BIOPESTICIDE PRODUCTION

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Summary

There are two aspects of economic problems caused by insects. One concerns the loss of production that results from damage to crops and to the health of human and domestic animals, the other concerns the cost of attempt to prevent or control such production losses. Mosquitoes and black flies are a constant threat to health and comfort, yet the chemical pesticides used to control them have created serious ecological problems. Population of resistant mosquitoes and black flies has evolved, beneficial insects and natural predators have been destroyed and environmental pollution has increased worldwide.

Therefore, scientists have sought new, environmentally safe technologies to combat mosquitoes and black flies.

Ecological problems created by chemical insect control methods and their relevance to human health are receiving serious attention everywhere. Various pathogens, including viruses, protozoa, fungi and nematodes can be used to regulate pest population. Biological control of pests and vectors has been studied to a limited extent for many years with several notable successes, of which microbiological control is one aspect. The development of insecticide resistance in pest and vector population, the damage caused to non-target organisms and the realization of other environmental hazards of chemical insecticides have led to an increasing interest in biological, including microbiological control methods.

At present some strains of *Bacillus thuringiensis*, nuclear polyhedrosis virus, fungi and the nematode parasite of mosquitoes are commercially available. Biological control methods, especially those using an ecological approach, are arising interest in developing countries. It is important that microbial control of insect pests be further developed and that entomologists should be able to quantify and make contributions to the regulation of insect populations by naturally occurring pathogens. The emphasis given to each subject area reflects the efforts of individuals; hence the purpose of this publication is to provide a record as an instruction in microbial control of insects and biopesticide production.

1. Introduction

For the past five decades humans have almost been wholly dependent upon synthetic/organic insecticides. Agriculture has been revolutionized by the use of chemicals for crop protection, which started in the last 1800 with the introduction of arsenical insecticides and Bordeau mixtures as grape fungicide, and progressing to the very sophisticated compounds available now. Today, fewer people produce more food at less cost than ever before. The effect of synthetic chemicals on agriculture has been so dramatic that conventional agriculture now means using chemicals. Despite the immense benefits, they are used in increasing quantities designed to kill living organisms. However, the very properties that give these chemicals useful-long residual action and high toxicity for a wide spectrum of organisms, have given rise to serious environmental problems. Furthermore, the emergence and spread of increasing resistance in many vector species, concerns over environmental pollution, and the ever increasing cost of the new chemical insecticides, make it apparent that vector and pest control can no longer be safely based upon the use of chemicals alone.

Consequently, increasing attention has been directed toward natural enemies such as predators, parasites, and pathogens. Unfortunately, none of the predators or parasites can be mass produced and stored for long periods of time, since they all must be raised in vivo. It has become evident that there is an urgent need for a biological agent, possessing the desirable properties of a chemical pesticide making it highly toxic to the target organism, which can be mass produced on an industrial scale, has a long shelf life and can be safely transported.

In the mid seventies, WHO and other international organizations initiated studies into existing biological control agents and the development of new ones. Today, biological control is widely regarded as a desirable technique for controlling insects, due to its minimal environmental impact and its avoidance of problems of resistance in the vectors and agricultural pests.

2. Biological Control

Of the nearly one million known species of insects, about 15,000 species are considered pests and about 300 require some form of control. Fortunately, most insect pests have pathogenic microorganisms associated with them.

Entomopathogens have been suggested as controlling agents of insect pests for over a century, and belong to species of fungi, viruses, bacteria, and protozoa.

Insect pathology per se probably had its beginning in the nineteenth century under the stimulus of Bassi and Pasteur. A significant contribution to microbial control of insects was made by Mechnikoff in 1879 and Krassilnikow in 1888, who were the first to document that an entomopathogen, a muscordine fungus, *Metarrhizium anisopliae* could be mass produced and applied as a microbial insecticide to control the grain and the sugar beet pests. The control of insect pests with bacteria was probably first attempted by d'Herelle in 1914, approximately 35 years after Pasteur's description of silkworm diseases. Apparently the control was not consistent and therefore interest in bacterial pathogens was curtailed.

However, after a lag period of nearly 30 years, White and Dutky succeeded in 1940 in demonstrating a control of the Japanese beetle by distributing spores of the milky disease bacterium *Bacillus popilliae.* This success stimulated further investigations of bacteria and literature began appearing on the effectiveness of *Bacillus thuringiensis*. The issuing of eight patents between 1960 and 1963 for *B.thuringiensis* led to a revived interest in bacterial insecticides. The use of viruses to control insect pests was stimulated by the studies of Balch and Bird in 1944 and Steinhaus and Thompson in 1949, respectively. This initial interest is presently having a rebirth, as is evidenced by the recent registration of the first viral pesticide in the United States by the Environmental Protection Agency (EPA).

Of these, bacteria, viruses and some fungi, because of their known effectiveness and relative lack of toxicity or pathogenicity to nontarget animals and plants, have been developed into commercial products. Biological control is generally man's use of a specially chosen living organism to control a particular pest. This chosen organism might be a predator, parasite or infectious disease, which attack the harmful insects. Biological control methods can be used as part of an overall integrated pest management program to reduce the legal, environmental, and public safety hazards of chemicals. In addition, it may be a more economical alternative to some insecticides.

Some biological control measures can actually prevent economic damage to agricultural crops. Unlike most insecticides, biological controls are often very specific for a particular pest. There is less danger of impact on the environment and water quality and they offer a more environmentally friendly alternative to chemical insecticides. They could also be used where pests have developed resistance to conventional pesticides. Unfortunately, research and development of biological insecticides attracts very little financial support compared to that given toward the discovery of chemical pesticides.

It is becoming clear that more attention needs to be given to the selection of broad spectrum biopesticides and improvements in the production, formulation and application technologies. Efforts need to be made to optimize the impact of these agents by integrating them with other novel crop protection strategies. Successful use of biological control requires a greater understanding of the biology of both the pest and its enemies. In some cases, biological control may be more costly compared to the use of pesticides. Often the results of using biological control are not as dramatic or quick as it is with chemical pesticides. Most natural enemies attack only specific types of insects unlike broad-spectrum insecticides which may kill a wide range of insects.

Today biological control is regarded as a desirable technique for controlling insects, due to its minimal environmental impact and preventing the development of resistance in vectors. Specific biotoxin-producing strains of Bacillus thuringiensis var. israelensis or B.sphaericus have been used throughout the world to suppress or eliminate the larval stages of mosquitoes, particularly where malaria, filariasis or certain arboviruses are present. Bacillus thuringiensis var. israelensis is also effective against the larval stages of Simulium spp., vectors of river blindness in man (onchocerciasis) in tropical Africa, and the cause of severe ‘fly worry’ in domestic livestock in several regions of the world. Depending on the specific control programmed, chemical larvicide's may precede or alternate with the use of Bacillus. Host treatment for onchocerciasis or filariaisis may also be performed. Studies conducted to date have shown no significant effect of these bacteria and their toxins on vertebrates and only minimal effects on some non-target arthropods and crustaceans. Development of resistance is apparently less of a problem than with chemical pesticides. These and other potential problems are continuously being monitored and investigated.

Other potential tools which could be used in the future for area-wide biological control programme against insect vectors/pests of veterinary importance include species-specific sex pheromones. These are presently being used as attractants for trapping and monitoring of insects and for mating disruption. In addition, several parasites and pathogens of vector/pest species are under continuous investigation and are providing promising results. Hopefully, the not too distant future will witness the development of further biological control techniques of sufficient scope to free entire regions of pathogenic agents or vectors which cause or transmit significant diseases not only of domestic livestock and humans, but also of free-living wildlife. Virtually all pest populations are affected by natural enemies to some extent. In many cases, natural enemies are the primary regulating force of the pest populations. Natural controls include effects of natural enemies (predators, parasites, pathogens), other biotic (living) factors such as food availability and competition, and abiotic (non-living) factors such as weather and soil. In pest management, biological control usually refers to the action of parasites, predators or pathogens, on a pest population which reduces its numbers below a level causing economic injury. Herbivorous insects and pathogens that attack pest weeds are also considered bio- control agents. Biological control is a part of natural control and can apply to any type of organism, pest or not, and regardless of whether the bio -control agent occurs naturally, is introduced by humans, or manipulated in any way. Biological control differs from chemical, cultural, and mechanical controls in that it requires maintenance of some level of food supply (e.g., pest) in order for the bio-control agent to survive and flourish. Therefore, biological control alone is not a means by which to obtain pest eradication. Biological control is defined as the action of natural enemies. It can be divided into 2 broad categories, natural biological control and applied biological control. Natural biological control occurs where native or co-evolved natural enemies reduce native arthropod populations, whereas applied biological control involves human intervention to enhance natural enemy activities. Applied biological control can be further separated into (a) classical biological control, where exotic natural enemies are introduced against an exotic or native pest, or (b) augmentative biological control, where human intervention occurs to enhance the effectiveness of the natural enemies already present in an area through manipulation of the environment.

Numerous species of plant-feeding insects have been evaluated for control of pest weeds. The greatest successes have been in rangelands, forests, and other natural habitats where other weed control approaches (e.g., herbicides, cultivation) are impractical or uneconomical. Some pathogens have also been looked at as weed biocontrol agents (e.g., plant rusts). The goal, while using the weed biocontrol agents, is generally to reduce the weed population and not to eradicate it. Importation of a biocontrol agent from the region of origin of the weed has been the most common approach. It is generally a long-term process which requires sustained efforts, but which can reap long-term benefits. A few of bacteria are highly effective at killing insects. The most important of these is Bacillus thuringiensis (Bt). It occurs naturally in insect-rich locations, including soil, plant surfaces and grain stores. It kills a range of insect orders and is the most widely used microbial biopesticide. It is also used in transgenic crops. There are over 40 Bt products available worldwide for control of caterpillars, beetles and blood-feeding flies such as mosquitoes. Together, these account for 1% of the world insecticide market.

As part of its life cycle, Bt produces protein crystals which have insecticidal properties. When ingested, the crystals paralyze the digestive tracts of insects, often killing them within 24-48 hours. Different Bt strains produce crystals with slightly different properties, and the crystals from each strain are specific for a small number of related insect species.

Over 1600 viruses have been recorded from more than a thousand species of insects. A family of viruses called baculoviruses is the most popular choice for microbial control as they are distinct from any type of virus recorded from vertebrates. They have been used regularly for pest control since the 1950s, particularly in forestry where they have been highly effective at controlling sawflies. Baculoviruses are very species, mostly caterpillars and sawflies, but also some species of beetle and flies. Baculoviruses infect their hosts through ingestion. Virus particles invade the cells of the gut before colonizing the rest of the body. Infection reduces mobility and feeding and insects are killed in five to eight days. Mass production of baculoviruses can be done only in insects, but this is economically viable for larger hosts such as caterpillars, and formulation and application are straightforward. At present, there are approximately 16 products available for use, or under development, mostly for control of caterpillar pests. Commercial products are available in Switzerland, Germany and Spain for the control of codling moth and the summer fruit tortrix. Products are also available in the USA for the control of tobacco bollworms on vegetables, ornamentals, tomatoes and cotton.

Over 750 species of fungi kill insects. Entomopathogenic fungi invade their hosts using spores that grow through the cuticle, and hence they are particularly suited for control of pests with piercing mouthparts, such as aphids and whiteflies, which are unlikely to acquire pathogens through feeding. Infection requires high humidity at the insect surface, but this can be overcome using oil-based formulations.

About 20 products are available worldwide for managing sap-feeding insects, beetles, caterpillars, flies and locusts. In the USA, and some countries in Europe, products based on the fungus Beauveria bassiana are becoming available for the control of a range of glasshouse pests.

Entomopathogenic nematode worms are just visible to the naked eye, being about 0.5 mm in length. Juvenile nematodes parasitize their hosts by directly penetrating the cuticle of through natural openings. They then introduce symbiotic bacteria, which multiply rapidly and cause death by septicaemia, often within 48 hours. The bacteria break down the insect body, which provides food for the nematodes. After the insect has died, the juvenile nematodes develop to adults and reproduce. A new generation of infective juveniles emerges 8-14 days after infection.

Unlike other entomopathogens, nematodes are exempt from registration and so have been popular choices for commercialization. Over 60 products are available in Europe. Nematodes require moist conditions to operate and have been marketed predominantly against soil pests, such as vine weevil and sciarid fly larvae. However they may also control foliar pests, for example Nemasys (Becker Underwood) which can be used to control western flower thrips. Like other natural enemies, nematodes are affected by environmental conditions.

Protozoan diseases of insects are ubiquitous and comprise an important regulatory role in insect populations. They are generally host specific and slow acting, most often producing chronic infections. The biologies of most entomopathogenic protozoa are complex. They develop only in living hosts and many species require an intermediate host. Species in the Microsporida are among the most commonly observed. Their main advantages are persistence and recycling in host populations and their debilitating effect on reproduction and overall fitness of target insects. As inundatively applied microbial control agents, only a few species have been moderately successful. The grasshopper pathogen Nosema locustae Canning is the only species that has been registered and commercially developed. The main disadvantages of the protozoa as inundatively applied microbial control agents are the requirement for in vivo production and low levels on immediate mortality.

3. Microbial Insecticides

Microbial insect control utilizes pathogenic microorganisms isolated from diseased insects during naturally occurring epidemics. Typically, such epidemics only occur when pest population densities are high and usually after appreciable damage have been done to crops. Over 400 species of fungi and more than 90 species of bacteria which infect insects have been described including *Bacillus thuringiensis*, varieties of which are manufactured and sold throughout the world primarily for the control of caterpillar pests and more recently mosquitoes and black flies.

Among fungal pesticides, five have been introduced since 1979, and three in 1981. Many countries with centrally planned economies have been using fungal pesticides successfully for many years. Sofar, more than 40,000 species of *Bacillus thuringiensis* have been isolated and identified as belonging to 39 serotypes. These organisms are active against either *Lepidoptera*, or *Diptera* or *Coleoptera*.

3.1. Bacillus thuringiensis

Maximizing the potential for successfully developing and deploying a biocontrol product begins with a carefully crafted microbial screening procedure, proceeds with developing mass production protocols that optimize product quantity and quality, and ends with devising a product formulation that preserves shelf-life, aids product delivery, and enhances bioactivity. Microbial selection procedures that require prospective biocontrol agents to possess both efficacy and amenability to production in liquid culture increase the likelihood of selecting agents with enhanced commercial development potential. Scale-up of biomass production procedures must optimize product quantity without compromise of product efficacy or amenability to stabilization and formulation. Formulation of Bacillus spp. for use against plant pathogens is an enormous topic in general terms but limited in published specifics regarding formulations used in commercially available products. Types of formulations include dry products such as wettable powders, dusts, and granules, and liquid products including cell suspensions in water, oils, and emulsions. Cells can also be microencapsulated. Considerations critical to designing successful formulations of microbial biomass are many fold and include preserving biomass viability during stabilization, drying, and rehydration; aiding biomass delivery, target coverage, and target adhesion; and enhancing biomass survival and efficacy after delivery to the target.

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Biographical Sketch

Dr. Nasrine Moazami began her research career as a self-described 'Medical Microbiologist Biotechnologist' in 1976 after receiving her PhD from the University of Laval in Canada. Dr. N. Moazami is the pioneer of biotechnology in Iran. She has 16 years experience in biopesticide research and production, work she started in 1980. She opened up vast ever-expanding possibilities of agriculture, industry, medicine and public health for solving problems through the introduction of biotechnology in Iran.

Dr. Nasrine Moazami is also the founder of the 'Persian Type Culture Collection (PTCC), which is mainly a collection of microorganisms of industrial importance. This Culture Collection is an affiliated member of WFCC [World Federation of Culture Collections] and MIRCEN International Network since 1985. She established the first marine biotechnology center in Queshm Island in the Persian Gulf in the south of Iran.

On July 27, 1995, Dr. Moazami was presented with the Prestigious French Award, the 'Chevalier dans l'ordre des palmes Academiques', a citation given for outstanding professional research.

Dr. Moazami is also the recipient of the February 9, 1989 International Kharasmi Science Festival first prize for the research on and production of biological pesticides.

On November 2, 1996, the President of Iran presented her the National Governmental Award for

Research.

At present she is head of the Institute of Advanced Technology of the Iranian Research Organization for Science and Technology as well as Director of the Tehran MIRCEN.