Allelopathic Potential of Rapeseed Cultivars on Germination and Seedling Growth of Weeds

I. UREMIS*, M. ARSLAN†, M.K. SANGUN‡, V. UYGUR§ and N. ISLER† *Department of Plant Protection, Mustafa Kemal University, 31034 Hatay, Turkey E-mail: iuremis@mku.edu.tr; iuremis@yahoo.com*

Allelopathic suppression of weeds is receiving greater attention as a possible alternative for weed management. Rapeseed (*Brassica napus* L., var. *oleifera*) contains allelochemicals that inhibits germination and growth of weed species. Allelopathic potential of 25 rapeseed cultivars on the seed germination, shoot and root growth of *Amaranthus retroflexus* L. (redroot pigweed), *Solanum nigrum* L. (black nightshade), *Portulaca oleracea* L. (common purslane), *Physalis angulata* L. (cutleaf ground cherry) and *Echinochloa colonum* (L.) Link. (junglerice) were investigated with shoot and root extracts. All rapeseed cultivars examined inhibited seed germination, shoot and root growth of tested weed species. However, inhibition rates significantly varied among rapeseed cultivars. Significant reductions in seed germination, shoot and root growths were observed as the extract concentration increased. Extracts from both above and below ground parts of rapeseed had inhibitory effect on the tested weed species, but shoot extracts had slightly higher inhibition rates than that of root extracts. Root exudates had also inhibited germination of the tested weed species, but inhibitory effects are not as high as shoot and root extracts. Relative to the germination inhibition of weed seeds, the rapeseed cultivars were categorized as having highly, moderately and low allelopathic potential. Cultivar Westar was found to be highly allelopathic while cultivars Jumbuck, Tobin, Lisoune and Galant were found to be less allelopathic on the tested weed species. Rapeseed cultivars differed in isothiocyanate benzyl and isothiocyanate allyl. Cultivars containing higher level of isothiocyanate benzyl and isothiocyanate allyl had stronger allelopathic capacity. The result of this study showed that a great deal of success could be obtained by incorporation of highly allelopathic rapeseed cultivars into crop rotations to control weeds.

Key Words: Allelopathy, Aqueous extract, Bioassay, *Brassica napus***, Isothiocyanates, Rapeseed.**

INTRODUCTION

Rapeseed (*Brassica napus* L., var. *oleifera*), an annual oil crop in the Brassica family, is the third major edible source of vegetable oil in the world after soybean

[†]Department of Field Crops, Mustafa Kemal University, 31034 Hatay, Turkey.

[‡]Department of Chemistry, Mustafa Kemal University, 31034 Hatay, Turkey.

[§]Department of Soil Science, Mustafa Kemal University, 31034 Hatay, Turkey.

and palm oil. It is utilized for vegetable oil (human consumption), animal feed and biodiesel. Rapeseed production increased about 10-fold for the last 20 years and passed peanut, sunflower and most recently, cottonseed in worldwide production. World rapeseed production was 46.3 million tones in 2006/2007. The leading producers are China, India, Canada and European Community¹.

Allelopathy is expected to be an important part of integrated weed management as a supplementary tool for weed control in the agro-ecosystems because of increasing public concern about harmful effects of pesticides on the environment and human health as well as increasing rate of weed resistant to known chemicals. Recently, genetic improvement of the crops to enhance allelopathic potential to control weeds become one of the objectives of breeding programs^{2,3}. Germplasm assessments of some known allelopathic crops were screened to detect allelopathic accessions⁴⁻⁸. However, there have not been any extensive studies on the accession of allelopathic potential of rapeseed cultivars or germplasms collections.

The species of Brassica family had great attention as source of allelochemicals and often used as green manure or cover crops for weed suppression⁹⁻¹⁵. Most of the species of Brassica like rapeseed produces glucosinolates that have been reported to have allelopathic activity after hydrolysis by the enzyme myrosinase $16,17$. In addition to isothiocyanates, other physiologically active less toxic breakdown products of glucosinolates (nitriles, thiocyanates and oxazolidinethiones) can also occur, depen- α ding on various factors¹⁸. Although allelopathic potential of Brassica species are well documented, allelopathic differences within the species have not been studied well. Allelopathic potential of rapeseed, widely cultivated species in the temperate northern regions or at higher elevations as an oil seed crop, may vary among cultivars. A great deal of success on controlling weeds can be achieved by the integration of highly allelopathic rapeseed cultivar into cropping systems, as green manure or cover crops.

The present research was conducted to determine allelopathic potential of different rapeseed cultivars on germination and seedling growth of *Amaranthus retroflexus* L. (redroot pigweed), *Solanum nigrum* L. (black nightshade), *Portulaca oleracea* L. (common purslane), *Physalis angulata* L. (cutleaf ground cherry) and *Echinochloa colonum* (L.) Link. (junglerice).

EXPERIMENTAL

Weed seed collection: The fruits of *S. nigrum* and *P. angulata* were collected from infested of farmer fields in October 2004. The fruits were shade dried in the laboratory at ambient temperature (20-25 ºC) for 30 d and then the seeds were hand separated and floated in distilled water to remove thrashes. After rinsing with distilled water, the seeds were dried on the filter papers at ambient temperature in the laboratory for 7 d. The panicles of *A. retroflexus* and *E. colonum* were collected from the infested areas and dried at room temperatures for 7 d. The panicles were shaken gently to make the mature seeds fall into the paper sampling bags. Trash was removed

from the seeds by floating them in distilled water. The plants of *P. oleracea* were shaken gently into the paper sampling bags to have mature seeds. To break dormancy for junglerice, the seeds were stored for 3 d at 40 $^{\circ}$ C in the dark.

Extract preparation: Rapeseed cultivars for extract preparation (Table-1) were grown at the Mustafa Kemal University farm (36º 15' N; 36º 30' E, 60 m altitude) of which the soil was developed from alluvial deposits of river terraces, is typical for the Eastern Mediterranean region of Turkey and is classified as Chromoxeret by USDA¹⁹. Soil taxonomy¹⁹ and Vertisol by FAO/UNESCO²⁰ having relatively high clay content with the predominant clay minerals smectite and kaolinite. The soil of the experimental plots was a clay silt loam with a pH of 7.6, 1.7 % organic matter, 0.13 % total nitrogen content and water holding capacity of 0.34 cm^3 . The plot size was 10 m^2 with six 5 m rows. Rapeseed species were planted 0.35 m apart in November 2004. On the basis of soil analysis and local recommendations, fertilizer was applied prior to planting at a rate of $25{\text -}25{\text -}0$ kg ha⁻¹ NPK. Total precipitation during the plant growing period was 378 mm. The maximum and the minimum air temperature were about 26-12 °C during the cropping period (November 2004-April 2005) while the maximum and minimum relative humidity were 25-90 %.

Rapeseed cultivars were uprooted at the middle of the flowering stage for each cultivar during April 2005 and taken immediately to the laboratory where they were washed throughly with tap water and separated into root and shoot. After rinsing with distilled water, shoots and roots were separately chopped into small pieces with clippers then grinded with a batch mill with the help of Ika M 20 Universal mill with M 23 Star-shaped cutter. Grinded fresh shoot and root samples were separately pressed with a modified hydraulic bottle jack to have shoot and root extracts. The extracts were filtered through a double layer of muslin cloth and then centrifuged (1500 g) for 2 h. The supernatant was filtered again using a 0.2 mm filter ware unit to give the final shoot and root extracts. The extracts were divided in to two halves. Half of the extracts was frozen in 100 mL plastic caps at -24 ºC for the future germination test, the other half was diluted to obtain a series of solutions with different concentrations (2, 4, 8, 16 and 32 %). Electrical conductivity and pH of shoot and root extracts were measured using a combined meter by Hanna Instruments model HI 255.

Germination bioassay: Hundred seeds of each species (*i.e. A. retroflexus, S. nigrum, P. oleracea, P. angulata* and *E. colonum*) were placed evenly on filter paper in sterilized 90 mm petri dishes after surface sterilization with water:bleach solution (10:1). Treatments were consisted of 10 mL of different concentrations of aqueous extracts (2, 4, 8, 16 and 32 %) and distilled water was used as a check. The experimental design was randomized plots with 4 replications. Weeds were in main plots, rapeseed cultivars were in sub-plots and extract doses were in sub-subplots. Two experiments (root and shoot extracts) were conducted separately. All petri dishes were placed in a growth chamber at 28/32 ºC for 12/12 h and dark/light period for 16/8 h. Distilled water was added equally in the petri dishes during

experiments when needed. Germinated seeds were counted at 3, 5, 7, 14, 21 and 28 d after incubation and removed from media. The per cent germination inhibition was calculated using

$$
GI = \frac{CG - TG}{CG} \times 100
$$
 (1)

where, $GI = per cent germination inhibition$ (%); $CG = number of germinated$ seeds in check without extract; $TG =$ number of germinated seeds in treatments with extract.

All experiments were conducted twice in a completely randomized design with 4 replications. Analysis of variance was performed for all data using a general linear model procedure²¹. Data from two experiments were pooled and mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level.

Growth bioassay: Ten seedlings (*ca.* 2 mm in height) of each species which had been pre-germinated on filter paper in a growth chamber at 28/32 ºC for 12/12 h and dark/light period for 16/8 h, were planted in 90 mm petri-dishes filled with sterilized quartz sand. Aqueous extracts as 10 mL were added in different concentrations (2, 4, 8, 16 and 32 %) for treatments and distilled water was used as a check. Petri dishes were, then, incubated in an illuminated growth chamber at 30 ºC. The shoot and root length of seedlings were measured on 7 d after treatment. The per cent growth inhibition was calculated for shoot and root lenghts separately using following equation:

$$
GRI = \frac{LC - LT}{LC} \times 100
$$
 (2)

where, $GRI =$ per cent growth inhibition $(\%)$; $LT =$ shoot or root length of seedlings for treatments with extract (mm); $LC =$ shoot and root length of weed seedling in untreated check (mm).

All experiments were conducted twice in a completely randomized design with 4 replications. Analysis of variance was performed for all data using a general linear model procedure²¹. Data from two experiments were pooled and mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level.

Soil test for root exudutes on weed germination: The effects of root exudes were studied by taking soil samples (10 cm in depth) from the plots of rapeseed cultivars Bounty, Comet, Synergy and Westar after uprooting the plants at the middle of the flowering stage. The soil taken from the outside of the plots was used as a check. Soil samples were placed in the petri dishes (25 g soil/petri) to assess the existence of allelopathic root exudes of each cultivar. Fifty seeds of *A. retroflexus*, *S. nigrum, P. oleracea, P. angulata* and *E. colonum* were planted in each Petri dish. Each treatment, redroot pigweed, black nightshade, common purslane, cutleaf ground cherry and junglerice was replicated 4 times and arranged in a completely randomized design. All experiments were conducted twice.

Germinated seeds were counted at 3, 5, 7, 14, 21 and 28 d after incubation. The per cent germination inhibition due to exudutes was calculated as

$$
GIE = \frac{CGE - TGE}{CGE} \times 100
$$
 (3)

where, $GIE =$ per cent germination inhibition (%); $CGE =$ number of germinated seeds in check without extract; TGE = number of germinated seeds in treatments with extract.

Analysis of variance was performed for all data using a general linear model procedure²¹. Data from two experiments were pooled and mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level.

Analysis of isothiocyanates: Isothiocyanates of selected rapeseed cultivars (Bounty, Comet, Synergy and Westar) were analyzed using Shimadzu, LC-10AT vp HPLC with SPD-M20A prominence DAD (diode array detector). The methods and analysis procedure was modified from Petersen *et al.*22 and analysis were done in Mustafa Kemal University Science Applied and Research Center Laboratories. The column was a H5ODS-12318 (5 µm, Hichrom) with a 25 % acetonitrile and 75 % of 10 mmol phosphate-buffer ($pH = 2.4$) were detected at 275 nm.

RESULTS AND DISCUSSION

Inhibition rates of shoot or root extracts of rapeseed cultivars on *A. retroflexus, S. nigrum, P. oleracea, P. angulata* and *E. colonum* germination were significantly different (Table-1). The germination inhibition of shoot extracts from 25 cultivars on *A. retroflexus, S. nigrum, P. oleracea, P. angulata* and *E. colonum* varied between 20.75 and 59.80 %, 21.0 and 60.85 %, 21.85 and 61.23 %, 21.13 and 67.13 %, 20.43 and 61.35 %, respectively. Germination of the tested weed seeds was less sensitive to shoot extracts of some cultivars than others. The highest inhibitory rates on the germination of all tested weed species were obtained from the cultivar Westar, while the lowest was obtained from Jumbuck, Galant, Lisoune, Goldrush and Tobin. Root extracts had the similar inhibition rates on the germination of all tested weed species. However, inhibitory effects of root extracts on the germination of all tested weed species were slightly lower than that of shoot extracts. Root extract from Westar had the highest germination inhibition on all of the tested weed species while root extracts from Jumbuck, Galant, Goldrush, Lisoune and Tobin had the lowest inhibition on *A. retroflexus S. nigrum, P. oleracea* and *P. angulata* germination, respectively. Among the 25 rapeseed cultivars, Jumbuck, Tobin and Lisoune are belong to *Brassica rapa* var. *oleifera* and the rest of are *Brassica napus* var. *oleifera*. Both shoot and roots extracts from cultivars in the *B. rapa* had always lower inhibition rates on germination, shoot and root growth of the tested weed species than that of cultivars in *B. napus*.

TABLE-1

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Shoot extracts reduced both shoot and root growths of the tested weed species (Table-2). Shoot and root growth inhibitions of test species significantly varied among rapeseed cultivars. Shoot extract from Westar had the highest while extracts from Jumbuck, Galant, Goldrush, Tobin and Lisoune had the lowest shoot and root growth inhibitions on the weed species. The tested weed species responded differently to the shoot extracts of the rapeseed cultivars. On the average, shoot growth inhibition rates varied between 20.12 and 47.73 % among the weed species. The root growth inhibition of the weed species, varied between 15.53 and 44.80 % among the weed species had lower than that of the shoot growth inhibition.

Like shoot extracts, root extracts from 25 rapeseed cultivars significantly reduced shoot and root growth of test species (Table-3). Shoot and root growth of the tested weed seeds were less sensitive to root extracts of some cultivars than others. Cultivar Westar exhibited markedly different level of inhibitions on the shoot and root growth of the tested weed species. The highest shoot growth inhibition on *A. retroflexus* (33.68 %), *S. nigrum* (39.34), *P. oleracea* (30.56 %), *P. angulata* (37.94 %) and *E. colonum* (28.52 %) were obtained from cultivar Westar. Tobin and Lisoune had the lowest shoot growth inhibition rates on *A. retroflexus* (17.48 and 17.61 %), respectively. On the other hand Galant and Jumbuck had the lowest shoot growth inhibition rates on *P. oleracea* (18.95 and 19.34 %), respectively. Cultivar Jumbuck had the lowest inhibition rates on *S. nigrum* (17.56 %), *P. angulata* (16.11 %) and *E. colonum* (16.71 %). The inhibition rates of root extracts of rapeseed cultivars on root growth inhibition were similar to shoot growth inhibition rates. The same cultivars had the highest (Westar) and the lowest (Tobin, Galant, Lisoune and Jumbuck) root growth inhibition rates on the tested weed species.

Inhibition rates of shoot and root extracts increased with the increasing rate of extract concentrations for all cultivars, but the dose response data was given for only cultivar Westar. A progressive increase in the inhibition of germination was recorded for shoot extract of Westar with the increasing extract concentration on the tested weed species (Fig. 1). All the applied concentrations of cultivar Westar shoot and root extracts (2, 4, 8, 16 and 32 %) suppressed the germination of the test weeds. However, the root extract did not have the same pattern of germination inhibition on the test species. Except for *P. angulata*, germination inhibition of *A. retroflexus, S. nigrum, P. oleracea* and *E. colonum* slightly varied between the lowest and the highest extract doses.

Surprisingly, shoot inhibition pattern of both shoot and root extracts of cultivar Westar on the tested weed species were similar (Fig. 2). Shoot and root extract inhibited shoot length with the increasing concentration of the extracts except for *P. angulata* and *A. retroflexus*. There was not a remarkable change in the inhibition of shoot length between 2 and 4 % extract concentrations of shoot and roots for both weed species. Shoot and root extracts of cultivar Westar inhibited the germination of the tested weed species (Fig. 3). Again, allelopathicity increased with increase in shoot and root extract concentrations and was greatest with shoot extracts and lowest with root extracts.

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Fig. 1. Inhibitory effect of shoot and root extracts from cultivar Westar on *Amaranthus retroflexus, Solanum nigrum, Portulaca oleracea, Physalis angulata* and *Echinochloa colonum* germination

Fig. 2. Inhibitory effect of shoot and root extracts from cultivar Westar on *Amaranthus retroflexus, Solanum nigrum, Portulaca oleracea, Physalis angulata* and *Echinochloa colonum* shoot length inhibition (%)

Fig. 3. Inhibitory effect of shoot and root extracts from cultivar Westar on *Amaranthus retroflexus, Solanum nigrum, Portulaca oleracea, Physalis angulata* and *Echinochloa colonum* root length inhibition (%)

To determine root leakage of rapeseed cultivar, soil samples were taken from rapeseed grown plots. Germination inhibitions of 4 rapeseed cultivars grown soil on the test weed species were significant, except for *S. nigrum* and *E. colonum* (Table-4). The highest germination inhibition on *A. retroflexus* (52.69 %), *S. nigrum* (21.61 %), *P. oleracea* (24.84 %), *P. angulata* (42.52 %) and *E. colonum* (21.95 %) were obtained from Westar, Comet, Westar, Bounty and Synergy, respectively. *Amaranthus retroflexus* and *P. angulata* were the most affected weed species from the root leakage. Germination inhibition of the root leakage of cultivars on *A. retroflexus* varied between 30.91 and 52.69 %. The lowest and the highest germination inhibition rates were obtained from cultivar Synergy and Westar, respectively. Cultivar Westar and Bounty had the lowest and the highest inhibition rate on the germination of *P. angulata*, respectively.

 $NS = Not$ significant at p 0.05.

The pH of selected rapeseed cultivars were between 7.05 and 7.81 for shoot extract and between 7.36 and 8.44 for root extract (Table-5). The pH range of both shoot and root extracts were within the germination pH range for the tested weed species. Shoot extracts of Bounty, Comet, Synergy and Westar had higher electrical conductivity, benzyl isothiocyanate and allyl isothiocyanate than that of root extracts (Table-5).

TABLE-5 pH, ELECTRICAL CONDUCTIVITY, BENZYL ISOTHIOCYANATE AND ALLYL ISOTHIOCYANATE OF BOUNTY, COMET, SYNERGY AND WESTAR EXTRACTS

Rapeseed cultivar	pH		$EC(S m-1)$		Benzyl isothiocyanate (mg/L)		Allyl isothiocyanate (mg/L)	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Bounty	7.60	8.14	895	430	4.67	4.62	8.59	11.44
Comet	7.05	8.44	1527	1112	4.72	4.68	9.13	8.25
Synergy	7.39	7.41	1802	625	4.57	4.54	6.47	5.66
Westar	7.81	7.36	1412	626	10.26	9.56	34.99	24.64

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Brassica species can be used to control weeds in cropping systems because of their allelopathic potential. However, cultivation of some Brassica species especially consumed as vegetable is limited due to the limited demand of grocery markets. Unlike vegetable Brassicas, rapeseed is widely cultivated species in the temperate northern regions or at higher elevations as an oil seed crop. Therefore, it can be considered one of the candidate allelopathic crops to control weeds in cropping systems of the mentioned regions. Allelopathic potential of Brassica species may vary both among and within the species. The inhibition of *A. retroflexus, S. nigrum, P. oleracea, P. angulata* and *E. colonum* seed germination, shoot and root growth by shoot and root extracts may reflect allelopathic potential of each cultivar. As expected, both shoot and root extracts of rapeseed cultivars varied in their allelopathicity to the test species germination, shoot and root growth (Tables 1-3). The present study showed that shoot extracts had slightly higher inhibitory effect on seed germination, shoot and root growth of the tested weed species. Higher allelopathic potential of the shoot tissues could be attributed to the higher isothiocyanates content of the shoot tissues (Table-5). But the opposite result was reported by Gardiner *et al.*17 that above ground parts of rapeseed cultivars produced very little isothiocyanates, whereas below ground parts produced a pronounced flush of isothiocyanates during the first 4 d after incorporation. These results are in accordance with previous studies which reported that the allelopathicity may vary among plant parts²³⁻²⁶. Although, above ground parts of the plants are considered as a main source of allelochemicals, it was suggested that considerable attention should be given to the roots as a primary source of allelochemicals²⁷ while breeding a crop having high allelopathic potential.

The current study showed that there were remarkable allelopathic differences among rapeseed cultivars. According to the results of shoot extracts inhibition rates on the germination of tested weed species, rapeseed cultivars were grouped into 3 categories: First group (germination inhibition > 40 %) consists of 2 cultivars including Westar and Comet. Second group (30-40 % germination inhibition) consists of 2 cultivars including Bounty and Maluka. Third group (20-30 % germination inhibition) consists of 21 cultivars (Table-1). Cultivar or genotypic differences for allelopathic potential were also reported for rice²⁸⁻³⁰, wheat³¹, sorghum³² and cucumber⁴. In the present study, cultivar differences in allelopathic potential were attributed to the different rates of isothiocyanates in the cultivars (Table-5). The results of germination and bioassay studies showed that both shoot and root extracts from cultivar Westar. Westar were more toxic to all weed species than the other cultivars. Higher level of allelopathicity of Westar was resulted from higher contents of isothiocyanates in shoot and root tissues (Table-5). Variation in plant glucosinolate content can be attributed to genetic, environmental and husbandry factors^{33,34}. It can be concluded from the HPLC analysis that chemical screening could be another way to access allelopathic potential of crop cultivars and it helps explanation of the results of germination and bioassay studies.

Rapeseed cultivars had the similar patterns of dose response curve for the tested weed species. Although the dose response studies were done for all of the tested cultivars, we depicted the dose response curve only for cultivar Westar (Figs. 1-3) since it was one of the highest allelopathic rapeseed cultivars. The figures showed that seed germination, shoot and root growth were inhibited even at the lowest extract concentrations, but the inhibition rate increased with the increased extract concentrations (Figs. 1-3). The results are in agreement with the previous investigations in that the activity of extracts was directly related to the concentration of extract rates $35-38$. Cultivar Westar may provide important genes for breeding highly allelopathic cultivars. It has been proposed that allelopathic potential of plants is genetically controlled^{39,40}. Thus, understanding of genetic control of allelopathy can enhance development of allelopathic cultivars that can be incorporated into crop rotation systems for improved weed control. Increasing allelopathic potential in combination with breeding for competitive plant types could result in crop cultivars with superior weed-suppressive ability. Therefore, allelopathic potential would be one of the valuable traits to breed cultivars that can be used for weed control in cropping systems.

Weeds especially small seeded weeds could be controlled with the incorporation of rapeseed greens in the soil. The present study showed that suppression of weeds could be increased with the selection of highly allelopathic rapeseed cultivars. Although the breeding approach alone cannot overcome the weed problems, an increase in the allelopathic potential of cultivars will likely have a great impact on weed control in agro-ecosystems. Another importance of rapeseed in rotation is reduce initial weed density, so that later on, following crops canopy can smother the weeds and excellent weed control can be achieved only a few application of herbicides. Moreover, allelopathy-based technology as a supplement of integrated weed management is also more easily transferable to organic production systems where tillage is the major weed control method. Germination studies with the rapeseed grown soil showed that root exudates of rapeseed had also inhibits germination of the tested weed species. But, inhibitory effects of root exudates on seed germination are not as high as shoot and root extracts. The allelopathic potential of rapeseed extracts on the germination inhibition of soybean, peanut and corn that can be planted after rapeseed as a second crop has also been tested. No significant germination differences between control and the treatments have been observed (data not given).

Conclusion

Shoot and root extracts of rapeseed cultivars inhibited seed germination, seedling and root growth of *A. retroflexus, S. nigrum, P. oleracea, P. angulata* and *E. colonum* are investigated in proportion related to the concentration of extracts. When considering rapeseed as an allelopathic crop, cultivar differences were important. Cultivars containing higher level of allelopathic chemicals had also stronger allelopathic

capacity. A great deal of success on controlling weeds could be achieved only by the integration of highly allelopathic rapeseed cultivar into cropping systems. Beside cultivar differences, the degree of allelopathicity also depends on temperature, moisture content, microbial activity and nutrient status of the soil and amount of the plant incorporated. For final decision about the allelopathic potential of rapeseed cultivars, future studies are needed to evaluate allelopathic activities of rapeseed under field conditions.

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