RECENT ACHIEVEMENTS IN MICROBIOLOGICAL PLANT PROTECTION

Leonid Voloșciuc, Boris Pânzaru, Natalia Lemanov, Arcadii Nicolaev, Tatiana Șcerbacov, Svetlana Nicolaev, Pantelimon Zavtoni, Liviu Moraru

Institute of Genetics, Physiology and Plant Protection of the ASM

Introduction

Plant pests need to be controlled to maintain the quantity and quality of food and feed produced by growers around the world. Different approaches may be used to prevent, mitigate or control of plant pests. Beyond good agronomic practices, growers often rely heavily on pesticides that have contributed significantly to the improvements in crop productivity the past years [6, 8].

However, the environmental pollution caused by use of pesticides, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture. Under such circumstances exploitation of living organisms to reduce the activities of crop pests causing activity of useful micro-organisms seems to be the most appropriate alternative to chemicals. Biological control refers to the purposeful utilization of introduced or resident living organisms to suppress the activities and populations of one or more plant pest [4, 6].

There are the following trends in bio-pesticides development:

» Bio-pesticides are not new they are in use since ages but grower/ industry acceptance happened in last 7 – 8 years.
» Global bio-pesticide market was $ 1.3 billion in 2011, and 63 % of it was microbial basis active ingredients.
» Projected to grow to 3.2 billion by 2017 (15.8 %).
» North America dominates bio-pesticides market with share of 40 %.
» Asia pacific and Europe are expected to be fastest growing market in near future due to stringent regulations for pesticides and increasing demand for organics.
» Overall bio-pesticides represent 2 % of pesticides market.
» Growth is hindered by well established crop protection chemical market, variable efficiency of bio-pesticides, less awareness among growers.

The overwhelming advantages of bio-pesticides are their high selectivity to target pests and safety to non-target and beneficial organisms. In the sustainable intensification of agriculture through green economy, the biopesticides have an immense role. They are amenable to bio-intensive pest management and ideally suited for organic niche products including export-oriented commodities. They can also be tailored to IPM programmes for increased efficacy, higher yield and lower chemical load. These are also effective as pesticide resistance management tools in order to prolong the life span of precious green chemical pesticides. The biopesticide development must also be targeted for integrated cropping systems. They are renewable, sustainable, offer an improved impact profile, and reduce pesticide residues.

Materials and methods

The ultimate success of bio control depends on how well the searching and screening process is done. There is no single way to search or screen. Both depend on the target pathogen, the crop and the cropping system. These isolates are then screened for their activity against the pathogen in laboratory and green house conditions. Any suitable isolate found are then evaluated for their efficacy under the field conditions [8, 9].

The isolates that fail to perform well in the field are again subjected to the in vitro evaluation to ascertain the cause of their failure before they could be rejected.

Testing in laboratory and field experience was carried out in four repetitions ran-

domized respectively, in accordance with the general requirements of experiences of this kind [10].

Results and discussions

The role of useful microorganisms in the biological protection of plants. Plant protection has immensely contributed to the success of Green Revolution and sustained production of food and feed. Due to intensification of

agriculture, loss of biodiversity, reliance on monocropping, and biotic stresses due to pests and pathogens have increased and led to several problems like pesticide residues in food stuff, environmental pollution, disorder of ecological equilibrium. For these reasons, management of pests will continue to play a pivotal role in sustaining production and productivity in agriculture [3, 4].

United States Environmental Protection Agency (EPA) for characterizations of microorganisms, whether indigenous or introduced are an important component of the environment, have proposed the following classification of Bio-pesticides as certain types of pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals:

1. Microbial pesticides – consist of micro-organism (fungi, bacteria, viruses and protozoa) as active ingredient.
2. Biochemical pesticides – plant extracts, pheromones, soaps and fatty acids, natural plant growth regulators. Avermectin, Pyrethrins, Spinocid from natural products but not bio-pesticides.
3. Natural enemies – parasitoids, predators and pathogens of pests.

Bio-pesticides use is directed in:

» First deployed on speciality high value crops, vegetables and greenhouse crops to manage residue.

» Now they are being applied on all type of broad acreage crops (cereals, oilseed, sugar, fiber, forage grains).

» To date 400 plus active ingredients have been registered across globe and 1250 plus products based on these active ingredients have been registered.

» Products based on various *Bacillus thuringensis* strains dominate the market.

» Other major products are based on *Beauveria, Metarhizium, Trichoderma, Bacillus subtilis, Pseudomonas flourescens* and entomopathogenic NPV.

Commercialization of biological preparations. Although the number of bio control products in plant disease management is increasing, these products still represent only 1% of the agricultural control measures while fungicides account for 15% of total chemicals used in agriculture [1, 2, 5]. In recent years many small and large entrepreneurs have entered into the commercial production of bio control agents resulting into the entry of various bio- control products into the world market.

Commercialization of bio-control products is a multi-step process involving a wide range of activities [5, 6, 7]: isolation of micro- organism from the natural ecosystem;

• Evaluation of bio-agent both in vitro and under glass house conditions; testing of the best isolate under field conditions; mass production; formulation; delivery; compatibility, registration and release.

Although the development and approval of a biological preparation activities are needed expensive long, though the world were recorded several biological means (table 1).

Recent Achievements microbiological plant protection in Moldova. Based on the severity of the phytosanitary issues, caused by the action of pests on the background of worsening ecological situation resulting from the application of pesticides to combat them, now the need to develop alternative means of plant protection increases, among which a more important one being the biological products made of various useful microorganisms. Microbiological protection recorded remarkable results in controlling

various pests (pathogenic agents of diseases, insect and mite pests, and weeds), permanently extending the range of the useful agents that are used, as well as the spectrum of the protected crops. There is a growing awareness that microbial pesticides are inherently different from chemical pesticides, with fundamentally different modes of action and that they should therefore be assessed on their own merits and problems and data requirements should be set accordingly. However, the basis for the proposed data requirements needs improved scientific justification.

Table 1. List of bio control products

No.	Bio control agent	Product	Target disease/ organism	Crop	Manufacturer
1	*Ageobacterium radiobacter* strain 84	Galtrol	*Agrobacterium tumefaciens*	Ornamentals, Fruits, Nuts	AgBioChem, USA
2	*Ageobacterium radiobacter* strain K 1026	Nagol	*Agrobacterium tumefaciens*	Ornamentals, Fruits, Nuts	Bio-care
3	*Bascillus subtillus* strain GB34	GB34	*Rhizoctonia, Fussarium*	Soyabean	Gustafon, USA
4	*Bascillus subtillus* strain GB03	Kodiac, companion	*Rhizoctonia, Aspergillus*	Wheat, barley, peas	Growth products, USA
5	*Pseudomonas aureofaciens* strain TX-1	Bio–jet, spot less	*Pythium, Rhizoctonia solani*	Vegetables and Ornamentals in green houses	EcoSoil system
6	*Pseudomonas fluorescence* strain A506	Frostban	Fire blight, bunch rot	Fruit crop, Tomato, Potato	Plant Health Technologies
7	*Streptomycine griseoviridis*	Mycostop	Soil borne pathogens	Ornamentals, Tree seedlings	Kemira Oy, Finland
8	*Trichoderma harzianum* T-22	Root shield, plant shield	Soil borne pathogens	Green house nurseries	Bio works, USA
9	*Trichoderma harzianum* T-39	Trichodex	*Botrytis cinerea*	Most of the food crops	Bio works, USA
10	*Ampelomyces quisquallis* isolate M-10	AQ10	Powdery mildew	Fruits, Ornamentals, Vegetables	Ecogen, USA
11	*Aspergillus flavus* AF36	Alfa guard	*Aspergillus flavus*	Cotton	Circleone globa,USA
12	*Gliocladium catenulatum* strain JI446	Prima stop soil guard	Soil borne pathogens	Vegetables, Herbs, Spices	Kemira Agro Oy, Finland

The scientists of the Institute of Genetics, Physiology and Plant Protection of Academy of Sciences of Moldova, by isolating, identifying and determining biological particularities of various useful microorganisms (viruses, bacteria and fungus) have developed original technological procedures of production and application and submitted for approval some biological preparations effective in controlling pests with the most severe impact on crops. These were submitted for approval or extension of the scope of use of National Council for Approval of Products for Phytosanitary Usage and Fertilizers.

Baculoviral preparation Virin-HSP was elaborated to fight *Helicoverpa armigera* which in recent years recorded expanding the area of spreading, as well as the spectrum

of attacked crops. The product is made on the basis of nuclear polyhedrosis virus with a high degree of specificity of the insect host and has titer of 6 billion polyhedra/g in the form of paste. The preparation has specific action on insects and causes noctuide epizootic phenomena acting on subsequent generations and protecting crops. In the Republic of Moldova it is recommended to protect vegetables (tomatoes, peppers), technical crops (sugar beet, sunflower, and tobacco), and cereals (corn), decorative and medicinal plants. Consumption norm – 0,2 kg/ha.

Paurin - contact bactericide obtained under bacterium *Pseudomonas fluorescens* BKM CP 330 D expected to combat the pathogen *Agrobacterium tumefaciens* Sm. fnd Town. in orchards and grapes, as well as root rots in vegetable crops, soybeans (*Fusarium gibbosum, Rhizoctonia solani, Pytium debaryanum, Alternaria sp., Penicillium sp., Aspergillus sp*.) and in potato (*Fusarium solani, Pectobacterium carotovorum*).

Trichodermin Th-7F SC - fungicide constituted under *Trichoderma harzianum* strain Th-7F (CNMN F-16) expected to combat pathogens in vegetable crops (*Rhizoctonia solani Kuechn, Botrytis cinerea* Pers, *Sclerotinia sclerotiorum* de Bary, *Myrothecium verrucaria, Ascochyta cucumis* (*melonis*) Fautr. Et Roum, *Colletotrichum lagenarium* E. et H., *Fusarium spp., Streptomycetes, Pythium debaryanum* Hesse); decorative (*Rhizoctonia solani* Kuechn, *Botrytis cinerea* Pers, *Sclerotinia sclerotiorum* de Bary; *Fusarium spp., Verticillium dahliae* Kleb.); tobacco (*Fusarium spp., Vericillium dahliae* Kleb., *Thielaviopsis basicola* Ferr, *Pythium debaryanum* Hesse, *Botritys cinerea* Pers); grape-vine (*Botrytis cinerea*) Pers.

Trichodermin SC proposed as liquid fungicide constituted under *Trichoderma lignorum* strain M-10 expected to fight pathogens in sunflower (*Sclerotinia sclerotiorum*), soybean (*Fusarium spp.*), grape-vine (*Botrytis cinerea*), and ensuring high biological, economic and ecological effectiveness. The preparations enhance also the biological indicators of crop development.

Gliocladin SC - fungicide obtained on the basis of the active substance of *Trichoderma virens* strain 3X, expected to fight white rot in sunflower (*Sclerotinia sclerotiorum*), soybean (*Fusarium spp*.), grape-vine (*Botrytis cinerea*). The preparation ensuring high biological, economic and ecological effectiveness.

Prospective directions of crop production by applying useful microorganisms

Induced Systemic Host Resistance. Induced resistance is the most indirect form of antagonism. Induced resistance can be local or systemic. Salicylic acid (SA) and non-expressor of pathogenesis-related genes1 (NPR1) are key players in systemic acquired resistance *Trichoderma harzianum* when inoculated on to roots or on to leaves of grapes provides control of diseases caused by *Botrytis cineria* on leaves spatially separated from the site of application of the bio control agent. Many classes of compounds are released by the *Trichoderma sp*. into the zone of interaction and induce resistance in plants. The first class is proteins with enzymztic or other activity. Fungal proteins such as xylanase, cellulases and swollenins are secreated by *Trichoderma* species. *Trichoderma endochitinase* can also enhance defense, probably through induction of plant defense related proteins [1, 8].

Other proteins and peptides that is active in inducing terpenoid phytoalexin biosynthesis and peroxidase activity in cotton, e.g., the small protein, SM1, which has hydrophobin-like properties, were found to be produced by strains of *T. virens*. Another hydrophobin-like protein produced by T22 that induces both enhanced root development and disease resistance was identified. Another group of proteins that induce defense mechanisms in plants are the products of avirulence-like (Avr) genes.

Infectious diseases of insects. Infectious agents are living units that must invade the insect host in order to initiate an infection. Unlike parasites and predators, pathogens do not always kill the hosts. Infection usually involves reproduction of the agent. The specific characteristics of the infective stages of pathogens greatly influence how

they contact and infect their hosts. The infectious agents responsible for transmission of the pathogen are susceptible to many environmental factors. Survival of the infective stage of insect pathogens outside the host is a major factor in the development of microbial insecticides. Some pathogen species may be very host specific, while others may be able to infect a wide range of insect species. The host range of a pathogen is especially important when considering a non-indigenous pathogen for introduction into a new habitat. Sub-lethal infections are not uncommon and these may include behavioural and developmental changes as well as a decrease in the fecundity of infected adults. Insects are infected by an incredibly large number and diversity of pathogen species. Most insect pathologists believe that there are actually more species of insect pathogens than there are species of insects. The major pathogen groups containing species that infect insects are: viruses, bacteria, fungi, protozoans, microsporidia, and nematodes.

Insect pathogens are used in biological control in at least three different ways: inundative applications, inoculative releases, management of naturally occurring pathogens, and introduction of exotic pathogens as classical biological control agents.

• Inundative applications are those in which insect pathogens are applied in large quantities with the goal of killing as many individuals of the pest population as quickly as possible. Replication of the pathogen in the host and production of additional infectious propagules may be desirable, but is not usually required for microbial insecticides to be effective.

• Inoculative applications are those in which small quantities of insect pathogens are applied or released into an insect host population. The goal is to produce infections in at least a few hosts, which will, in turn, produce numerous infectious propagules that will infect many more susceptible hosts.

• Management of naturally occurring pathogens are important components of the natural enemy complex of many insect species, including pest species. Some groups of pathogens, such as microsporidia, may not always maintain host insect densities below economic thresholds, but they suppress the rapid increase of pest populations. Insects pathogens and are often responsible for the decline of populations that have exceeded the economic threshold. In most cases the major goal for managing naturally occurring insect pathogens is to elicit an epizootic earlier in the season, before the host densities have exceeded the economic threshold. This can be accomplished by inoculative releases of the pathogen or by changing cultural and phyotosanitary practices to promote an epizootic.

Conclusions

Biological control is the best alternative to pests suppression. Bio agents themselves being non-pathogenic to plants need to be formulated in a way that favors the activity and survival of microbe it contains.

The pace of biopesticide research is increasing. From the beginning of 2006 through early April 2015, 4 biological preparations had been registered with National Council for Approval of Products for Phytosanitary Usage and Fertilizers. Many of these are agriculture related and registered for use as such. The value of microbiological means of protection developed by the scientists of the institute does not consist just in their considerable biological, environmental and economic effects, but also their possibility to be included in conventional and organic farming systems.

Achieving maximum efficiency of biological preparations may be registered at the establishment IPM, which is a kind of management using different strategies and techniques such as cultural, biological and chemical in controlling insect pests and diseases in agricultural crops.

In further research and development on biopesticides must be given high priority and agriculturists in general and policy makers in particular must be educated about the dangers of handling and application of chemical pesticides, and importance of sustain-

able agriculture to feed ever growing population.

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