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Effect of irrigation and adjuvant on residual activity of pendimethalin and metazachlor in kohlrabi and soil

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Abstract: Metazachlor and pendimethalin are the most used herbicides in kohlrabi. The main objectives of the present study were to evaluate the residual activity of herbicides pendimethalin and metazachlor in kohlrabi and soil under different irrigation regimes and to evaluate the effect of soil adjuvants on the residual activity of tested herbicides. Pendimethalin dissipation half-life (17.3–38.3 days) was higher than metazachlor dissipation half-life (12.1–16.8 days). The pendimethalin half-life was not affected by an adjuvant, irrigation, and an experimental year. Pendimethalin mobility in the soil was affected more by natural precipitation than by irrigation. The use of adjuvant did not affect pendimethalin leaching in dry weather conditions. In wetter natural conditions, a higher pendimethalin leaching was found at early-irrigated plots treated by an adjuvant (9.39% of the applied dose was detected in the soil layer 5–10 cm). Metazachlor dissipation half-life was not affected both by an adjuvant and by irrigation. In the soil layer 5–10 cm, metazachlor was detected only in 2016 on intensively irrigated plots without the use of adjuvant (0.072 μg/g). A concentration of pendimethalin in kohlrabi tubers ranged between 2 and 7 μg/kg. The highest concentration of pendimethalin was detected in tubers, which were intensively irrigated shortly after the application of herbicides without an adjuvant, especially when natural precipitation was high. Metazachlor was not detected in any of the tested kohlrabi samples.

Keywords: post-emergence herbicide application; low residual production; herbicide persistence and leaching

Kohlrabi (*Brassica oleracea* var. *gongylodes)* is a common brassica vegetable crop, which is grown in Europe and North America (Biesiada 2008). Use of herbicides is a common weed management strategy in brassica vegetables (Miller et al. 2003). Metazachlor and pendimethalin are the most used herbicides in kohlrabi.

Pendimethalin is a soil activated herbicide that controls many broadleaf and grass weeds and is used in many crops, including brassica vegetables. Pendimethalin belongs to dinitroaniline herbicide group, which inhibits polymerization of tubulin basic units and the creation of protofilaments. Consequently, it also interrupts the production of microtubules and the entire spindle apparatus (Vaughn and Lehnen 1991). In brassica vegetables, pendimethalin is recommended only on the transplanted canopy, and it can be applied prior or after to planting at an application rate up to 1200 g/ha. Pendimethalin efficacy increased if irrigation occurred shortly after its application, especially under dry conditions

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(Jursík et al. 2019). Persistence of pendimethalin in the soil is relatively long, and pendimethalin residues are therefore common in many soils (Saha et al. 2015, Karasali et al. 2016). Pendimethalin persistence is longer in soils with lower pH (Chen et al. 2018) and when a transparent nonwoven fabric covers the soil (Jursík et al. 2017). According to Kočárek et al. (2018), the adjuvant significantly affected pendimethalin behavior in laboratory conditions but had no effect in field conditions.

Metazachlor is a specific inhibitor of very-long-chain fatty acids biosynthesis affecting alkyl chains longer than C_{18}, and its site of action is outside the chloroplast (Boger 2003). Metazachlor is used in kohlrabi before and after sowing or planting, but residues of metazachlor are often detected in underground water (Karier et al. 2017). Therefore, restrictions on metazachlor use in the European Union are expected shortly. Alginate controlled release formulation of metazachlor reduced the vertical mobility of this herbicide in the soil in comparison with suspension concentrate (Wlodarczyk 2014).

Adjuvants are substances without the biological activity of their own, but that enhance the effectiveness of herbicides (Baratella et al. 2016). The behavior of herbicides in the soil can be influenced by the addition of adjuvants (Locke et al. 2002). Adjuvants can reduce leaching of soil-active herbicides (Reddy 1993). Some adjuvants influence the concentration of herbicide residues in soil, as well as herbicide delivery, uptake, redistribution, and persistence and thus, the final biological efficacy (Cabrera et al. 2010). Oil and surfactant adjuvants decreased metazachlor decomposition in soil contaminated by heavy metals (Zajaczkowska and Kucharski 2017).

Leaching and persistence of herbicides in the soil are affected by precipitation or irrigation. According to Renaud et al. (2004), pesticide leaching is affected mainly by the preferential flow, soil sorption capacity, pesticide half-life, and diffusion inside the soil aggregates. The risk of herbicide leaching is higher on the soils with lower sorption capacity. The concentration of herbicide in the soil solution is higher in these soils compared to soils with a higher sorption capacity. Important properties of pendimethalin and metazachlor in the environment are shown in Table 1.

Pendimethalin is a common contaminant of vegetables (Baša et al. 2012). Esturk et al. (2014) detected pendimethalin residues in parsley (95% of samples) and lettuce (93% of samples). Metazachlor residues in the vegetable are less frequent, but Gonzáles-Martín et al. (2017) detected metazachlor in a few samples of propolis. The maximum residue level (MRL) established by Regulation (EC) No. 396/2005 for kohlrabi tubers is 300 μg/kg of pendimethalin and metazachlor (including relevant metabolites). Degradation of pesticide residues in brassica vegetables (especially in head cabbage) relatively speeds – the half-life of tested pesticide residues ranged between 1 and 8 days (Kocourek et al. 2017).

The main objectives of the present work were to evaluate the residual activity of herbicides pendimethalin and metazachlor in kohlrabi and soil under different irrigation regimes and to evaluate the effect of a soil adjuvant on the residual activity of tested herbicides in same experiments. This study completes the study of Jursík et al. (2019), who assessed the biological efficacy and selectivity of pendimethalin and metazachlor in the same experiments.

MATERIAL AND METHODS

Site description. Kohlrabi (cv. Lech) was planted in plot trials in Prague, the Czech Republic, Central Europe (300 m a.s.l., GPS: 50°7'N, 14°22'E), in 2015 and 2016. This region is characterized by a temperate climate (annual average air temperature around 9°C,

Table 1. Important properties of tested herbicides in the environment (source: Footprint Database 2018)

		Pendimethalin	Metazachlor
Solubility in water at 20°C (mg/L)		0.33	450
GUS leaching potential index		–0.32	2.17
Soil degradation (aerobic)	field DT_{50} (days)	100.6	6.8
	lab at 20°C DT_{50} (days)	182.3	10.8
Adsorption strength by Freundlich	K_f	220.1	1.02
	K_{fOC}	13 792	79.6

GUS – groundwater ubiquity score; DT_{50} – pesticide half-life; K_f – Freundlich adsorption coefficient; K_{fOC} – Freundlich organic carbon adsorption coefficient

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total average annual precipitation nearly 500 mm). The soil of experimental fields was classified as a Haplic Chernozem consisting of 19% clay, 26% sand, and 55% silt (silt loam soil) with the sorption capacity of 210 $mmol_{+}$/kg and soil pH_{KCl} of 7.3. Nutrient content (Mehlich 3) in the top layer (0–25 cm) was as follows: 2.7 mg N/kg, 136 mg P/kg, 289 mg K/kg, 142 mg Mg/kg and 6858 mg Ca/kg. C_{ox} (oxidizable carbon) 2.0%.

Experimental setup. Before kohlrabi planting, the soil was fertilized with 60, 27, and 53 kg/ha of N, P, and K, respectively. During the growing season, 40 kg N/ha was applied. Maize was the previous crop in both experimental years and was treated with tank mix combination of bromoxynil (375 g/ha; Pardner 22.5 EC®, 225 g/L) + tembotrione (66 g/ha; Laudis OD®, 44 g/L). The trials were arranged in a split plot design with herbicide and adjuvant treatment as the main plot and irrigation regime as the subplot. Three replications per herbicide/adjuvant treatment were organized in a randomized complete block design. The area of the main plot was 21 m^2 (2.1 × 10 m).

Pendimethalin (Stomp 400 SC; 400 g/L; manufactured by BASF SE, Ludwigshafen, Germany) was applied at a rate of 1200 g/ha. Metazachlor (Butisan 400 SC; 400 g/L; manufactured by BASF SE, Ludwigshafen, Germany) was applied at a rate of 800 g/ha. Soil adjuvant Grounded (732 g/L of refined paraffin oil, aliphatic hydrocarbons, hexahydric alcohol ethoxylates, and C_{18}–C_{20} fatty acids; manufactured by Helena Chemical Company, Collierville, USA) was applied at a rate of 0.4 L/ha. Herbicides and adjuvants were applied one week after kohlrabi planting, i.e., 21.04. in 2015 and 28.04. in 2016. Herbicides were applied using a small-plot sprayer with Lurmark 015F110 nozzles at a spray volume of 300 L/ha and pressure of 0.25 MPa. Row spacing was 30 cm and in-row plant spacing 30 cm. The second day after the herbicide application, 40 mm of water were irrigated by a special irrigation frame with micro-irrigate sprinklers on subplots (10 m^2). Terms of kohlrabi planting, harvesting, weather, and irrigation conditions in both experimental years are given in Table 2.

Samples collection. Soil samples for determination of pendimethalin and metazachlor concentration were collected from two soil layers (0–5 cm and 5–10 cm) using soil cylinders (immediately after the herbicide application and then 5, 14, and 35 days after the herbicide application in 2015; and 7, 15, 25 and 46 days after application in 2016). Three kohlrabi tubers were collected from the central part of each plot (2.5 m^2) for a determination of the pendimethalin and metazachlor concentration at harvest (4.6. 2015, resp. 11.6. 2016).

Herbicide determination in soil. The pendimethalin and metazachlor concentration in methanol extracts were determined using high-performance liquid chromatography (HPLC) whose parts were in details described in the previous study (Kočárek et al. 2016). The wavelengths for detection of metazachlor and pendimethalin were 225 nm and 240 nm, respectively. The retention time was 2.4 and 9.3 min. for metazachlor and pendimethalin. Limit of a detection (LOD = 3 × σ/slope) and limit of a quantification (LOQ = 10σ/slope) of metazachlor was 0.0250 and 0.0833 μg/cm^3. LOD and LOQ of pendimethalin were 0.0056 and 0.0189 μg/cm.

Concentrations of pendimethalin and metazachlor in both soil layers (0–5 cm and 5–10 cm) during the experiment were used to calculate their dissipation rate constant (k) using the first order equation:

$$C = C_0 e^{-kt} \quad (1)$$

The herbicide half-life (DT_{50}) was then calculated using the equation of:

$$DT_{50} = \frac{0.6932}{k} \quad (2)$$

Herbicide residues determination in kohlrabi. Kohlrabi samples were tested in the certified laboratory of the Department of Food Analysis and Nutrition at the University of Chemistry and Technology Prague. Analysis of target herbicides was a part of a multiresidue analytical method that had been fully validated by ISO 17025, 2016. Extraction of pesticide residues was based on QuEChERS method. Pesticides were

Table 2. Weather conditions and irrigation during growing seasons

Year	Date of planting	Date of harvest	Mean temperature (°C)	Total natural precipitation (mm)	Total irrigation (mm)	
					heavily irrigated plots	low irrigated plots
2015	14.04.	04.06.	13.28	54.4	100	60
2016	20.04.	11.06.	13.69	93.3	85	45

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extracted from a portion of the homogenized sample (5 g) after water addition by acetonitrile. After separation of aqueous and acetonitrile layers (induced by addition of anhydrous $MgSO_4$ and NaCl salts) an aliquot of the upper organic layer was transferred into a vial for LC-MS/MS. For the final identification and quantification of pesticides residues, the U-HPLC system coupled to a triple quadrupole mass spectrometer with electrospray ionization in positive ion mode (ESI+) was used. The generated data were processed by MassLynx software version 4.1 (Milford, USA).

Pendimethalin and metazachlor certified standard and triphenyl phosphate (TPP, internal standard) were purchased from Fluka (Seelze, Germany). Working standard solutions were diluted with acetonitrile to prepare matrix-matched calibration standards. Organic solvents for pesticide residue analysis were of the highest purity grade: acetonitrile from Sigma-Aldrich (St. Louis, USA) and methanol from Merck (Darmstad, Germany). Ammonium formate, formic acid, and anhydrous magnesium sulfate were obtained from Sigma-Aldrich (St. Louis, USA). Sodium chloride was from Penta (Prague, Czech Republic). Milli-Q water (Millipore, USA) was used for preparing mobile phases.

Recovery was evaluated using blank sample homogenate spiked with a pendimethalin and metazachlor standard (concentrations of 40 and 4 μg/kg) and then processed as described above. The performance characteristics of the analytical method employed were as follow recovery 81–90%, relative standard deviation (six replicates) 1–6%, limit of quantitation was 2 μg/kg.

Statistical analyses. Using Statistica version 12 (StatSoft, Tulsa, USA), results were tested by analysis of variance (ANOVA) followed by Tukey's post hoc comparisons to corresponding controls once the differences among mean values had been determined. ANOVA effects and differences were considered significant at $P < 0.05$.

Calculated herbicide half-live values (using Eq. 2) for both pendimethalin and metazachlor showed a normal distribution. *T*-test was used for a comparison of pendimethalin and metazachlor half-life. The effect of irrigation and adjuvant on pendimethalin and metazachlor half-lives was studied using multifactor ANOVA.

The pendimethalin occurrence in both tested soil layers (0–5 cm and 5–10 cm) showed a normal distribution after a logarithmic transformation. Metazachlor half-life showed a normal distribution. Multifactor ANOVA was used to evaluate the effect of irrigation, adjuvant, and year of application both metazachlor and pendimethalin half-life at the end of vegetation. *T*-test was used to compare pendimethalin and metazachlor half-lives. A simple regression was used to compare the concentration of pendimethalin in the soil layers (0–5, 5–10 and 0–10 cm) and the pendimethalin half-life and its concentration in kohlrabi.

RESULTS AND DISCUSSION

Herbicide dissipation half-life. Pendimethalin dissipation half-life in the soil layer 0–10 cm ranged from 17.3 to 38.3 days (Figure 1). Jursík et al. (2017) observed a similar pendimethalin half-life in the same

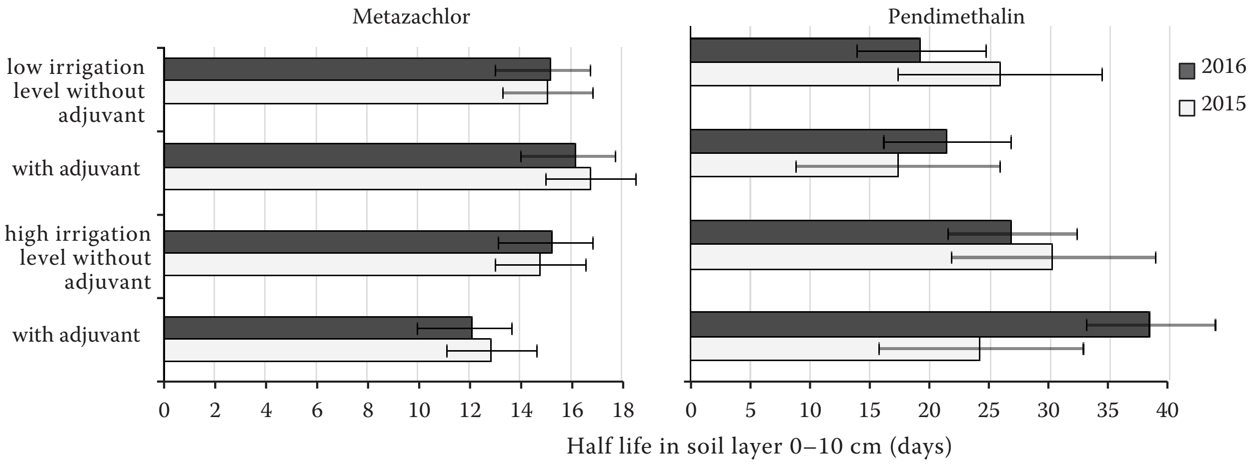

Figure 1. Half-lives of pendimethalin and metazachlor in the soil layer 0–10 cm in dependence on an irrigation level and use of adjuvant Grounded (the error bars indicate standard deviation of pendimethalin and metazachlor half-lives in 2015 and 2016)

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soil ranging between 23.0–85.3 days in treatments covered by non-woven fabric and 17.7–51.0 days in no covered treatments. Tandon (2015) determinated a half-life of pendimethalin in the soil between 11.7 and 34.1 days for a recommended application rate and between 9.2 and 46.8 for a double rate. In our study, the pendimethalin half-life was not affected by an irrigation ($P = 0.1028$), an adjuvant ($P = 0.9615$) and an experimental year ($P = 0.6613$). No effect of an adjuvant and irrigation on a pendimethalin half-life (ranging from 43.0 to 44.6 days) in Haplic Chernozem under field condition was documented by Kočárek et al. (2018). On another hand, a dimethenamid half-life (ranging from 8.8 days to 12.9 days) was significantly prolonged by the use of an adjuvant and by dry conditions. Walker and Bond (1977) studied the pendimethalin half-life in different soil moistures in laboratory conditions. They found a longer pendimethalin half-life under dry soil conditions than in wet soil. A similar effect was described by Odero and Shaner (2014). These results don't match with results of our study because, at treatments irrigated shortly after the pendimethalin application, a longer pendimethalin half-life was detected compared to treatments irrigated later. The longer pendimethalin half-life at early irrigated treatments can be caused by the pendimethalin incorporation from the soil surface into the soil profile (in our case not deeper than 5 cm) which reduces its photodecomposition and evaporation. The reduction of the pendimethalin half-life due to its incorporation into the soil was also reported by Odero and Shaner (2014).

The calculated dissipation half-life of metazachlor in the soil layer 0–10 cm ranged from 12.1 to 16.8 days (Figure 1) and was significantly lower ($P < 0.001$) than the pendimethalin half-life. Metazachlor half-life was neither affected by an irrigation ($P = 0.1154$) and an adjuvant ($P = 0.6080$) nor by an experimental year ($P = 0.8518$). Kucharski and Sadowski (2011) reported a shorter metazachlor half-life. These authors also observed that the addition of oil and surfactant adjuvants slowed down the degradation of metazachlor in soils. The half-life for a mixture of metazachlor + oil and surfactant adjuvants was about 8–16 days longer in comparison with the metazachlor, half-life applied solo (26 days). Sadowski et al. (2012) detected the metazachlor half-life in three different soils ranging from 22 to 35 days. The high concentration of a clay fraction in the soil texture and the high organic carbon content increased the metazachlor degradation. Mantzos et al. (2017) reported a metazachlor half-life in three soil types in the range of 13–18 days under sunlight and 39–78 days under dark conditions. Authors noted that the organic carbon content and the transition metals concentration were the major soil constituents affecting indirect photolysis of metazachlor rates under sunlight conditions.

Herbicide distribution in soil layers. Herbicides mobility in the soil was evaluated on the base of its concentration in the soil layer 5–10 cm and as the percentage of its concentration from an applied dose in the soil layer 5–10 cm at the end of the experiment shortly before kohlrabi harvest (Figure 2). Pendimethalin concentration in the soil layer 5–10 cm was 12–30 times (2015), resp. 3–8 times (2016) lower compared to the pendimethalin concentration in the soil layer 0–5 cm (Table 3). Higher mobility of pendimethalin was observed in 2016 (higher natural precipitation) on plots, which were irrigated shortly after the application (pendimethalin leaching was 6.73% from an applied dose without adjuvant and 9.39% from an applied dose with adjuvant). The pendimethalin mobility in this study was lower than we reported

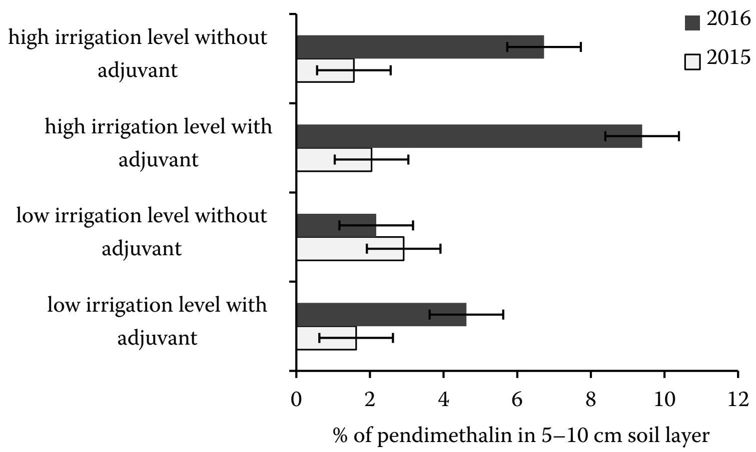

Figure 2. Ratio (% from the applied dose) of pendimethalin concentration in the soil layer 5–10 cm at the end of the experiment to the concentration of pendimethalin immediately after the application in the soil layer 0–5 cm. The error bars indicate standard deviations in 2015 and 2016

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Table 3. Pendimethalin concentration in soil (µg/g) layers 0–5 cm and 5–10 cm at the end of the experiment (mean ± standard deviation)

Adjuvant	Irrigation level	2015		2016	
		0–5 cm	5–10 cm	0–5 cm	5–10 cm
No	low	1.050 ± 0.198[a]	0.087 ± 0.029[a]	0.366 ± 0.101[a]	0.045 ± 0.154[a]
	high	1.363 ± 0.200[a]	0.047 ± 0.031[a]	0.456 ± 0.059[a]	0.141 ± 0.152[a]
Yes	low	0.674 ± 0.550[a]	0.046 ± 0.051[a]	0.396 ± 0.166[a]	0.099 ± 0.032[a]
	high	1.005 ± 0.125[a]	0.058 ± 0.020[a]	0.867 ± 0.099[b]	0.203 ± 0.045[a]
A: Adjuvant (*P*-value)		0.0514	0.4943	0.0351	0.9722
B: Irrigation level (*P*-value)		0.5315	0.5077	0.0116	0.4065
A × B		0.2178	0.4669	0.002	0.6701

Values within a column with the same letter are not significantly different at the 5% *HSD* (honestly significant difference) (*P* = 0.05) level

in our previous study conducted in lettuce (Jursík et al. 2017). In this study, the highest concentration of pendimethalin in the soil layer 5–10 cm (0.141, resp. 0.203 µg/g) was detected at the end of the growing season in 2016 on plots which were irrigated shortly after the pendimethalin application. Kočárek et al. (2018) documented no effect of an adjuvant and irrigation on a pendimethalin soil mobility in field conditions.

The mobility of metazachlor was relatively low. In the soil layer 5–10 cm, metazachlor was detected only in 2016 (4.74% of its concentration from applied dose, Table 4) on plots which were irrigated shortly after the application and adjuvant was not used (0.072 µg/g). On another hand, the lower incidence of metazachlor in the soil layer 5–10 cm at the end of a growing season can also be attributed to its faster degradation. Similar results were presented by Jursík et al. (2013) in case of acetochlor in the same soil. A higher metazachlor mobility and the significant effect of irrigation on metazachlor mobility was documented by Włodarczyk (2014).

Herbicide residues in kohlrabi. The concentration of pendimethalin in kohlrabi tubers ranged between 2 and 7 µg/kg in both experimental years. The MRL of pendimethalin stated for kohlrabi (300 µg/kg) was not exceeded in any of the samples tested. Similar results were shown by Sondhia (2013), who detected low levels of pendimethalin residues in cauliflower and radish crops after a pre-emergence application, and by Tsiropoulos and Miliadis (1998), who detected pendimethalin residue levels below the MRL after a post-emergence application in onion. On the contrary, Kaur and Bhullar (2015) did not detect any residues of pendimethalin in harvested cabbage.

In 2015, the concentration of pendimethalin in kohlrabi was significantly affected neither by an irrigation level nor by a used adjuvant (Table 5). In

Table 4. Metazachlor concentration in soil (µg/g) layers 0–5 cm and 5–10 cm at the end of the experiment (mean ± standard deviation)

Adjuvant	Irrigation level	2015		2016	
		0–5 cm	5–10 cm	0–5 cm	5–10 cm
No	low	0.357 ± 0.094[a]	nd	0.146 ± 0.019[a]	nd
	high	0.350 ± 0.123[a]	nd	0.198 ± 0.002[a]	0.072 ± 0.110
Yes	low	0.540 ± 0.077[a]	nd	0.184 ± 0.031[a]	nd
	high	0.402 ± 0.026[a]	nd	0.124 ± 0.122[a]	nd
A: Adjuvant (*P*-value)		0.0513		0.5876	
B: Irrigation level (*P*-value)		0.1996		0.2697	
A × B		0.0925		0.5017	

nd – not detected. Values within a column with the same letter are not significantly different at the 5% *HSD* (honestly significant difference) (*P* = 0.05) level

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Table 5. Pendimethalin residue concentrations in tubers (μg/kg) at kohlrabi harvest in experimental years

Adjuvant	Irrigation level	2015	2016
No	low	4.5[a]	3.47[a]
	high	3.38[a]	6.63[b]
Yes	low	4.17[a]	2.38[a]
	high	3.17[a]	3.48[a]
A: Adjuvant (*P*-value)		0.5932	0.0158
B: Irrigation level (*P*-value)		0.0307	0.0148
A × B		0.9038	0.1345

Values within a column with the same letter are not significantly different at the 5% *HSD* (honestly significant difference) (P = 0.05) level

2016, a significantly higher concentration of pendimethalin (6.6 μg/kg) was detected in tubers which were intensively irrigated shortly after the application of herbicides without the adjuvant Grounded compared to other tested pendimethalin treatments (2.4–3.5 μg/kg). Irrigation level significantly affected the concentration of pendimethalin in kohlrabi tubers in both experimental years. Adjuvant significantly decreased the concentration of pendimethalin only in 2016 (Table 5).

Residues of metazachlor were not detected in any of the tested kohlrabi samples. Koleva-Valkova et al. (2016) detected residues of metazachlor in plants of oil-seed rape 28–68 days after the pre-emergence application. In the case of post-emergence foliar application, metazachlor residues were detected only 8–28 days after the application. In our study, kohlrabi was harvested (metazachlor was analyzed) 45 days after a post-emergence application. No significant relationship (data not shown) was found between pendimethalin concentration in kohlrabi and its half-life in the soil layer 0–10 cm (and its concentrations in soil layers 0–5 cm and 5–10 cm), though significantly negative relationship between pendimethalin half-life and its concentration in lettuce was found in the previous study (Jursík et al. 2017).

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