POTENTIAL APPLICATION OF BIODEGRADABLE POLYMER – POLY(3-HYDROXYBUTYRATE) – IN DESIGNING OF NEW GENERATION FORMULATIONS OF AGROCHEMICALS

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ABSTRACT

The work summarizes the results of research into fundamentals of design and application of slow-release and ecologically safe forms of agrochemicals used for protecting plants from pathogens and weeds. The potential of degradable polyester poly(3-hydroxybutyrate) [P(3HB)] for designing long-term formulations of fungicides (tebuconazole) and herbicides (metribuzin) is described. Experimental forms of agricultural preparations were compared with the commercial fungicide Raxil Ultra containing tebuconazole and Sencor Ultra, a herbicide containing metribuzin. Polymer/active ingredient blends in the form of solutions, emulsions and powders were used to construct slow-release formulations shaped as microparticles, microgranules, films and pellets and loaded with different amounts of agrochemicals. Release profiles of agrochemicals from experimental formulations were examined in aqueous and soil laboratory systems with respect to the geometry of formulations, type of embedded chemicals, load of polymer bases and degradation rate of polymer matrices. Efficiency of the obtained formulations with the model plants and weeds *Triticum aestivum*, *Latuca sativa*, *Raphanus sativus*, *Agrostis stolonifera*, *Setaria macrocheata*, *Chenopodium album*, *Melilotus albus*, *Amaranthus retroflexus* was studied in laboratory ecosystems. Soil microecosystems with crops (*Triticum aestivum*) infected by root rot pathogens *Fusarium* were used to assess the efficacy of the fungicides. The research has produced new data on degradable P(3HB) as a material for constructing slow-release formulations of agrochemicals. The studies showed that the slow-release herbicide and fungicide formulations were effective against weeds and plant pathogens and their effects were comparable with those of free forms of agrochemicals or even stronger. This research may be a promising avenue for constructing environmentally friendly and targeted controlled-release formulations of pesticides to protect crops against pests and pathogens.

Keywords: slow-released formulations, poly-3-hydroxybutyrate, antifungal activity, herbicidal effect, controlled release

INTRODUCTION

Increased accumulation of toxic and unrecyclable waste products caused by uncontrolled use of chemicals is one of the main global environmental problems. A way to meet this challenge is to expand the use of tools and methods of biotechnology, which may help to protect beneficial biota and enhance productivity in agriculture as well as reduce toxic impacts of chemicals on agroecosystems and the whole biosphere [1]. Intensive farming involves the use of enormous amounts of various chemicals to control pests, weeds, and pathogens of crops. However, most of these substances are accumulated in biological objects, contaminate soil and water environments, harm living organisms and upset the balance in natural ecosystems [2]. The newest trend in research is development and application of environmentally friendly pesticides with targeted and controlled release of active ingredients embedded in biodegradable polymer bases or covered with biodegradable coatings [3, 4]. In the natural environment, polymer matrices are transformed by soil microflora into products which are harmless to the living and nonliving nature and gradually release incorporated chemicals into the environment [5]. The use of novel formulations can reduce the amounts of chemicals applied to soil and enable their sustained and controlled delivery over the plant growing season preventing sharp releases into the environment that occur when plants are treated with free pesticides. These formulations can be only constructed if materials with the following properties are readily available: degradability, safety and compatibility with the environment and global biosphere cycles, long-term presence in the soil (for weeks and months) and controlled degradation into non-toxic products. Chemical compatibility with pesticides or fertilizers and processability by the available methods of production allow creating a range of biopolymer materials suitable for "green biotechnology" [6]. Advancements in science and technology determine a wider use of products synthesized in biotechnological processes. Polymers of hydroxy-derived alkanoic acids, polyhydroxyalkanoates, are valuable products of biotechnology that have a number of useful properties including biocompatibility and biodegradability [7, 8]. These polymers are promising materials for fabricating biomedical devices, controlled drug delivery systems, degradable packaging for food and drinks, products for agriculture and public utilities [9-11].

PREPARATION OF SLOW-RELEASE AGROCHEMICAL FORMULATIONS

This work is aimed at developing fundamentals of designing slow-release environmentally friendly formulations of pesticides using the advanced biodegradable polymer P(3HB) to protect crops from pathogens and weeds. During the first stage of the investigation, two agrochemicals were selected based on the literature review and preliminary studies – a herbicide containing metribuzin (MET) and a fungicide containing tebuconazole (TEB). These agrochemicals meet the following criteria: high efficiency, applicability on a large scale in agriculture, suitability for soil applications, compatibility with polymer matrices in different phase states, stability in nonpolar organic solvent solutions and amenability to analysis by spectrophotometric methods (chromatography-mass spectrometry, high-performance liquid chromatography and infrared spectroscopy).

Two-component systems (polymer/active ingredient) were used to construct slow-release formulations shaped as films, microgranules and pellets loaded with different amounts of pesticides. Film-shaped forms were prepared from the mixture of P(3HB) and corresponding agrochemical solutions by evaporation of the solvent. Granules were obtained by precipitation of the mixture in hexane. For microparticles the emulsion technique was used. Pellets were cold pressed from powdered blends of the polymer and agrochemicals.

An examination of initial substances (polymer and chemicals) and experimental formulations by IR-spectroscopy, differential scanning calorimetry and X-ray structure analysis showed that loading the polymer base with chemicals did not produce any noticeable effect on its physicochemical properties. The pesticide and polymer formed a stable mechanical mixture and no chemical binding of the components occurred.

The kinetic of polymer samples degradation depended of the shape and method of preparation. In view of this, the release profiles of agrochemicals from experimental formulations were examined with respect to the geometry of formulations, type of embedded chemicals, load of polymer bases and degradation rate of polymer matrices in soil laboratory systems. Embedding metribuzin [P(3HB)/MET] and tebuconazole [P(3HB)/TEB] into degradable bases enabled a slow burst-free release of active ingredients into soil (Fig. 1).

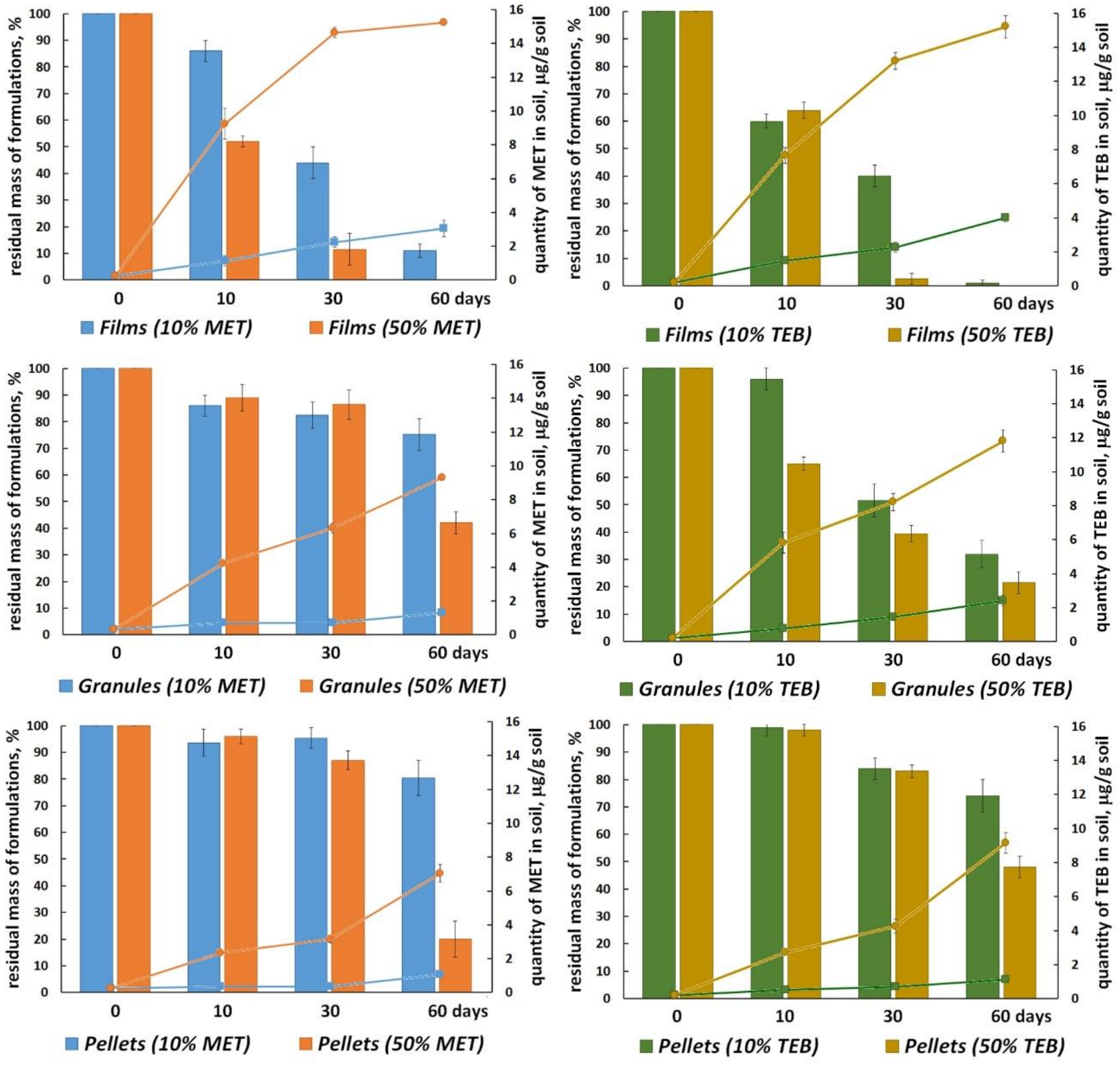

Fig. 1. Degradation dynamics of formulations (%) and cumulative release of metribuzin (MET) and tebuconasole (TEB) into soil in laboratory soil microecosystems

The highest release rate of agrochemicals was detected in microparticles. Granules and pellets degraded at slower rates, which affected MET and TEB release. Destruction of pellets was the slowest as high density formulations made by pressing impeded polymer base degradation and reduced active ingredients release.

By varying the composition of the matrix, it is possible to regulate the release rate of the active ingredient within a wide range [9]. To control P(3HB) degradation and to increase availability of this polymer, composites P(3HB) with polyethylene glycol, polycaprolactone and birch sawdust were prepared. The composition of the matrix had a substantial effect on the release of agrochemicals. This way, use of fillers can accelerate polymer base degradation and make this process suitable for development of novel agropreparations with different action time.

THE EFFECT OF SLOW-RELEASE AGROCHEMICALS ON SOIL MICROFLORA

To evaluate the experimental slow-release formulations, laboratory soil microecosystems with agrogenically transformed soil were constructed and described. During the second stage of the research, the structure of soil microbial communities was examined and the dominant microbial species were identified including primary degraders that demonstrated P(3HB)-depolymerase activity. It was shown that P(3HB) is a substrate both attractive for microorganisms and biodegradable by soil bacteria and fungi. According to RNA sequence analysis, most of P(3HB)-degrading bacteria belong to the phyla *Proteobacteria* (*Variovorax, Acidovorax, Mitsuaria, Achromobacter*) and *Actinobacteria* (*Nocardia*, *Streptomyces*); fungi were identified as *Penicilluim* and *Fusarium*. Introduction of P(3HB)-samples into soil increased the total number of microorganisms by a factor of 100 or more. A gradual destruction of polymer samples due to the action of soil microorganisms was detected. First it manifested itself through a decrease in molecular weight and an increase in polydispersity. At later stages, it was observed in visible thinning of samples, their fragmentation and, finally, in their complete disappearance. The degradation rate of P(3HB) depended on the type of soil and the structure of microbial community. For instance, in field soil with low content of biogenic elements and low abundance of copiotrophic microorganisms the weight of polymer samples decreased by 50% after 30 days, whereas in garden soil characterized by high content of biogenic elements and the number of microorganisms, the polymer samples decreased by the same percentage after 10 days.

An analysis of fungicidal action of P(3HB)/TEB formulations on phytopathogenic fungi *Fusarium verticillioides* (former *F. moniliforme*) showed an effective suppression of them in soil during the 30 days of the experiment, at the rate comparable with the effect of the commercial fungicide Raxil Ultra at similar concentrations of TEB (Fig. 2).

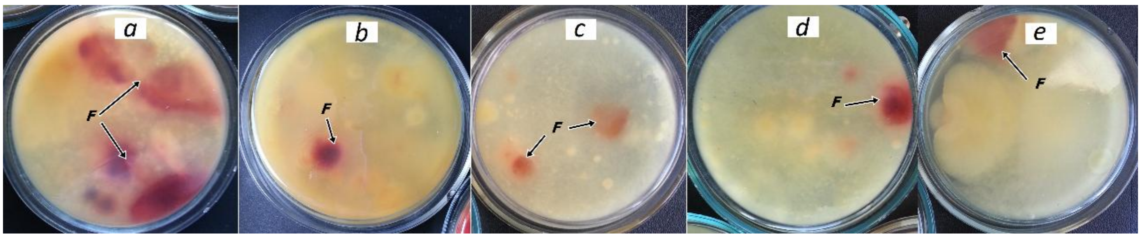

Fig. 2. The growth of *Fusarium verticillioides* colonies; *a* – control, *b* – Raksil Ultra, *c* – P(3HB)/TEB-films, *d* – P(3HB)/TEB-pellets, *e* – P(3HB)/TEB-granules. Arrows indicate colonies of Fusarium

A slight decrease in the total number of fungi in soil samples treated by P(3HB)/TEB formulations was also observed, while the number of bacteria did not change significantly.

A two-month study showed that use of embedded forms of metribuzin did not inhibit the development of saprotrophic soil microorganisms in the course of the experiment. Moreover, the numbers of microorganisms increased compared to those in the initial soil samples. The same concentration of a free form of the herbicide reduced the number of microorganisms in the soil after 60 days. In the rhizosphere of test plants (lettuce *Latuca sativa*, radish *Raphanus sativus*, wheat *Triticum aestivum*), growth of microorganisms was not suppressed in the presence of P(3HB)/MET formulations.

This research project resulted in developing methods and techniques for designing novel formulations of agrochemicals based on the degradable polyhydroxyalkanoate P(3HB). The studies addressed the effects of various factors (geometry, degree of loading, chemical composition of polymer base, type of soil and microbial community) on degradation of formulations in soil and release profiles of the active ingredients. Major factors that regulate these processes were determined. Based on the results, it can be concluded that these formulations are suitable for slow release of herbicides and fungicides.

EFFECT OF SLOW-RELEASE AGROCHEMICALS ON PLANTS

At the final stage of the research, the efficiency of the formulations developed was studied on test plants in laboratory soil ecosystems. For studying herbicidal activity, five types of weed plants (*Agrostis stolonifera*, *Setaria macrocheata*, *Chenopodium album*, *Melilotus albus*, and *Amaranthus retroflexus*) were exposed to experimental formulations P(3HB)/MET and compared with free form metribuzin in the commercial pesticide Sencor Ultra. In the herbicide-treated ecosystems the density of weed plants and the aboveground biomass were significantly reduced. As a result, complete suppression of the growth of weeds was achieved (Fig. 3). During the first 10 days of the experiment, the efficiency of P(3HB)/MET formulations was lower or comparable to that of the commercial pesticide. However, after 30 days, a prolonged effect and higher activity of the experimental formulations as compared to Sencor Ultra were observed.

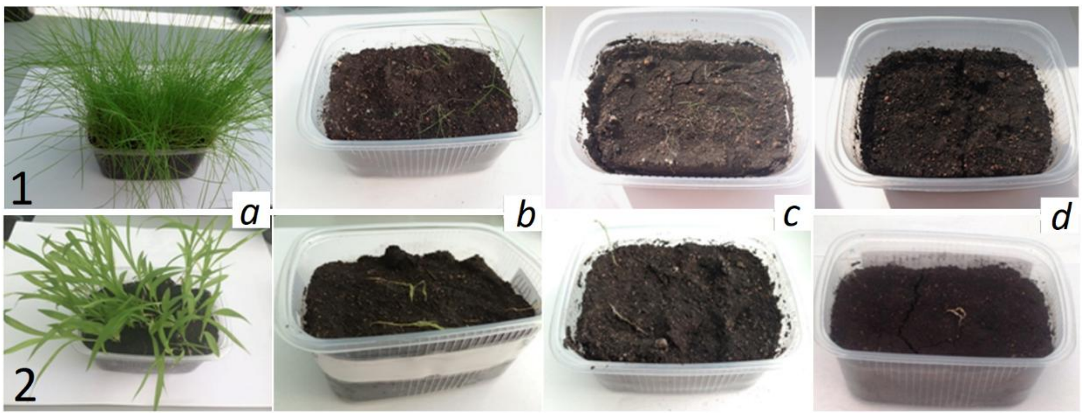

Fig. 3. The growth suppression of weeds *Agrostis stolonifera* (1) *and Setaria macrocheata* (2) by different metribuzin formulations: control without herbicide (*a*), Sencor Ultra (*b*), P(3HB)/MET-films with 10% (*c*) and 50% (*d*) of metribuzin

A similar effect was observed in co-cultivation of wheat (*Triticum aestivum*) and weeds (*Melilotus albus*). After 50 days, there was no growth of weeds in all versions of metribuzin formulations, both embedded and free forms (Fig. 4).

Fig. 4. The growth suppression of *Melilotus albus* by different metribuzin formulations: control without herbicide (*a*), Sencor Ultra (*b*), P(3HB)/MET-films (*c*) and P(3HB)/MET-microparticles (*d*)

The efficiency of the experimental forms of tebuconasole P(3HB)/TEB was assessed by infection rate of fungal root rot pathogens in wheat roots. It was shown that the proportion of roots infected with *Fusarium*, *Alternaria*, and *Bipolaris* fungi decreased by 10% when embedded tebuconazole was used. Furthermore, the fungicidal effect was prolonged and, after 30 days, was comparable to the effect of the commercial fungicide Raxil Ultra (Fig. 5). Experimental forms of the fungicide showed a strongly pronounced healing effect on the wheat root system in the case of additional contamination of soil with *F. verticillioides* spores in concentrations of up to $1{\cdot}10^6$ g^{-1}. After 30 days, the degree of infected roots was reduced by 34%, and the fungicidal effect exceeded the effect of the commercial fungicide.

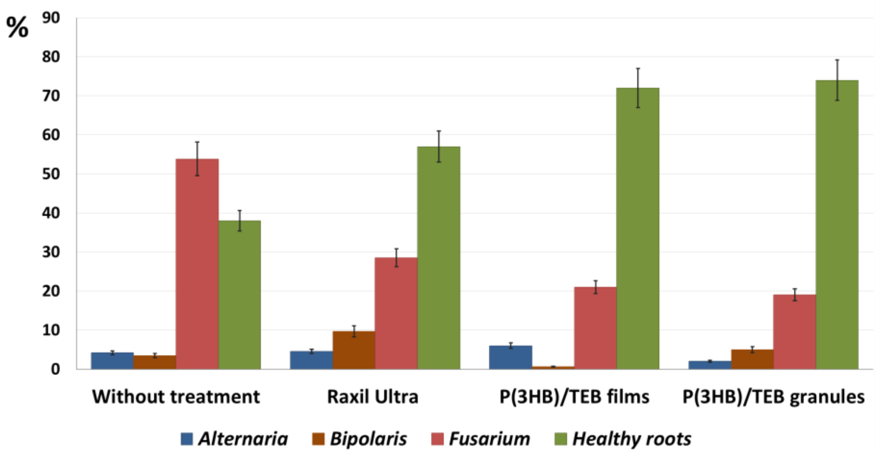

Fig. 5. The degree of infected roots (%) of *Triticum aestivum* at 30 days after tebuconazole treatment

The obtained results suggest that the use of polyhydroxyalkanoates, natural polyesters, as a degradable polymer matrix is an effective approach to constructing slow-release formulations of agrochemicals. An essential next step should be field testing of the experimental formulations.

CONCLUSION

The studies have allowed to determine the nature of interaction between agrochemicals and P(3HB) in different phase states and, based on these results, to develop ways of loading chemicals into variously shaped polymer bases and design agricultural formulations intended to suppress plant pathogens and control weeds.

A series of experimental slow-release formulations was produced using different methods and their structure and physicochemical properties were studied. The kinetics of active ingredients release from a polymer base depending on the geometry of formulations, concentration of agrochemicals, type of soil and the structure of soil microbial community were obtained in laboratory soil microecosystems. The studies revealed that the slow-release herbicide and fungicide formulations were effective against weeds and plant pathogens and their effects were comparable with those of free forms of agrochemicals or even stronger. The obtained results suggest that the use of polyhydroxyalkanoates, natural polyesters, as a degradable polymer matrix is an effective approach to constructing slow-release formulations of agrochemicals. An essential next step should be field testing of the experimental formulations.

Thus, the research has produced new data on degradable polyhydroxyalkanoates as a material for constructing slow-release formulations of agrochemicals. The fundamentals have been provided for constructing environmentally friendly and targeted controlled-release formulations of pesticides to protect crops against pests, pathogens and weeds. The development of this research direction can help manage the risks of accumulation and uncontrolled distribution of pollutants in the environment and replace dead-end synthetic plastics with degradable materials capable of entering the biospheric cycles.

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