

Magdalena JAWORSKA¹

SOME RESEARCH PROBLEMS IN MODERN PESTICIDE MANUFACTURING

NIEKTÓRE PROBLEMY BADAWCZE W PRODUKCJI NOWOCZESNYCH PESTYCYDÓW

Abstract: Efficient plant protection involves the application of all methods available within the IPM (Integrated Pest Management) programme. The chemical plant protection method is undergoing considerable transformations since not only new efficient biologically active substances are sought but the requirements concerning their biological safety for non-target organisms and the whole environment have increased. No success is possible without a proper functional form. New formulations and microcapsules, oil dispersions or agrogels should be biodegradable and cannot pollute the environment. Scientific research aimed at seeking substances enhancing the pesticide effect, such as capsaicine, which is a natural component of pepper (paprika), increasing toxicity of some insecticides. Environment-friendly preparations are products based on chitosan destruction, useful particularly for the protection of organic crops and valuable ornamental plants against diseases. Biotechnological methods are used in Poland and in the world to develop biopesticides, such as preparations containing new species and strains of live entomopathogenic nematodes for plant protection against pests.

Keywords: chemical pesticides, biopesticides, environment protection

Insects (*Insecta*) constitute the most numerous phylum among the arthropods (*Arthropoda*). To this day over 2 million insect species have been identified and new ones are discovered and described every year.

Simultaneous evolution of insects and plants, lasting for over 300 million years formed a strict interdependence between these organisms. It is particularly apparent between flower plants and pollinating insects, which have a specialized sucking and licking mouthparts which enables them to suck up nectar. Insects – plant pests have specialized mouthparts of the chewing type (capable of crushing hard terrestrial vegetation), piercing-sucking type for piercing plants and sucking up saps and other modifications.

About 50 % of insect species are phytophages. Accompanying the humans from time immemorial they provide serious food competition and make people seek various meth-

¹ Department of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31-120 Kraków, Poland, email: rrmjawor@cyf-kr.edu.pl

ods of protection. These were different, starting from mechanical measures through biological ones (eg the use of ants in ancient China), the use of plant extracts to prayers and spells, and even court sentences. These endeavours usually proved little effective, which was also due to limited technical abilities and isolation between human communities.

Pesticides were first used successfully on a wide scale only in the second half of the 19th century. At first they were inorganic compounds with a strong effect, such as lead arsenate, calcium arsenate and fluorine compounds, which at present have only historic importance as insecticides of the first generation. This group also comprised preparations of plant origin with contact activity such as pyretrum and nicotine used as a tobacco concoction.

A rapid development of pesticides started with production of synthetic preparations. In 1942 insect killing properties of DDT were discovered and soon after this compound initiated a new era in plant protection. In 1946 mass application of organic phosphorus insecticides started, which still make up a considerable share, and then the next II generation preparations were introduced, such as carbamates and pyrethroids. III generation insecticides (antifeedants, hormones and feromones) and IV generation insecticides (antihormones) appeared in the seventies.

Insecticides currently in use make up the smallest group of plant protection means applied in Poland; however, their role is still very important (eg in fighting potato beetle, rapeseed pests or orchard pests).

They are also present on farms as household chemicals used for sanitary and veterinary hygiene.

Organic compounds of anthropogenic origin, including also crop protection chemicals pose the main threat not only to human health but also to flora and fauna. The important role of plant protection chemicals results mainly from the wide scale of their application, their persistence in the natural environment and toxic properties. Diminishing the environmental impact of plant protection chemicals is one of the objectives assumed by the European Union in the Sixth Environment Action Programme of the European Community 2002–2012 (EAP6) (Decision No. 1600/2002/WE of the European Parliament and the Council adopted on 22nd July 2002). Based on four priorities, the programme made the European Commission responsible for the preparation of seven Thematic Strategies, including sustainable application of pesticides. The subject belongs to priority 3 EAP6 (“Environment and Health and Life Quality”), which sets the following goals:

- minimizing hazards and threats to human health and the environment resulting from pesticide application;
- decreasing levels of active harmful substances, particularly through replacement of the most dangerous substances by safer alternatives
- promoting farming systems using small amounts of pesticides or pesticide-free plantations.

Achieving these goals is possible only by introducing new active substances or developing new pesticide preparations (functional forms, formulations).

The number of biologically active substances applied has been decreasing systematically, which is connected with very high costs of research, especially concerning the

assessment of the impact of these compounds on the environment and human health. Currently, the total costs of developing a new, original active substance which requires between 30 and 50 thousand synthesis reactions is 120 million dollars.

Pesticide biologically active chemicals are generally organic compounds revealing considerable biological activity and relatively small water solubility. Moreover, they are able to penetrate insect or plant cuticle to some degree. For these reasons they are used for plant protection only as the right functional forms called pesticide preparations. The time which elapses from synthesis of a biologically active substance to marketing its commercial preparation is ca 10 years. It is so because the manufacturer must properly select the components to ensure the stability of biologically active pesticide substances during storage and to enable obtaining stable dispersions in the fluid phase during spraying. According to the law in force, the manufacturer of a plant protection chemical is obliged to submit to the Plant Protection Institute detailed documentation of the chemical he manufactures, comprising among others results of tests on behaviour and decomposition of the biologically active substance in soil, water and air together with the results of an ecotoxicological test, comprising data on acute toxicity for fish, daphnias and algae.

Measures undertaken to reduce the environment pollution, mainly of surface and groundwaters, should start at the stage of developing proper functional forms of the preparation. Scientific research on both the stability of a biologically active substance and its toxicity but also on paths of degradation, sorption properties and identification of potential ecological side effects should concern preparations including the effect on the above-mentioned agents applied in the process of adjuvant formulation.

Therefore, efficient plant protection does not only involve the application of a proper biologically active substance but its functional form is also important. Under conditions of increasing requirements for modern plant protection a necessity arises to create new improved variants of well-known types of formulations and new concepts of the effect which the preparation components have on active substance behaviour in various compartments of the environment. It is anticipated that the obtained insecticide preparations will reduce their dose and widen the spectrum of the optimal temperature of the preparation application. Results of testing the effect of various surficially active compounds on the processes of mass exchange in modified dispersive systems of modified forms of EC and WP insecticides from pyrethroids group will be a basis to obtain new formulations of these compounds as microcapsules, agrogels and oil dispersions [1, 2].

Preparation of appropriate formulations as products ready for sale is mainly the task of relevant departments of large chemical plants. Therefore, a great amount of data concerning technologies of formulation exists as patents [3].

The available literature contains a number of papers confirming greater persistence and toxicity of active substances used as preparations in comparison with the same properties determined for a pure active substance. It is also known that application of various types of oil adjuvants causes intensification of active substance durability and considerably contributes to decreasing the environmental pollution level.

So, scientific research aims at developing functional forms of plant protection chemicals which would be less harmful for the natural environment and human health. At this point a reference should be made to more efficient pest control in agriculture and

diminishing the amount of applied active substances, which is an important factor in multifunctional development of agriculture, especially organic farming.

Because of an increasing quantity of chemical insecticides accumulating in the environment and growing pest resistance to some of them, particularly to pyrethroids, new substances which would support their effect are sought intensively. Such synergistic substances include, among others, capsaicine, a natural component present in pepper. It affects insect thermoreceptors diminishing the efficiency of their work, meaning that insects do not feel threatened by temperature. Therefore it may be assumed that capsaicine and its chemical analogues disturbing the insects natural physiological mechanism leading to an increase in the insecticide toxicity, as was demonstrated on an example of potato beetle larvae [4]. It has been common knowledge for over 40 years that the model of ionic balance, described in the physiology handbooks, causing depolarization in neuron and formation of action potential, is not the only model occurring in the whole nature. Because it has been shown that in different insect taxonomic groups, genesis of both rest and action potential is different and associated with the presence of some determined channels in the membrane, a xenobiotic affecting a determined kind of ionic channels will differently affect an insect in which the importance of these ionic channels is crucial and the one where these membrane channels are of lesser importance. This fact provides a basis for a selective, for some insect groups, insecticidal effect of pyrethroids, particularly indenooxadiazines [5].

Nowadays, plant protection abandons purely chemical pest control and more often uses biological methods – Ecologically Based Pest Management (EBPM) and Integrated Pest Management (IPM) [6]. Recommendations and requirements of Good Agricultural Practice concerning application of plant protection chemicals are legally based on the Act on Crop Protection. Meeting these requirements is most important not only from the perspective of efficacy and efficiency of plant protection measures, but even more important from the environment protection and human health viewpoint (Ministry of Agriculture and Rural Development, 2005). However, while introducing biological preparations containing live entomopathogenic nematodes to crops, one should consider their impact on biodiversity. One of the main hazards to biodiversity is penetration of alien species (including their accidental or planned introduction), which often causes the competitionally inferior native species to drop off from the contest. Therefore, biological plant protection should necessarily apply the preparations manufactured on the basis of local species. The nematodes most frequently isolated from soils in Poland include: *Steinernema feltiae*, *S. affinis* and *Heterorhabditis megidis* [7]. Introduction of nematodes as biopreparations to crops may efficiently protect crop production reducing economic losses with a simultaneous decrease in applied chemical pesticides.

Biological methods may not only reduce a large pest population, but also maintain the pest population on a low level, safe for the crops. It is most important, since small numbers of insects settling individual niches prevent a much more dangerous invasion of other agrophagous species [6].

Entomopathogenic nematodes and their symbiotic bacteria are safe for the environment, mammals and other organisms which are not their aim [8–10].

Another crucial asset of these organisms is the morphological and physiological ability of infective juveniles (L3) to adapt to stressful conditions in soil: low temperatures,

low moisture, pH, pollutants [11–16] but also long survival period of larvae without the host-insect [17]. In overdried soil an infective juvenile may survive in moisture below the level causing plant withering [8]. *H. bacteriophora* larvae resistance to pressure of 2000 kPa (290 psi) or *S. carpocapsae* and *H. megidis* resistance to the pressure of 1380 kPa (200 psi) is another asset of these organisms [18]. Owing to these properties they may be applied using the same equipment as for chemical measures: sprayers with mesh diameter bigger than 50 µm, helicopters or irrigation systems. Nematodes can also be applied using injection nozzles and pumps, which are the standard equipment for pressure application.

Moreover, nematode potential is not usually weakened when chemicals are used at the same time [19].

Nematodes have been cultured for over 70 years [8] and currently they are cultured on a large scale using three methods: *in vivo*, *in vitro* on solid media and *in vitro* in liquid media. Each of these methods has its advantages and disadvantages influenced by production costs, required technical skills or product quality. Moreover, each of them reveals potential for development. However, reduction of production costs through optimization of parameters and factors affecting final efficiency of nematodes are the most important.

Present biological substances containing *Steinernema* and *Heterorhabditis* nematodes are manufactured on a large scale by numerous firms in the world [17, 20, 21]. From among many species belonging to these two families, only 6 of them are used for commercial production.

Primary nematode lines are kept in liquid nitrogen until the start of production. The next stage is activation of these organisms when the nematodes in cuvettes are placed for 18 days in incubators where the temperature required for individual species is maintained. Initiating of the nematode reproduction process lasts for the subsequent 18 days and for this reason they are placed in cuvettes containing plant fermentation products (the composition is secret). The subsequent 18 days are devoted to final production in two containers of 6 thousand liter volume and two 40 thousand liter ones. Proper medium pH values, pressure and temperatures are maintained. Because of growing demand for that product BU, an additional container, 40 thousand liter volume, is planned for the next year. The whole production process is conducted in sterile conditions and the process is fully computerized. Proper functioning of the apparatus is monitored by four persons. The next stage involves filtering and washing the whole mass filling the containers in order to obtain a pure product containing 100 % of nematodes, whose vitality and infectivity are controlled in a laboratory. If the product meets all required quality standards it is placed together with the substance protecting these organisms against moisture loss, in unit packagings. The packed nematodes may survive at 2 °C for about two months. No more than 24 hours elapse from the moment of placing an order (by a farmer) for a nematode batch to its realization (supply to the farmer) [22]. Production of biopreparations with live nematodes is conducted also in Poland by “Owiplant” Horticultural enterprise on the basis of native nematode species. The only Polish preparation Owinema containing *Steinernema feltiae* nematodes was obtained on solid medium.

Entomopathogenic nematodes (EN) are alternatives for chemical control of sciarid flies, especially on mushroom farms. There are in Poland 8–9 week cycles of mush-

room production and over such a long period chemical pesticides are ineffective. In 1998 on two Polish mushroom farms in Krakow and Rzeszow production was protected from sciarid flies using EN: commercial biopesticides “Entonem” (Dutch), “Nemasys” (English) and “Owinema” (Polish selected strain of *Steinernema feltiae*) [23]. Chemical standard pesticides: teflubenzuron, Nomolt, and diflubenzuron, Dimilin, were used for comparison. On both mushroom farms sciarid flies, *Lycoriella solani*, were the main trapped insect pests. Biological protection of mushroom against sciarids was improved. Mean yield was increased from 1.10, when chemical protection was applied, to 2.20 kg/m². The index of treatment profitability, the index of cost coverage and the index of percentage refund of expenditures of treatment, were the best on treatment with Polish “Owinema” biopesticide. The cost of sciarid’s control by “Owinema” was the lowest (0.48 PLN/m² 3.9 PLN = 1US\$). The data confirmed the suitability of EN commercial products as an alternative for chemical mushroom protection against sciarid flies. Biological control with Polish bio-pesticides “Owinema” was superior.

As was demonstrated in research by of Jaworska et al [24, 25], nematodes are resistant to heavy metals, whose ions contaminate soil increasingly [26]. In laboratory conditions most metal ions, except lead (Pb(II)), copper (Cu(II)) and zinc (Zn(II)) in medium concentrations do not reveal a toxic effect on entomopathogenic nematodes. However, invasive abilities of nematodes in water ion solutions weaken already after 96 hours, particularly in case of lead (Pb(II)), chromium (Cr(VI)), vanadium (V(V)), cadmium (Cd(II)) and nickel (Ni(II)) ions. Further research [27] revealed that this negative effect may be mitigated by an addition of Mn(II) (400 mg · dm⁻³/L) or Mg (160 mg · dm⁻³/L) or lithium to the solution.

A new biopreparation has been planned on the basis of *Heterorhabditis megidis*. These nematodes are the most frequently isolated from the soils of Poland. Nematodes of this species efficiently control not only *Lepidoptera* caterpillars, *Hymenoptera* larvae or *Diptera*, but also beetles. Preparations obtained from the liquid *in vitro* culture guarantee better productivity so they may provide competition for foreign preparations, registered in Poland (Table 1) [24].

Table 1

List of plant protection means containing entomopathogenic nematodes licensed for sale and use in Poland

No.	Name of plant protection mean	Manufacturer of plant protection mean	Name and characteristics of living organism	Licence valid until
1.	Entonem	KOPPERT Biological Systems B.V. – The Netherlands	<i>Steinernema feltiae</i>	11.05.2010
2.	Larvanem	KOPPERT Biological Systems B.V. – The Netherlands	<i>Heterorhabditis bacteriophora</i>	11.05.2010
3.	Owinema	“OWIPLANT” Ltd. Horticultural Enterprise – Owińska	<i>Steinernema feltiae</i>	26.11.2009
4.	Steinernema System	BIOBEST N.V. Biological Systems – Belgium	<i>Steinernema feltiae</i>	15.03.2010

Assumptions for the technological process of manufacturing a modern, environment-friendly biopreparation based on chitosan destruction products have been developed. It will be useful for plant protection against fungal, bacterial and virus diseases [25]. The biopreparation will be particularly serviceable for organic crops for pro-health food production. Synthetic plant protection means currently available on the market reveal toxicity both for humans and animals posing a hazard to the environment. They are unable to simultaneously combat various fungi, bacteria and virus strains which makes necessary the application of various pesticides or their combinations depending on present threat. The use of synthetic pesticides leads to pathogen immunization to their activity. So far, no biocides which would efficiently control fungal, bacterial or virus disease at a wide spectrum of their effect have been known.

The faults of commercial pesticides, leading to environment degradation and causing, among others, genetic changes in living organisms, including the human organism, arouses growing interest in natural plant protection means, including biopreparations. The preparation based on products of chitosan destruction (derivatives of chitin, the natural polymer) should meet these requirements.

Chitosan, due to its unique properties, such as biodegradability, bioactivity, biocompatibility, fiber forming and film forming abilities and good blending ability with other polymers, finds many applications in various fields, including medicine, agriculture, environment protection and food industry.

The purposefulness of developing a plant protection biopreparation with chitosan oligomers results also from ecological reasons, since such a preparation does not cause environmental pollution. Natural origin, no toxicity for humans, animals or plants, no phytotoxic activity provides a safe alternative for toxic pesticides. It may be expected that the new preparation may reveal activity comparable with vaccines in people and animals, improving their immunity to fungi and bacteria activity. The optimal method of chitosan destruction allowing for highly productive formation of water soluble oligomers will probably be an enzymatic degradation or a combination of various methods. A new, easily applicable mean with a wide activity spectrum for cultivational applications, will be developed soon. It will be serviceable for protection against diseases caused by fungi, bacteria and viruses.

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NIEKTÓRE PROBLEMY BADAWCZE W PRODUKCJI NOWOCZESNYCH PESTYCYDÓW

Katedra Ochrony Środowiska Rolniczego
Uniwersytet Rolniczy w Krakowie

Abstrakt: Skuteczna ochrona roślin polega na zastosowaniu wszystkich dostępnych metod w programie IPM (Integrated Pest Management). Metoda chemicznej ochrony ulega znacznym przekształceniom, poszukuje się nie tylko nowych skutecznych substancji biologicznie czynnych, ale również wzrosły wymagania co do ich bezpieczeństwa biologicznego dla organizmów niedocelowych i całego środowiska. Bez właściwej formy użytkowej nie można liczyć na sukces. Nowe formułacje i mikrokapsułki, dyspersje olejowe czy agrozele mają być biodegradowalne i niezanieczyszczające środowisko. Badania naukowe idą również w kierunku poszukiwania substancji wspomagających działanie pestycydów, jak np. kapsaicyna, naturalny składnik papryki, zwiększająca toksyczność niektórych insektycydów. Preparaty przyjazne dla środowiska to produkty na bazie degradacji chitozanu, przydatne szczególnie w uprawach ekologicznych i ochronie cennych roślin ozdobnych przed chorobami. Biopestycydy, jak np. preparaty zawierające żywe nicienie owadobójcze do ochrony roślin przed szkodnikami, tworzone są w Polsce i na świecie z wykorzystaniem nowych gatunków i szczepów, przy udziale metod biotechnologicznych.

Słowa kluczowe: pestycydy chemiczne, biopestycydy, ochrona środowiska