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Effects of allelochemicals in *Brassica napus* L. residues on the germination of weeds

R. Cepuliene, Z. Kriauciuniene*, R. Velicka, A. S. Maruska,
M. Stankevicius, A. Marcinkeviciene and R. Kosteckas
Vytautas Magnus University, Studentu str. 11,
LT-53361 Akademija, Kaunas Dist., Lithuania
E. Mail: zita.kriauciuniene@vdu.lt, audrius.maruska@vdu.lt

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ABSTRACT

We investigated the inhibitory influences of winter oilseed rape residues on weeds. Residues after harvest on the germination of *Galium aparine* L. (i). 3-months after residues decomposition in soil on the germination of *Sinapis arvensis* L. and *Tripleurospermum perforatum* L. (ii). 7-months after decomposition on the germination of *Sonchus arvensis* L. The total contents of phenolic compounds in soil in decomposing rape residues significantly decreased after 3-months. The glucosinolates content was higher in rape stubble compared to upper plant part of threshed crop residues at harvest. The content of volatile organic compounds in rape residues 7-months after decomposition was the highest, comparing with other decomposition periods, except threshing remains. These results suggested that decomposing winter oilseed rape residues in soil were allelopathic and hence, influenced the agroecosystems in the following two-years period.

Key words: Allelochemicals, *Brassica napus*, crop residues, decomposition, germination, glucosinolates, phenolic compounds, seedling growth, volatile compounds, winter oilseed rape.

INTRODUCTION

Oilseed rape (*Brassica napus* L.) is raw material for edible oil, biofuel and fodder. It significantly influences the soil properties, activates microbiological processes, reduces weeds and soil sickness (50). It synthesises the allelopathic compounds (diverse and abundant amount of phenolic compounds) called allelochemicals, released into the environment through the leaves volatiles, roots residues and decomposition of residues (20,35). The phenolic compounds provides biocontrol of weed and pest control through crop rotations for sustainable agriculture (3,40,38). The enzymatic hydrolysis products of glucosinolates (31,37,54) present in *Brassicaceae* family plants, isothiocyanates and other volatile compounds, can be used to control the diseases and pests (2,8,28). Till now about 130 glucosinolates are identified in *Brassicaceae* plants (1,29). Glucosinolates are not toxic; however, when plant cells become damaged, enzymatic hydrolysis of glucosinolates begins (52), during which biologically active and mainly toxic volatile compounds are formed (11,41,49).

Plant residues and oil production waste from the *Brassicaceae* family plants inhibits the weed seeds germination (19). Petersen et al. (37) in *Brassica rapa* identified 5-isothiocyanates [2-phenethyl, n-butyl, 3-butenyl, benzyl and allyl isothiocyanates] which inhibited the germination of *Sonchus aspera* (L.) Hill, *Matricaria inodora* L., *Amaranthus hybridus* L., *Echinochloa crusgalli* (L.) Beauv., *Alopecurus myosuroides* Huds seeds. Brown and Morra (12) observed that the volatile compounds and products of microbial residue decomposition emitted during glucosinolate hydrolysis in oilseed rape residues reduced the seed germination capacity. Weeds reduces crop yields due to their allelopathic

*Correspondence author

effects on crops (56). In plastic tunnel studies, *Brassicaceae* family plants as cover crops reduced *Cyperus esculentus* (≤ 39 %) and *Sorghum halepense* (≤ 46 %), thus, they could be used in an integrated weed control system (5).

Variation in results between the experiments and the plant species requires detailed research of the allelopathic interactions between plant species (8) and a detailed analysis of allelopathic compounds (14,42). Comprehensive studies of the allelopathic effect of oilseed rape on crops and weeds may provide scientific explanation for its influence on soil fertility, plant growth when oilseed rape is included in crop rotation. In oilseed rape crop the *Sinapis arvensis* L., *Tripleurospermum perforatum* (Merat) M. Lainz., *Galium aparine* L. and *Sonchus arvensis* L. are major weeds (50). This study aimed to (i). determine the allelochemicals in plant parts residues (threshing remains, stubble and roots) of winter oilseed rape (*Brassica napus* L.) at harvest and (ii). to determine the quantitative and qualitative changes in the biomass after various decomposition periods in soil (3,7,14,19 and 26 months) and (iii). to determine the influence of these residues on germination and seedling growth of test weeds.

MATERIAL AND METHODS

I. Plant materials

Winter oilseed rape (*Brassica napus* L. spp. *oleifera biennis* Metzg.) residues (threshing remains, stubble and roots) were collected in August, 2014 after the crop harvest from Experimental Station of our University (54º52′ N, 23º49′ E). We collected 12 kg threshing remains, 5 kg stubble and 4 kg roots. These rape residues were chopped into 2-3 cms size chaffs. Twenty g chaff were kept in 9 × 12 cms plastic mesh bags. These bags were buried 20 cms deep in ploughed soil at 20 cms distance from each other. Different numbers of crop residues bags, according to their decomposition duration and rate, were buried into the soil for various periods (0,3,7,14,19,26 months). A total of 560 crop residues bags of different plant parts were buried into the soil (Table 1).

The soil at the experimental site was Hapli Epihypogleyic Luvisol (Drainic) (22), The soil pH_{KCl} : 6.6-6.8, total nitrogen : 0.13-0.21%, humus : 2.2-3.0%, mobile phosphorus : 242-265 mg kg^{-1}, and the mobile potassium : 139-147 mg kg^{-1}. The maximum and minimum temperature during the experimental period was +26.3 °C and –23.0 °C, respectively, and average rainfall per year was 680 mm.

The field experimental treatments consisted of two factors: I. Residues of oilseed rape: 3 [(i). Threshing remains (stems with branches and siliques), (ii) stubble (30 cms from root collar), (iii). Roots]; II. Duration of residue decomposition in soil: 6 (0,3,7,14,19,26 months). The treatments were replicated 4 times in Complete Randomised Design.

II. Bioassays

We determined the effects of different concentrations of aqueous extracts of winter oilseed rape residues [(threshing remains, stubble and roots) immediately after harvest (0 months) and after different time of decomposition in soil (3,7,14,19 and 26 months)] on the germination of 4-test weeds. The test weeds were : scentless mayweed (*Tripleurospermum perforatum* (Merat) M. Lainz.), field mustard (*Sinapis arvensis* L.), catchweed bedstraw (*Galium aparine* L.), field sow thistle (*Sonchus arvensis* L.). The following two-factorial laboratory experiment with four replications was done: I Residues of oilseed rape. II.

Aqueous extracts concentrations. We prepared 6-concentrations: [Control (distilled water), 1 : 10, 1 : 50, 1 : 250, 1 : 1250, 1 : 6250] of oilseed rape residues and water.

Table 1. Number of bags with crop residues ploughed into the soil for the decomposition experiment

Decomposition period (Months)	Number of soil buried bags
Rape threshing remains	
3	30
7	30
14	57
19	57
26	232
Stubble	
3	9
7	9
14	12
19	12
26	42
Roots	
3	9
7	9
14	12
19	12
26	30
Total	560

The allelopathic effects of aqueous extracts of various parts of rape, decomposed in soil for different periods were determined in Petri dishes (diameter 9.5 cms, height 2 cms) on the germination of weeds on filter paper in 4-replications. Fifty seeds each of 4-Test weeds were placed in Petri dishes lined with 2-filter papers, these Petri dishes were placed in the climate chamber [RUMED 1301 at 23 ^{o}C temperature and 65 % humidity]. The weed seeds germination was recorded after seven days.

III. Phenolic compounds analysis

Phenolic compounds in the winter oilseed rape residues (threshing remains, stubble and roots) were determined (i). immediately after harvesting and (ii). after decomposing in the soil for 3, 7, 14, 19 and 26 months. Fifteen bags of threshing remains, stubble and roots were randomly selected after removing from the soil.

The total phenolics content was estimated as per Folin-Ciocalteau method (40) using gallic acid as standard. For phenolic compounds extractions, 1 g of testing material was weighed in 15 ml tube and extracted with 10 ml 70 % methanol solution. The reaction mixture contained 250 µl of biomass extract, 250 µl of diluted Folin-Ciocalteu reagent and 500 µl of saturated sodium carbonate solution. The mixture was diluted up to 5 ml with distilled water, the contents were mixed and kept in dark for 30 min. The mixture was centrifuged at 6000 rpm for 10 min, and the absorbance of samples was read at 725 nm in spectrophotometer (UV-Vis 1601PC; Shimadzu, Kyoto, Japan). The total phenolic content was expressed as milligrams of gallic acid equivalents per g dry sample (mg g^{-1} dry weight) and was calculated as gallic acid equivalent using the average molar absorptivity of gallic acid. Chemical analyses of total phenolic content were done in five replications.

IV. Glucosinolates

The qualitative and quantitative analysis of glucosinolates was done by reversed-phase high-performance liquid chromatography (HPLC) (Shimadzu LC-2010AHC) with elution gradient and UV detector according to the standard ISO 9167-1:1992 (21). The chromatographic column Luna 3u C18(2) 100A, 150 x 3 mm (manufacturer Phenomenex, USA) was used for the analysis. Glucosinolates were detected by UV detector (wave length : 229 nm). Individual glucosinolates were identified according to desulfoglucosinolate retention duration. The content of glucosinolates (µmol g^{-1}) was determined as per the chromatographic curve of sinigrin as internal standard. The analysis of glucosinolates was performed in the residues (threshing remains, stubble and roots) of winter oilseed rape immediately after harvesting and after decomposition in soil for 3 and 7 months. Nine bags of each type of tested residues were selected randomly after removing from the soil. Samples for research were taken from three bags selected randomly. Three replicates were used for glucosinolates determination.

V. Volatile compounds

The content of volatile compounds in the aqueous extracts of oilseed rape residues was determined by the method of gas chromatography (GC-MS) based on the methodology proposed by Al-Gendy and Lockwood (4). Three bags containing different type of crop residues were randomly selected after removing them out from the soil and samples for analysis were taken. Ten g rape residues was infused with 100 ml distilled water and left for 24 hours at 18 ºC temperature for the enzymatic hydrolysis. Volatile compounds from obtained extracts were collected by dichloromethane. The organic solvent was separated by centrifugation, dried by sodium sulphate and concentrated by nitrogen up to 100 µl. One microliter of prepared extract used for the GC-MS analysis by the Shimadzu GC 2010 gas chromatograph with the Shimadzu GCMS-QP 2010 mass spectrometer. RTX-5MS fused-silica capillary column (30 mm x 0.25 mm) was used. Helium gas 9.0 (AGA, Latvia) was used as a mobile phase, at 2.0 ml·min^{-1} linear velocity in the column. The injector temperature was set at 260 ºC. Injection was carried out using split mode 1 : 10. Ion source temperature was set at 200 ºC, and interface temperature was 260 ºC. Column temperature gradient was starting from 50 ºC hold for 2 min. then rising to 200 ºC at 5 ºC min^{-1} velocity and maintained for 5 minutes. Electron ionization with 70eV was used for ionization of the compounds, scanning mass range 40-400 m/z. Volatile compounds were identified by their retention duration, while concentration was ascertained in equivalent units by peak area of obtained compounds in the chromatogram. Three replicates were used for each test.

VI. Statistical analysis

The research data were statistically evaluated using quantitative one and two-factorial analysis with ANOVA from the software pack SYSTAT 10 (46). There was no significant interaction between the results of separate years during the three-year studies; therefore, the averages of the years were compared in the analysis.

RESULTS AND DISCUSSION

(I). Weed seeds germination

(i). ***G. aparine* :** The aqueous extracts of oilseed rape residues after harvest inhibited the seeds germination of *G. aparine* (Fig. 1 a). However the seeds were tolerant to low

concentrations [(1 : 6,250; 1 : 1,250) and 1 : 50] of extracts of threshing remains. The higher concentration aqueous extracts of stubbles were most inhibitory to seed germination. At the same concentration of aqueous extract the seed germination was lower in other species (*Sinapis arvensis* and *Sonhus arvensis*) than in *G. aparine*. The germination in control was 60.0 %.

(ii). ***Sonchus arvensis* :** The aqueous extracts concentrations of threshing remains, stubble and roots of winter oilseed rape residues that decomposed in the soil for 3-months significantly ($P \leq 0.05$) inhibited the seeds germination of *Sinapis arvensis* (Fig. 1 b). The seeds germination of *Sinapis arvensis* was inhibited (43.5 to 100 %) over control (distilled water). The roots extract of highest concentration (1: 10) completely inhibited the weed seeds germination.

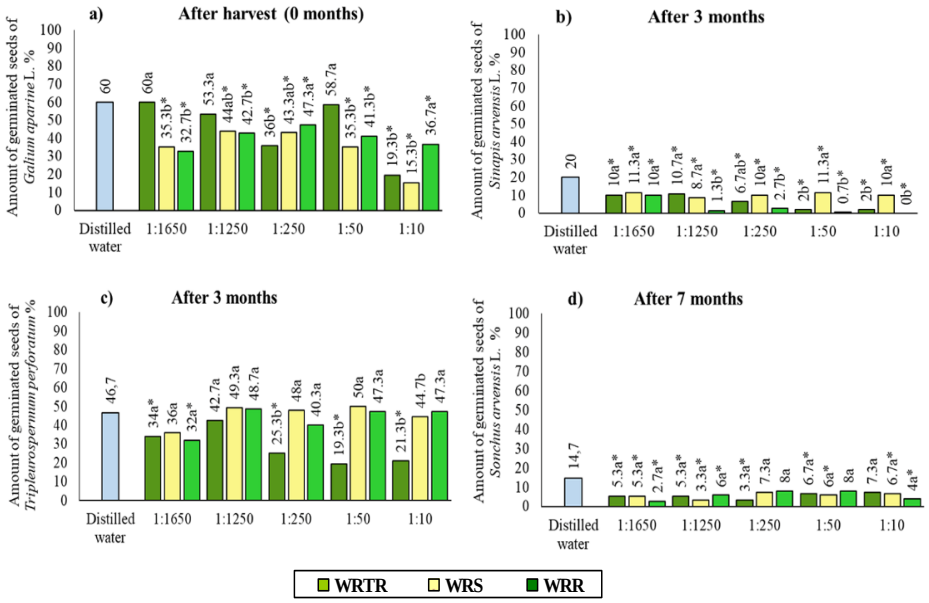

Figure 1. The influence of aqueous extracts of winter oilseed rape residues after harvesting and different period of decomposition in the soil on the germination of *Gallium aparine* L. (a), *Sinapis arvensis* L. (b), *Tripleurospermum perforatum* (Merat) M. Lainz (c) and *Sonchus arvensis* L. (d).
Note: Factor I - rape residues: WRTR/Threshing remains of winter oilseed rape, WRS/Stubble of winter oilseed rape, WRR/Roots of winter oilseed rape; Factor II - concentrations of aqueous extracts. Mean values of factor A not sharing the same letter (a, b) and mean values of factor B sharing the asterisk (compared with distilled water) are significantly different; $P \leq 0.05$.

(iii). ***T. perforatum***: All concentrations of extracts of threshing remains of winter oilseed rape after three months of decomposition [except 1 : 1,250 concentration] significantly ($P \leq 0.05$) reduced (27.2-58.7 %) the seeds germination of *T. perforatum* (Fig. 1 c) than control.

(iv). ***S. arvensis***: The aqueous extracts of rape residues that decomposed in the soil for 7-months inhibited the seeds germination of *Sonchus arvensis* than control (Fig. 1 d). Only the extracts of winter oilseed rape stubble and roots with medium concentration

(1 : 250) the roots extracts with 1 : 50 concentration and the highest concentration (1 : 10) extract of threshing remains did not influence the seed germination of *Sonchus arvensis* significantly ($P \leq 0.05$).

(II). Phenolic compounds

The total contents of phenolic compounds in the threshing remains and stubble of winter oilseed rape at harvest were similar, but were 7.4 % - 10.7 % lower in the roots (Fig. 2). The total phenolic compounds contents in the residues of various plant parts of rape that decomposed in soil for 3- and 7-months were similar. However, their contents in the threshing remains that decomposed in soil for 14 months was significantly higher (19.7 to 24.6 %) than in the stubbles or roots after the same period. This may be because of faster decomposition of rape threshing remains than stubble and roots. Faster decomposition of high-molecular weight phenolic polymer lignin begins 14-months after decomposition of rape residues in soil (23). Even 14.5 months after decomposition of rape residues in soil, about 56 % organic carbon was degraded in the threshing remains and 50 % in the stubbles and roots. During this time, the dry matter in threshing remains was decreased (88 %), stubble (47 %) roots (38 %) (24). In our present study, the total content of phenolic compounds in decomposed (19-26 months) parts of rape did not differ significantly.

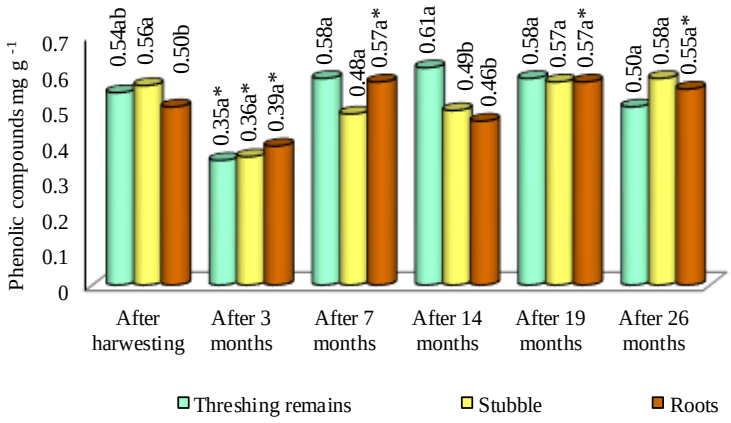

Figure 2. The total content of phenolic compounds in the residues of separate morphological parts of winter oilseed rape after harvesting and after decomposing in the soil during a different period.
Note: mean values not sharing the same letter (a, b) and the asterisk (compared with rape residues after harvesting) indicate significant difference; $P \leq 0.05$.

The contents of phenolic compounds in rape threshing remains, stubble and roots, 3-months after decomposition in soil significantly decreased (22.0 to 35.2 %). Such decrease in phenolic compounds may be due to the leaching of these compounds. During the 7-months of residues decomposition in soil, the soil microorganisms and enzymes hydrolysed their higher-molecular mass compounds (proteins, lipids, lignin, etc.), therefore the total content of phenolic compounds in rape residues was higher. The content of phenolic compounds in biomass were 33-66 % higher than after 3-months of decomposition in soil

and 7-17 % higher to compare with the residues after harvest. Decomposition process was more intense during the summer. Therefore, the content of phenolic compounds in the threshing remains, 14-months after incorporation in soil was highest, than their contents after other periods of decomposition. The content of phenolic compounds in the stubble to compare with decomposed in soil for 7-months remained similar, but decreased in roots (19.3 %). Thus rape roots decomposed more slowly and the leached phenolic compounds content was lower. Nineteen and 26 months after the incorporation of rape residues in the soil, the content of phenolic compounds in the threshing remains and stubble did not differ than their content at harvest, whereas their contents were 14.0 and 11.0 % higher in roots, respectively. Thus the generation of phenolic compounds and their transfer has settled, in threshing remains and stubble, whereas the decomposition of lignin, cellulose and hemi-cellulose began in roots after 14 months of incorporation into soil (23).

Plant roots decompose more slowly in soil than aerial plant parts, because the root tissues are rich in slow-decomposing compounds (lignin and cellulose), than starch, proteins and sugars, which hydrolysed easily (53). The phenolic compounds content in the threshing remains and roots that decomposed in the soil for 19 months was 16.3 and 23.9 % higher, respectively, than their content in residues decomposed for 14 months. However, the phenolic compounds contents in the residues of winter oilseed rape that decomposed in the soil for 19 or 26 months was similar.

Glucosinolates

The glucosinolates content is one of the main indicators of the allelopathic potential of rape. These compounds are found in all plant parts, but their contents differed in various plant parts and varies with stage of plant growth and biotic and abiotic environmental factors (20). The highest glucosinolate contents are found in flowers and seeds (44,48). In seedlings, their content is highest in cotyledons but in mature plants, the roots have more glucosinolates than leaves (47) and the youngest leaves are richest (27). The glucosinolates content is highest in fully mature seeds of oilseed rape (10). Our results indicated that the glucosinolates contents differed in harvested winter oilseed rape residues (roots, stubble, and threshing remains) and changed during their decomposition in soil (Table 2). At harvest, roots were richest in glucosinolates. The total content of glucosinolates was 6.9 times higher in roots and 6.3 times higher in stubble than in threshing remains. The content of glucosinolates in winter oilseed rape roots was similar to stubble.

After the qualitative analysis of glucosinolates in the harvested winter oilseed rape residues, 3-individual glucosinolates (progoitrin, gluconasturtiin, and 4-hydroxyglucobrassicin) in threshing remains, 3-in stubble (progoitrin, gluconapin, and gluconasturtiin) and 5-in roots (progoitrin, epi-progoitrin, glucoraphanin, gluconapin and gluconasturtiin) were identified. In the breakdown products of indole glucosinolates, there were no volatile compounds, whereas the breakdown products of aliphatic and aromatic glucosinolates contained volatile compounds [isothiocyanates and nitriles (20)]. Aliphatic glucosinolate progoitrin was found in all residues of winter oilseed rape, it was 20 % higher in stubbles than in threshing remains and roots. Glucosinolate gluconasturtiin was found in all harvested oilseed rape residues and it was high in stubbles (72.3 % of the total content of glucosinolates). Van Dam *et al*. (47) reported that the glucosinolate concentration and diversity was higher in plant roots than its aerial parts in 29-*Brassicaceae* family plants. The quantitative and qualitative composition of glucosinolates in harvested winter oilseed rape

residues differed than 3-months after decomposition in soil. The glucosinolates contents in roots decomposed for 3-months in soil was lower (12.5 %) than stubble. The contents in threshing remains was identical to stubble and/or roots. Three types of glucosinolates were found in the 3-months decomposed residues of winter oilseed rape: two were aliphatic glucosinolates (glucoallysin and gluconapin) and one was aromatic glucosinolate (4-hydroxyglucobrassicin). The aliphatic glucosinolates were found in all rape residues, while the aromatic glucosinolate was found in the threshing remains (14.3 %) and stubbles (10.7%).

Table 2. The qualitative and quantitative content (μmol g^{-1}fresh weight) of glucosinolates in residues of different parts of winter oilseed rape

Glucosinolates	Residues of winter oilseed rape		
	Threshing remains at harvest	Stubble	Roots
After crop harvest (0-months)			
Progoitrin	0.113	0.138	0.113
Epi-progoitrin	-	-	0.181
Glucoraphanin	-	-	0.202
Gluconapin	-	0.320	0.212
Gluconasturtiin	0.081	1.195	1.095
4-Hydroxyglucobrassicin	0.015	-	-
Total contents	0.209b	1.653a	1.803a
3-months after decomposition in soil			
Glucoallysin	0.249	0.305	0.184
Gluconapin	0.205	0.215	0.325
4-Hydroxyglucobrassicin	0.076	0.062	-
Total content	0.503ab	0.582a	0.509b

Note: mean values of winter oilseed rape not sharing the same letter (a, b) are significantly different; $P \leq 0.05$.

Poulsen *et al.* (39) investigated the biological availability of glucosinolate degradation products in the soil after their hydrolysis. The glucosinolate hydrolysed quickly and isothiocyanates and other toxic compounds were formed and these were included into the matrix of less biologically available compounds in the soil, e.g. humic substances, strong surface complexes and microbial biomass. Other experiments demonstrated that organic matter is the main isothiocyanate sorbent in soil (17).

Volatile organic compounds

Oilseed rape, other plants of *Brassicaceae* family and their residues emit a mixture of volatile organic compounds into the environment. The mixture contains different alcohols, ketones, terpenoids, aldehydes, esters, carboxylic acids, sulphides, nitriles and isothiocyanates (16). The nitriles and isothiocyanates are glucosinolate hydrolysis products and are typical secondary metabolites produced by *Brassica* plants (9). Unstable intermediate compounds of glucosinolate degradation, depending on their side chain composition forms different volatile organic compounds (18,45).

We found that the volatile organic compounds contents differed in the aqueous extracts of winter oilseed rape residues at harvest and in residues decomposed in soil. In threshing remains it was highest 3-months after decomposition in soil (Table 3). Their concentration was 60.6 % higher in threshing remains after 3-months of decomposition than

in aqueous extracts of threshing remains after harvest. The n-pentacosane was found in the volatile compounds of aqueous extracts of 3-months decomposed winter oilseed rape threshing remains and was 35.4 % of the total content of volatile compounds present in this extract. The 2-phenethyl isothiocyanate, important in allelopathy, was also found in the aqueous extracts of 3-months decomposed threshing remains of winter oilseed rape. It is product of the enzymatic hydrolysis of aromatic glucosinolates (20). The concentration of 2-phenethyl isothiocyanate in relative units was 1.33. It was also found in the aqueous extracts of 19-months decomposed threshing remains of winter oilseed rape and its concentration was 0.64 in relative units

Table 3. The volatile compounds concentration in aqueous extracts of threshing remains of winter oilseed rape after harvest and after decomposition in the soil over different periods

Volatile compounds	Release time (min) of compounds	Concentration of volatile compounds (in relative units) Duration of decomposition (Months)					
		After harvest	3	7	14	19	26
3-Octanone	7.490	-	-	-	12.60	-	-
3-Octanol	7.727	-	-	-	12.90	-	-
2-Ethylhexanol	8.649	-	1.19	-	12.90	0.42	-
n-Dodecane	9.395	-	-	0.46	-	-	0.69
p-Cresol	10.042	-	-	0.80	-	-	-
2-Phenethyl alcohol	11.044	3.12	-	-	-	-	-
Phenyl acetate	14.089	-	11.30	-	-	-	-
1-Isocyano-2-methyl benzene	16.168	-	3.09	-	-	-	-
Tridecanol	16.366	-	-	0.31	-	-	0.51
n-Tetradecane	18.829	-	-	0.50	0.70	0.48	-
n-Octadecane	20.368	-	-	-	0.44	0.64	0.35
2-Phenethyl isothiocyanate	20.581	-	1.33	-	-	0.64	-
n-Pentadecane	21.311	-	-	0.37	0.31	0.30	0.42
n-Hexadecane	23.661	-	1.19	-	0.90	0.71	-
4-Methylcyclohexyl ester	23.129	3.40	-	-	-	-	-
n-Tricosane	22.275	-	-	0.30	-	-	0.42
Menthofuran	25.005	15.0	-	-	-	-	-
4-Methyl-1-oxaspiroundecene	25.743	10.3	-	-	-	-	-
n-Heptadecene	25.893	-	-	-	0.38	-	-
Methyl hexadecanoate	30.583	-	-	0.84	-	-	-
Pentadecanolide	33.862	-	-	5.39	-	-	-
1-Tetradecene	37.253	-	-	1.86	-	-	-
n-Pentacosane	46.185	-	33.0	9.73	-	-	3.17
Total contents		31.82	51.10	20.56	41.13	3.19	5.56

The total contents of volatile organic compounds found in the aqueous extracts of threshing remains of winter oilseed rape that decomposed for 14 months were 2-folds higher than those decomposed for 7 months. The concentration of volatile compounds in threshing remains was 29.3 % higher than the concentration in the aqueous extract of threshing remains after harvest. Three compounds (3-octanone, 3-octanol and 2-ethylhexanol) with small molecular mass constituted 93.4 % of the total volatile organic compounds. The total

content of volatile organic compounds in winter oilseed rape threshing remains that decomposed for 19 and 26 months in the soil significantly decreased. Amount of volatile organic compounds declines when intensive crop residues decomposition and weight loos begins (6).

The highest content of volatile organic compounds in the aqueous extracts of stubble of winter oilseed rape at harvest was in 7-months decomposed stubble (Table 4). Pentadecanolide compound was dominant (80.5 % of the total content of volatile organic compounds). The contents of volatile organic compounds in stubble 7-months after decomposition were 6.1-folds higher, and was 2.3-folds higher after 3-months of decomposition, it was 2.3-folds higher than the stubble at harvest. In winter oilseed rape stubble 3-months after decomposition, n-hexadecane was dominant (58.1% of the total content of volatile compounds). The content of volatile compounds in the aqueous extracts of winter oilseed rape stubble that decomposed in the soil for 14, 19 and 26 months was 57.8, 18.6 and 27.1 % lower, respectively, than stubble at harvesting. This may be as a result of reduced availability of organic compounds which after hydrolysis may form volatile organic compounds (39). 2-Phenethyl isothiocyanate was identified in the extract of stubble that decomposed in the soil for 19 months. Its concentration was 8.5 % of the content of volatile compounds in this extract.

Table 4. Volatile concentration compounds in the aqueous extracts of stubble of winter oilseed rape after harvesting and after decomposition in the soil over different periods

Volatile compounds	Release time (min) of compounds	Concentration of volatile compounds (in relative units) Duration of decomposition (Months)					
		After harvest	3	7	14	19	26
3-Octanone	7.490	-	-	-	-	-	0.47
3-octanol	7.727	-	-	-	0.31		1.36
2-Ethylhexanol	8.649	-	-	-	0.69	0.42	
Benzyl alcohol	8.814	-	-	-			2.51
n-Dodecane	9.395	-	-	-	0.31	0.33	0.39
2-Phenethyl alcohol	11.044	6.87	-	-	-	-	-
1-Isocyano-2-methyl benzene	16.168	-	4.76	-	-	-	-
Tridecanol	16.366	-	-	-	-	-	0.32
n-Tridecane	16.201	-	-	-	0.52	-	0.32
n-Tetradecane	18.829	0.82	-	-	0.78	0.57	-
Dimethyl phthalate	20.295	-	1.35	-	-	-	-
2-Phenethyl isothiocyanate	20.581	-	-	-	-	0.66	-
n-Pentadecane	21.311	0.63	-	-	0.43	-	0.48
n-Hexadecane	23.661	1.23	12.6	-	0.99	0.74	-
Pinacol	22.096	-	1.04	-	-	-	-
Methyl hexadecanoate	30.583	-	-	8.36	-	-	-
Methyl linoleate	33.758	-	-	3.01	-	-	-
Pentadecanolide	33.862	-	-	46.9	-	-	1.11
n-Pentacosane	46.185	-	1.94	-	-	1.86	-
	Total contents	9.55	21.69	58.27	4.03	7.77	6.96

The highest content of volatile organic compounds was found in the aqueous extract of roots that decomposed for 7-months (Table 5). In these residues, pentadecanolide was 67 % of the total volatile organic compounds. The content of volatile organic compounds in the roots of oilseed rape, 7-months after decomposition in the soil was 30.6 % higher than roots at harvest. The 2-phenethyl isothiocyanate was identified in the aqueous extract of winter oilseed rape roots at harvest. Its concentration was 38.4 % of the total content of volatile compounds and was highest than other volatile organic compounds. The total content of volatile organic compounds in the root extracts of winter oilseed rape that decomposed in the soil for 3,14,19, 26 months was 86.0, 90.8, 75.1 and 92.1 % lower, respectively, than at harvest.

Table 5. Volatile compounds concentration in the aqueous extracts of roots of winter oilseed rape after harvesting and after decomposition in the soil over different periods

Volatile compounds	Release time (min) of compounds	Concentration of volatile compounds (in relative units) Duration of decomposition (Months)					
		After harvest	3	7	14	19	26
3-Octanol	7.727	-	1.08	-	-	-	-
2-Ethylhexanol	8.649	-	-	-	0.39	-	-
n-Dodecane	9.395	-	-	1.39	-		1.34
2-Phenethyl alcohol	11.044	15.72	-	-	-	-	-
1-isocyano-2-methyl benzene	16.168	7.42	3.32	3.60	-	-	-
Tridecanol	16.366	-	-	-	-	-	0.78
n-Tetradecane	18.829	0.67	-	1.52	0.81	1.64	-
n-Octadecane	20.368	-	-	-	0.72	-	-
2-Phenethylisothiocyanate	20.581	19.00	-	-	-	-	-
n-Pentadecane	21.311	-	-	1.26	0.44	-	0.64
n-Hexadecane	23.661	1.08	1.00	-	0.97	2.48	1.15
6-Methoxyquinoline-1-oxide	25.643	4.94	-	-	-	-	-
n-Heptadecane	25.893	-	-	-	0.42	0.96	-
Methyl hexadecanoate	30.583	-	1.53	9.94	-	-	-
Methyl linoleate	33.758	-	-	3.59	-	-	-
Pentadecanolide	33.862	-	-	43.30	-	-	-
n-Pentacosane	46.185	-	-		-	6.10	-
Total content		48.83	6.93	64.60	3.75	11.18	3.91

Biochemical compounds produced by plants and their decomposing residues helps in control of nematodes, fungi, pathogens and weeds. *Brassica* plant residues incorporated into the soil inhibits the weeds growth for 2-weeks (25,26), thus, *Brassicaceae* family plants are important in crop rotations. The phytosanitary action of these plants varies among species and varieties and depends on the geographical location and conditions of growth (13). The oilseed rape residues may be used for plant protection, due to great influence of the emissions of isothiocyanates that the released from the incorporated residues (32). The soil incorporated winter oilseed rape emits isothiocyanates (15), their concentration did not exceed 1 nmol g^{-1} of dry soil mass. The incorporation of finely chopped mustard biomass into the soil with abundant water resulted in allyl isothiocyanate concentration of 100 nmol

g^{-1} of dry soil mass (33). Uniformly distributed and evenly incorporated plant residues controls the root-knot nematodes *Meloidogyne incognita* (43). Low concentration isothiocyanates, which are emitted for prolonged period, e.g., from ploughed crops, significantly reduces some soil-borne pathogens, e.g. *Alternaria alternata, Colletotrichum dematium, Cylindrocarpon destructans, Fusarium oxysporum, Pythium ultimum, Phytophthora cactorum, and Rhizoctonia fragariae* (34). Such mechanism of indirect defence against pests is environmentally friendly and ecologically sound plant protection strategy for plant protection systems in agriculture.

CONCLUSIONS

All winter oilseed rape residues after harvesting were inhibitory to seeds germination of *Galium aparine*. Threshing remains of winter oilseed rape after three months of decomposition in the soil had inhibitory influence on the germination of seeds of *Sinapis arvensis* and *Tripleurospermum perforatum*. Winter oilseed rape residues that decomposed in the soil for 7-months were most inhibitory to seeds germination of *Sonchus arvensis*.

The phenolic compounds contents in the residues that decomposed in the soil for 3-months was lowest (0.37 mg g^{-1}); subsequently, it increased to highest content (0.57 mg g^{-1}) in winter oilseed rape residues that decomposed in soil for 19-months. The highest content of water-soluble volatile organic compounds was found in the threshing remains of winter oilseed rape that decomposed in soil for 3-months. The decomposition of winter oilseed rape stubble and roots was slower; thus, the highest content of volatile organic compounds in these residues was 7-months after decomposition. The total content of glucosinolates in winter oilseed rape roots and stubble that decomposed in the soil for three months was, respectively, 2.8 and 3.5 times lower, whereas in the threshing remains, the content was 1.9 times higher than the residue after harvesting.

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