Contribution of Research in Africa
Biological Control Opportunities

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Abstract

Natural biological control agents have a profound effect on the regulation of pest populations. However, their impact on the suppression of pests can be severely compromised by prevailing agricultural practices, the environment and the use of chemical pesticides. Biocontrol agents include macro-organisms (macrobials) and relatively smaller micro-organisms (microbials). The major macrobials are parasitoids, predators, invertebrates, reptiles, birds and mammals while microbials are mainly bacteria, viruses, fungi, nematodes, protozoans and rickettsia. The potential for utilizing biocontrol agents in pest management with an emphasis on agriculture is discussed.

Introduction

Pests (arthropods, diseases and weeds) coexist with their natural enemies, which determine their numbers and the degree of damage they would cause to crops, livestock and human health. However, natural regulation is severely disrupted by human activities on the ecosystem or natural catastrophes, such as bad weather, providing the pests an opportunity to increase beyond the economic threshold. Certainly, the economic threshold depends on the value of the crop where even low numbers of pests may be significant in terms of crop loss. Biological control agents may therefore not forestall or prevent economic losses but are an integral part of pest management. Their effectiveness will depend on their relative ability to maintain pest populations at non-damaging levels. Compatibility with other pest management strategies is a crucial element in determining the contribution of biocontrol agents in any crop production system.

The main biological control agents can be classified as macro-organisms (macrobials) which include parasitoids, predators, invertebrates, vertebrates (birds and mammals). In addition micro-organisms (microbials) regulate pest number by causing direct mortality or by their toxins. These include viruses, bacteria, nematodes, protozoa and rickettsia. Some of the microbials do not cause death but out-compete the pathogenic organism as antagonists and consequently ameliorate the effect of the disease on the crop, e.g. *Trichoderma* spp. and *Fusarium oxysporum* (biological fungicides).

The downside of biological control agents is their specificity as opposed to being broad spectrum pesticides, underscoring the need to use more agents or other intervention strategies to manage the variety of pests which occur on any one crop.

Macrobial Biocontrol Agents

Some macro-organisms are non-specific predators of arthropod pests that exert considerable suppression of these pests, especially when they appear in large numbers. These include spiders, praying mantis, birds, reptiles and small mammals. Some of
these generalists are also able to disseminate arthropod diseases after feeding on infected pests as the infective organisms pass through their gut without being inactivated. Birds are known to do this very effectively as they are highly mobile.

More specific macrobials include predators and parasitoids that can be manipulated to confer optimum pest suppression by in situ conservation, introduction or augmentation. In situ conservation of endemic natural enemies can be enhanced by habitat management such as provision of refugia, increasing food and shelter or multiple cropping including flowering plants. However use of chemical pesticides often decimates natural enemies. It is conservatively estimated that 52 per cent of total pesticide imports (6383.6 tonnes in 2000) goes to horticultural crops pest management.

Where exotic pests are involved it is often more prudent to introduce appropriate natural enemies from the pest area of origin. This approach is referred to as classical biological control. Once the natural enemies are introduced they may establish and continue to reproduce and suppress the target pests for a long time.

Augmentation of natural enemies may entail introduction of small quantities, inoculative release, or frequent timed introductions, inundative release, or massive releases during a critical stage of the cropping season. The latter approach is appropriate for introducing pest pathogens that readily suppress the pests in a similar manner to that of chemical pesticides. Greathede (1971) discusses some of the successful biocontrol agents within the Ethiopian region.

The success of any natural enemies to suppress pests depends on the ability to search for the pest, reproduction capacity, survival and host specificity.

Some of the predators that have had considerable suppression of crop pests are:

- **Beetles**
  - Carabids
  - Coccinellids (ladybirds)
- **Flies**
  - Syrphids (hover flies)
- **Bugs**
  - Anthocorids (*Orius*)
  - Lygaeids
  - Mirids
  - Reduviids
- **Wasps**
  - Sphegids
  - Vespids
- **Predatory mites**
  - Phytoseiids
- **Lacewings**
  - Chrysopids
- **Praying mantis**
  - Mantids
- **Ants**
  - Formicids

Some important parasitoids of crop pests include:

- **Wasps**
  - Inchneumonids (*Diadegma, Trichogramma*)
  - Braconids (*Cotesia, Aphidius*)
  - Eulophids (*Tetrastichus, Diglyphus*)
  - Pteromalids (*Antestiopsis*)
  - Scelionids (*Telenomus*)
  - Encyrtid (*Copidosoma, Anagyrus*)
  - Eupelmids (*Eupelmus*)
Chalcidids (*Brachymeria*)
Aphelinids (*Encarsia*)
- Flies
  - *Fladenmyia*
  - Agromyzids.

**Microbial Biocontrol Agents**

Unlike their macrobial counterparts, microbials behave in a similar way to chemical pesticides as they are quantifiable in terms of infective units or concentration of toxins and may thus be referred to as biopesticides. The most important arthropod pest pathogens include:

- **Viruses** – ingested and cause mortality in 3–10 days, propagated *in vitro* and safe to higher mammals
- **Bacteria** – ingested, cause mortality in 30 minutes to 1 day, many propagated *in vitro*, most safe to mammals and beneficial arthropods
- **Fungi** – enter host through cuticle, cause mortality in 4–7 days, propagated *in vitro* not totally safe to mammals and beneficial arthropods
- **Protozoa** – acquired orally, chronic infections, propagation *in vivo*, safe to mammals and useful arthropods
- **Rickettsia** – transmitted via eggs, propagation *in vivo*, many very virulent, safety to mammals doubtful as well as to some beneficials, cause variable mortality but may reduce fecundity
- **Nematodes** – some propagated *in vitro*, sometimes cause epizootics, act slowly and best for pests living in cryptic habitats, safe for mammals but may harm beneficial arthropods.

The most exploited organisms, in order of importance, are entomopathogenic viruses, fungi, bacteria and nematodes.

**Entomopathogenic Viruses**

Major families of insect pathogenic viruses include:

- *Baculoviridae* (nucleopolyhedrovirus, NPV, and *granulosis virus*, GV)
- *Reoviridae* (*cytoplasmic polyhedral virus*, CPV)
- *Entomopoxviridae* (EPV)
- *Iridoviridae* (*iridio virus*, IV)
- *Ascoviridae*
- *Birnaviridae*
- *Caliciviridae*
- *Nodaviridae*
- *Paroviridae* (*denso virus*, DNV)
- *Picornaviridae*
- *Polydnaviridae*
- *Rhabdoviridae*
- *Tetraviridae*
- *Oryctes virus* (now *Baculoviridae*).

Baculoviruses that include *granulosis virus* (GV) and nucleopolyhedrovirus (NPV) are most studied and offer the best opportunity for arthropod pest control. Their virus particles develop within a crystalline-protein structure, occlusion body (OB), which protects the virion outside the host. Once ingested the alkaline insect gut dissolves the
protein envelope. This releases the virions, which rapidly multiply in the haemocoel killing the host in 1–3 days, and their bodies rupture releasing millions of OBs.

One of the salient features of baculoviruses is their multiple modes of dispersal by adult pests (auto dissemination), by restless sick larvae climbing to tops of plants to die, by aerial drift of larvae by silk threads (ballooning), and by birds, predators or casual humans. The viruses also survive through predators and birds, which disseminate the inoculum through droppings. Soil and crop litter are good reservoirs of the viruses while soil inhabitants feeding on organic matter can recycle the viruses. The viruses are however inactivated by ultra violet light and heat. They may also be inactivated by physical-chemical properties of leaves on certain plants. Wind and rainwater could cause attrition of the OBs from crops. In order to enhance these biopesticides formulation should include adjuvants, wetters, spreaders, stickers and UV masking agents.

There are several biopesticides based on baculoviruses e.g.

- *Anagapha falcifera* NPV
- *Spodoptera exempta* NPV
- *Helicoverpa armigera* NPV.

Recently KARI/CAB International identified and tested the *Diamondback moth granulosis virus* (PxGV). From this work it was found that baculoviruses vary serologically and in efficacy and should be precisely determined to strain before being developed into pesticides. It is also equally important to stabilize the final product in order to achieve the desired level of pest suppression. The conditions and time of application may also be crucial to ensure that the biopesticide remains active on the target surface for a considerable period.

**Entomopathogenic Bacteria**

Bacteria, especially the spore forming *Bacillus* species, infect arthropod pests after ingestion. During sporulation, *Bacillus thuringiensis* (Bt) cells produce a large protein crystal in addition to a thick-walled endospore. The crystal is an inert toxin (endotoxin) which, after ingestion by a suitable host, is dissolved by the alkaline gut, thereby releasing the toxin which infects the gut and the haemocoel inducing lethal septicaemia. Hosts die within a few days from a milky disease and, as they decay, bacterial spores are released into the soil where they persist as reservoirs for the next host. Some commercial products based on entomopathogenic bacteria are:

- *Bacillus thuringiensis* subsp. *kurstaki* (Thuricide)
- *Bt* subsp. *aizawai* (Xentari).

An endotoxin from *Bt* has also been produced as a commercial insecticide (*Bacillus thuringiensis* delta endotoxin). *Pasteuria penetrans* also suppresses root knot nematodes.

**Entomopathogenic Fungi**

Several entomopathogenic fungi are found in the subdivisions Mastigomycotina, Zygomycotina, Ascomycotina and Deuteromycotina. Arthoropod infesting fungi almost invariably penetrate the host cuticle directly using complex enzymes. The host usually dies from mycosis caused by extensive mycelial colonization of the haemocoel but in higher fungi mortality is caused by a toxin released by the yeast phase.
Some of the fungal products which are under development or are commercialized include:

- **Beauveria bassiana**
- **Zoophthora radicans**
- **Metarhizium anisopliae**
- **Paecilomyces fumosoroseus**
- **Verticillium lecani (Pochonia glaminosporium)**
- **V. chlamydosporium**
- **Trichoderma sp.**
- **Fusarium oxysporum**

**Entomopathogenic Protozoa and Nematodes**

When ingested by insects some protozoa multiply, destroying the normal functions of the host. The infection is chronic and would kill only when the organisms are too numerous. The most important entomopathogenic phylum is Microspora, which significantly reduces host development and fecundity. **Nosema** and **Vairimorpha** are important genera, which can be explored for use as biocontrol agents.

Entomopathogenic nematodes, mainly in the genera Steinernematidae and Heterorhabditidae, are important parasites of arthropod pests. Their juveniles enter the host via the mouth, anus or cuticle and multiply in the haemocoel, killing the host. Some of the locally isolated nematodes, more than two hundred isolates, have been found to be pathogenic to local pests. The association of nematodes with bacteria in the genus *Xenorhabdus* and *Photobacterium* increases their pathogenicity.

Some of the local isolates that have been characterized include:

- **Steinernema karii** sp.n
- **Heterorhabditis bacteriophora**
- **H. indica**.

**Conclusion**

Biocontrol agents vary from large macrobials to smaller microbials. Toxins may enhance their direct effect on hosts. Conventional chemical pesticides depress the activity of biocontrol agents. Use of selective and benign pesticides such as insect growth regulators, fermentation products and hormonal mimics can be compatible with biocontrol agents in an integrated pest management strategy. Semiochemicals and allelochemicals, especially attractants, pheromones and kairomones which influence the behaviour of the arthropod pest, can enhance the effectiveness of biocontrol agents and biopesticides.

**Reference**

Discussion

Comment
What industry desperately needs is alternatives to chemical pesticides. We must reduce the use of pesticides. We need products which will replace pesticides. It is an urgent issue to inform and develop legislation to enable the full potential of IPM to be realized. KARI has an exceptional track record of research in biological control and a vast number of stored isolates. Let’s cooperate in the commercialization of these and allow Dudutech access to these to compare their efficacy with that of the Dudutech isolates. In this way KARI may be able to realize the commercial value of these isolates. At present Dudutech is not even allowed to sell indigenous predators and parasites, of local origin but which have been used successfully for decades throughout the world.

Let’s do less talking about IPM and implement programmes that will enable predators, parasites, entomopathogenic nematodes and finally biopesticides to be made available to growers.

Question
Do most products have temporary legislation?

Answer
No, but the allocation of temporary legislation depends on how important the missing data are for consideration of use. If very important, then registration will not be provided.
Baculoviruses and Bacteria as Potential Tools in Crop Protection

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Abstract

Some insect pathogens have potential for use as biopesticides. These include bacteria, fungi, viruses, nematodes and protozoa, which could be produced in vivo or in vitro for small-scale or large-scale application in management of target pests. Although they do not give a lasting solution, biopesticides are used in the same way as insecticides, thus making them highly adaptable to many established pest management programmes. Advances in biotechnology would allow production costs to decrease and efficiency to increase, thus making them even more appealing. In Kenya, there are several insect pests susceptible to these biopesticides. Several Bacillus thuringiensis (Bt) commercial formulations have been registered and are available in the market for use in controlling such pests, but their use is still limited due to the cost, lack of knowledge on their existence by the potential users, and non-existence of regulation and registration guidelines. Presently less than one per cent of biopesticides is sold in the world market. The issues of registration and perceived risks affect the progress in development of biopesticides and the sooner this is resolved, the better for the industry. The development and use of bacterial and viral biopesticides in crop protection is discussed.

Introduction

In recent years, microbial insecticides or biopesticides have emerged as significant pest management components and rapid development has taken place in terms of research and commercialization. This is partly because consumer markets are becoming increasingly aware of the environmental concerns and are making demands on the industry to move towards a more ecologically rational approach to pest management. Several insect pathogens bacteria, fungi, viruses, nematodes, rickettisia and protozoa have potential for use as biopesticides and have been tested for their ability to control insect pests. Naturally, these micro-organisms often cause epidemics in insect populations that help in regulating them. Because of ease of handling, most of them have been used or studied for use and formulated into baits, dusts, granules, and sprays and delivered in ways similar to those of conventional chemical insecticides to control target pests. Such biological control preparations have label directions like insecticides and are registered with the appropriate authority. To date, the pathogens most widely used as microbial insecticides are bacteria, fungi and viruses; the rest are not used extensively as they have the disadvantage of being slow to kill insects.

According to Butt et al. (2001), recent rapid advances in biopesticides technology have concentrated on developed country markets and a high-tech approaches. This technology can however be effectively adapted to meet African needs and conditions through some innovative stages: characterization of effective local pathogens for African pests; development of appropriate pathogen application technology;
development of novel biopesticides formulations based on locally available agricultural by-products; and development of novel and appropriate technology for small-scale and commercial production of biopesticides. Research studies in recent years indicate existence of several local Bacillus thuringiensis (Bt) strains and some baculoviruses. This, together with the availability of production materials locally, and readily available production technology, offers an opportunity for launching a successful process of producing biopesticides as in Latin America and Asia. With a cost-effective production system, the potential for biopesticides use in Kenya is enormous. Encouragement of small-scale in vivo production by organizations such as NGOs, farmers groups and research institutes, using locally isolated strains, may help to pave way for future commercial production.

Bacterial and viral based biopesticides have been produced commercially in some developed countries for use on agricultural and forestry pests, especially those belonging to the order Lepidoptera. Their use is also rapidly expanding to other developing countries. Among bacteria, Bt has been the most exploited commercially, while members of baculoviruses have been the most used among the viruses, because of their virulence, specificity to insect pests and safety to man and the environment. The future of biopesticides appears to be assured considering the increasing problems of resistance and environmental contamination with conventional insecticides, which is creating a compelling need for safer alternatives. However, it would be important to initiate a kind of government innovative foundation that would take up the role of formulating, producing and selling these products locally, without necessarily looking for huge profit margins, in order to encourage and sustain their use.

Biopesticides have the potential for the management of key African insect pests of food and cash crops; they are environmentally safe, host specific and non-persistent and can be substitutes for the expensive imported pesticides. They can also improve the quality of export and local market produce by eliminating the risks of pesticides.

Bacterial and Viral Biopesticides

Bacillus thuringiensis (Bt)
Known bacterial biopesticides are Bacillus thuringiensis, B. popilliae, and B. sphaericus (Table 1). B. thuringiensis Berliner is a widely distributed, rod-shaped, spore-forming, aerobic, gram-positive bacterium. It is the most widely used pathogen for microbial control of insect pests and has been tested against a wide spectrum of insects including Lepidoptera and Diptera in the laboratory and field (Krieg and Langenbruch, 1981).

Table 1: Main bacterial control agents of insects

<table>
<thead>
<tr>
<th>Species</th>
<th>Active component</th>
<th>Principal insect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus thuringiensis var. kurstaki</td>
<td>Spores and crystal endotoxin</td>
<td>Lepidoptera</td>
</tr>
<tr>
<td>Bt israelensis</td>
<td>Spores and crystal endotoxin</td>
<td>Diptera (mosquitoes blackflies)</td>
</tr>
<tr>
<td>B. sphaericus</td>
<td>Spores, some toxin</td>
<td>Diptera (mosquitoes)</td>
</tr>
<tr>
<td>B. popilliae</td>
<td>Spores</td>
<td>Coleoptera (Scarabaeidae)</td>
</tr>
</tbody>
</table>

It is a complex species divisible into more than 20 varieties (or H serotypes) by serological and biochemical tests. Bt is reported to produce a proteinaceous parasporal
Baculoviruses & Bacteria as Potential Tools in Crop Protection

body delta-endotoxin or crystal toxin during sporulation (Hanny, 1953), which is extremely toxic to target insects. However, it causes little or no harm to humans, most beneficial insects and other non-target organisms. It is the principal insecticidal component of the commercial preparations. The Bt toxin exists in three size molecules designated 125–138, 65–75, and 25–28 kilodaltons and are encoded by CryI and CryIV; Cry, CryIII and CryIV and the Cyt genes, respectively (Haider and Ellar, 1989). They are further proteolytically converted into smaller toxic polypeptides (Hofte and White, 1989).

Nucleopolyhedroviruses (NPVs)
More than 600 baculoviruses have been isolated from arthropods, mainly insects, and some have been successfully used for the control of many lepidopteran, hymenopteran and coleopteran pests. They have a circular double-stranded DNA and are members of the Baculoviridae family. This family is divided into two subfamilies, Eubaculovirinae and Nudibaculovirinae. The Eubaculovirinae consist of the occluded baculoviruses, which include the nucleopolyhedrovirus (NPV) and granulovirus (GV) genera (Table 2).

<table>
<thead>
<tr>
<th>Table 2: Members of the Family Baculoviridae</th>
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<tbody>
<tr>
<td>Genus</td>
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<tr>
<td>---------------</td>
</tr>
<tr>
<td>NPV</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>GV</td>
</tr>
<tr>
<td>NOB</td>
</tr>
</tbody>
</table>

OB = occlusion body; NPV = nucleopolyhedrovirus (or nuclear polyhedrovirus); SNPV = single nuclear polyhedrovirus; MNPV = multiple nuclear polyhedrovirus
AcMNPV = Autographa californica nuclear polyhedrovirus
BmSNPV = Bombyx mori nuclear polyhedrovirus
PiGV = Plodia interpunctella granulovirus
HZNOB = Helicoverpa zea nonoccluded baculovirus.
From: McIntosh and Grasela, 1994

The NPV replicate within the nuclei of invertebrate cells and occlude virions (virus particles) within occlusion bodies (OBs), a proteinaceous matrix, also known as polyhedra. The NPV genus is further subdivided into two subgenera: single nuclear polyhedrovirus (SNPV) in which virions or enveloped nucleocapsids are packaged into OB singly, and multiple nuclear polyhedrovirus (MNPV) in which two or more viruses are embedded into the OB. The GV replicate partially within the nucleus and cytoplasm and are individually occluded and singly enveloped. The Nudibaculovirinae consist of the non-occluded baculoviruses, which are singly enveloped and do not produce OBs. NPVs are the mostly studied because of their ease to grow in cell culture.

Host Range of Bt and NPV

Bt
Since the discovery of Bt, several infectivity tests have been conducted in the laboratory to find out the susceptibility of different pests. Most Bt serotypes have been found to be pathogenic of larvae to Lepidoptera. Krieg et al. (1982) outlined arthropod susceptible to Bt, and also the variety of Bt involved in the laboratory or in the field. Some Bt strains have also been found to be pathogenic to some insects belonging to
Diptera and Coleoptera. Some of the local crop pests that have been found to be susceptible to Bt include: *Chilo partellus*, *Busseola fusca*, *Maruca testulalis*, and *Plutella xylostella* (Kariuki, 1987; Oketch, 2001; Thumbi, 2001). The host range and toxin composition for some strains of Bt is as indicated in Table 3.

**Table 3: Host range and toxin composition for some strains of Bt**

<table>
<thead>
<tr>
<th>Strain or subspecies</th>
<th>Insect host</th>
<th>Delta endotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>kurstaki</em> HD-1 (Btk)</td>
<td>Lepidoptera</td>
<td>CryIA(a), CryIA(b), CryIA(c), CryIIB</td>
</tr>
<tr>
<td><em>kurstaki</em> HD-73</td>
<td>Lepidoptera</td>
<td>CryIA(c)</td>
</tr>
<tr>
<td><em>thuringiensis</em> HD-2 (Bti)</td>
<td>Lepidoptera</td>
<td>CryI(A), CryIB</td>
</tr>
<tr>
<td><em>aizawai</em> (Bta)</td>
<td>Lepidoptera</td>
<td>CryIA(a), CryIA(b), CryIC, CryID</td>
</tr>
<tr>
<td><em>entomocidus</em> (Bte)</td>
<td>Lepidoptera</td>
<td>CryI(A), CryIB, CryIC</td>
</tr>
<tr>
<td><em>tenebrionis</em> (Btn)</td>
<td>Coleoptera</td>
<td>CryIIA</td>
</tr>
<tr>
<td><em>israelensis</em> (Bti)</td>
<td>Diptera</td>
<td>CryVA, CryIVB, CryIVC, CryIVD, CytA</td>
</tr>
</tbody>
</table>

From: Tabashnik, 1994

**NPV**

Baculoviruses have been isolated from a wide variety of insect pests from several orders, including Lepidoptera (with most isolates) and Coleoptera (Goodman and McIntosh, 1994; Adams, 1991). Individual baculoviruses have a limited host range, usually only infecting the target insects and a few closely related insect species in particular ecosystems. They therefore have minimal potential for damaging the environment (Goodman and McIntosh, 1994). The MNPVs in general have a wider host range than SNPVs and other baculoviruses in the family Baculoviridae, as evidenced in both in vivo and in vitro systems (McIntosh and Grasela, 1994; Harper, 1976). Among MNPVs, AcMNPV and AfMNPV have the widest in vitro and in vivo host range respectively (McIntosh and McIntosh, 1994). A recently isolated MNPV of diamondback moth, *Plutella xylostella*, PxMNPV, has also been shown to be infective to several lepidopteran hosts and their derivatives cell line (Kariuki and McIntosh, 1999). Some of the in vivo and in vitro host range of baculoviruses is as indicated in Table 4.

**Mode of Action of Bt and NPV**

**Bt**

Upon ingestion of Bt, the right combination of pH, salts and enzyme in the digestive system breakdown and activate the highly insoluble crystals. Following activation, the Bt toxins bind to high affinity receptors (glycoproteins) on the midgut epithelium, which result in generation of pores on the cell membrane, thus disturbing cellular osmotic balance and causing the cells to swell and lyse by the process of ‘colloid-osmotic lysis’ (Adang, 1991). This results in leakage of the alkaline gut contents into the haemocoel, which might be severe enough to kill the larvae and may cause changes within the larvae, which allow growth of Bt or other organisms, resulting in septicaemia. Damage to the larval digestive tract also causes it to stop feeding in 15 minutes to 1 hour after Bt ingestion. Combinations of the leakage, lack of feeding and septicaemia usually kills the insect within one to several days depending on the dose.
NPV
Nucleopolyhedroviruses are highly virulent and infection occurs after susceptible insect larvae eat food contaminated with the virus. After ingestion, the OBs are dissolved in the insect gut lumen by the alkaline environment in Lepidoptera and Hymenoptera and digestive proteases, releasing the enveloped virions. The virus enters the midgut cells, especially the columnar epithelial cells, by fusion with the membranes. The nucleocapsids are released, enter the cell and migrate to the nucleus through the nuclear pores. Replication of the virus follows infection of major tissues such as body fat, trachea, hypodermis and haemocytes, and the massive destruction of the body tissues that accompanies production of OB kills the insect in 3–10 days. Before death, infected larvae may gather in a typical way at the tip of the plants, cease feeding, the integument changes in colour and lustre, and the insect becomes flaccid and fragile. Upon death the insect rapidly darkens and the body ruptures to release millions of OBs.

Production of Biopesticides

* Bt is an ideal organism for large-scale commercial production because it grows easily in submerged cultures using conventional fermentation equipment. Stock cultures are best preserved as freeze-dried samples. In addition, spores also retain their viability on agar slants for a long period. For mass production of *Bt* the following general steps are involved:

1. Culture storage and maintenance
2. Propagation
3. Fermentation
4. Down-stream processing
5. Formulation and storage.

The first commercial product containing *Bt*, Sporein, was produced in France before 1938. Present worldwide production is in the order of several million tonnes with

### Table 4: In vivo and in vitro hosts of AcMNPV, AfMNPV and PxMNPV

<table>
<thead>
<tr>
<th>Insect host</th>
<th>Larval host</th>
<th>Cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Autographa californica</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Plutella xylostella</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Heliothis zea</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>H. subflexa</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>H. virescens</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Trichoplusia ni</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Spodoptera frugiperda</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>S. exigua</em></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

AcMNPV = *Autographa californica* multiple nuclear polyhedrovirus
AfMNPV = *Anagapha farcifera* multiple nuclear polyhedrovirus
PxMNPV = *Plutella xylostella* multiple nuclear polyhedrovirus

From: McIntosh and Grasela, 1994; Kariuki et al., 2000
leading manufacturers in the US and Europe. Some of the products available in the market include: Thuricide, Dipel, Certan, Xentari, Greenguard.

Production of NPVs can be achieved through in vitro and in vivo methods. These methods are quite often used in the laboratory for production of a small quantity of the virus. In vitro production involves the inoculation of susceptible insect cells with NPV after harvesting of OBs from cells by sonication and centrifugation to concentrate them. The in vivo method involves the use of whole insect larvae to produce the virus. The larvae are fed on artificial/natural diet inoculated with the virus and the OBs are harvested from dead infected larvae by homogenizing the carcasses and sieving through cheesecloth, followed by a series of differential centrifugation of the OB suspension (Kariuki, 1996). Commercial production of NPVs depends upon mass rearing and infection of host insect larvae as described in the in vivo method above. Occlusion bodies collected from diseased last-instar larvae are concentrated, purified and formulated with various adjuvants into wettable powder for subsequent field use (McIntosh and Ignaffo, 1981). Commercial in vitro production has not been possible as it depends on mass culturing on insect cells in a high-cost serum containing medium. Unlike Bt, production of NPV in vitro is rather expensive and this is one of the factors that has hampered their development. However, recent development of serum-free media has resulted in greatly reduced medium costs (Shuler et al., 1990; Agathos, 1994).

Some of the NPV products that have been produced commercially include: Gemstar (Helicoverpa zea NPV), Gypcheck (Lymantra dispar NPV), SPOD-X LC (Spodoptera spp. NPV), Biotnel (Trichoplusia ni NPV), Neocheck (Neodiprion sertifer NPV) etc.

Role of Bt and NPV in Pest Control

Because of their ability to kill insects considered harmful, Bt and NPV have undergone fast development for the purpose of use in the field. Today they are being used in integrated pest management (IPM) programmes for short-term control of pests. Both can successfully and safely be used in augmentative biological control where they are actively produced and released repeatedly (inundative) to control pest populations. Bt and NPV are used for short-term control because they are short-lived in the environment. They are therefore applied repeatedly like conventional chemical insecticides to control a wide range of lepidopterous defoliators in agricultural crops and forests. Although the use of these biopesticides is not intensive in Africa, studies in Kenya have demonstrated their potential in control of many agriculturally important pests such as Helicoverpa armigera, Plutella xylostella, Phthorimaea operculella, Chiolo partellus, Busseola fusca, and Maruca testulalis (Kariuki, 1987; Baya et al., 2001; Brownbridge, 1990; Kibata et al., 1999).

The major disadvantage of Bt and NPV is the rapid disappearance of their activity in the field and inability to spread in insect populations. This is due to inactivation by solar radiation (UV). Also the narrow host range is a disadvantage in comparison to chemical insecticide. To circumvent these problems, advanced technology involving genetic engineering is applied as discussed below.

Improvement of Biopesticides

This is usually done in order to improve their host range, virulence and delivery. This can be achieved by using them synergistically in combination with other microbial or chemical insecticides. Genetic engineering is also done, especially with NPVs, with the main goal of enhancing their marketability. They are therefore modified to enhance
virulence – since NPVs would require several days to kill insects, during which time they are still actively feeding and causing crop damage. Development of faster-acting viruses enhances their overall effectiveness as insect control agents in agricultural settings (Goodman and McIntosh, 1994).

A variety of genes have been inserted in the NPV genome that have the potential for disrupting physiological processes such as digestion or moulting. Such genes are: Bt delta endotoxin gene, insect hormone genes, insect enzyme genes, insect venom genes and invertebrate neurotoxin. NPVs are also modified in order to expand their host ranges. This is because many of them infect only insects within one family or genus, which limits their marketability. The usefulness of Bt has been increased partly due to technical innovations that allow expression of Bt toxin genes in transgenic crop plants (Tabashnik, 1994), thus enhancing its infectivity and delivery.

Regulation and Registration

According to Goettel et al. (2001), many regulation and registration requirements serve the purpose of ensuring the safety of the agent and efficacy. However, these have not been encouraging to the development and registration of biopesticides because the biological control agents have been put together with other acts dealing with plants, noxious weeds etc. Many are also regulated by legislation initially designed for chemical pesticides, and a separate review system for biopesticides from that for conventional pesticides will be needed. Many countries in Europe and America including organizations such as the Food and Agricultural Organization (FAO) have been drawing up new regulations for biopesticides. Different countries also have different data requirements for registration and harmonization is needed in order to reduce the cost of registration of the product.

General data requirements for registration of microbial pathogens (Adapted from OECD, 1996) are as follows:

- Identity
- Physical, chemical and biological properties
- Function, mode of action and handling
- Manufacturing, quality control and analytical methods
- Residues
- Efficacy
- Toxicology
- Ecotoxicology
- Effect on environment.

Although biopesticides do not possess properties that would make them potentially hazardous, assessment of their potential risk is important. This is also important because the intention of developing them is to commercialize them, and so registration and regulation are needed to protect all the stakeholders involved.

Conclusions

The use of biopesticides in Kenya is at the moment on the low side, but potential exists considering the huge horticultural industry (major user of chemical pesticides) which is second only to tea in terms of export. A greater percentage of vegetable export is
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destined for the European Union market which has recently introduced the pesticide maximum residue levels (MRLs) requirement that all horticultural produce to EU market has to meet. With the anticipation of an introduction of zero tolerance sooner or later, Kenya has to start embracing environmentally safe methods of pest control now, if it is to continue enjoying and sustaining monopoly in this market. A delay in implementing such methods in pest management will result in the loss of market to other competing countries that are anxiously taking on board modern and safe methods of pest management.

The rapid development of biopesticides in some countries such as India and those in SE Asia was partly driven by the urgent need of safer alternatives as a result of massive pesticides resistance that nearly brought horticultural production to a halt. We will not need to wait for that to happen and it is therefore advisable to start now before the situation gets out of hand as cases of resistance to pesticides have been on the increase, especially with diamondback moth, african bollworm, red spider mite and others.

In order to hasten the utilization of biopesticides, the prevailing policy environment needs to be enabling and conducive. For instance it would be important for the Government to implement an Integrated Pest Management (IPM) Policy especially in the horticultural industry so that the producers and other stakeholders feel obliged to adopt environmentally friendly methods for pest management in production. In time, this will change the industry from total dependence on chemical pesticides as a panacea to pest problems, to one driven by the need for production of quality food for both local and export markets. The presence of an efficient and thorough registration and regulation mechanism will also be important in development of biopesticides. The registration should be encouraging to local commercial producers who are willing to venture into production of biopesticides using locally isolated strains or plant materials.

Production of biopesticides is expensive and require government support/intervention to kick off before interested private commercial entrepreneurs could join in. Through the IPM Policy, the Government could levy manufacturers and sellers of chemical pesticides a small feed that could fund the development of pesticides in the country. For instance, through bilateral assistance, the Government could create an innovative foundation under any of the institutes (such as university, research institute etc.) to develop, build and run a biopesticides production plant in the country using locally identified pathogens. Products of this plant could be used for field trials in IPM programmes for a wide range of pests, and to train its technical staff and scientists in biopesticides use. This will ensure that biopesticides are integrated into unified crop protection programmes at affordable cost to growers.

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Development, Registration and Commercialization of Biopesticides: The Case Study of ‘Green Muscle®’

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Abstract

Locusts and grasshoppers cause enormous damage to crops in many countries, especially in Africa. Attempts to control them have relied heavily on the use of synthetic insecticides. The LUBILOSA (Biological Control of Locusts and Grasshoppers) project was formulated to develop a biopesticide against these pests. Surveys in West and North Africa identified the fungus Metarhizium anisopliae var acridum isolate IMI 330189 as the most promising biological control agent. The complex processes of developing the biopesticide product e.g. formulation, storage, production were successfully implemented and the route to develop a commercial product was agreed upon. Biological Control Products (BCP), based in South Africa was identified as the most suitable commercial company to manufacture, market and sell the product (eventually known as ‘Green Muscle®’). Problems of disclosure, sharing and exchange of information were solved mutually.

Introduction

Appropriate management of intellectual property (IP) generated by public sector research and development (R&D) organizations is an increasingly important issue as institutes struggle to balance their public service role with the opportunities for benefit from their IP through the commercial sector. The need for the exploitation of IP has emphasized the importance of collaboration between the public sector R&D institution, client, development partner and commercial sector. The ‘Biological Control of Locusts and Grasshoppers’ (LUBILOSA), a collaborative programme between CAB International (CABI), International Institute of Tropical Agriculture (IITA), DFPV (Programme Majeure Formation, Protection des Végétaux), Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) and CILSS (Interstate Committee for Drought Control in the Sahel), provides a case where all partners’ interests were catered for in the exploitation of an IP developed by the public sector and marketed by the commercial sector.

Scientific papers on the identity, production storage etc. of the product were freely published, whereas technical procedures required for formulation to use the product were patented by CABI and transferred to Biological Control Products (BCP) under strict Confidentiality and Licensing Agreement. Benefits arising from the development of Green Muscle® include access to the technology and the environmental, economic and social benefits that accrue from it, capacity building through training and royalties generated from the sale of Green Muscle. The monies generated from the royalties on the sale of Green Muscle in Africa are deposited into a Trust Fund, to support collaborative initiatives associated with promoting biopesticide development and use in Africa.
The Challenge

Appropriate management of IP generated by public sector R&D organizations is an increasingly important issue. The difficulties are perhaps most evident with public organizations involved in development assistance work, i.e. their mandate to make all information freely available is not consistent with commercialization of IP. The advent of 'biotechnology', with its potential benefits, has highlighted the need for public sector R&D institutions to be able to work with sponsors, clients and commercial companies but these new relationships can create conflict between the needs of the different stakeholders.

There are, however, some success stories.

The LUBILOSA Programme

- Biological control was considered to be such an alternative
- A collaborative programme entitled 'Biological Control of Locusts and Grasshoppers' (LUBILOSA) was developed in 1989
- Collaborators in the programme were: IITA, DFPV, CILSS, GTZ and CAB International
- Donors were: CIDA, DGIS, ODA (now DFID), SDC, USAID.

LUBILOSA Project Cycle

- Phase 1 (1989–91) – Established the principle of using oil formulations of fungi (\textit{Metarhizium anisopliae}) against locusts.
- Phase 2 (1992–94) – Established that the oil formulation of the isolate IMI 330189 was effective. Mass production initiated at IITA.
- Phase 3 (1995–98) – large-scale field trials, toxicological, ecotoxicological and economic studies carried out. These studies indicated that commercialization offered the most favourable route to implementation. LUBILOSA licensed the technology to two commercial partners (BCP in South Africa and NPP in France). Registration of the product has been granted in South Africa.

Origin, distribution and access to LUBILOSA isolate IMI 330189

Product Development
The process of taking a fungal isolate and turning it into a marketable product involves a process of:

- Identification and characterization of collected isolates
- Laboratory bioassay to determine virulence
- Formulation
- Storage tests
- Mass production
- Small-scale application trials
- Large-scale trials
- Operational level trials
- Assessments of ecotoxicology and mammalian and fish toxicity
- Scale-up of production
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- Preparation of registration and submission of dossier
- Identifying commercial companies to manufacture, distribute and sell the product
- Confidentiality
- Commercial, regulatory, technical and quality standards
- Identifying appropriate industrial partners
- Gathering market information
- Licensing of the technology to the private sector
- Disbursement of benefits accruing from the successful commercial exploitation of the product.

To address the above, it is essential to establish appropriate partnerships with R&D collaborators, sponsors and commercial companies. LUBILOSA provides an example of the type of problems that need to be addressed and some of the options that are available to deal with the commercialization of public sector generated intellectual property.

**Green Muscle: the Product**

*Active Material*
The active material of the mycoinsecticide Green Muscle is based on the fungus *Metarhizium anisopliae* var. *acridum*. The standard isolate is IMI 330189. This isolate has been found to be effective against a wide range of locusts and grasshoppers.

*Formulations*
Laboratory assays have shown that the formulation of *M. anisopliae* conidia in oil improved the efficacy and speed of kill in comparison with water-based suspensions, especially at low humidity. The programme has developed a unique oil-miscible flowable formulation.

*Storage Characteristics*
Long-term storage of *Metarhizium* conidia is possible provided that the moisture content is kept low (below 6%). No loss of virulence is observed after 12 months at 30°C.

*Application*
The LUBILOSA mycoinsecticide is compatible with all ULV spraying equipment likely to used for operational application. Rates of application of 0.5 l/ha (50 g/ha) have been successfully used.

*State Registration*
Green Muscle is registered in South Africa for control of Brown Locust and has been recommended by FAO Desert Locust Pesticide Referee Group for use in conservation and environmentally sensitive areas.

*Public/Private Partnerships for Developing Green Muscle*
The basic principle on which LUBILOSA has approached the development of Green Muscle for locust and grasshopper control has been to meet the ‘public need’ with a basic, workable product and system for production, which has been made readily available to the public domain. However, the LUBILOSA manufacturing process was assessed to be uneconomic. LUBILOSA is transferring its technical knowledge of
production and formulation to two companies which bring-to-bear their experience in large-scale manufacturing expertise of biopesticides.

Biological Control Products (BCP), based in South Africa, is currently licensed to manufacture, market and sell Green Muscle. It is a small-medium enterprise (SME) whose core business is to manufacture, market and sell biological control agents for use in the control of plant pathogens. Their main product is the nematicide, based on the fungus *Paecilomyces lilacinus* for the control of nematodes of tomatoes and related crops, that is registered in South Africa as ‘PL Plus’. The *Paecilomyces* production plant in Pinetown utilizes a similar solid substrate system to that required for industrial scale production of *Metarhizium*.

**Disclosure, Sharing and Exchange of Information**

In general, public disclosure of information is considered unhelpful to commercialization of a product but the decision to, or not to, disclose information generated by a research team also has implications for individual scientists and the donors funding the research, for example:

- Public sector scientists in general
- Research workers
- Donors supporting R&D projects
- Commercial companies
- Dealing with confidentiality at the project level
- Ownership of the IP.

**Commercial Company Collaboration**

Interaction with a commercial company is a two-way process. The commercial company needs to be convinced that the product is commercially viable and the licensee needs to be sure that the commercial company has the wherewithall to register, manufacture, market and sell the product to the required standards and price.

Key issues for both parties to collaborate on mycoinsecticide commercialization include:

- **Product specification**: Sufficiently broad spectrum for there to be a sizeable market for the product, high virulence, good speed of kill, good storage capability, use of a conventional formulation utilizing existing application equipment.

- **Production**: Utilization of an established production process, conventional packaging and storage.

- **Markets and demand**: A number of large, regular, well-established markets, few competitive products, a specific product advantage for which there is an established demand or a well-defined niche market presently unexploited that provides an economically attractive opportunity.

- **Distribution and sales**: Product must fit within existing networks of distribution; wholesale and retail sales outlets and mechanisms need to be well established.
• **Toxicology and ecotoxicology:** Product should ideally be environmentally friendly and have low vertebrate toxicity.

• **Registration:** Product should require first tier testing only, or before entering a fast track registration process.

• **Economics:** Favourable toxicological attributes reduce development and registration costs, use of an established production process reduces development costs – production, packaging, storage and transport costs need to be low, and competitive price.

**Which Companies?**

There are a number of companies that already have mycoinsecticide products on the market and a number of others have the capability to produce and market them. The decision as to which companies LUBILOSA should approach depended on their ability to meet the following criteria:

• A small to medium-sized enterprise (<50 employees)
• Production capability that can be readily adapted to *Metarhizium*
• Access to donor funding
• An existing distribution system in appropriate regions
• Access to capital
• A track record in registering, producing, marketing and selling biopesticides
• Willingness to enter into an appropriate licensing agreement.

**Basis of LUBILOSA Collaboration with Commercial Companies**

Prior to entering into any discussions with commercial companies, confidentiality agreements were signed to protect LUBILOSA intellectual property. Licensing agreements were negotiated on the basis of the following:

• A non-exclusive basis incorporating a specific geographical, pest species or cropping system jurisdiction
• Transfer of liability
• An advance payment (licence fee) – scale dependent on company and royalty
• A royalty payment in the range 2.5–7.5%
• A non-assignable agreement and acceptable accounting procedures.

In turn, LUBILOSA provides the commercial company with:

• Toxicological and ecotoxicological information relevant to, but not necessarily wholly inclusive of, requirements for registration of the product.
• Relevant efficacy data and results
• Know-how and expertise to assist in the production process, registration, labelling and marketing of the product.

The IPR transfer agreement and the licensing agreement with a commercial company included clauses to safeguard the interests of the donors, in particular with warranties with regard to acknowledgement of the donors and their statutory obligations, and product liability and use. The licensing agreement between CABI and the commercial
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company includes clauses that reflect the requirements of these warranty and liability statements.

Access to Benefits Arising from LUBILOSA

A large number of agencies and organizations have participated in and contributed to the development of the LUBILOSA mycoinsecticide. It is essential that every effort is made to ensure the adoption of this technology by relevant groups in Africa. At the same time an appropriate mechanism is required to distribute the benefits arising from the technology in accordance with the Convention of Biological Diversity (CBD).

Benefits arising from the development of the mycoinsecticide include:

- Access to the technology and the environmental, economic and social benefits that accrue from its use
- Capability building through the LUBILOSA training programme
- Royalties generated from sale of the mycoinsecticide.

The licences issued to the companies include clauses that ensure the following conditions apply:

- Good commercial practice with recourse to the appropriate transfer of public sector technology to the commercial sector
- Reasonable price charged for the sale of the product; the price must be competitive with other similar products to ensure general use and accessibility
- Reasonable availability of the mycoinsecticide within sponsor core countries requiring such products
- Monies generated from the commercial exploitation of the mycoinsecticide shall be credited to a Trust Fund and used in accordance with the declared objectives of the Fund for disbursement within Africa.

Royalties will be generated by the sale of the bioinsecticide but the amounts of money generated are unlikely to be large. If these royalties are split between all the agencies and organizations involved in the LUBILOSA Programme, then the amounts paid to each will be insignificant and of little practical value. For this reason, it was proposed that the money generated from royalties on the sale of the bioinsecticide in Africa be accrued in a Trust Fund. The purpose of the Trust will be to support collaborative initiatives associated with promoting biopesticides development in Africa.

The Trust Fund document specifies the purposes and principles of disbursement, the Trustee and powers, the Trust account, duration and taxation issues.

Recommendations

Some recommendations on the development of biopesticides are:

- Define at the outset of each development assistance R&D project whether a product will result from project outputs. Where this is the case consider:
  - Implications for exploitation
  - Non-commercial and commercial routes available
  - Market potential of the expected product
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- Need for commercial advice and input
- Potential links with commercial companies.

- Ensure each partner in a project establishes and agrees to a publication policy and scrutinizes all R&D outputs to identify commercially exploitable know-how.

- Ensure that all information that is not required to commercialize the product is made freely available.

- Standardize Material Transfer Agreements (MTAs) based on the CGIAR (Consultative Group for International Agricultural Research) germplasm exchange MTA but with appropriate modification for the possibility of a subsequent commercial implementation route.

- Establish the principle of confidentiality agreements as part of the collaborative process between all partners.

- Engage commercial companies as early as possible in the product development process.

- In multi-donor projects, thought needs to be given in the first year as to how the IPR issues should be dealt with. Multi-sponsor agreements provide a relatively simple solution to the problem.

- Identify and establish clear unequivocal mechanisms for the disbursements of benefits arising from commercial exploitation which should include research collaboration, access to the final product and monetary benefits derived from its licensing and sale.

Discussion

Comment
Patenting of formulated biopesticides is alright but the patenting and storage conditions should be something that is suitable for the public.

Response
The storage patenting is done before the researcher enters into an agreement with the commercial firm that would trade in it.

Question
What is the use of withholding information even after applying and obtaining patent rights?

Answer
To ensure that the production, marketing and selling is done by commercial company with whom LUBILOSA programme has signed a secrecy agreement as well as a licensing agreement. This ensures that LUBILOSA’s intellectual property rights are protected, the liabilities are transferred to the commercial company which in turn has to remit royalties as well as pay annual licensing fee so the secrecy agreement ensures that only one commercial company derives direct benefits, besides LUBILOSA programme.