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RESEARCH ARTICLE

Efficacy of embedded metribuzin and tribenuron-methyl herbicides in field-grown vegetable crops infested by weeds

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Abstract

The purpose of the present study was to investigate the efficacy of the experimental formulations of the metribuzin (MET) and tribenuron-methyl (TBM) herbicides embedded in the matrix of degradable poly-3-hydroxybutyrate blended with wood flour in field-grown tomato and beet crops infested by weeds. There is a necessity to develop environmentally friendly and effective means to protect plants because of the shortcomings of the free herbicide forms such as the environmentally unsafe spray application of solutions and suspensions of the widespread metribuzin and tribenuron-methyl herbicides, removal from soil during watering events and rains, and transport to natural aquatic environments, where the herbicides accumulate in the trophic chains of biota. Free TBM is also rapidly inactivated in soil and metabolized to nontoxic products in plants. The efficacy of experimental formulations of metribuzin and tribenuron-methyl embedded in the matrix of degradable poly-3-hydroxybutyrate blended with wood flour was tested in field-grown tomato and beet crops infested with weeds. Application of metribuzin resulted in the highest productivity of tomatoes (2.3 kg/m^2) and table beet (3.4 kg/m^2), improved biometric parameters of tomato fruits and beet roots, and caused reduction in nitrate nitrogen concentrations in them. The mode of herbicide delivery did not affect sugar contents, but application of both metribuzin and tribenuron-methyl induced a 1.7-fold and 1.4-fold, respectively, increase in vitamin C concentrations in tomato fruits and beet roots relative to the vegetables grown on the subplots treated with free herbicides and the intact plants. Embedded herbicides can be used as preemergence herbicides in the field.

Keywords Metribuzin · Tribenuron-methyl · Embedding · Degradable P(3HB) · Weed growth inhibition · Quality of tomato fruits and beet roots

Introduction

Vegetables constitute an essential component of human diet. Vegetable crop yields can be enhanced by applying proper agricultural practices, supplying nutrients and water to plants and controlling plant pathogens, pests, and weeds (Rao et al. 2016; Li et al. 2017).

Tomato is one of the most popular vegetables (Savić et al. 2008), cultivated both in green houses and in the field. Production of tomatoes is growing rapidly, as they make a major contribution to vegetable consumption (Bao and Li 2010). Tomato is the most important vegetable crop after potato; in 2015, the global production of tomatoes reached 164 million tons (Gennari 2015; Vaccari et al. 2015). Tomato fruits are high in antioxidants such as lycopene, phenols, and vitamin C (Toor et al. 2006), which are beneficial to human health, and their levels, as well as the amounts of sugars, in tomatoes can be enhanced by choosing proper growing conditions.

Another important vegetable crop is table beet, and beet industry has been expanding recently. Table beet is cultivated as a major source of betacyanins, which have antioxidant and anti-inflammatory properties, beneficial to human health

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(Szalaty 2008; Georgiev et al. 2010). The expanding beet industry, however, faces the challenge of minimizing the risk of crop loss. Diseases may reduce plant density and homogeneity of the crop stand, shorten the duration of time foliage is healthy, and decrease the yield of marketable roots (Pethybridge et al. 2018). Thus, as the production of table beet is increased, higher efficiency of plant pathogen and weed control is required.

Application of herbicides, which constitutes over 40% of the pesticides used in agriculture, inevitably results in incorporation of these chemicals into the food chains, posing threat to natural ecosystems and human health. The situation is compounded by the inability of a number of commercial herbicides to control the weeds that have developed resistance to them. For example, *Chenopodium album* L. is resistant to acetolactate synthase, and *Ambrosia artemisiifolia* L. and *Abutilon* are resistant to triazine herbicides (Liebl et al. 2008). These are the incentives to pursue research aimed at developing new and more effective means for weed control. Another aspect that researchers should focus on is the need to reduce herbicide application rates and minimize their damage to the beneficial biota and the entire biosphere.

A new line of research, which is aimed at reducing the risk of uncontrolled spreading of pesticides and accumulation thereof in the biosphere, is the development and agricultural use of environmentally safe new-generation pesticides with targeted and controlled release of active ingredients embedded in biodegradable microparticles, microcapsules, microgranules, etc. (Oliveira et al. 2018, 2019). The crucial component of constructing such preparations is the availability of appropriate materials with the following properties (Sarkar et al. 2018): ecological compatibility with the environment and global biospheric cycles, i.e., degradability; safety for living and nonliving nature; long-term (weeks and months) presence in the natural environment and controlled degradation followed by formation of nontoxic products; chemical compatibility with pesticides and fertilizers; and processability by available methods, compatible with pesticide and fertilizer production technologies. The materials extensively studied as matrices for embedding agrochemicals are synthetic nondegradable polymers (polystyrene, polyacrylamide, polyethylene acrylate, polyamide, polyurethane, polycyanoacrylate) (Sarkar et al. 2018) or polymers covered with biodegradable coatings (Grillo et al. 2014; Roy et al. 2014). In the natural environment, polymer matrices are transformed by soil microflora into products that are harmless to the living and nonliving nature, and the embedded chemicals are gradually released to the environment (Ong and Sudesh 2016).

Among biodegradable materials, special attention is given to biopolymers synthesized by microorganisms in biotechnological processes: polyhydroxyalkanoates (PHAs). These polymers are thermoplastic, mechanically strong, and slowly degradable in biological media (Chen 2012; Koller et al. 2017; Volova et al. 2019). As these polymers decompose via truly biological degradation and do not undergo hydrolysis in liquid media, the products made from them may function, e.g., in soil, for months. The available literature data on using PHAs to construct environmentally safe pesticide formulations are limited, and the research in this area has been started only recently. Poly-3-hydroxybutyrate [P(3HB)]—the best studied PHA—has been used as degradable matrix in slow-release pesticide formulations (Volova et al. 2019, 2020). Metribuzin formulations were prepared in the form of films, microgranules, and microparticles based on P(3HB). Those formulations were found to be effective herbicides in experiments with weed model (Volova et al. 2016a, b). They were also used to control weeds in the laboratory wheat stands (Zhila et al. 2017). Microparticles of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) loaded with the trifluralin herbicide were found to have a similar effect (Cao et al. 2019).

In order to make PHA polymers, whose cost is rather high now, more easily accessible, and less expensive, research was conducted in which pesticides were embedded in matrices of degradable P(3HB) blended with natural materials (peat, clay, and birch wood flour) (Thomas et al. 2020). Laboratory studies showed the efficacy of those formulations in the stands of cereal crops (winter wheat and barley) infected by plant pathogens and infested by weeds (Volova et al. 2019; Kiselev et al. 2020).

The purpose of the present research was to study the efficacy of the embedded metribuzin and tribenuron-methyl herbicides in field-grown vegetable crops infested by weeds.

Materials and methods

Herbicides

Two herbicides with different modes of action were studied: metribuzin (MET) and tribenuron-methyl (TBM). The metribuzin and tribenuron-methyl herbicides used in experiments with plants were supplied by Xi'an Tai Cheng Chem Co., Ltd. (China); the content of the active ingredient in metribuzin was 97.2% and in tribenuron-methyl—95.5%.

Metribuzin [4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one] is a systemic selective herbicide of the class of 1,2,4-triazines, having a broad spectrum activity against some dicots and grass weeds. MET has a long-lasting effect, acting via both leaves and soil. The mode of action is based on inhibiting the Hill reaction (water photolysis) and photosynthetic electron transport between primary and secondary electron acceptors in photosystem II. MET effectively protects soybean, maize, cereal, potato, and tomato crops from annual dicots and grass weeds.

Tribenuron-methyl [methyl ester of 2-(6-methyl-4-methoxy-1,3,5-triazin-2-yl(methyl)carbamoylsulfamoyl) benzoic acid] is a systemic selective herbicide of the sulfonylurea family. The mode of action is based on inhibiting acetolactate synthase, which takes part in biosynthesis of branched-chain amino acids (valine, leucine, and isoleucine), causing a decrease in the levels of these amino acids in plant tissues followed by disruption of protein and nucleic acid synthesis. TBM effectively protects cereal crops from dicots and grass weeds (Granstar Pro. DuPont Manufacturer's Manual).

Materials for embedding herbicides

The herbicides were embedded into the matrix of the poly-3-hydroxybutyrate/birch wood flour blend. P(3HB) polymer samples were synthesized using the *Cupriavidus eutrophus* B10646 strain and proprietary technology; the polymer had the following properties: degree of crystallinity 75%, melting point 176 °C, thermal decomposition temperature 287 °C, and molecular weight (M_w) 590 kDa (Volova et al. 2020). Wood flour was used as a filler. Wood flour was produced by grinding wood of birch (*Betula pendula* Roth) using an MD 250–85 woodworking machine ("Stanko Premier," Russia). Then, it was dried at 60 °C for 120 h until it reached constant weight, and 0.5-mm mesh was used to separate the particle size fraction; onset of thermal decomposition was 220 °C.

The polymer and wood flour were pulverized by impact and shearing action in ultra-centrifugal mill ZM 200 (Retsch, Germany). To achieve high fineness of polymer grinding, the material and the mill housing with the grinding tools were preliminarily cooled at − 80 °C for about 30 min in an Innova U101 freezer (New Brunswick Scientific, USA). Grinding was performed using a sieve with 2-mm holes at a rotor speed of 18,000 rpm. The fractional composition of the polymer and filler powders was determined using vibratory sieve shaker AS 200 control (Retsch, Germany). Then, polymer powder was mixed with the filler powder in benchtop planetary mixer Speed Mixer DAC 250 SP (Hauschild Eng., Germany); the blend time was 1 min, and the speed was 1000 rpm. The procedure and the properties of the blend were described in detail elsewhere (Thomas et al. 2020). Herbicide granules were prepared using polymer paste wetted with ethanol and mixed with birch wood flour and the herbicide in screw granulator (Fimar, Italy). The formulations contained the following percentages of the components: P(3HB)/wood flour/herbicide—50/30/20 (wt.%).

Vegetable crops

Early- and mid-maturing cultivars were chosen for growing in the field in the zone with continental subarctic climate (Krasnoyarsk Krai, South Siberia, Russia). An ultra-early-maturing tomato cultivar, "Zagadka," with the time from sowing the seeds to harvesting the first fruits of 83–87 days, was cultivated. The plant is a determinate cultivar, with reduced stature. The fruits are round-shaped, slightly ribbed, dense, and meaty and have a good flavor.

The table beet cultivar "Tsilindra" is included in the State Register of Selection Achievements, and it is recommended for cultivation in all climatic environments in Russia. This is a mid-maturing cultivar, requiring 120 days to reach maturity. The roots are dark red, cylindrical, and thin-skinned. As the cultivar is resistant to species-specific diseases, it is a heavy yielder.

Characterization of conditions of vegetable crop cultivation in the field

Micro-field experiments were carried out on the meadow-chernozem thick heavy clay-loam soil at the field laboratory of Krasnoyarsk State Agrarian University located at the city of Krasnoyarsk during the growing season of 2019. The arable land in Krasnoyarsk Krai is seasonally frozen. Characterization of the soil temperature regime in the annual cycle (based on measuring mean annual soil temperature at a depth of 0.2 m) suggests that this is a cold subtype soil. The agrochemical examination of the soil plot and the soil diagnostics were conducted in May 2019, before sowing of seeds, using conventional techniques. The soil was characterized by neutral pH, low hydrolytic acidity, and very high, more than 10%, humus content (Table 1). The high cation exchange capacity (72 mmol/100 g) was associated with the very high humus content and the heavy clay-loam soil texture. As the soil contained a high percentage (99%) of bases, lime treatment was not necessary. Calcium was the major absorbed base. The Ca:Mg ratio in the 0–20-cm soil layer was 6.3—a typical value for the soils of this genetic type. The agrochemical characterization of the soil suggested that it was high in nitrate nitrogen and moderate in ammonium nitrogen. The soil was high in mobile phosphorus and exchangeable potassium. Thus, the meadow-chernozem soil of the experimental plot was potentially and effectively fertile.

Table 1 Chemical characterization of the meadow-chernozem soil (0–20 cm)

No.	Parameter	Value
1	pH_{H2O}	7.2
2	Humus, %	10.7
3	Hydrolytic acidity, mmol/100 g	0.75
4	Total absorbed bases, mmol/100 g	71.0
5	Cation exchange capacity, mmol/100 g	71.8
6	Base saturation percentage, %	99.0
7	Nitrate nitrogen, mg/kg	16.0
8	Ammonium nitrogen, mg/kg	10.1
9	Mobile phosphorus, mg/kg	239.0
10	Exchangeable potassium, mg/kg	110.7
11	Exchangeable calcium, mmol/100 g	27.2
12	Exchangeable magnesium, mmol/100 g	4.3

During the growing season of the vegetable crops, moisture content of the 0–20-cm soil layer was determined by thermogravimetric analysis; the temperature conditions of the soil were investigated in the 0–10-cm and 10–20-cm layers from May through September in five test subplots within the experimental plot (soil temperature and moisture content were measured every 12–20 days).

In 2019, the growing season was warm and subhumid. The amount of precipitation from May through September was 46–85% of normal precipitation. The average air temperatures in May and June were 1–2 °C above the long-term average temperatures (Table 2). The temperature in the first 10 days of August was particularly high, with an average daily temperature reaching 23 °C. In July, the air temperature was close to the normal temperature, but precipitation was just 38 mm, and that was 53% below normal.

With each vegetable crop, two herbicides—metribuzin (MET) and tribenuron-methyl (TBM)—were used as follows:

1 Negative control, «-» (plants were grown without herbicide application)
2 Positive control, «+» (plants were sprayed with MET and TBM aqueous solutions)
3 Treatment (embedded MET and TBM (granules) were buried in soil simultaneously with sowing beet seeds and transplanting tomato seedlings): embedded MET, P(3HB)/wood flour/MET = 50/30/20 (wt.%); embedded TBM, P(3HB)/wood flour/TBM = 50/30/20 (wt.%). In the positive control, free herbicides were applied following the recommended application rates (Rakitsky 2011). The necessary concentrations of MET or TBM embedded in the matrix were achieved by varying the amounts of the granules.

The area of each subplot was 1 m^2; experiments were performed in triplicate; a systematic spacing design was used. Tomato seeds were planted on March 25; tips were removed in the phase of two true leaves, on April 4; seedlings were transplanted outdoors on June 14. The system of planting was 4 plants per m^2. Beet seeds were sown in late May, using the 25 × 7 cm planting pattern. Tomatoes were harvested on August 20 and beetroots on September 11.

Vegetable crops were visually examined and photographed weekly, and samples for analysis were collected monthly, to compare the effects of different herbicide forms. During the experiment, vegetable crops were not watered; no fertilizers were added to soil.

Chromatographic determination of pesticides

Residual herbicide contents in vegetable and soil were measured by chromatography in accordance with the guidance document of Rospotrebnadzor of RF ("Determination of residual amounts of pesticides in food products…" 2006). To determine metribuzin and tribenuron-methyl, residual amounts of the herbicides were extracted from the samples with acetone, the specimen was transferred from the acetone-water fraction to dichloromethane, the extract was purified on the column with silica gel, and the final re-extract was concentrated. A 10.0 ± 0.1 g analytical sample of biomaterial was placed into a 250-mL flat-bottom flask, and 50 mL of acetone was added; the sample was shaken for 80 min in an incubator shaker at a temperature of 25 °C. The extract was purified on a chromatographic column with silica gel. Twenty-five milliliters of the resulting extract was concentrated using a Rotavapor R/210 V rotary evaporator (Büchi, Switzerland), and the residue was re-dissolved in 2 mL acetonitrile. Quantitative determination of metribuzin was carried out using a hardware-software complex for medical research on the basis of a gas chromatograph "Chromatek-Crystal 5000", a capillary column with a stationary phase, type SE-30, tribenuron-methyl—by HPLC using a UV detector (Ultimate 3000 with UF, FLU detectors). Detection was done at the wavelengths located at the absorption maxima of the herbicides: 298 nm for metribuzin and 220 nm for tribenuron-methyl. The range of the concentrations detected was between 1 and 500 μg mL^{-1} or higher. Calibration curves were plotted by using calibration solutions of high-purity

Table 2 Temperature and precipitation over the growing season in 2019 (the data obtained at the "Krasnoyarskoye Opytnoye Polye" weather station)

Month	Air temperature (°C)				Precipitation		Long-term average air temperature (°C)	Long-term average precipitation (mm)
	Ten-day periods			Monthly average	Monthly total, mm	% of normal		
	I	II	III					
May	9.2	7.3	13.5	10.1	20	45.5	8.9	44
June	15.7	18.9	19.6	18.1	51	85.0	15.9	60
July	20.1	19.2	17.3	17.3	38	46.9	18.4	81
August	22.5	16.3	16.2	18.2	58	77.3	15.1	75
September	11.8	10.4	10.0	10.3	62	131.9	9.1	47

compounds: chemically pure metribuzin [$C_8H_{14}N_4OS$] (99.7% pure) was used (State Standard Sample 7713-99—the state standard accepted in Russia (Blok-1, Moscow) and chemically pure tribenuron-methyl [$C_{15}H_{17}N_5O_6S$] (98.3% pure) (State Standard Sample 8628-2004—the state standard accepted in Russia (Blok-1, Moscow).

The efficacies of different ways of herbicide delivery in controlling weeds

Weeds were photographed, and the dates of the onset of plant death and beginning of the death of most plants were determined by counting their number per m^2 during the experiment; mortality rates of different species were estimated. As the number of developing, flowering, and dying crops and weeds varied, the number of samplings during the experiment was not the same. Sampling of vegetable crops for determining biometric parameters and chemical analysis was done during harvesting. Weeds were photographed, and the dates of the onset of plant death and beginning of the death of most plants were determined by counting their number per m^2 during the experiment. The total number of weed plants was counted on May 31, June 27, July 22, and August 20.

The effects of different herbicide formulations on the productivity and quality of tomatoes and beetroots

The tomato fruit yield was estimated quantitatively and qualitatively on August 20, 2019. The following parameters were determined: fruit mass per m^2, number of fruits per m^2, number of fruits per plant, and an average mass of the fruits on one plant. The beet root yield was estimated quantitatively and qualitatively on September 11, 2019. The following parameters were determined: root mass per m^2, number of roots per m^2, and an average mass of one beetroot.

Quantitatively biochemical characterization of tomato fruits and beetroots included determination of nitrate nitrogen using a "Soex" nitrate meter, dry matter (GOST 28561-90), sugar according to M 04-69-2011, and vitamin C according to M 04-86-2016, using a Kapel-105M capillary electrophoresis system. Results of analyses and yield data were processed using dispersion analysis (Dmitriev 1995).

Statistical analysis

Results were expressed as the average of three replicates. Statistical analysis of results was performed using the standard software package of Microsoft Excel. Average values and standard deviations were determined at a chosen confidence level, $a = 0.95$, using data filtering. The data are presented as the means ± standard error of the means.

Results and discussion

The study of the structure of vegetable crop yields showed that yield parameters varied depending on the effectiveness of the weed control by different herbicide formulations.

Concentrations of metribuzin and tribenuron-methyl in soil

Herbicide concentrations in soil were determined during the micro-field study of table beet (Fig. 1). Results of measurements show the MET and TBM concentrations at definite dates, without taking into account removal of the herbicides from the fields with drainage water and herbicide degradation by soil microflora. Significant differences were noted between concentrations of the free and embedded herbicides and between MET and TBM concentrations, as these herbicides differ in solubility and application rates. MET is a highly soluble herbicide (1350 to 2040 mg/L, depending on the soil pH and type). Throughout the experiment, metribuzin was detected in

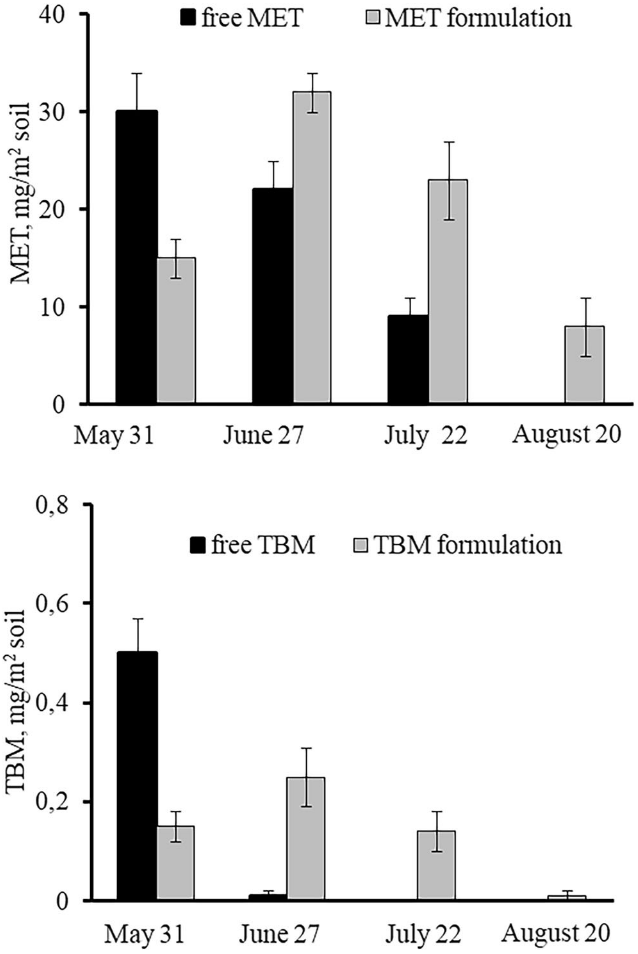

Fig. 1 The concentrations of metribuzin and tribenuron-methyl in soil table beet culture at different time points

soil on the treatment plot. MET was gradually released from granules, and on May 27 (1 week after seeds and MET granules were buried in soil), its concentration in soil was 15 mg/m^2; on June 27, it was the highest (34 mg/m^2); and then it decreased, reaching 15 mg/m^2 on August 20. MET can remain active in soil 1 to 3 months, depending on the soil type and weather (Rakitsky 2011). The gradual decrease in MET concentration can be attributed to the degradation of the herbicide by soil microflora and the high solubility of MET and removal from soil. As noted above, during that period, there were many very heavy rains. TBM has much poorer solubility than MET (2.04 mg/L), and TBM application rates are almost 20 times lower; this herbicide is inactivated in soil very quickly, within days. After being taken up by plants, it is metabolized to form harmless products (Ganiev and Nedorezkov 2006).The concentration of the poorly soluble TBM was about 0.15–0.20 mg/m^2 from May 31 to July 22, and only traces were detected on August 20.

Different results were obtained for the free herbicides. MET concentration was lower than in the treatment throughout the experiment, decreasing gradually; on July 22, it was about 10 mg/m^2, or three times lower than at the beginning of the experiment (May 31); on August 2, only traces were detected. TBM, which was quickly inactivated in soil, was only detected on May 31.

Thus, embedded herbicides, in contrast to their free forms, are gradually released to soil and persist in it, controlling weeds over extended periods of time.

The effects of herbicide formulations on weed development

The first weeds emerged on June 14 (22 days after beet seeds were sown and 7 days after tomato seedlings were planted). The experimental plot was heavily infested with weeds such as saltbush (*Atriplex*), red-root amaranth (*Amaranthus retroflexus*), wallflower (*Erysimum*), dandelion (*Taraxacum*), yellow cress (*Rorippa*), field milk thistle (*Sonchus*), chickweed (*Stellaria media*), and grass (*Elytrigia*). Weeds in the tomato and beet crops were dominated by saltbush (reaching 70 and 40% of the total weed plants, respectively). During the growing period, the total number of the weeds changed. They reached their maximum at the end of June, and then, their number varied depending on the herbicide applied and the form of the herbicide (Table 3 and Fig. 2).

In the beet and tomato crops, the highest total numbers of weeds in the negative controls in June were 177 and 254 plants/m^2, respectively. The weeds in the tomato crops were represented by saltbush, 74%; red-root amaranth, 7%; chickweed, 6%; dandelion, 4%; field milk thistle, 3%; grass, 2%; and wallflower, 2%. The weed species composition of the beetroot crops was as follows: saltbush, 39%; wallflower, 19%; red-root amaranth, 12%; chickweed, 11%; dandelion, 8%; grass, 6%; yellow cress, 4%; and field milk thistle, 1%.

Table 3 The total number of weeds in vegetable crops with different herbicide applications

Treatment/control	Number of weeds (plants/m$^{2)}$			
	May 31	June 27	July 22	August 20
Tomato cv. “Zagadka” crops				
Control «-»	0	254	224	192
Control «+» TBM	0	78	77	70
Control «+» MET	0	92	88	68
P(3HB)/wood flour/TBM	0	160	93	62
P(3HB)/wood flour/MET	0	165	136	56
Table beet cv. “Tsilindra” crops				
Control «-»	0	177	123	81
Control «+» TBM	0	47	56	44
Control «+» MET	0	74	68	56
P(3HB)/wood flour/TBM	0	77	44	27
P(3HB)/wood flour/MET	0	61	16	9

The effect of herbicide application became evident in June, when the number of weed plants dropped dramatically relative to the negative control. In June, the average number of weeds on the plots with tomatoes treated with free MET and TBM was 80 plants/m^2, and that was 1.5–2.0 times less than in the treatments with embedded herbicides. The number of weeds infesting beet crops in June was lower as well: 74 plants/m^2 on the plot with free MET and 47 plants/m^2 on the plot with free TBM. Then, until the end of the experiment, the number of the weeds had not changed on the plot with free TBM and had been somewhat reduced on the plot with free MET (Table 3). A possible reason for the more effective weed control by metribuzin compared with tribenuron-methyl is that in soil, TBM is rapidly inactivated, and in plant tissues, it is metabolized to form products harmless to plants. Therefore, a single application of the TBM spray was effective for weed control over 1 month, but the herbicide did not produce a prolonged effect on the development of weeds infesting vegetable crops. The most effective weed control was achieved on the plot with embedded MET (Table 3). The effect was observed 2 weeks after application, and it lasted throughout the growing season, reducing the number of weeds.

Later on, in the positive controls, the number of weed plants remained almost the same, but they were growing steadily and their mass increased. By contrast, on the treatment plots, the embedded herbicides decreased the number of the weeds considerably and inhibited their growth and development. Photographs (Fig. 1) show that in July, on the negative control subplots, the weeds were tall enough to shade the crops. At the same time, on the subplots treated with

Fig. 2 Photographs of the beet cv. "Tsilindra" (**a**) and tomato cv. "Zagadka" (**b**) crops with different herbicide applications during the experiment (field season of 2019)

herbicides, there were considerably fewer tall weeds, as their growth was slower, and on the subplots with embedded herbicides, there were very few if any tall weeds. In late August, the largest number of weeds was present on negative control plots with the beet and tomato crops: 81 and 192 plants/m^2, respectively; saltbush plants constituted 55–60% in tomato crop and about 30% in beet crop. Considerably fewer weeds were present on the plots with embedded MET (9 plants/m^2 in the beet crop and 56 plants/m^2 in the tomato crop; at that time, there were very few saltbush plants on treatment plots). Thus, embedded MET and TBM were effective against all weed plants present on the field including the prevailing saltbush.

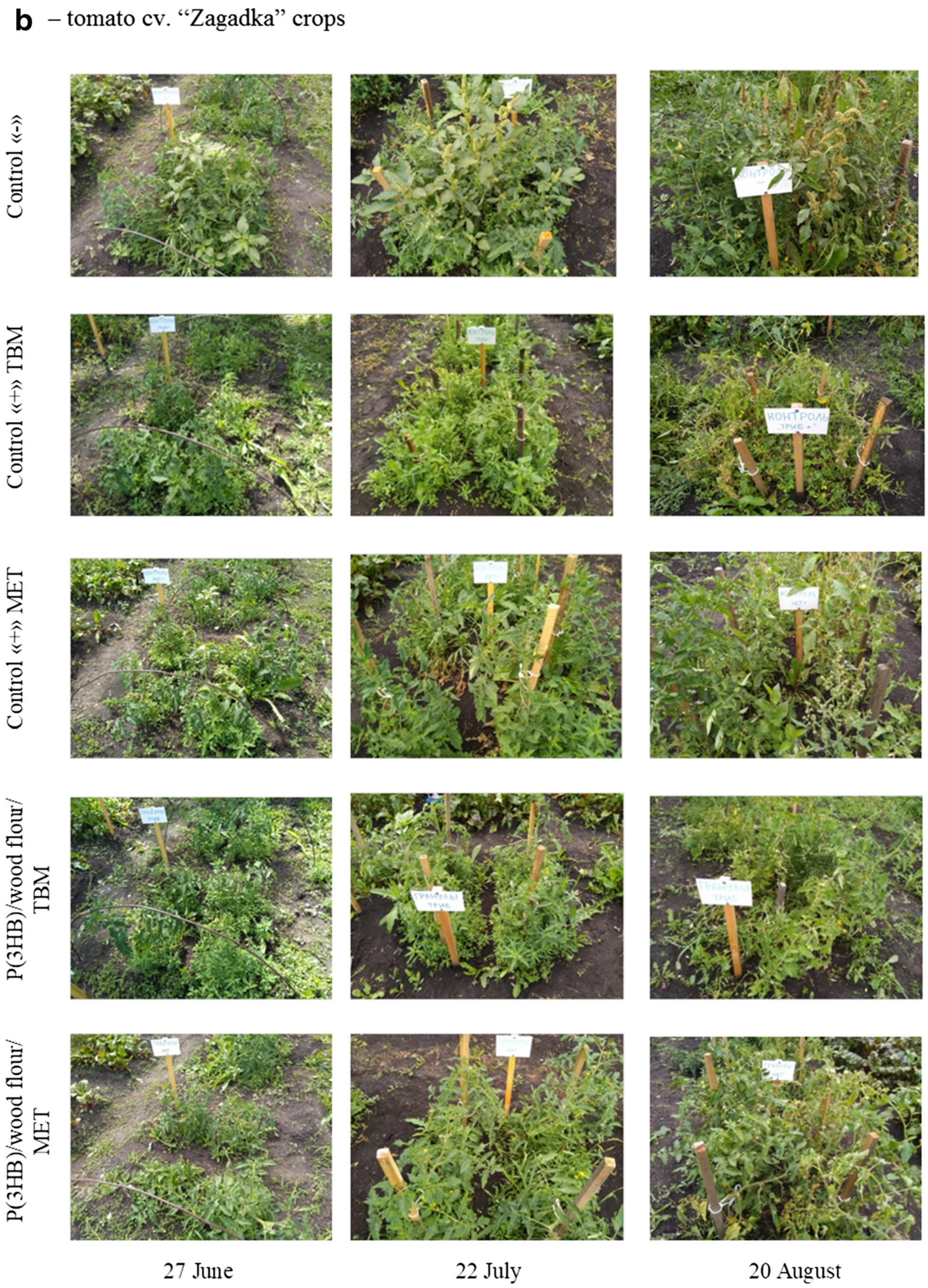

Fig. 2 (continued)

The effect of the herbicide formulations on biometric parameters of vegetable crops

Tomatoes were harvested and their biometric parameters were determined on August 20, 2019, as fruit maturation was delayed. Applications of the tribenuron-methyl and metribuzin herbicides as sprays and as granules affected the number of fruits, percent of nonstandard fruits, and the average mass of the fruits (Table 4).

The height of tomato plants is a grading factor. In the micro-field experiment, the ultimate height of the tomato plants ranged from 61 to 80 cm. The average height of the plants grown on the subplot untreated with herbicides was greater than on the subplots treated with the herbicides. Thus, herbicides could produce an unfavorable effect on vegetable crops. In the negative control, the average number of fruits was 6 per plant, the average mass of the fruit was 43 g, and crop productivity was 1.4 kg/m^2; only 20% of the fruits

Table 4 Biometric parameters of tomatoes and table beetroots in experiments with different herbicide formulations

Treatment/control	Plant height (cm)	Number of fruits/roots per plant	Average mass of the fruit/root (g)	Nonstandard fruits/roots (%)
Tomato cv. "Zagadka"				
Control «-»	80.1 ± 0.2	8	43.4 ± 2.1	20
Control «+» TBM	61.1 ± 0.3	5	34.5 ± 1.8	52
Control «+» MET	77.1 ± 0.5	7	38.6 ± 2.0	45
P(3HB)/wood flour/TBM	76.6 ± 0.2	6	27.6 ± 1.4	53
P(3HB)/wood flour/MET	77.4 ± 0.4	11	77.4 ± 3.1	10
Table beet cv. "Tsilindra"				
Control «-»		28	94.9 ± 2.4	0
Control «+» TBM		25	69.3 ± 1.8	10
Control «+» MET		30	99.3 ± 2.7	0
P(3HB)/wood flour/TBM		31	79.3 ± 3.0	7
P(3HB)/wood flour/MET		31	107.8 ± 2.9	0

were substandard (below 4 cm). Parameters of tomato fruits produced on the subplots treated with the free herbicides were inferior to the parameters of the fruits on intact plants: the number of the fruits and their mass were considerably lower and the number of substandard fruits higher, especially on the subplots treated with TBM (Table 4).

The best productivity parameters for tomatoes were obtained on the subplot treated with embedded MET, where the number of the weeds was the lowest. In that treatment, the number of the fruits per plant was 3–4 fruits greater, and the average mass of a fruit 34–39 g higher than in the negative and positive controls, and the number of substandard fruits was the lowest—10. The productivity achieved on the subplot treated with embedded MET was substantially higher than on the other subplots—2.3 kg/m^2 (Fig. 3).

Different productivity parameters of table beet were achieved on the subplots treated with the free and embedded MET and TBM as well (Table 4). The number of beetroots per m^2 was significantly ($p = 0.04$) greater on the subplots treated with embedded MET and TBM (31 roots/m^2) and free MET (30 roots/m^2) compared with the negative control. On the subplot treated with free TBM, this parameter was lower (25 roots/m^2), and the number of substandard roots was higher (10), while the average mass of the beetroot was the lowest (59.3 g). The highest average mass of one beetroot was achieved on the subplots treated with free and embedded MET: 99.3 and 107.8 g, respectively.

The largest yield of beetroots (3.4 kg/m^2) was harvested from the plot with embedded MET (Fig. 3). Application of TBM considerably decreased beet productivity. The number of roots was the lowest, the spacing was the greatest, the average mass of the roots was the lowest (59 g), and 10% of the roots were substandard (of length less than 5 cm).The improved biometric parameters of the vegetable crops in the treatments, where embedded herbicides were used, resulted from the more effective suppression of weed growth and reduction in the number of weed plants compared with the

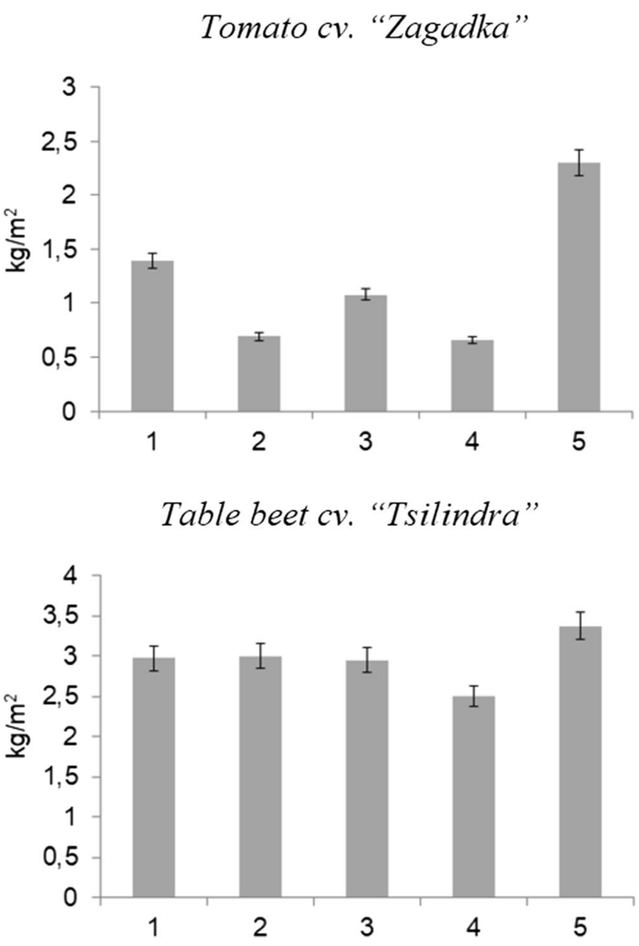

Fig. 3 Productivities of the tomato cv. "Zagadka" (**a**) and table beet cv. "Tsilindra" (**b**) in the experiment: 1, control «-»; 2, control «+» TBM (spray); 3, control «+» MET (spray); 4, P(3HB)/wood flour/TBM granules; and 5, P(3HB)/wood flour/MET granules

control plots. The number of the weeds, which competed with the crops for nutrients and water, was decreased, and, hence, the supply of nutrients to crops was enhanced, resulting in better conditions for plant growth and development of tomato fruits and beet roots.

The favorable effect of embedded metribuzin in the form of granules on productivity of vegetable crops is associated with its high efficacy in weed control: the herbicide is gradually released to soil and functions there over long time periods. The half-life of tribenuron-methyl is 6 days, while metribuzin may remain active in different soil types for 1 to 3 months, i.e., throughout the entire growing season.

Biochemical parameters and quality of vegetable produce

In addition to the productivity of vegetable crops, an important parameter is their quality and nutritional value. It is important to know how the quantitative and qualitative biochemical composition of vegetables varies to effectively grow and preserve vegetable crops.

Nitrate nitrogen is accumulated in vegetable crops with different rates, and, thus, concentrations of nitrogen compounds in vegetables may vary between 10 and 150 mg/kg. Analysis showed that nitrate concentrations in tomato fruits were 2–3 times below maximum allowable concentration (MAC) (Table 5). Tomato fruits grown on the subplot treated with embedded MET contained reduced concentrations of nitrate nitrogen—51 mg/kg. In the treatments, where slow-release herbicide formulations caused higher mortality rates of the weeds, both biometric parameters were enhanced, and nitrate nitrogen was decreased, which was associated with the effects of growth conditions on nitrogen assimilation and metabolism in plants. Nitrogen assimilation is plant uptake of nitrogen compounds from soil, transport of these compounds to plant organs, and their participation in intracellular metabolic reactions—transformation of inorganic nitrogen into organic nitrogen. Assimilation of nitrogen by plants results in not only formation of the active (metabolic) pool of organic compounds but also passive accumulation of nitrogen in plant organs and tissues, i.e., formation of nitrogen store. The metabolic (active) to storage nitrogen pool ratio in plants varies widely depending on a number of internal and external factors. Under favorable growth conditions, including nitrogen nutrition, intracellular metabolism and formation of organic compounds occur at high rates. By contrast, under unfavorable conditions, the rates of biochemical reactions slow down, and the inorganic nitrogen taken in by plants is not metabolized but is rather passively accumulated in plant tissues and organs as a storage pool of nitrates and other nitrogen forms. In our opinion, the lower rate of festation of crops with weeds on the treatment plots minimized the competition between crops and weeds for water and nutrients and created more favorable growth conditions for crops. Thus, an optimal balance was established between the metabolic and storage pools: most of the nitrates were assimilated and metabolized by plants without being accumulated as the storage pool. A similar nitrate decrease was noted by the authors. Biochar, which improves soil structure and increases availability of water to plant sunder water deficit, was applied to soil and caused a similar reduction in inorganic forms of nitrate and ammonium nitrogen in tomato fruits, enhancing their quality and productivity (Agbna et al. 2017). Application of slow-release nitrogen fertilizers produced a favorable effect on yield and size of tomatoes and decreased nitrate content in the fruits (Li et al. 2017).

Beet roots accumulate considerable concentrations of nitrate nitrogen, which are often higher than MAC, because of specific metabolism of beet. Beet grown in the micro-field experiment without application of nitrogen-based fertilizers accumulated in significant amounts of nitrate nitrogen in its roots (53–103 mg/kg), and they were below MAC (Table 5). In the treatment with embedded MET, nitrate nitrogen concentration in beetroots was 35 mg/kg lower (52.7 mg/kg) than in the negative control beetroots and 48 mg/kg lower than in the positive control. Results obtained for tomato fruits and beetroots were comparable, suggesting that embedded MET produced the same effect on nitrate accumulation in different vegetable crops.

An important indicator of the quality of vegetables is their chemical composition, including the contents of dry matter, sugars, and vitamin C. The micro-field experiment showed that dry matter concentration in tomato fruits varied depending on the type of herbicide delivery (Table 6).

Table 5 Nitrate concentrations in tomato fruits and beetroots in experiments with different herbicide formulations

Treatment/control	Tomato cv. “Zagadka” Nitrates, mg/kg (MAC = 150 mg/kg)	Table beet cv. “Tsilindra” Nitrates, mg/kg (MAC = 1400 mg/kg)
Control «-»	69.3 ± 3.5	87.3 ± 4.0
Control «+» TBM	80.0 ± 4.2	61.0 ± 3.4
Control «+» MET	83.0 ± 4.7	100.3 ± 4.9
P(3HB)/wood flour/TBM	68.7 ± 3.6	102.7 ± 5.0
P(3HB)/wood flour/MET	51.0 ± 2.8	52.7 ± 3.1

Table 6 Chemical composition of tomato fruits and beetroots in experiments with different types of herbicide delivery

Treatment/control	Dry matter (%)	Sugars (%)	Vitamin C (mg/100 g)
Tomato cv. "Zagadka"			
Control «-»	5.60	2.51	7.44
Control «+» TBM	5.66	2.60	8.06
Control «+» MET	5.57	2.53	8.29
P(3HB)/wood flour/TBM	5.55	2.76	14.40
P(3HB)/wood flour/MET	5.98*	2.89	13.03
Table beet cv. "Tsilindra"			
Control «-»	12.40	11.87	13.12
Control «+» TBM	13.00	12.77	13.75
Control «+» MET	13.40	12.52	12.94
P(3HB)/wood flour/TBM	14.60	12.66	16.75
P(3HB)/wood flour/MET	12.50	12.13	17.24

Dry matter increased significantly, reaching 5.98%, in the treatment with embedded MET compared with the intact plants and positive control. Concentration of sugars in tomato fruits was related to growing conditions and the form of herbicides, being higher in the treatments with embedded TBM and MET. The herbicide application technique affected accumulation of vitamin C in tomato fruits even stronger. In the treatments with embedded TBM and MET, vitamin C concentration reached 13.03 and 14.40 mg/100 g, respectively, being 1.6–1.7 times higher than in the negative and positive controls. Thus, not only did metribuzin produce the herbicidal effect but it also induced improvement of the quality of tomato fruits. Similar beneficial, non-pesticidal, effects were reported in a number of studies. Strobilurin, a broad spectrum fungicide used in tomato crops, enhanced tomato productivity, mitigating the effects of unfavorable factors such as moisture deficit (Bartlett et al. 2002). That pesticide is known to have other than fungicidal effects (Cantore et al. 2016): it enhances tomato productivity by regulating hormone levels, retaining water in the fruits, and slowing down senescence. Strobilurin-based agrochemicals were found (Cantore et al. 2016) to be useful in treating tomato diseases, as they enhanced tomato productivity and the effectiveness of water use by plants under deficit irrigation and induced an increase in sugars and antioxidants in the fruits. Application of polymer-coated nitrogen-based fertilizers, which were slowly released to soil, produced a favorable effect on tomato productivity and fruit size and increased the contents of soluble sugar and lycopene in the fruits (Li et al. 2017).

Table 7 Results of chromatographic analysis for pesticides in beetroots and tomato fruits in experiments with different types of herbicide delivery

Treatment/control	Herbicide concentration in the sample (μg/kg)	Regulatory compliance
Table beet cv. "Tsilindra"		
Control «+» TBM	< 0.1	Conforms to standard
Control «+» MET	< 0.1	Conforms to standard
P(3HB)/wood flour/TBM	< 0.1	Conforms to standard
P(3HB)/wood flour/MET	< 0.1	Conforms to standard
Tomato cv. "Zagadka"		
Control « + » TBM	< 0.1	Conforms to standard
Control « + » MET	< 0.1	Conforms to standard
P(3HB)/wood flour/TBM	< 0.1	Conforms to standard
P(3HB)/wood flour/MET	< 0.1	Conforms to standard

In all treatments and controls, the beetroots contained similar weight percentages of dry matter, about 12.5–14.6% (Table 6). Sugar contents in the roots were comparable, too. Vitamin C concentrations in the beetroots grown on the subplots with embedded herbicides were 1.4 times higher than in the controls. Thus, the herbicide type and the mode of application determine not only the crop productivity but also the quality of the vegetable produce.

Pesticides can potentially accumulate in the crops. Therefore, vegetables grown in experiments with different types and modes of delivery of herbicides were analyzed for TBM and MET by chromatography (Table 7).

Metribuzin and tribenuron-methyl contents in beetroots and tomato fruits, regardless of the delivery mode (free or embedded herbicide), were below 0.1 μg/kg, i.e., conformed to the standards.

Conclusion

The efficacy of experimental formulations of metribuzin and tribenuron-methyl embedded in the matrix of degradable poly-3-hydroxybutyrate blended with wood flour was tested in field-grown tomato and table beet crops infested with weeds. The reduction in weed density was beneficial for crop productivity and improved biometric parameters of the vegetables. Application of embedded metribuzin resulted in the highest productivities of tomatoes (2.3 kg/m^2) and beetroots (3.4 kg/m^2) and caused reduction in nitrate nitrogen concentrations in tomato fruits and beetroots. Application of both embedded metribuzin and tribenuron-methyl induced a 1.7-fold and 1.4-fold, respectively, increase in vitamin C concentrations in tomato fruits and beetroots.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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