Microbial Control of Insect Pests: is it an effective and environmentally safe alternative?

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Abstract

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Entomopathogenic viruses, bacteria and fungi are currently used as alternatives to traditional insecticides. Its use should not be generalized because each pest has its own case. In specific cases, viruses proved very effective in managing populations of certain pests as for Lepidoptera and Hymenoptera forest pests in Europe and those introduced in forests in the USA and Canada; also, for controlling the cotton leafworm, potato tuber worm and greater wax moth larvae. They are specific to target insects and highly safe to mammals and the environment. Bacterial diseases like the Milky disease (*Bacillus popilliae*) successfully controlled the Japanese beetle grubs for 10 years after only one soil treatment. Each of the three subspecies of *B. thuringiensis* attacks larvae of a specific order, i.e., *B.t. kurstaki* for Lepidoptera, *B.t. israelensis* for Diptera and *B.t. tenebionis* for Coleoptera. All commercial products of *B.t.* are free from exotoxins that pose hazards to man and all organisms in the environment. Some fungi are effective microbial control agents against certain insect pests only under conditions of high R.H. and temperature that are available in glass houses. That is why fungi are also effective at tropical and sub-tropical areas as against cacao insect pests in Brazil. In some instances, fungi (*Beauveria bassiana*) cause allergy to man. They attack non-target insects, adult parsitoids and predators. Thus, fungi have low specificity and pose hazards to biodiversity.

Key words: Entomopathogens, BT toxins, non-target species, biological control.

Introduction

Insects are known since long ages to become infected with different entomopathogenic microorganisms that form an important factor of the natural mortality. In some cases, such pathogens were responsible for the natural outbreaks of specific epidemien among population of certain insect species. Thus, they could play a distinct role in the collapse of an insect population under certain conditions. This phenomenon inspired Bassi (5) to propose the idea of using the insect pathogenic microorganisms for controlling the agricultural insect pests. The first successful large scale microbial control application using coidiospores of the fungus *Metarhizium anisopliae* was carried out in the Russian Ukraine against the beet weevil, *Bothynoderes punctiventris,* (64), needed amounts of pure conidiospores were produced in the laboratory for this purpose.

In the last 50 years, microbial control of pests and plant diseases showed an amazing development associated with pronounced good results under optimized laboratory conditions, followed many times by disappointing results in the field applications. Thus, we need to understand the important concepts required to produce reliable, effective, and safe entomo-pathogens for microbial control. It was stated that "it is thus surprising that, while research directed to these major targets with a number of common goals, very little attention has previously been given to the integration of research effort (12). Disciplines such as pathology, genetics, physiology, mass production, formulation and application strategies are essential components in all three targets in making the necessary advances to enable an entomopathogenic microorganism to become registered and commercialized", and accordingly, to be an effective and safe mean in the microbial control arsenal. Following is a short overview pointing out the effectiveness and safety of the entomopathogenic viruses, bacteria and fungi as candidates for microbial control of insect pests.

Effectiveness of Entomopathogenic Viruses

Entomopathogenic viruses are obligate intracellular parasites having either DNA or RNA encapsulated into a protein coat known as capsid to form the virions or nucleocapsids. The latter, when found embedded in a protein matrices, then they belong to the termed group of occluded viruses like those of NPVs, CPVs and GVs. Nucleocapsids that naturally lack such matrices termed as free viruses (FVs). The occluded viruses of the families Baculoviridae, Reoviridae, and Entomopoxviridae could withstand abiotic factors of the surrounding environment. Meanwhile, free virus members of the Rhabdoviridae that are associated with their host all the time avoid such environmental factors being vertically transmitted from generation to another (87). Otherwise, the rhabdovirus degrades in less than a week if left unprotected in the environment (45).

The effectiveness of these viruses as microbial control agents depends on their persistence in nature and the mode of dispersal in the surrounding environment (2, 3, 29, 31, 76). The unique successful control of the coconut palm rhinoceros beetle, *Oryctes rhinoceros* with the virus, *Rhabdionvirus oryctes* discovered by Hüger (47) in Malaysia is attributed to the vertical transmission of the virus and its teratological effects (68). The infected adults secrete the virus into feces in feeding sites at the crown of coconut palms as well as in breeding sites in decayed palm logs in which they lay eggs, and thus infecting the next larval generation. Longevity of infected adults reached 30 days, among which they disseminate the disease to other individuals in the close agroecosystem (38, 47, 61, 62, 91, 92, 93), with the subsequent reduction in the level of damage (6, 7, 8, 51). Successful microbial control actions against this pest in different countries are shown in table 1.

On the other hand, successful control of many lepidopteran pest larvae with occluded viruses of the family Baculoviridae is mainly related to the induced horizontal dissemination by application techniques specially when applied with airplanes, and in some cases to the behavior of infected larvae in some forest pests. Some species of infected larvae move to positions high in the plant canopy, a behavior that facilitates the horizontal transmission of the virus through food contamination and light (88).

Effective pest control actions were recorded against lepidopteran and hymenopteran pests in many countries (29, 31). Fact is that the NPVs infect and replicate in different host tissues (fat bodies, hypoderm, trachea, and blood cells). In sawflies (Symphyta : Hymenoptera), NPVs infect only the midgut tissue. Such differences influence greatly the number of virions produced per infected host individual, affecting both the dynamics of horizontal transmission in nature and the economics of commercial virus production (88). In USA and Canada, the sawfly, *Diprion* (*Gilpinia*) *hercyniae,* was introduced from Europe and became a dominant forest pest.

By all means including use of the common chemical insecticides, the control of this pest was not successful. The microbial control with the virus, *Borrelinavirus diprion* was the only effective measurement that suppressed the pest population. Aerial applications of NPV-suspensions were carried out on large scales over the infected forests. The same action was repeated in 1950 in the same area against the sawfly, *Neodiprion sertifer*, which was also introduced from Europe. Both viruses were imported from Europe where they kept the two pests under control, and then they were mass-produced, formulated, and applied by airplanes over forests where they kept the populations of the two sawflies under control. Effective microbial control of other pests using commercialized entomoviruses is mentioned in Table 2.

Table 1. Introductions of *Rhabdionvirus oryctes* into populations of *O. rhinoceros.* (Updated)

Country	Year	Release method	Reference
W. Samoa	1967	Contaminating breeding sites	61
Tonga	1970	Contaminating breeding sites	91
Fiji	1970	As above and release of infected adult beetles	6
Wallis Island	1970	Contaminating breeding sites	39
Tokelau Island	1970	Contaminating breeding sites	86
Mauritius	1970	Contaminating breeding sites	68
Palau	1970	Contaminating breeding sites	86
American Samoa	1972	Release of infected adult beetles	86
Papua New Guinea	1978	Release of infected adult beetles	36
Maldives	1984	Release of infected adult beetles	93
Sultanate of Oman	2000	Release of infected adult beetles	51

Effectiveness of Entomopathogenic Bacteria

Insect pathogenic bacteria are present in the families Pseudomonadaceae. Entrobacteriaceae, Lactobacillaceae, Micrococcaceae and Bacillaceae. Based on safety to non-target organisms and humans, only the members of Bacillaceae (Order Eubacteriales) were the most studied, commercialized, and successfully used in microbial control of lepidopteran, dipteran and coleopteran insect pests.

The first microbial control treatment using an entomopathogenic bacterium was that against the grubs (larvae) of the Japanese beetle, *Popillia japonica*, a pest introduced from East Asia into North America. The Bacterium *Bacillus popilliae*, the causative agent of the milky disease was produced by injection into grubs collected from the fields. Processed formulations were applied in the field or turned into soil. The disease suppressed the pest population for more than 10 years after one application. The dead grubs became an infection source in soil for other healthy individuals. In fact, this unique successful example could be attributed to the presence of *B. popilliae* spores in soil far from the destructive effect of the sun rays (UV).

Table 2. Effective microbial pest control with virus preparations commercialized in Europe.

Pest insect	Virus Type	Trade Name	Company	Registered country
Adoxophyes orana	GV	Capex	Andermatt-Biocontrol AG	Switzerland
Agrotis segetum	GV	Agrovir	Saturnia-Copenhagen (R+P)	Denmark
Cydia pomonella	GV	Madex Granupom	Andermatt-Biocontrol AG Hoechst AG	Switzerland Germany
		Carpovirusine	Pforzheim (P) Calliope SA	France
Mamestra brassicae	NPV	Mamestrin	Calliope SA	France
Neodiprion sertifer	NPV	Monisärmi-ovirus Virox	Kemira-OY, Espoo (R+P) Microbial Resources Ltd	Finland UK
Spodoptera exigua	NPV	Spod-X	Birnkmann BV	Netherlands
Spodoptera littoralis	NPV	Spodopterin	Calliope SA	France

In Western Europe, the coleopteran *Melolantha melolantha* was also successfully controlled by *B. popilliae* var. *melolanthae*. *B. popilliae* is a highly specific entomopathogen; its use in Europe to control *M. melolanthae* was not successful.

The discovery of *Bacillus thuringiensis* by Dr. Ernest Berliner in Gemany in 1915 (8) drew the attention of Dr. Edward Steinhaus in California, USA, during the late 50s, *B. thuringiesis* (*B.t.*) was used on large scale applications successfully to control the larvae of the Luzerne moth, *Goliath philodice* in California (84). Since that time, many types of *B.t.* were isolated (9) and showed variable differences in efficacy against many lepidopteran, dipteran and coleopteran insect species, depending on the type of endotoxins and exotoxins produced by the *B.t.* isolate. The production of exotoxins limited the use of *B.t.* in the early 60s as it is a general non-specific toxin presenting hazards to human and environment. The selection of the new subsp. *B. thuringiensis kurstaki* strain HD-1 (Serotype H3a:3b) that does not produce exotoxins, launched the commercialization of this strain world wide (19). Thus, effective use of *B.t.kurstaki* was recorded by many authors all over the world for controlling different agricultural lepidopteran pests (81). For example, in the Arab countries, Egypt started application of *B.t.* in 1960 against young larvae of the cotton leafworm, *Spodoptera littoralis* in cotton. This pest was also successfully controlled in clover fields, *Trifolium alexandrinum* L. (22, 67). The efficacy of *B.t.* commercial products was highly increased by the addition of feeding stimulant (sugar or molasses) to the sprayed suspension (21, 23, 50, 56) or chitinase (81). The *B.t.* local product "Protecto" is widely used in Egypt for controlling *Ephestia* spp. in palm dates, and other lepidopteran pests on vegetables, field crops and grapes (67, 71, 82).

Hence the intensive use of *Bacillus sphaericus* induced resistance in larvae of mosquitoes (4, 80), the discovery of the subspecies *B.t.israelensis*, (35), a specific bacterium against dipteran larvae, posed new safe alternative to chemical insecticides used in water bodies for controlling mosquito larvae (1, 17, 25, 64, 69, 89).

In addition, the discovery of the subspecies *B.t.tenebrionis* (43, 44, 58), an effective and specific bacterium against coleopteran larvae, extended the host range of *B.t.* to pests of the order Coleoptera (58). Table 3 shows some of the commercialized *B.t.* subspecies and varieties registered by the Environmental Protection Agency (EPA), USA, for use and recorded an effective control against target insect pests.

Table 3. Some of the bacteria registered after approval of the EPA (USA)

The bacteria	The host
Bacillus popilliae Dutky *B. lentomorbus* Dutky	Japanese beetle larvae
B.thuringiensis subsp. *Kurstaki*	Lepidopteran larvae
B.thuringiensis subsp. *Israelensis*	Dipteran larvae
B.thuringiensis subsp. *Tenebrionis*	Coleopteran larvae
B.thuringiensis subsp. *san diego*	Coleopteran larvae
B.thuringiensis subsp. *kurstaki* strain EG 2348	Lepidopteran larvae
B.thuringiensis subsp. *kurstaki* strain EG 2424	Lepidopteran/coleopteran larvae
B.thuringiensis subsp. *kurstaki* strain EG 2371	Lepidopteran larvae
B.thuringiensis subsp. aizawa strain GC-91	Lepidopteran larvae
B.thuringiensis subsp. Aizawa	Lepidopteran larvae
B.sphaericus Neide	Dipteran larvae

Effectiveness of Entomopathogenic Fungi

There are more than 400 species recorded as entomopathogenic fungi from which only about 20 species with potential capability for use in microbial control of insect pests (95). Most of them are included in 11 genera, *i.e., Lagnidium, Entomophthora, Neozygites, Erynia, Ashersonia, Verticillium, Nomuraea, Hirsutella, Metarhizium, Beauveria, and Paecilomyces* (73).

The early successful application with an entomopathogenic fungus dated back to 1878 by the Russian scientist Metchnikoff (64) using *Metrhizium anisopliae* against the wheat leaf beetle, *Anisoplia austriaca* and the beet weevil *Cleonus punctiventris* in sugar beet fields (70).

Many field trials were carried out with entomopathogenic fungi to control agricultural insect pests (20, 27, 90), as well as insects of veterinary and medical importance. Most results are not satisfactory and few recorded an effective and successful control. The latter occurred mostly with certain fungi (*Verticillium lecanii, Beaveria bassiana,* and *Paecilomyces fumosoroseus*) used in glass houses against aphids, depending on the inside dominating high temperature and relative humidity (42, 47, 78). Other effective applications in open fields were only effective in areas and crops where high temperature and high relative humidity are present, *e.g.,* in tropical and subtropical countries. The use of *B.* bassiana and *M. anisopliae* in Brazil was very effective for controlling the cacao coleopteran and lepidopteran insect pests (30). In addition, fungi were effective microbial control agents in crops with vegetation contributes to the presence of high R.H. in the micro climate between plants as in sugar beet fields in Europe and northern Asia, or in clover fields in the Mediterranean countries (22). In the Pacific, *M. anisopliae* was successfully used in combination with the virus *Rhabdionvirus oryctes* for suppressing populations of the coconut palm rhinoceros beetle (6, 16).

In the Arab countries, mostly located in arid or semi-arid zones, small field trials were carried out to experiment the efficacy of different entomopathogenic fungi against different insect pests in several crops (26, 28, 63, 76). The low efficacy of entomopathogenic fungi in the open fields in the arid and semi-arid areas could be mainly related to the low levels of R.H., where germination and penetration of the conidiospores attached to the insect bodies require high R.H. (90–100%). This important factor will be more unsuitable for microbial control with entomopathogenic fungi in such areas among the next 5 decades due to the induced dryness of the ecosystem as side effect of the 50-years global "Shield Project" started in 2000 for decreasing the "Global Warming" phenomenon. When the clouds of aluminum oxide - (and barium salts) sprayed by jets in the stratosphere to reflect the sun heat in the outer space – settled down to the troposphere, the oxides grasp the air humidity turning into aluminum hydroxide and thus dryness of the ecosystem proceeds. Accordingly, the R.H. decreased and already reached 35-50% which is not sufficient for germination of the conidiospores of the entomopathogenic fungus.

Safety of Microbial Control

Safety of entomopathogenic microorganisms used in microbial control is a major concern associated with the increased needs for biocontrol agents desired in the exploitation of IPM programs for agricultural, veterinary, and medical insect pests (10, 11). Particular attention is paid to the following aspects by registration of the bioproducts based on microbes: 1) allergic properties, 2) risks of toxic metabolites, 3) genetic recombination and displacement of natural strains, and 4) effect on biodiversity, *i.e.,* on non-target organisms. These aspects have been covered in depth (59). In fact, most of the microbes used as biological control agents occur naturally in the different ecosystems (18), even in large densities when causing epizootics. It is worth to know that the general absence for infection of man with such microbes in the medical literature is an important evidence that these agents do not pose a significant human health risk (88), or for plants (13), fish and crustaceans (83), birds (53), mammals (77, 79), and non-target insects and mites (33, 74, 75). The exception is that fungi show les specificity posing risk to beneficials like predatory insects, parasitoids, bees and pollinators. The level of specificity required for safety depends on the characteristics of both the targeted area and the taxonomic groups of involved organisms. In fact, each case must be considered individually because actions that may be safe in one habitat may be undesirable in another (15, 40, 46).

Safety of Viruses

A literature summary concerned with safety of entomopathogenic viruses to human and environment was published (85, 88). It reported that the majority of viruses which have been considered for use in pest control are either NPVs or GVs of the family Baculoviridae that are specific to arthropods. Their safety was tested against more than 24 vertebrate species including different species of mammalian, avian, and fish. None of these viruses was able to cross infection to any of the tested animals (11, 38, 72).

Concerning safety to beneficial arthropods, immature larvae of parasitoids in infected hosts may die not due to virus infection, but relatively to premature loss of the host or to alteration in quality of the host (34).

Entomopathogenic viruses are typically limited in their host range such that only species in one genus or in related genera in one family are infected (85). Thus, not only the distantly related types of invertebrates but also vertebrates are not at risk from virus applications (34). The NPV-*Autographa californica* with the widest known host range (34 lepidopteran species) proved safe to beneficials and environment. Similar results were recorded for the CPV commercialized for use in Japan; one of them has a host range broader than most NPVs. The NPV infecting the lasiocampid larvae *Dendrolimus spectabilis* Butler infects larvae in several genera of Lepidoptera (88).

Safety of Bacteria

In the very early stage of using *B .thuringiensis* in the 50s, there was a concern for its toxicity to mammals (31, 32, 37) and non-target organisms (54, 55, 57) due to the presence of the exotoxins produced by the vegetative cells either in the production medium or in the body of infected lepidopteran larvae remain in the ecosystem. Thus, some concern has been raised in the past about potential *B.t.* contamination of honeybee (54, 57), and of drinking water supplies by exotixins in Germany due to its close relation to *B. cereus*, a bacterium implicated in food poisoning in humans (9, 37, 41, 60) .The picture is completely different in the last 3 decades, since the discovery of the isolates that do not produce exotoxins which are currently the base for commercial production of *B.t.* (mostly *B. t.* subsp. *kurstaki*) in different countries. The literature is very rich with results proving the safety of *B.t.* and *B. sphaericus* to mammals and non-target organisms (*e.g*., 18, 32, 37, 62, 66, 77, 79). The infected lepidopteran prey could affect the predatory insects (24).

The silkworm, *Bombyx mori*, and immature parasitoids inside target pests are likely to be killed if exposed to *B.t* (72). Data on such effects are recorded (34, 74, 75, 82). The prohibited use of *B.t.* as microbial pest control agent in India at the areas of silk production (71) is an understandable special case to avoid infection of larval colonies mass reared on the mulberry trees in open fields.

Also, when *B.t.* subsp. *israelensis* was applied to aquatic systems, it showed little effect on non-target invertebrates, with the exceptions of those in the dipteran families Chironomidae, Dixidae, and Ceratopognidae that were killed (34). Some effects were also recorded on Ephemeroptera and Odonata, but much less than that caused by application of chemical pesticides in the same circumstances (94). It could be related in case of *B.t.i.* to suppression of mosquito larval population as prey for immatures of Odonata (94).

Safety of Fungi

Most of the entomopathogenic fungi developed for commercial use in microbial control of insect pests showed no infectivity to man or other vertebrates (30, 72). Safety tests with *Nomuraea rileyi* (48, 49), *Hirsutella thompsonii* (65), *Verticillium lecnii* (72) and *Lagenidium giganteum* (53) assured negative findings to different mammalians and birds (52). On the other hand, *Beauveria bassiana* has been reported to cause allergies in humans (90) and is at least an opportunistic pathogen to man and other mammals (10).

Concerning non-target invertebrates, high mortality appear when they contacted or ingested spores of the entomopathogenic fungi. Larvae of the coccinellid *Cryptolaemus montrouzieri* suffered 50% mortality when fed Boverin-t, a commercial conidiospore preparation of *B. bassiana* (34). Honey bee workers experienced 29% mortality when fed spores of *H. thompsonii* (14). Both *B. bassiana* and *Metarhizium anisopliae* infect *B.mori,* and also killed honey bees following field applications (72). Besides, the parasitized hosts of some species showed increased susceptibility to entomopathogenic fungi and those populations of some over wintering predacious carabid beetles and other invertebrates are killed by fungi showing an increased risk if large amounts of fungal inoculums are added to soils as a consequence of agricultural microbial pest control (34). Infection of different beneficial natural enemies were recorded (27, 28, 48), *e.g.*, adults of the braconid parasitoid *Apanteles* sp., the coccinellid predator *Cydonia vicina isis*, the earwig *Labidura riparia*, and the syrphid fly *Syrphus corollae* in fields of sugar beet treated with conidiospores of both *B. bassiana* and *M. anisopliae.*

الملخص

الحسيني، منير. 2006. المكافحة الميكروبية للآفات الحشرية: هل هي بديل فاعل وأمين بيئياً؟ مجلة وقاية النبات العربية. 42: 162-169.

تستخدم بعض الفيروسات والبكتريا، والفطور الممرضة للحشرات كبدائل لمبيدات الآفات التقليدية في المكافحة الميكروبية للآفات الحشرية. ولايجب تعميم إستخدامها، إذ لكل آفة حالتها الخاصة. وقد أثبتت حالات محددة نجاح وفعالية الفيروسات الممرضة للحشرات في مكافحة بعض آفات أشجار الغابات من حرشفيات وغشائيات الأجنحة في أوروبا والمدخلة منها الى أمريكا وكندا، كذلك فى مكافحة دودة ورق القطن، فراشة درنات البطاطس/البطاطا، ودودة الشمع الكبيرة. وهذه الفيروسات متخصصة على الحشرات المستهدفة وجدّ أمينة على الثدييات والبيئة. كذلك أثبتت البكتريا *Bacillus popilliae* نجاحاً كبيراً في مكافحة الخنفساء اليابانية بمعاملة واحدة للتربة إمتد تأثيرها لعشرة سنوات متتالية، ويختص كل من تحت الأنواع الرئيسية الثلاثة للبكتريا *B. thuringiensis* فى إصابة يرقات رتبة محددة حيث يختص *B.t. kurstaki* بحرشفيات الأجنحة، *B.t. israelensis* بذات الجناحين، *B.t .tenebrionis* بغمديات الأجنحة. وتعتمد المستحضرات التجارية على الأنماط التي لا تنتج السم الخارجي exotoxin لأنه سم عام غير متخصص يهدد الإنسان وكافة الكائنات في التربة وبالتالي فهوغير آمن بيئياً. أما بالنسبة للفطور، فيقتصر استخدام بعضها تحت ظروف الرطوبة العالية والحرارة اللازمتين لإنبات الأبواغ/الجراثيم الكونيدية والتي تتوافر في الزراعات المحمية تحت الفيئات الزجاجية لمكافحة المنّ والتربس والذباب الأبيض. كما ينجح إستخدامها ضد الآفات الحشرية فى المناطق المدارية وتحت المدارية كما فى مكافحة آفات الكاكاو فى البرزيل. وقد تسبب بعض الفطور حساسية للإنسان، ونظراً لعدم تخصصها فهى تصيب الحشرات غير المستهدفة مثل المتطفلات والمفترسات الحشرية البالغة تحت ظروف التطبيق الحقلى، والفطور ذات تخصص ضعيف وقد تشكل خطراً للتنوع الحيوي.

كلمات مفتاحية: ممرضات، سموم BT، أنواع غير متخصصة، مكافحة حيوية، مدى الأمان

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