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Biotechnological Approach in Biopesticides-An overview

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ABSTRACT

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. Biopesticides are very effective in the agricultural pest control without causing serious harm to ecological chain or worsening environmental pollution. There is a requirement to develop alternatives to chemical pesticides for crop protection, due to the evolution of pesticide resistance in some pest species and concerns about the safety of chemical residues. One solution is the use of biopesticides (pest control agents based on living organisms) as an alternative in food production. The aim of this paper was to improve understanding of the environmental and regulatory sustainability of biopesticides as alternatives to chemical pesticides for crop protection. This paper reports on categories of biopesticides, biological control of aflatoxin, production of biopesticides using engineering techniques and its development.

Introduction

Biotechnology with its promise to revolutionize agriculture around the world is assuming an increasingly greater role in India's agricultural research. The indiscriminate use of chemical pesticides has affected humans and their environment and insect pests remains to be one of the major limiting factors in sustaining the productivity of various crops. Biotechnology has opened an exciting frontier in agriculture. Agriculture is one of the most important sectors of the developing countries. Indian economy is basically agriculture oriented and country's economic development is largely dependent upon the development of agro culture. Agriculture represents 16.67% of India's GDP and almost 70% of the population in the country depends primarily on agriculture, which provides employment to about 60% of the work force.

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.

Biological Control

It is not intended to provide an exhaustive survey of forms of biological control, but simply to give an indication of the major alternatives available. It is interesting that stakeholder organizations do not hold a common view on the definition of biopesticides, although PSD make a clear distinction between the regulations covering macrobiological and microbiological agents.

Indeed, it is worth noting that there is some disagreement within the bioscience community as to the definition of biological control. As the definition of Eilenberg [1] is widely accepted, and defines biological control as 'The use of living organisms to suppress the population of a specific pest

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organism, making it less abundant or less damaging than it would otherwise be'. However, other authors have widened the definition to include also genes and gene products. This broader definition includes substances such as semi chemicals and pesticidal substances obtained from plants (e.g. *Neem tree* extract), but it is not scientifically rigorous. To resolve problems associated with these definitions, the term 'biologically – based control' is favoured by many to distinguish between living organisms and their genes and gene products. The agents used are sometimes referred to as 'biorational' agents. In this paper, we use Eilenberg's definition and distinguish between biological control and biologically-based control. The biological control agents of arthropod pests are classed as predators, parasitoids and pathogens. Predators consume their prey. Parasitoids are insects that have a free living adult stage, but which have a parasitic larval stage. Pathogens are micro-organisms that cause disease in the host. Our project is concerned with naturally occurring fungal pathogens of insects.

As stated previously, biologically-based control agents include genes and gene products. Important groups are semiochemicals, which are 'chemicals emitted by plants, animals and other organisms … that evoke a behavioural or physiological response in individuals of the same or other species. They include pheromones and allelochemicals.' They have been used both for detecting and monitoring pests and for mating disruptions. Other compounds include plant extracts with pesticide action, and genes from naturally occurring bacteria, such as *Bacillus thuringiensis*, that code for insect specific toxins and which can be genetically engineered to be expressed in plants, making the plant resistant to insect attack.

Biological control has a range of strategies, based on exploiting the ecology, behaviour and other attributes of the control agent:

- Conservation control refers to efforts to conserve and exploit naturally occurring populations of natural enemies by enhancing their habitat. This can be done by stopping deleterious practices, such as withdrawing sprays of broad spectrum pesticides that reduce natural enemies, or by introducing new practices to enhance the environment, such as creating refuges. 'One of the most successful applications of conservation biological control is the establishment of permanent strips of natural vegetation within cereal fields, so-called "beetle banks" to provide a long-term home for natural enemies.' [2]. One issue is over how large an area the beneficial effects are felt.
- Augmentation refers to the introduction of natural enemies into an environment (the original concept was that the applied agents 'augment' natural enemies already resident in the habitat. It is based on the idea that the pest has become separated from the full range of its natural enemies and attempts to re-establish pre-existing relationships). It has two different types, inoculation and inundation. In inoculation, natural enemies are released at low levels and are expected to reproduce within the target environment, although they are not expected to become permanently established and top up releases are usually required. For inundation, natural enemies are mass released into the target habitat and there is little expectation that they will be self sustaining. The natural enemies may already be present in the environment, albeit in low numbers, and hence the phrase 'introduction of natural enemies' – which is widely used – is somewhat misleading. In practice, inoculation and inundation form a continuum.
- Classical biological control is the specific case of the intentional introduction of exotic natural enemies for the control of an exotic pest, with the goal of permanent establishment of the control agent. This method can be highly successful but there is evidence that it is falling out of favour with regulators. 'Regulatory restrictions on their introduction have nearly eliminated classical biological control with exotic pathogens of introduced insect pests in the United States.'[3]

Biologically-based agents are applied in a range of ways depending on the chemical properties of the agent and the ecology of the pest. For example, pheromones may be used in traps to lure pests away from crops, while plant-based pesticides can be sprayed onto crops as curative treatments.

Chemical pesticides: the problem

The production of crops is significantly reduced by invertebrate pests, plant diseases, and weeds. At present, crop protection relies heavily on chemical pesticides. However, consumers are deeply suspicious of the possible health effects of pesticide residues on food, a concern that is picked up by retailers who are in many cases pushing for levels of reduction in pesticide use that go beyond what is required by regulators. The latest available report of the Pesticides Residues Committee reveals that in 2003 tests on 4,000 samples of both imported and home produced food found that only 0.7 per cent of the produce tested exceeded the MRL.

It is important to recognize that the elimination of pesticides would have a substantial impact on the quantity and quality of food available and its price. How can one reconcile the need to sustain levels of food production and farm income with the declining availability and acceptability of chemical pesticides? The broad solution is to use the ecologically based pest control management strategy known as Integrated Pest Management where 'the basic goal is to use control tactics against pests only when necessary.'[4]. 'IPM suggests using methods for control only if the pest population is causing damage above the economic injury level. IPM thus always requires a good understanding of the pest system.' There is a role for chemical pesticides in IPM when infestations cannot be controlled by any other means, but they should be used as the last rather than the first resort. There is also scope for increased use of alternatives to chemical pesticides such as biological controls and in particular biopesticides.

Categories of Biopesticides

(1) Microbial pesticides consist of microorganism (e.g., bacterium, virus, fungus and protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests although each separate active ingredient is relatively specific for its target pests. For example, there are fungi that control certain weeds, and other fungi that kill specific insects.

The most widely used pesticides are subspecies and strains of Bacillus Thuringiensis or Bt. Each strain of bacterium produces different mix of proteins and specifically kills one or a few related species of insect larvae. While some Bt's control moth larvae found on plants, other Bt's are specific for larvae of flies and mosquitoes. The target insect species are determined whether the particular Bt produces protein larvae that can bind to larval receptor thereby causing insect larvae to starve

(2) Plant Incorporated protectants (PIP) are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the Bt pesticidal protein, and introduce the gene into the plant's own genetic material. Then the plant, instead of the Bt bacterium, manufactures the substance that destroys the pest. The protein and its genetic material, but not the plant itself, are regulated by EPA.

(3) Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances, such as insect sex pheromones, which interfere with mating, as well as various scented plant extracts that attract insect pests to traps. Because it is sometimes difficult to determine whether a substance meets the criteria for classification as a biochemical pesticide, EPA has established a special committee to make such decisions.

Biopesticide Production

Use of New Genetic-Engineering Technology

Biological control is the most important alternative to chemical pesticides in protecting crops from pests, pathogens, and weeds. Major breakthroughs in molecular biology and biotechnology since the early 1980s indicate that quick improvement in the competitive ability of biological control methods is possible, and that biopesticides can play a major role in crop protection in the future. It has become possible to improve some of the critical properties that earlier hampered the usefulness of many biocontrol agents. Valuable genes from completely unrelated organisms can now be utilized for biological control purposes. Biological control using recombinant DNA (genetic engineering) technology can be achieved in several different ways: control agents may be improved; crop plants can be engineered to carry better resistance genes; or organisms associated with the plant may be modified to provide protection. All these approaches have successfully been used in several different ways experimentally. Product development has been very active in the area of incorporating resistance genes mainly from *BT*-directly into plants. Successes include potato, tomato, tobacco, and cotton. General root colorizing bacteria of plants have also been engineered to produce insecticidal toxins, which protect against pests such as the corn rootworm. Another bacterium living in the vascular tissues of corn has also been modified to give protection against the corn borer. None of these modified plants or associated organisms is available commercially yet. Similar approaches are used for the biological control of plant pathogens and weeds, but research has been most active in the area of insect control. In the wake of the enthusiasm about the new possibilities, some serious doubts have arisen. How safe are these organisms for actual use? How do they affect the environment or humans? Therefore, a very critical approach is necessary toward the use of genetic-engineering technologies in agriculture. In principle, genetic engineering can be used for biological pest control in two ways: one is improving the properties of the biological control agents, and the other is engineering crop plants to be resistant to pests.

Engineering Biological Control Agents

The genetic improvement of biological agents is a relatively new concept. For this, a great deal must be known about the biology, ecology, and behavior of the organism. This is a very crucial step.

Engineering Crop Plants

The first published reports of successful engineering of crop plants to produce insecticidal or antifeedant proteins appeared in 1987. The crop plants were tobacco and tomato, producing the delta endotoxin of *Bacillus thuringiensis* to make them resistant against caterpillars. To date, transgenic crop plants have been produced of at least 27 different species, including potato, cabbage, sugar beet, rice, soybeans, corn, rapeseed, sunflower, walnut, and poplar. Within only two years of the first reports, at least 53 field trials in seven countries were conducted, involving eight plant species. Instead of being inserted directly into the crop plant genome, the protective insecticidal genes can be engineered into associated organisms. Two bacteria have been successfully tested for this purpose. *Pseudomonas fluorescens*, which colonizes the root systems of crops, has been engineered to express *Bacillus thuringiensis* (Bt) toxins, and thus provide continuous protection against such pests as corn rootworm. The genes for all the major proteins that account for the insecticidal properties of *Bt* have been cloned and sequenced. Now we have nucleotide sequences for more than 20 *Bt* genes that encode proteins active against leopidopterans, eight genes encoding proteins active against dipterans, and two genes encoding proteins active against coleopterans. To increase the environmental stability and effectiveness of the various *Bt* toxins in the field, genes encoding proteins active against beetles and caterpillars have also been cloned into the rhizobacterium *Pseudomonas fluorescens*. After fermentation, the bacteria are killed and the cell walls hardened chemically. The endotoxins are thereby microencapsulated, resulting in insecticides with greatly enhanced residual activity. Large-scale field trials with this product have been performed, and the product obtained full registration in 1991.Similar strategies have been employed to develop mosquitocidal species of algae. Even more significantly, several major crop species, including cotton, tobacco, and soybeans, have been transformed with *Bt* genes, becoming resistant to attack by caterpillars and beetles. Through genetic-engineering techniques, the *Autographa californica multinucleocapsid nucleopolyhedrosis virus* (AcMNPV) has been engineered to kill insects more quickly by expressing either enzymes or toxins soon after host invasion. Of particular interest is the possibility of making viruses produces insect neurohormones, which can cause rapid physiological disruptions in minutely defined target hosts. This strategy is in its early stages of development, but there is little doubt that within the very near future we will have viruses with extended or specifically designed host ranges, capable of killing insects within 24 to 48 hours. These genetically engineered viruses should have an advantage for use against hosts that are not easily controlled by *Bt*. Very little is known about the genetics of entomopathogenic fungi. The first transformation system for an entomopathogenic fungus was developed using *Metarhizium anisopliae* protoplasts mixed with a fungicide-resistant plasmid. A benomyl-resistant strain of *M.anisoplieae* has thus been obtained. Fungal enzymes involved in the penetration of the insect cuticle have now been identified. Knowledge of these genes and gene products will eventually lead to the possibility of genetic alteration of fungal pathogens that possess those genes. Transformation systems for some fungi exist already and may soon be applied to the entomopathogenic species. Para sexual recombination not only facilitates genetic analysis in asexually reproducing fungi, but also provides an important tool in strain improvement of bioprotectant fungi. Entomopathogenic fungi, which are facultative parasites, are subjected to many environmental factors, and the host insect can exert a selective pressure by favoring one or a few genotypes. Pathogenicity tests showed that some strains are selective hosts for virulence. The distinct genetic homogeneity of this population could be the sign of an evolutionary history with particular adaptation of pathogenicity towards this host insect. Nuclear markers will allow a reexamination of host specificity and characteristics of populations in terms of evolutionary history, and maybe co-evolution. The first applications are likely to be the utilization of various *Bacillus thuringiensis* toxins, of some insect baculoviruses, and of some antagonists for plant-disease control. Other applications appear remote for the time being. The formulation distinguishes pathogens from chemical pesticides, where the method of exposure is less critical and can be reliant on indirect means such as translocation leading to systemic action of activity via the vapor phase. This requirement for direct contact places unique and strong demands on the formulation and application methods for microbial pesticides. In fact, the operational concept of delivery is much more useful than that of application. The microbial active ingredient must be placed or brought into contact with the target, delivered to it, as it were. The principle commercially relevant genera of entomopathogenic fungi, *Metarhizium* and *Beauveria*, are contact pesticides. The infective fungal conidia penetrate the insect cuticle to initiate the infection process, leading to insect death. Nonetheless, most efforts to employ them as insecticide active ingredients have been based on the use of a particular isolate against a particular insect pest, usually in isolates found associated with the pest in some natural infection.

Biopesticide in India

Biopesticides represent only 2.89% (as on 2005) of the overall pesticide market in India and is expected to exhibit an annual growth rate of about 2.3% in the coming years (Thakore, 2006). In India, so far only 12 types of biopesticides have been registered under The Insecticide Act, 1968. Neem based pesticides, *Bacillus thuringensis*, NPV and *Trichoderma* are the major biopesticides produced and used in India. Whereas more than 190 synthetics are registered for use as chemical pesticides. Most of the biopesticides find use in public health, except a few that are used in agriculture. Besides, i) transgenic plants and ii) beneficial organisms called bio-agents: are used for pest management in India. Consumption of biopesticides has increased from 219 metric tons in 1996-97 to 683 metric tons in 2000-01, and about 85% of the biopesticides used are neem based products. Consumption of chemical pesticides has significantly fallen from 56,114 MT to 43,584 MT during the same period.

Annual availability of biopesticides in India: [6]

Biopesticides/Bioagents	Quantity/annum (approx)
Neem 300 PPM	1,000,000 L
Neem 1500 PPM	250,000 L
Bt	50,000 kg
NPV (liquid)	500,000 Le
Beauveria	Meager
Pheromone traps	500,000 nos.
Lures	2 million
Trichogramma	1 million
Chrysoperla & other biocontrol insects	Meager
Trichoderma	500 T

Biological Control of Aflatoxin Contamination of Crops

Aflatoxin contamination of crops compromises the safety of food and feed supplies and causes significant economic losses each year. Of the many research approaches being studied to reduce and, ultimately, eliminate aflatoxin contamination, biological control is one of the more promising, particularly for the near-term. Numerous organisms have been tested for biological control of aflatoxin contamination including bacteria, yeasts, and nontoxigenic strains of the causal organisms, *Aspergillus flavus* and *A.parasiticus*. Most of the field successes to date have been achieved by applying certain nontoxigenic strains of *A.flavus* and *A.parasiticus* to soil of susceptible crops, such as peanuts, cotton, and corn. The applied strains occupy the same niche as the naturally occurring toxigenic strains and competitively exclude them when crops are susceptible to infection. Various formulations have been used to apply the nontoxigenic strains to soil, but the most effective methods have been to combine the desired strain with a carrier/substrate, such as a small grain. This was done either by minimally growing the desired strain on sterilized grain or by coating the surface of the grain with conidia of the strain. After application to the field and uptake of moisture, the fungus completely colonizes the grain, and abundant sporulation provides inoculum levels sufficient to achieve a competitive advantage for the nontoxigenic strain. In several years of field studies, particularly with peanuts and cotton, significant reductions in aflatoxin contamination in the range of 70-90% have been achieved consistently. Two separate products have recently received EPA registration as biopesticides to control aflatoxin contamination in cotton (AF36) and peanuts (afla-guard).

Research and Development of Biopesticides

Over the past 10 years, with the rapid development of new techniques, such as molecular biology, genetic engineering, protein engineering and others, all gradually improving the biopesticides production, the field had developed excellent application prospects, with extensive social and economical benefits. The superior characteristics of biopesticides attracted more attention than ever before and made them a hot spot of research in biotechnology institutions and companies. The research and application of biopesticides had been well developed and biopesticides gradually replaced the highly toxic pesticides in the market. In recent years, chemical pesticides' production declined by 2% per year [6] while biopesticides' output increased at the annual rate of 20%. In Canada, between 1972 and 2008, the Pest Management Regulatory Agency approved registration of 24 microbial active substances with 83 formulations. The majority of the registrations (55/83) occurred since 2000 and at the beginning of 2008 there were 10 new products (a combination of new active substances, strains, formulations, and uses) under regulatory evaluation [7]. The main varieties are *B.t.* pesticides, botanical pesticides (rotenone, saponin, etc.), viral pesticides (*Heliothis Armigera Nuclear Polyhedrosis Viruses*, etc.), fungal pesticides (*Trichoderma*, etc.) and plant growth regulation pesticides (gibberellin, etc.). There had been about 30 kinds of commercialized biopesticides in the world [8] up to 2009. In 1997, the sales of *B.t.* products reached $ 984 million and went up to $ 3.6 billion in 2005. In 2006, the global leading species of biopesticides were as follows: *B.t. CryF1*, *NRRL21882* (*Aspergillus flavus*), *Bacillus licheniformis* strain *SB3086*, etc [9]. Developed countries pay great attention to the projected rapid pace of the development of biopesticides. In the early stage, few kinds of biopesticides were registered in developed countries; only 16 were registered in America in 1996, while 1090 products had been registered by the end of 2003, with product sales of nearly $ 2.2 billion. As of October 2008, there were 327 biopesticides registered in China, accounting for 1.6% of total registered pesticide products (Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA), 2008). [10]. In India, by 2006 only 12 biopesticides (such as *B.t.*, *Trichoderma*, *Pseudomonas*, and *Beauveria* species) had been registered, but 194 substances were listed as chemical pesticides [11]. The new developed and registered biopesticides are increasing at a rate of 4% each year and the market share of biopesticides will rise to 30%.

Conclusion

In an agricultural system, where the human goal is to maximize the food value of the plant for human or animal consumption, any organisms in the system that impede this objective are called pests or diseases. The most common way to reduce their impact on food production has been to use chemical sprays (insecticides and fungicides) that destroy the pests but not the food plants. Biological control uses a different approach to pest management, focusing on natural enemies of plant pests and diseases to manage their populations. The need to develop "safer pesticides" has become a priority of both the current administration and the Environmental Protection Agency (EPA). The current EPA policy is to facilitate the testing and registration of pesticides which have "reduced risks". It became evident that there was an urgent need for a biological agent that possessed the desirable properties of a chemical pesticide, which is highly toxic to the target organism, able to be mass-produced on an industrial scale, have a long shelf. The delivery of biocontrol technologies or the method of application of biocontrol is a very important aspect for the success of the biopesticide technology. There is also a need for greater attention to be given towards product formulation. Greater emphasis needs to be placed on developing formulations specific to the needs of the “active ingredient” of entomopathogens for greater success in the field with biological agents. The successful adoption of biocontrol needs a high level countrywide farmer training. In particular farmers need to learn about augmentative biocontrol agents as living entities, their basic food and habitat requirements, and how to cater for these needs by providing alternative foodsources/hosts. [12].

References

1. Eilenberg J, Hajek A, Lomer C. ‘Suggestions for unifying the terminology in biological control’, *BioControl.* 2001 *46*: 387-400.
2. Hajek N. *Natural Enemies: An Introduction to Biological Control.* 2004 (Cambridge: Cambridge University Press).
3. Lacey L A, Frutos R, Kaya H.K, Vail P. ‘Insect Pathogens as Biological Control Agents: Do They Have a Future?’. Biological Control 2001 21, 230-48.
4. Thakore Y. The biopesticide market for global agricultural use. Industrial Biotechnology, Fall. 2006: 194-208.
5. Kalra A, Khanuja SPS. Research and Development priorities for biopesticide and biofertiliser products for sustainable agriculture in India. *In*. Business Potential for Agricultural Biotechnology. Asian Productivity Organisation. 2007. 96-102.
6. Cheng XL, Liu CJ, Yao JW. The Current Status, Development Trend and Strategy of the Bio-pesticide Industry in China. Hubei Agric. Sci. 2010 49: 2287-2290.
7. Bailey KL, Boyetchko SM, Längle T. Social and economic drivers shaping the future of bisological control: A Canadian perspective on the factors affecting the development and use of microbial biopesticides. Biol. Control, 2010 52: 221-229.
8. Xu YZ. The Application of Biological Pesticides and Industrial Development Measures. Chin. Agr. Sci. Bull. 2008 24(8): 402-404.
9. Wang YX. Report of Chinese check of exportation bulwark and analysis. China Economic Press. 2006 67-91.
10. Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA),2008.http://english.agri.gov.cn/ga/amoa/iumoa/200906/t20090625_1212.html
11. Gupta PK. Status of biopesticides-Indian scene. Toxicol. Int. 2006 13: 65-73.
12. Sema Wahab. Biotechnological Approaches in the management of plant pests diseases and weeds for Sustainable Agriculture. Journal of Biopesticides, 2009 2(2): 115-134.