ARCHIVES OF ENVIRONMENTAL PROTECTION

SYNTHESIS AND EVALUATION OF EFFECTIVENESS OF A CONTROLLED RELEASE PREPARATION 2,4-D: A REDUCTION OF RISK OF POLLUTION AND EXPOSURE OF WORKERS

WITOLD J. KOWALSKI[1]*, IWONA ROMANOWSKA[2], MARZENA SMOL[3], ANDRZEJ SILOWIECKI[4], MARIOLA GŁAZEK[4]

[1]Institute of Chemistry, Environmental Protection and Biotechnology
Jan Długosz University, Częstochowa PL-42-200,
[2]Centre of Polymer and Carbon Materials, Polish Academy of Sciences
41-819 Zabrze,
[3]Department of Chemistry and Technology of Water and Wastewater
Faculty of Engineering and Environmental Protection
Częstochowa University of Technology, 42-200 Częstochowa
[4]Institute of Plant Protection, National Research Institute, Sośnicowice Branch,
Sośnicowice, 44-153
*Corresponding author's e-mail: wjk@interia.pl

Abstract: A novel herbicidal controlled release formulation composed of (2,4-dichlorophenoxy) acetic acid (2,4-D) chemically bonded to biodegradable (R,S)-3-hydroxybutyric acid oligomers was investigated. The synthesis of (R,S)-3-hydroxy butyric acid oligomers was carried out via the ring opening polymerization of β-butyrolactone initiated by 2,4-dichlorophenoxy acetic potassium salt in the presence of complexing agents. The formed oligomers were characterized by size exclusion chromatography, proton magnetic resonance and electrospray mass spectrometry in order to find out their molar mass distribution and molecular structures. An assessment of biological effectiveness of the obtained herbicidal formulation was carried out in the greenhouse vs. *Sinapis alba var. Nakielska*. A promotion of the controlled release formulation with decreased water solubility and with low vapor pressure of the active ingredient, instead of traditional formulations of 2,4-dichlorophenoxy acetic acid may help to ensure a greater safety for workers and reduce the risk of dissemination of the active ingredient in the soil profile.

INTRODUCTION

2,4-dichlorophenoxy acetic acid (2,4-D) and derivatives are widely used as a synthetic plant growth regulators (auxin) in the agro-chemical treatments [29]. 2,4-D is an anionic herbicide widely used to protect crops of wheat, maize and rice to control broad-leaf weeds. However, it can be considered as a potential pollutant of soils and ground

water [6, 8, 18]. Due to its relatively high water solubility, 2,4-D is weakly adsorbed by soil particles and has a high leaching potential and then poses a threat of surface and groundwater contamination [5, 12]. Formulations containing volatile or leachable herbicides very often entail undesirable side effects such as a contamination of the surrounding environment, as well as, an incorporation of plant protection chemicals or their metabolites into the food chain. Persistent and less persistent herbicides are inadequate because the former may be hazardous for humans, while the latter necessitate multiple applications with high exposure to operators and high costs. Controlled release formulations (CRF) of herbicides have been introduced in response to growing concern about ecological problems associated with increased use of plant protection chemicals required for intensive agricultural practices.

The following main CRF can be distinguished: coated granules, matrix systems containing physically trapped active agents, polymer systems containing covalently bound active agents and polymer membrane-pesticide reservoir systems, e.g. micro-encapsulations [13, 23]. CRF offer the following advantages over conventional formulations: longer residual biological activity, possible reduction of mammalian toxicity, control/reduction of evaporation of herbicide, possible reduction of phytotoxicity to crop, reduction of solvent usage in formulations and possible reduction of herbicide application rate [3, 19, 26]. Some natural polymers such as starch, cellulose, chitosan, alginate, and lignins and their derivatives have shown to be good carriers for active ingredients [7, 14, 25, 28]. Natural polymers are easily degradable and plentiful in resource, so their use as carriers for herbicides is of special interest in terms of both economic and sustainable development due to their low cost and ease of processing, as well as, no toxicity to the environment [24]. In this context, polymer micro- and nanoparticles have emerged among the new technologies under study as potential alternatives for the development of release systems in agronomy. Polymer micro- and nano-structured systems can act as transport media or bioactive ingredients, and are able to alter the physicochemical properties of the substances they incorporate. These systems can offer the following advantages: a reduction of the amount of chemical substance required for weed control; diminished risk of environmental contamination; reduction of energy consumption, since fewer applications are needed compared to conventional formulations, and an increased safety of the operating personnel. The synthetic polymers such as polyhydroxyalkanoates (PHA) [2], including polyhydroxybutyrate (PHB), hydroxyvalerate (PHV) and their copolymers (PHBV) [10, 20] are easily produced by a variety of bacteria and are completely biodegradable during natural biological processes, making them important for the production of release systems for bioactive materials [11]. Natural PHB are highly crystalline (55−80%), which decreases the speed of degradation in comparison to lactic and glycolic homo- and copolymers [21, 27]. The active ingredient is released from such formulations at a controlled rate, increasing the period of its effectiveness and obviating the need for an overdose. Biodegradable polymers have been extensively used as a matrix for the controlled release of a variety of pesticides [17]. The release mechanisms are primarily dominated by diffusion in such biodegradable polymer-based matrix formulations, which is characterized by the decrease of the overall release rate with time. A particular interest was focused on polymers containing herbicides as pendant groups (e. g., pentachlorophenol, 2,4-dichlorophenoxy-acetic acid and 4-chloro-2-methyl-phenoxyacetic acid) [1].

Bhattacharya *et al.* [4] synthesized a diolamide of 2,4-D, which on copolymerization with a number of dicarboxylic acids would give polymer systems releasing 2,4-D. The structures and physicochemical properties of these polymers varied over a wide range by using a number of various dicarboxylic acids and cross-linking agents. The labile ester functions in the backbone provided that polymeric herbicides were completely biodegradable by hydrolysis. Cross-linking with vinyl or acrylic monomers provided partially degradable polymeric herbicides. Natural aliphatic polyesters and their synthetic analogues gained more applications due to their nontoxic biodegradation products and the use of renewable materials for their production.

Controlled release systems loaded with 2,4-D were prepared by double encapsulation using solvent evaporation technique followed by the complex coacervation method [9]. The production of these systems was aimed at modifying the release rate of the active agent and at reducing the herbicide risks by dermal contact. The coated microspheres composed of ethylcellulose or cellulose acetate derivatives were proposed as microsphere matrix and with gelatin-arabic gum complex as envelope. The carboxymethylcellulose gels were suggested as carriers for the controlled release of 2,4-D with an addition of some modified bentonites in gel formulations [17]. The formulations may be useful to reduce the pollution caused by the anionic herbicides, as well as to improve the performance of herbicides to protect crops.

Jedliński *et al.* [15] proposed a ring-opening polymerization of cyclic lactones for the synthesis of polymeric materials containing functional groups that are transferred from the substrates used as initiators. The use of volatile herbicides containing carboxylic acid groups leads to a product capable of controlled release of active substances. They are much less volatile, obtain desirable levels of lipophilicity and are degraded by hydrolysis of ester bonds, especially in the presence of enzymes in the environment [22].

We intended to evaluate a novel herbicidal CRF composed of 2,4-D and biodegradable (R,S)-3-hydroxybutyric acid oligomers. The synthesis of functional (2,4-dichlorophenoxy) acetyl-(R,S)-3-hydroxy butyric acid oligomers was carried out via the ring opening polymerization of β-butyrolactone initiated by (2,4-dichlorophenoxy) acetate potassium salt in presence of a bulky complexing agent. The formed oligomers were characterized by the size exclusion chromatography (SEC), proton magnetic resonance (^{1}H-NMR) and mass spectrometry with ionization of the sample by spraying in an electric field (ESI-MS) in order to find out their molecular structures and molecular mass distribution. An assessment of biological effectiveness of the synthesized herbicidal formulation was carried out in the greenhouse using white mustard (*Sinapis arvensis var. Nakielska*).

MATERIALS AND METHODS

2,4-dichlorophenoxyacetic acid sodium salt monohydrate, was obtained from Rokita-Agro SA, Brzeg Dolny, Poland. The crude material was converted into a potassium salt, and purified by repeated crystallization. Pielik 85 SP (Rokita-Agro SA, Brzeg Dolny, Poland) containing the declared content of 85% w/v of sodium monohydrate salt of 2,4-dichlorophenoxyacetic acid sodium salt monohydrate was used without further purification as the reference herbicide.

Synthesis and structural characterization of mixture of oligomers of 2,4 dichlorophenoxy acetyl-(R, S)-3-hydroxybutyric acid

Potassium salt of 2,4-dichlorophenoxy acetic acid (2,4-D) was used as an initiator in ring-opening anionic polymerization of β-butyrolactone carried out in solution.

In the initialization phase, 2,4-D was bound chemically through ester linkage to the initial repeating unit of 3-hydroxybutyric acid (formed in the opening phase of β-butyrolactone ring opening polymerization. Then another β-butyrolactone molecules underwent ring-opening polymerization and produced a mixture of functional oligomers of 2,4-dichlorophenoxy acetyl (R, S)-3-hydroxybutyric acid with controlled molecular structure and molecular weight distribution (Fig. 1).
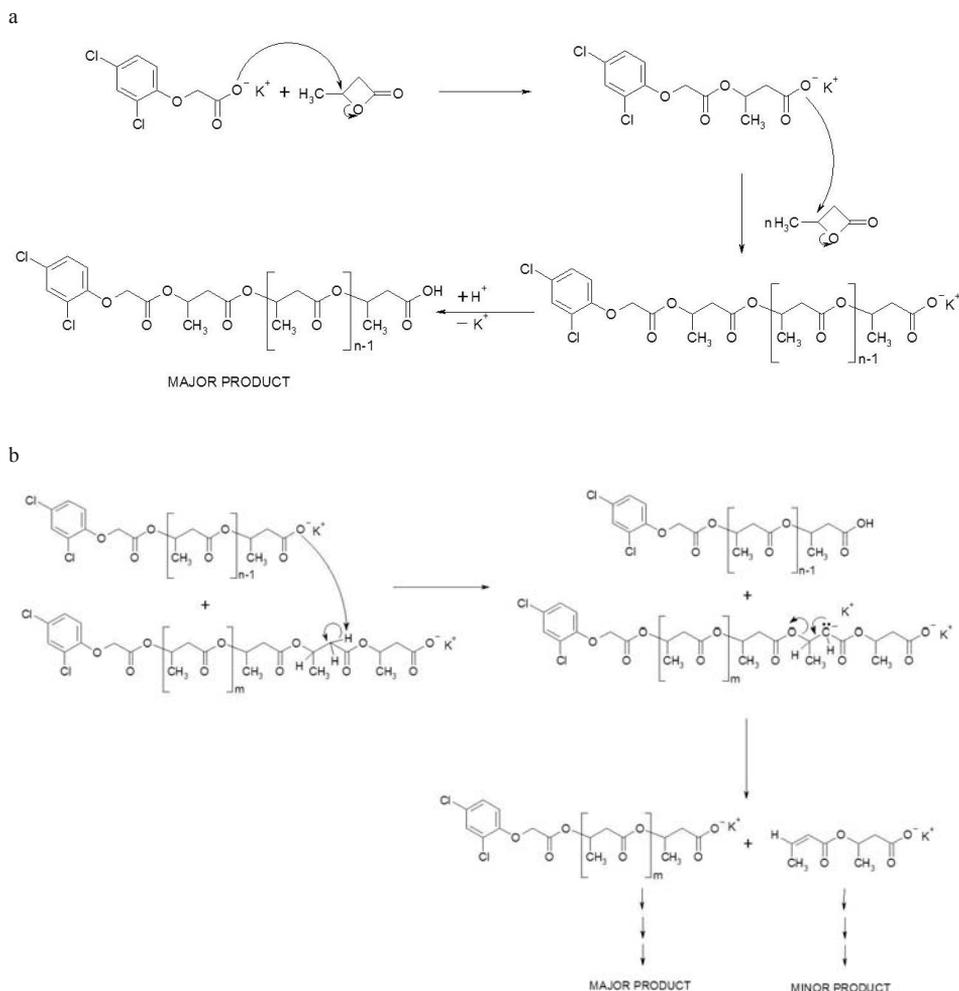

Fig. 1. Ring opening polymerization of β-butyrolactone initiated by potassium (2,4-dichlorophenoxy) acetate:
a. formation of 2,4-dichlorophenoxyacetyl-(R,S)-3 hydroxybutyric acid oligomers (the major products),
b. formation of minor products without the of 2,4-dichlorophenoxyacetyl radical

The extent of polymerization was monitored by Fourier transform infrared spectrometry (FT-IR) on the basis of intensities of stretching vibration bands of the carbonyl group in the β-butyrolactone monomer (1815 cm^{-1}) and in functional oligomers formed (1760 cm^{-1}). After completion of the reaction of the residual monomers, low molecular species and solvent were separated by evaporation under vacuum at 80°C.

The ^{1}H-NMR spectra were carried out to confirm the loss of signal intensity of protons characteristic of β-butyrolactone and increase the signal of protons in the formed mixture of oligomers of (R, S)-3-hydroxybutyric acid. Mixtures of functional oligomers of defined molecular structures and molar mass distribution were obtained by optimizing the synthesis parameters, i.e., the initiator to monomer ratio, temperature, type and amount of solvent. The mixtures were characterized by the size exclusion chromatography (SEC): the number-average molar mass Mn, weight average molar mass Mw, size average molar mass Mz were determined, as well as, the polydispersity index I, which is a statistical measure of the molar mass distribution of oligomers in the resulting mixtures (I = Mw/Mn) (Fig. 2).

$$M_n = \frac{\sum M_i \cdot N_i}{\sum N_i}; \ M_w = \frac{\sum M_i^2 \cdot N_i}{\sum M_i \cdot N_i}; \ M_z = \frac{\sum M_i^3 \cdot N_i}{\sum M_i^2 \cdot N_i}; \ I = \frac{M_w}{M_n}$$
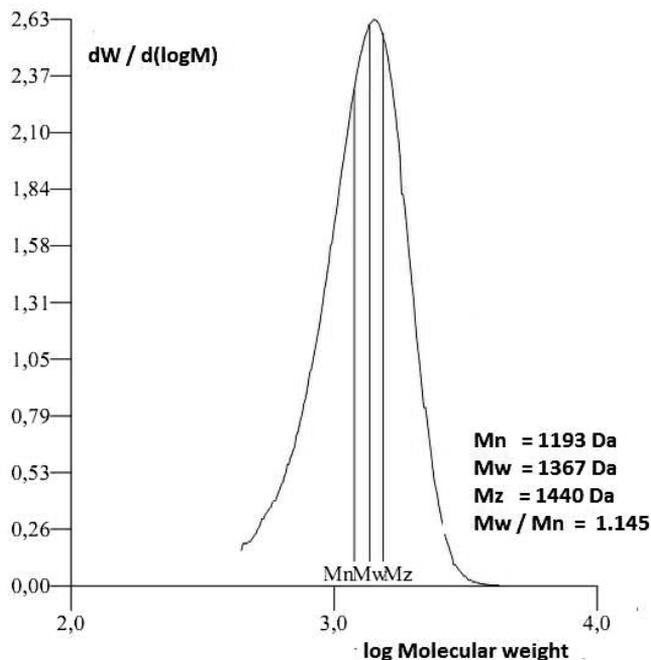

Fig. 2. Molecular weight distribution of 2,4-dichlorophenoxy)acetyl-(R,S)-3-hydroxybutyric acid oligomers obtained by size exclusion chromatography

The electrospray ionization-mass spectrometry measurements (ESI-MS), shown in Figure 3 have contributed to determine the sequences of molecular ions in the oligo(R,S)-3-hydroxybutyric acid chains.

The chemical structure and the molecular weight distribution were described by the mass to charge ratio, m/z, for characteristic ions observed in the mass spectrum. The occurrence of the differential value Δm/z = 86 Da corresponds to molar mass of the 3-hydroxy butyric acid ion, and the presence of multiple repeating units of 3-hydroxy butyric acid in the molecule corresponds to **n** Δm/z, where **n** is the number of repeating units. The presence of Δm/z = 220 Da corresponds to the molecular ion of 2,4-dichlorophenoxyacetic acid. The reaction products consisted of the (2,4-dichlorophenoxy) acetyl-(R,S)-3-hydroxybutyric acid oligomers and a minor quantity of the inactive oligo(R,S)-3-hydroxybutyric acid functionalized with a crotonate group formed when the ring opening polymerization was initiated by the β-butyrolactone molecule. In Figure 1b a mechanism of crotonate insertion in the final product is suggested.

Biological evaluation of the herbicidal effectiveness

An initial evaluation of biological effectiveness of the synthesized mixture containing 2,4-dichlorophenoxy)acetyl-(R,S)-3-hydroxybutyric acid oligomers was carried out in the greenhouse. The concentration of the active ingredient in the studied formulation was calculated from the integral signal intensities recorded in the ¹H NMR spectra and was equal to 0.21 gram per 1 gram of the synthesized product mixture. The conventional formulation *Pielik B85 SP* (containing 85% (m/m) of 2,4-dichlorophenoxy)acetic acid sodium salt was used as a reference. *Sinapis alba var. Nakielska* (white mustard, the Bayer's code: SINAL) was used as a test plant for the effectiveness evaluation. The studied formulation containing synthesized (2,4-dichlorophenoxy)acetyl-(R,S)-3-hydroxybutyric acid oligomers (2,4-D -HBAO) and a reference formulation containing equivalent amounts
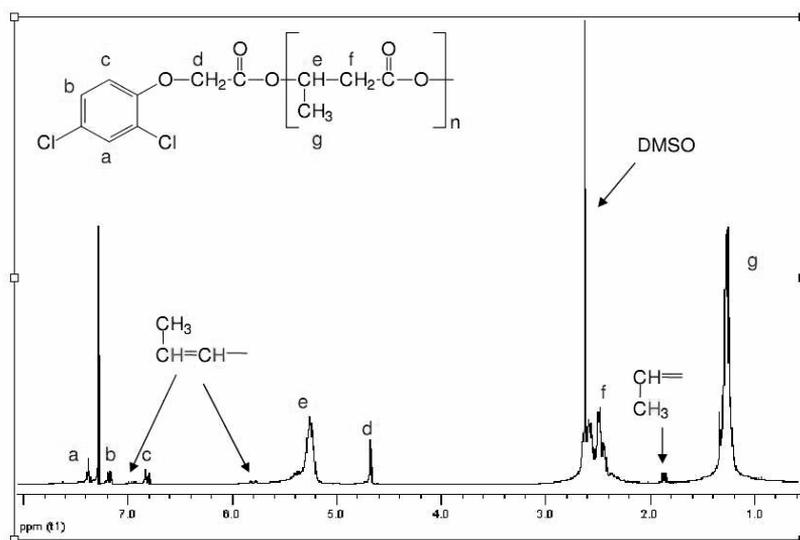

Fig. 3. ¹H NMR spectrum of oligomeric 2,4-dichlorophenoxy)acetyl-(R,S)-3-hydroxybutyric acid oligomers

of (2,4-dichlorophenoxy)acetic acid sodium salt monohydrate (2,4-D-Na-momohydrate). The experiment was repeated three times in three pots (the pot internal diameter: 13 cm, soil thickness: 4 cm). The soil composition was as follows: sand (13% v/v), peat low (13% v/v), humus (14% v/v), peat moss (60% v/v), chalk and fertilizers up to 100% v/v; the soil pH: 5,5−6,5); 30 test plant seeds were sown in each pot; watering with tap water (the water hardness was equivalent to 287 mg $CaCO_3$/1L H_2O). The greenhouse average air temperature ranged from 15−20°C. The spraying procedure: a volume of 20 mL of liquid formulations of 2,4-D-HBAO containing 8.40 g/L and 4.20 g/L, respectively, and the reference herbicide formulation (2,4-D-Na-momohydrate) containing 8.50 and 4.25 g/L active ingredients, respectively, were used. Spraying was performed with a pressure sprayer (operating pressure 0.5 bar).

Characterization of the oligomer formulation (2,4-D-HBAO)

FTIR spectra were recorded with an FTS 40-A Biorad spectrometer. ^{1}H NMR spectra were recorded at room temperature in CDCl3 with tetramethylsilane (TMS) as the internal standard using a Varian VCR-300 spectrometer with a 4499 Hz spectral width, an acquisition time of 1.998 s and 64 repetitions. Number of average molecular weight and molecular weight distribution (Mw/Mn) values of the synthesized functionalized oligomers were estimated by SEC experiments conducted in chloroform at 35°C at a flow rate of 1 $cm^{-1} \cdot min^{-1}$ using a Spectra-Physics 8800 solvent delivery system with a set of two PLgel 5 μm MIXED-C ultrahigh efficiency columns and the Shodex SE 61 refractive index detector. The samples (volume 10 μL solution in chloroform (concentration of 1% w/v) were injected. Polystyrene standards with narrow molecular weight distributions were used to generate a calibration curve. Electrospray mass spectrometry analyses were performed using an LCQ mass spectrometer (Finnigan, San Jose, CA). The samples were dissolved in a chloroform/methanol mixture (10/1 v/v). The solution was introduced to the ESI source by continuous infusion by means of an instrument syringe pump at a rate of 3 μL · min^{-1}. The ESI source was operated at 4.25 kV, and the capillary heater was set to 200°C. The experiments were performed in negative-ion mode.

RESULTS AND DISCUSSION

The target plants of *Sinapis alba var. Nakielska* (SINAL) reached the development phase of cotyledons (BBCH 10) according to the BBCH-scale for crops and weeds [16] when the spray was performed.

The observations were carried out for three weeks after spraying in order to assess the plant damage after 3, 5, 7, 10, 12, 14, 17, 19 and 21 days. The percentage of plant population damage was defined by visual evaluation of each plant and is presented in Tab. 1.

A lower effectiveness was observed at the beginning of the experiment for the oligomer mixture formulations when compared with the reference 2,4-D sodium salt formulations. The observed differences in the effectiveness have converged significantly after two weeks after spraying. Losses of more than 99% of white mustard plants have been observed for all plants treated with the oligomer formulations and the reference formulations within 19 days after spraying. The relationships between the herbicide effectiveness vs. time after spraying are shown in Figure 5.

Table 1. The effectiveness of studied formulations vs. *Sinapis alba var. Nakielska* (SINAL)

| No.	Formulation	Concentration [g/L]	Time delay after spraying (days)								
			3	5	7	10	12	14	17	19	21
			Development phases (BBCH units)								
			10	10-11	10-12	11-12	12	12	12	12-13	12-13
			Destruction in *Sisnapis alba* plant population [%]								
1.	*Blank*	-	0	0	0	0 a	0	0	0	0	0
2.	2,4-D-Na-monohydrate	*4.25*	25	45	94	84	91	95	100	100	100
3.	2,4-D-Na-monohydrate	*8.50*	30	53	72	88	94	97	100	100	100
4.	2,4-D -HBAO	*4.20*	15	27	41	63	77	88	97	99	100
5.	2,4-D -HBAO	*8.50*	19	33	48	71	83	92	99	100	100
Least significant differences calculated at p = 0.05			0	6,77	6,20	12,70	0,97	1,14	1,65	1,29	0,49
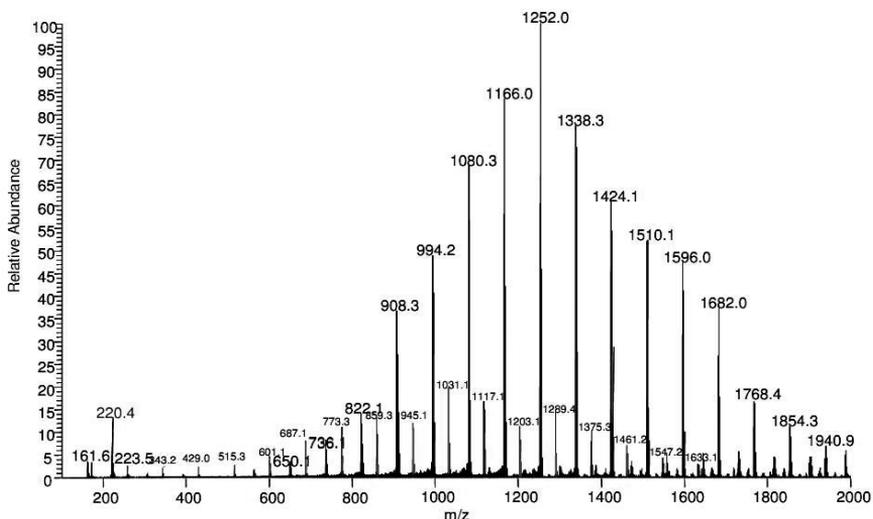
Fig. 4. Electrospray ionization mass chromatogram (ESI-MS) obtained in the negative ions mode. (The main intensity series represent the molecular structure of 2,4-dichlorophenoxy)acetyl-(R,S)-3-hydroxybutyric acid oligomers and the subtle intensity series: oligo((3-hydroxybutyric acid) with an unsaturated end group)
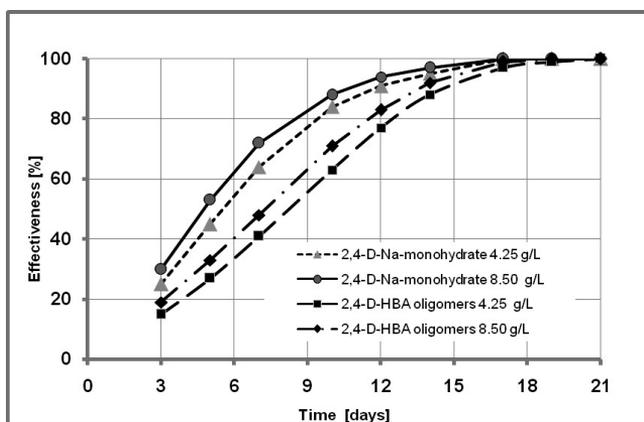

Fig. 5. Destruction of Sinapis alba var. Nakielska population vs. time delay after spraying for the studied and reference formulations

Two ranges of plot inclination could be distinguished on diagrams representing the relationship of the herbicide effectiveness vs. time:

1. plots depicting the formulations of oligomer mixtures ran rectilinearly from the day of spraying for two weeks and subsequently converged to the 100% effectiveness at the end of the third week,

2. plots of 2,4-D sodium salt conventional formulations rose steeply upwards for about one week, then followed a continuous reduction in inclination (the slope), and after two weeks they converged to the 100% effectiveness.

Small shifts downwards have been observed during the first two weeks in the case of the two times smaller concentrations of the studied formulations. All plots converged to the 100% effectiveness in the third week.

CONCLUSIONS

The mixture of oligomers of 2,4-dichlorophenoxyacetyl-(R, S)-3-hydroxybutyric acid has been proposed as a carrier of 2,4-D herbicide in controlled-release formulations containing amorphous analogue of natural oligomers of 3-hydroxybutyric acid. Their degradation products are identical as metabolites formed in plant cells and the physicochemical and operational parameters of the carrier could be adjusted by selecting the polymerization conditions.

The effectiveness of CR test product was comparable to the conventional formulations (2,4-D sodium salt reference), but the formulations composed of oligomer mixtures produced lower concentrations of active ingredients over an extended period of time due to initiation of the controlled release mechanism, which led to delays in the observed effectiveness.

The described formulation might be particularly useful in practical applications, where the prolonged herbicide duration is required, e.g., when the active ingredient concentrations in the conventional formulation fade away due to prompt evaporation or leaching out from the target site. The oligomer supported 2,4-D formulations might increase safety of the operating personnel, who is not exposed to high vapor pressures, and their extended biological activity might contribute to overcome serious environmental problems observed for conventional 2,4-D formulations.

REFERENCES

[1]   Akelah A.: *Novel utilizations of conventional agrochemicals by controlled release formulations*, Mater. Sci. Eng. C 4, 83 (1996).

[2]   Amass W., Tighe B.: *A review of biodegradable polymers: use, current developments in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polymers and recent advances in biodegradable studies*, Polymer. Int. 47, 89 (1998).

[3]   Bahadi M.: *Safe formulations of agrochemicals*, Chemosphere, **16**, 615 (1987).

[4]   Bhattacharya S., Sanya S.K., Mukherje R.N.: *Controlled-Release Polymeric Herbicide Formulations with Pendent 2,4-Dichlorophenoxyacetic Acids*, Ind. Eng. Chem. Prod. Res. Dev. 25, 585 (1986).

[5]   Buser H.R., Mueller M.D.: *Occurrence and transformation reactions of chiral and achiral phenoxyalkanoic acid herbicides in lakes and rivers in Switzerland*, Environ. Sci. Technol., **32**, 626 (1998).

[6]   Celis R., Koskinen W.C., Cecchi A.M., Bresnahan G.A., Carrisoza M.J., Ulibarri M.A., Pavlovic I., Hermosin M.C.: *Sorption of the ionizable pesticide imazamox by organo-clays and organohydrotalcites*, J. Environmental Science & Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, **34 (6)**, 929 (1999).

[7]   Cottefill J.V., Wilkins R.M., da Silva F.T.: *Controlled release of diuron from granules based on a lignin matrix system*, J. Controlled Release, **40**, 133 (1996).

[8]   Curwin B.D., Hein M.J., Sanderson W.T., Nishioka M.G., Reynolds S.J., Ward E.M., Alavanja M.C.: *Pesticide Contamination Inside Farm and Nonfarm Homes*, J. Occupational & Environmental Hygiene, **2 (7)**, 357 (2005).

[9]   Elbahri Z., Taverdet J.-L.: *Elaboration of microspheres and coated microspheres for the controlled release of the herbicide 2,4-D*, Polymer Bulletin, **59**, 709 (2007).

[10]   Focarete M.L., Scandola M., Jendrossek D., Adamus G., Sikorska W., Kowalczuk M.: *Bioassimilation of Atactic Poly[(R,S)-3-hydroxybutyrate Oligomers by Selected Bacterial Strains*, Macromolecules, **32 (15)**, 4814.

[11]   Garrido-Herrera F.J., Daza-Fernández I., González-Pradas E., Fernández-Pérez M.:*Lignin-based formulations to prevent pesticides pollution*, J. Hazard. Mater., **168**, 220 (2009).

[12]   Hyun S., Lee L.S.: *Quantifying the contribution of different sorption mechanisms for 2,4-dichlorophenoxyacetic acid sorption by several variable-charge soils*, Environ. Sci. Technol., **39**, 2522 (2005).

[13]   Knowles A.: *Recent developments of safer formulations of agrochemicals*, Environmentalist., **28**, 35 (2008).

[14]   Kulkarni A.R., Soppimatha K.S., Aminabhavi T.M., Dave A.M., Mehta M.H.: *Glutaraldehyde crosslinked sodium alginate beads containing liquid pesticide for soil application*, J. Controlled Release, **63**, 97 (2000).

[15]   Kurcok P., Kowalczuk M., Hennek K., Jedlinski Z.: *Anionic polymerization of β-lactones initiated with alkali-metal alkoxides: reinvestigation of the polymerization mechanism*, Macromolecules., **25 (7)**, 1992.