticide Programs. In Europe, to facilitate procedures for registration of new products, previously based on EU Directive 91/414, new criteria have recently been implemented by Regulation (EC) No 1107/2009. Besides, specific data requirements and the principles for evaluation and authorization of new plant protection products (PPPs) have been established by recent Regulation (EU) No 283 and 284/2013, and Regulation (EU) No 546/2011, respectively. On this basis, any new active substance is first authorized at the EU level, while the formulated product is subjected to approval at Member State level.

IV CONCLUSION

The need to feed a growing human population requires continuous advancements in pest management systems limiting production losses in major crops. On the other side, the land available for cultivation and farming on Earth is limited, which requires the development of new technologies supporting improvements in productivity. Safeguarding the environment and human health, and the need to manage the development of insect resistance to pesticides, are additional concerns. For all these reasons, the integration of bio-based insecticides in combination or rotation with synthetic formulations is strongly recommended. This is in line with the expectations of fruit and vegetable consumers and with the requirements of a modern legislative framework on the use of IPM in agriculture.

Following this trend, an increasing academic and industrial interest in the discovery and development of new bioinsecticides is being experienced by the scientific community working in the field of insect pathology. The relative market segments and the industrial interest in this field are also growing at a significant rate. As a result, researches on entomopathogenic bacteria are gaining momentum and new discoveries are expected in the near future. Due to the regulatory issues related to the pre-market authorization of new active substances, the availability of bio-based products, including entomopathogenic bacteria, is still limited to certain crop pests. However, both research funding bodies and industry are progressing in the direction of increasing investments in this field, which will result in a continuous expansion of the repertoire of insect pathogenic bacteria-based formulations available for integrated pest management.

REFERENCES

1. Feldhaar H. Bacterial symbionts as mediators of ecologically important traits of insect hosts. Ecol. Entomol. 2011;36:533–543. doi: 10.1111/j.1365-2311.2011.01318.x.

2. Vilcinskas A. Coevolution between pathogen-derived proteinases and proteinase inhibitors of host insects. Virulence. 2010;1:206–214. doi: 10.4161/viru.1.3.12072.

3. De Maagd R.A., Bravo A., Berry C., Crickmore N., Schnepf H.E. Structure, diversity, and evolution of protein toxins from spore forming entomopathogenic bacteria. Annu. Rev. Genet. 2003;37:409–433. doi: 10.1146/annurev.genet.37.110801.143042.

4. Ruiu L., Satta A., Floris I. Emerging entomopathogenic bacteria for insect pest management. Bull. Insectol. 2013;66:181–186.

5. Lacey L.A., Frutos R., Kaya H.K., Vail P. Insect pathogens as biological control agents: Do they have a future? Biol. Control. 2001;21:230–248. doi: 10.1006/bcon.2001.0938.

6. Glare T., Caradus J., Gelernter W., Jackson T., Keyhani N., Kohl J., Marrone P., Morin L., Stewart A. Have biopesticides come of age? Trends Biotechnol. 2012;30:250–258. doi: 10.1016/j.tibtech.2012.01.003.

7. Vega F.E., Kaya H.K. Insect Pathology. 2nd ed. Elsevier; London, UK: 2012. p. 504.

8. Pigott C.R., Ellar D.J. Role of receptors in *Bacillus thuringiensis* crystal toxin activity. Microbiol. Mol. Biol. Rev. 2007;71:255–281. doi: 10.1128/MMBR.00034-06.

9. Bravo A., Gill S.S., Soberon M. Mode of action of *Bacillus thuringiensis Cry* and *Cyt* toxins and their potential for insect control. Toxicon. 2007;49:423–435. doi: 10.1016/j.toxicon.2006.11.022.

10. Glare T.R., O'Callaghan M. Bacillus thuringiensis: Biology, Ecology and Safety. Wiley; Chichester, UK: 2000.

11. Ruiu L., Falchi G., Floris I., Marche M.G., Mura M.E., Satta A. Pathogenicity and characterization of a novel *Bacillus cereus sensu lato* isolate toxic to the Mediterranean fruit fly *Ceratitis capitata* Wied. J. Invertebr. Pathol. 2015 doi: 10.1016/j.jip.2015.01.010.

12. Baumann P., Clark M.A., Baumann L., Broadwell A.H. *Bacillus sphaericus* as a mosquito pathogen: Properties of the organism and its toxins. Microbiol. Rev. 1991;55:425–436.

13. Charles J.F., Silva-Filha M.H., Nielsen-LeRoux C. Mode of action of *Bacillus sphaericus* on mosquito larvae: Incidence on resistance. In: Charles J.F., Delecluse A., Nielsen-LeRoux C., editors. Entomopathogenic Bacteria: From Laboratory to Field Application. Kluwer Academic Publishers; London, UK: 2000. pp. 237–252.

14. Davidson E.A. Ultrastructures of American foulbrood disease pathogenesis in larvae of the worker honey bee *Apis mellifera*. J. Invertebr. Pathol. 1973;21:53–61. doi: 10.1016/0022-2011(73)90113-4.

15. Zhang J., Hodgman T.C., Krieger L., Schnetter W., Schairer H.U. Cloning and analysis of the cry gene from *Bacillus popilliae*. J. Bacteriol. 1997;179:4336–4341.