Brief overview of microorganisms used against agricultural insect pests

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Abstract
Entomopathogens used against insect pests as biological control agents include microorganisms such as fungi, bacteria, viruses, protozoan and nematodes. They cause diseases and eventual death in certain groups of insects. These organisms are also considered to be non-contaminants in agricultural environment as compared to conventional chemical pesticides. Basic descriptions of each representative entomopathogens are given, together with their availability and commercial use worldwide, to provide a broad overview on the current status of these organisms. As biological control entails ecologically sound and environmentally safe practice in agricultural production, continuous research and technological development will provide producers with better options as alternative pest control measures.

Keywords: baculovirus, *Beauveria bassiana*, *Bacillus thuringiensis*, *Metarhizium anisopliae*, *Yersinia entomophaga*.

Resumen
Los entomopatógenos que se utilizan contra insectos plagas como agentes de control biológico incluyen microorganismos como hongos, bacterias, virus, protozoos y nematodos. Estos organismos causan enfermedades y muerte eventual en ciertos grupos de insectos. También se consideran no contaminantes en el ambiente agrícola en comparación con los pesticidas químicos convencionales. Se dan descripciones básicas de cada grupo representativo de entomopatógenos, junto con sus disponibilidades y usos comerciales en el mundo, para proporcionar una reseña amplia del estado contemporáneo de estos organismos. A causa de que el control biológico conlleva prácticas ecológicamente sanas y seguras en cuanto al medio ambiente para la producción agrícola, las investigaciones continuas y desarrollo tecnológicos proporcionarán a los productores las mejores opciones como medidas de control alternativos.

Palabras clave: baculovirus, *Beauveria bassiana*, *Bacillus thuringiensis*, *Metarhizium anisopliae*, *Yersinia entomophaga*.

Introduction

Entomopathogenic microorganisms are gaining ever-increasing importance in Integrated Pest Management (IPM) as ecologically sound and environmentally safe components in contemporary agricultural production.

Miller *et al.* (1983) put forward a future perspective and principal guidelines for research and development on entomopathogenic organisms. A premise of decreased dependence on chemical control measures against insect pests was also within their scope.

Knowledge of ecology of pest species as well as their bio-rational controlling agents such as entomopathogens is fundamental for successfully implementing IPM (Fuxa 1987). Biotic and abiotic environmental factors must be taken into account along with other complex ecosystem characteristics (Lacey & Kaya 2007).

In a general statement, the U.S. Environmental Protection Agency (EPA) describes some characteristics of biopesticides which include entomopathogens. They state that 1) Biopesticides are by nature less toxic to people than conventional chemical pesticides, 2) they are generally effective only against specific target pest species, 3) when used as a principal component of IPM, they would significantly reduce the use of agrochemicals, and 4) they would rapidly reincorporate into the general environment thereby avoiding long-term ecological pollution (EPA 2010).

Hajek *et al.* (2005, 2007) reviewed and compiled information on pathogens and nematodes used as biological control agents against insects and mites. Fungi, bacteria, viruses, microsporidians and nematodes are included in the literature.

Commercially produced biological pesticides are now available worldwide. The contemporary commercial situation in world market, however, is in flux. It is a dynamic market. Continuous changes in availability and registration are the norm. Weinzierl *et al.* (2005) revised their 1995 list of microbial insecticides available in the US market with product names. They cited bacteria, fungi, protozoa, viruses and nematodes with basic biological characteristics and discussed the potential of each group with some comments on current market status (Weinzierl *et al.* 2005).

Kabaluk and Gazdik (2005) revised a directory of microbial pesticides registered among 30 member countries of the Organization of Economic Cooperation and Development (OECD), to which Mexico belongs as the only member state in Latin America. The aim of the document was to harmonize data requirements, test guidelines, and hazard and risk assessment methods within OECD countries. Although they contend that the directory is not exhaustive, a large volume of resources are listed according to different categories such as insecticide, miticides and nematicides among others. The list can thus be taken as a general market trend in contemporary microbial pesticides in various countries.

The objective of the present note is to briefly examine current status and use of major microbial insecticides in agricultural settings and to put these beneficial microorganisms into perspective in terms of contemporary agricultural pest control measures. The article does not attempt to exhaustively review the subject. Fungi, bacteria, viruses and nematodes in sequence are mentioned in this overview.

Fungi

Fungi require humid environment to proliferate and entomopathogenic fungi are no exception. They need high humidity field conditions to be effective. They are often directed against soil-inhabiting insects, but unless moist soil is insured by rainfall or otherwise, such applications may be in vain. Aside from strictly following instructions for use written in product labels, environmental conditions as well as time of application have to be carefully considered in relation to corresponding crop cycle (EPA 2012).

The OECD directory lists 5 species of fungi available in the market. They are: *Beauveria bassiana*, *Metarhizium anisopliae*, *Beauveria brongniartii*, *Verticillium lecanii* and *Paecilomycetes fumosoroseus*. The first two species, *B. bassiana* and *M. anisopliae*, constitute 39 % and 35 %, respectively, of the total fungi registered among member countries (Kabaluk & Gazdik 2005).

Meyling and Eilenberg (2007) suggested that *B. bassiana* are associated only with insect hosts above ground, while *M. anisopliae* is associated with hosts exclusively on or below the soil surface in temperate agroecosystems. Thungrabeab and Tongma (2007) reported that these two fungal species are generally not pathogenic to non-target species, including common natural enemies such as Coccinellids and Chrysopids found in Thailand. They tested *B. bassiana* and *M. anisopliae* against 3 natural enemy species and a beneficial soil Collembora. *Coccinella septempunctata* (Coloeptera, Coccinellidae), *Chrysoperla carnea* (Neuroptera, Chrysopidae), *Dicyphus tamanii* (Hemiptera: Miridae) and *Heteromurus nitidus* (Collembola, Entomobryidae) were used in the study. While *M. anisopliae* showed some pathogenicity to *C. carnea* and *H. nitidus*, *B. bassiana* had no pathogenic effect on all 4 species tested (Thungrabeab & Tongma, 2007).

Beauveria bassiana causes "white muscardine disease" in the infected insect which becomes covered in white mycelia. This is a ubiquitous soil-inhabiting fungus. Its spores prepared as a commercial product can be put into water for spraying. Upon contact with insect's external cuticle, spores germinate and invade the insect body with mortal effect (Groden 1999). This fungus has a relatively wide range of insect hosts, such as whiteflies (Hemiptera: Aleyrodidae), aphids (Hemiptera: Aphididae), weevils and borers (Coleoptera), grasshoppers (Orthoptera) and diamondback moth (Lepidoptera: Pluteridae, *Plutella xylostella*). Care must be taken to avoid application near colonies of honeybees (*Apis mellifera*, Hymenoptera: Apidae) or within their foraging range, as there is a potential risk of infection (EPA 2012).

The common name of the fungal disease caused by *Metarhizium anisopliae* is green muscardine disease, as the spores of the fungus give the infected insect a greenish appearance. This is another ubiquitous soil-inhabiting fungus (Cloyd 2010) for which preparation and application procedures as well as infective process are basically the same as for *B. bassiana*. However, the host range is more selective than *B. bassiana*, targeting principally against root weevils (Coleoptera), flies and gnats (Diptera), thrips (Thysanoptera) and various tick species (Arachnida: Ixodidae). Although slightly contradictory to studies reported by Thungrabeab and Tongma in 2007, EPA (2012) notes that many insects of human interest, such as honeybees and beneficial insects, namely, green lacewings (Neuroptera: Chrysopidae), lady beetles (Coleoptera: Coccinellidae) and parasitic wasps (Hymenoptera), are not affected by this fungus.

Bacteria

Similar abiotic conditions (high humidity) are required for bacterial entomopathogens as for fungi. These microorganisms are susceptible to dry environmental conditions (Weinzierl *et al.* 2005). Unlike fungi mentioned earlier, bacterial entomopathogens are required to be ingested by target pest insects. *Bacillus thuringiensis* is the predominant species commercially produced throughout the world. Many distinct subspecies and strains of *B. thuringiensis* are known to produce toxins with insecticidal properties.

The OECD directory lists numerous bacterial insecticides registered among member states as of 2005. With a few exceptions, the species most available in the market is *B. thuringiensis*. Different strains of *B. thuringiensis* infect distinct insect groups, namely Lepidoptera, Coleoptera, and Diptera. More than 75 % of the products in the list are based on *B. thuringiensis* subspecies *kurstaki*, followed by other subspecies *israelensis* and *aizawai*. Subspecies *B. thuringiensis tenebrionis* is used against coleopteran pests such as the Colorado potato beetle, while *B. thuringiensis israelensis* is specifically targeted against Dipterans such as mosquitoes and fungus gnats. *B. thuringiensis kurstaki* and *B. thuringiensis aizawai* are used against lepidopteran pests (Kabaluk & Gazdik 2005). Products combining endotoxins from both of these two subspecies are also available. With the advent of genetic engineering, *B. thuringiensis* toxin producing genes have been incorporated into such crops as potato, maize, and cotton (Brookes & Barfoot 2006). Roh and others (2007) summarized present state of knowledge on this widely studied bacterium. Further elaboration on this aspect of *B. thuringiensis* is, however, outside the scope of present article.

Hurst and others (2010) described a new species of entomopathogenic bacterium and proposed the name *Yersinia entomophaga* with the type strain MH96. It was found infecting a grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae) in New Zealand. The bacterium effectively killed a number of Lepidoptera and Coleoptera species within 3 to 5 days upon infection. A patent application has been submitted, delineating the use of the bacterium and its derivatives as biopesticides (Glare & Hurst 2010).

Yersinia entomophaga is now highly touted as a promising and effective biological control agent against major groups of insect pests. Readers are reminded, however, that another species of *Yersinia*, *Y. pestis* is arguably the cause of the infamous Black Death in human history. In a recent report, Haensch and others (2010) confirmed that *Y. pestis* was indeed the cause of the pandemic, upon extensive forensic DNA studies on European medieval mass graves sites.

Present author expresses a reserved opinion on *Y. entomophaga* as to its use in agricultural and general environment as well. The adaptive capacity of microorganisms in general is high as compared to more complex organisms, simply because their life cycle is short. Any mutant strain, which might not necessarily be beneficial to humans if not outright dangerous, can spread rapidly throughout the environment.

Virus

Viruses are obligatory parasites on living host cells. Viruses presently used in agriculture belong to two genera of the family Baculoviridae, namely, *Granulovirus* (GV) and *Nucleopolyhedrovirus* (NPV). To become infected, insect pests need to consume host plant with Baculovirus applied by a spray. Baculoviruses principally infect insects and other arthropods and do not affect vertebrates. Once infected, an insect larva may contain billions of replicated viruses within its body (Bonning & Nusawardani 2007).

As of 2005, OECD directory of microbial pesticides includes 14 registered Baculoviruses commercial products in various countries; 9 products under GV and 5 under NPV. Target pest insects are those of forestry, orchard, horticultural, ornamental, dried fruits and nuts, as well as grain crops including soybean (Kabaluk & Gazdik 2005).

Federici (1998) and D'Amico (2010) indicated a high cost of Baculovirus products, more than most chemical treatments, as being one of the main obstacles to adaptation of Baculovirus for pest control. The reason for the high price is the labor intensive nature of producing large quantity of Baculovirus for field use. However, a totally different panorama has noted in Brazil.

Anticarsia gemmatalis (Lepidoptera: Noctuidae), the velvet bean caterpillar, has a Neotropic distribution and is a voracious defoliator of soybean and other leguminous crops. Since 1980's, technical development and active use of Baculovirus against *A. gemmatalis* as the major pest of soybean crops in Brazil have been reported with prodigious positive results (Moscardi 1999, Correa-Ferreira *et al.* 2000). The virus in this case is denominated as *Anticarsia gemmatalis* Multiple Nucleopolyhedrovirus (AgMNPV). Initially, the practice was based on farmer-level monitoring in the fields, collection of infected larvae, on-site spray preparation and application using homogenate larval cadavers. Family members and relatives can be mobilized for such *in situ* activities. Labor situations and associated income are dissimilar in developing countries relative to industrialized nations. Kaolin, a type of clay, impregnated with the virus is now commercially available in Brazil at low cost. It is to be dissolved in water and the solution can be broadcasted using a simple backpack sprayer or large volume sprayers as well.

This elemental and low cost technology was transferred from Brazil to Paraguay in the early 1990's. Kokubu (1994) reported population dynamics of *A. gemmatalis* in soybean fields in eastern Paraguay in relation to the use of AgMNPV. Two salient findings were noted in the report: 1) virus applications effectively suppressed a resurgence of the pest population during the crop season, while typical chemical control measures continued to cause a second outbreak of the pest, 2) Insecticide cost was more than 700 % higher than that of viral insecticide (Kokubu 1994).

Probably on the ground of less expenditure on pest control, increase in soybean production in Paraguay has augmented exponentially, especially since 1995. The country now boasts as being one of the world's topmost exporters of the commodity (FAOSTAT 2012). Surplus income and savings made at the producer level would encourage further expansion in area of planting, augmenting more production.

Nematodes

Nematodes are metazoan animals of microscopic sizes, classified under the phylum Nematoda. They are ubiquitous in general environment; free-living, predacious, or parasitic to plants and animals including humans. Entomopathogenic nematodes belong to two particular families: Steinernematidae and Heterorhabditidae. Two genera, *Steinernema* and *Heterorhabditis*, are the groups used in contemporary biological control. (Georgis & Gaugler 1991, Grewal *et al.* 2005a).

One characteristic feature of these nematodes is that they are intimately related with bacterial species inhabiting within their bodies. This nematode-bacterium complex of mutualism provides the reason and mechanism for the insect killing capacity in such nematodes. *Steinernema* species harbor *Xenorhabdus* bacteria in their gut and *Heterorhabditis* are associated with *Photorhabdus* bacteria. Nematodes actively seek out host insects (Shapiro-Ilan *et al.* 2009). They first attach to and make inroads to the host insect body, therein releasing the bacteria. The bacteria then cause a lethal septicemia in host insect, which is rapidly killed within 24 to 48 hours (Stock & Goodrich-Blair 2008). Moreover, life cycle of these nematodes is completed within 2 to 3 days, reproducing thousands of progenies within a host body (Griffin *et al.* 2005). Third stage juveniles break out of the host to seek new hosts.

Several species of entomopathogenic nematodes are commercially available. The OECD list shows 30 commercial products as of 2005 among member nations. More than one third is based on *Steinernema feltiae* used primarily to control fly larvae. Two other species, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, are listed as the second most commonly used nematodes (Kabaluk & Gazdik 2005). Shapiro-Ilan and Gaugler (2010) listed 26 producers and suppliers of entomopathogenic nematodes; 24 in the US and one each in Germany and Switzerland. For the entomopathogenic nematodes to be effective in the field, certain biotic as well as abiotic conditions need to be met (Shapiro-Ilan & Gaugler 2002, Shapiro-Ilan *et al.* 2006). High environmental humidity is one critical factor. These nematodes are basically soil inhabiting organisms and demand moist soil for survival. They are thus highly susceptible to desiccation (Grewal *et al.* 2005b).

Narrow temperature range is yet another decisive factor in their effectiveness. Optimum temperature generally rests between 20 and 30 degrees Celsius, although some species may withhold below 15 °C or even above 35 °C (Jagdale *et al.* 2005, Grewal *et al.* 2005b, Jagdale & Grewal 2007). Also care must be taken of high temperature and its duration in holding tanks before spraying application (Shapiro-Ilan & Gaugler 2010).

Perceived Impediments

A number of critical factors hindering wider acceptance of biological pesticides have been pointed out (Weinzierl *et al.* 2005). In this article, we focus on three anthropocentric aspects.

1) Specificity of biological pesticides is akin to a two-edged sword. On the one hand, entomopathogenic microorganisms are often selected for their specificity. This intrinsic attribute could, on the other hand, limit uses in managing many other pest insects that may be in need of attention. As their host range is limited, a multiple number of microbial products may be necessary to control different species of pests in a particular situation. This in turn would impose additional costs on producers willing to opt for biological pesticides. Use of these biological products might be taken as economic burden to agriculturalists whatever their production scale may be.

2) Slow action is yet another unfavorable characteristic of biological pesticides. With the availability of fast-action toxic chemical pesticides, growers are accustomed to see an im-

mediate response to agrochemical applications. This mindset has been reinforced for the last two to three generations worldwide.

3) Lack of information may be another factor affecting the acceptance of microbiological insecticides. Although technical reports abound indicating relative swiftness and effectiveness of the entomopathogenic microorganisms, such information may not be readily accessible to farmers and producers. Moscardi (1999) emphasized the importance of education and knowledge dissemination at the farmers' level when he summarized AgMNPV use in Brazil. People in production fields are needed to be well-informed and convinced of the benefit and importance of the use of biological pesticides in terms of ecology and economy.

Disparity in many aspects of contemporary life among world's nations is evident. Social organizations and educational levels in the Americas, for instance, are not equally advanced. Latin American countries including Mexico may lack adequate socio-economic capacity to adopt such technologies as entomopathogens compared to Canada and the USA. To be accepted and adopted worldwide, innovative pest control methods or any technology for that matter has to be simple and at the same time economical. Formulation and application of entomopathogens are comparable to chemical pesticides. Equipment is fundamentally the same and precautions are less stringent (Burges 1998). It is therefore imperative to make this technology economically accessible and simple to use for all producers, not only in industrialized nations but also in developing countries as well.

Environmentally conscious and ecologically sound pest control methods are central to contemporary agricultural production. Reduced dependence on non-renewable resources and more usage of locally available resources are the emerging axioms in sustainable agriculture. Under the concept and practice of IPM, systematic use of entomopathogens in agricultural pest control is a viable and economically competitive component. Contemporary research and development of entomopathogens should be directed toward practical and feasible use of these organisms. Such development is vital to sustainable agricultural production. It is also an important role of scientific community to broadly communicate with the public by transmitting acquired knowledge.

Entomopathogenic microorganisms are here to stay. Their use will no doubt evolve as our needs in agriculture progress. Cautious and comprehensive ground work is, however, cardinal to safeguarding the well-being of a global ecosystem.

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