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*Full Length Research Paper*

# Allelopathic potentials of residues of 6 brassica species on johnsongrass [*Sorghum halepense* (L.) Pers.]

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**Johnsongrass (*Sorghum halepense* (L.) Pers.) is a troublesome weed species of many crops in Turkey as well as worldwide. Allelopathic potential of residues of some brassica species, which are round white radish (*Raphanus sativus* L.), garden radish (*R. sativus* L.), black radish (*R. sativus* L. var. *niger*), little radish (*R. sativus* L. var. *radicula*, turnip (*Brassica campestris* L. subsp. *rapa*) and rapeseed (*Brassica napus* L. *oleifera* DC.) on johnsongrass were investigated under both laboratory and field conditions. All species suppressed johnsongrass in field and laboratory conditions. The lowest suppression was from garden radish, which has already been used to control johnsongrass by few farmers in Turkey. It is concluded that the plants studied can be used to control johnsongrass. Higher amount of isothiocyanates (isothiocyanate benzyl, Isothiocyanate allyl) in black radish extract and lower amount of isothiocyanates at garden radish extract were determined. Parallel results for johnsongrass suppression and amount of isothiocyanates show that allelopathy play roles in johnsongrass suppression by brassica species.**

**Key words:** Johnsongrass, round white radish, garden radish, black radish, little radish, turnip, rapeseed, *Brassicaceae.*

## INTRODUCTION

Johnsongrass, *Sorghum halepense* (L.) Pers., is a perennial grass species, which is a native plant of mediterranean area (Holm et al., 1977) where Turkey is located. Johnsongrass is ranked as the 6$^{th}$ worst weed, which was reported by 53 countries as a weed in 30 different crops (Holm et al., 1977). It has been reported in many crop fields such as cotton, maize, wheat and vegetables, fruit plantations and waste areas in Turkey (Ulug et al., 1993). It is reported that 61% of cotton producing areas are infested with johnsongrass in Turkey (Gunes et al., 2008). Yield lost in cotton due to johnsongrass interference was calculated as 7 to 69% depending on johnsongrass density (Uludag et al., 2007). Although there have been several effective control methods to control johnsongrass in different crops, it is still considered a difficult weed to control (Uludag et al., 1998; Dalley and Richard Jr, 2008) because of its prolific reproduction by both seeds and rhizomes (McWhorter, 1971). In addition, resistance against most effective herbicide groups, which keep johnsongrass under control, has been evolved (Heap, 2009).

Arising problems due to current weed control techniques, expanding demand to organic crops and increasing public concerns on environmental issues require alternative farming systems which are less pesticide dependent (Singh et al., 2003; Waller, 2004). Allelopathy is one of the approaches which can be used as an alternative method to combat pests in cropping systems (Inderjit and Keating, 1999). Different plant species/families have been reported having allelopathic activity and could be used in agricultural/ecological systems (Rice, 1995). Among the families, *Brassicaceae* has had great attention (Campbell, 1959; Bell and Muller, 1973; de Almeida, 1985; Brown and Morra, 1997; Dehaan et al., 1997; Weston and Duke, 2003; Arslan et al., 2005). *Brassica*-

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*ceae* species have glucosinolate compounds (Larsen, 1981), which are group of plant secondary compounds whose biologically active hydrolysis products are produced when cells containing them are ruptured and the glucosinolates are hydrolyzed by the enzyme myrosinase (Vaughn, 1999). Glucosinolate amount and chemical composition vary among plant populations, species, varieties, years, plant stages and/or plant tissues (Larsen, 1981; Sang et al., 1984; Mason-Sedun et al., 1986; Carlson et al., 1987; Clossais-Besnard and Larher, 1991; Rosa et al., 1997; Eberlein et al., 1998; Kushad et al., 1999).

Different aspects of the allelopathic effect of *Brassicaceae* crops on weeds have been studied. Norsworthy (2003) reported that crops and weeds showed germination sensitivity to wild radish (*Raphanus raphanistrum* L.) extract at varying degrees and radicle growth of some weeds was inhibited. De Almeida (1985) found that extracts of winter sown turnip and rape residues inhibited germination of some weeds. Jimenez-Osornio and Gliessmann (1987) showed allelopathic potential of broccoli (*B. oleracea* L. var. *italica*) and wild mustard (*B. campestris* L.) using extracts of them on several indicator species. Leather (1983) concluded that rape leachates inhibited growth of velvet leaf and morning glory but not wild mustard. Mason-Sedun et al. (1986) revealed differences among *Brassicaceae* species and varieties on root and shoot growth of wheat used as test species. Turk and Tawaha (2003) showed that black mustard (*B. nigra*) aqueous extracts inhibited germination and seedling growth of wild oat (*Avena fatua*). Koseli (1991) concluded that regrowth of johnsongrass rhizomes were suppressed by incorporation of above and below ground parts of *R. sativus*. Petersen et al. (2001) determined isothiocyanates (ITC) released by turnip-rape mulch (*B. rapa* (Rapifera group-*B. napus* L.) and showed effect of ITCs on weed species.

It was reported that johnsongrass infestation was quite less when cotton was sown after the harvest of radish crops in the eastern mediterranean region of Turkey (Koseli, 1991). However, this system has not been widely applied by farmers because the demand for turnip is limited in grocery market and research to improve the system has not been sufficient. In addition, other *Brassicaceae* crops, which can be considered weed control agents, have demand in market in Turkey. Many radish and turnip species are consumed fresh in Turkey as well as other *Brassicaceae* crops such as cabbage, cauliflower, etc. Total production of *Brassicaceae* vegetables in Turkey is over 900, 000 ton per year (TUIK, 2006). Although rapeseed has been produced in little acreage for many years (FAO, 2009), an increase is seen recently in Turkey.

The objective of the current study was to determine the allelopathic potential of residues of 6 *Brassicaceae* species widely cultivated in Turkey which are round white radish (*R. sativus* L.), garden radish (*R. sativus* L.), black radish (*R. sativus* L. var. *niger*), little radish (*R. sativus* L. var. *radicula*), turnip (*B. campestris* L. subsp. *rapa*) and rapeseed (*B. napus* L. subsp. *oleifera* DC.) on johnsongrass control.

## MATERIALS AND METHODS

Six *Brassicaceae* crops, round white radish, garden radish, black radish, little radish, turnip and rapeseed were chosen to test their allelopathic potential on rhizome re-growth of johnsongrass. The effect of crop residues after harvesting was investigated under field conditions. The effect of extracts of above ground parts of crops on johnson-grass was studied in laboratory conditions. In addition, amounts of isothiocyanates in crop extracts were determined.

### Effect of plant residues

Field experiments were conducted in a naturally johnsongrass-infested field in Telkalis Research Farm of Mustafa Kemal University Hatay/Turkey, located in 36° 15′ North and 36° 30′ east Mediterranean region of Turkey in 2002 and 2003. The soil was a clay silt loam with pH of 7.5, 0.8% organic matter and water holding capacity of 0.34 $cm^3$. Experiments were set on different parts of the field each year. The experimental design was randomized complete block design (RCBD) with 3 replications. Plot size was 15 $m^2$ (3 m x 5 m). *Brassicaceae* species (seeding rate: radishes and turnip 15 kg $ha^{-1}$, rapeseed 20 kg $ha^{-1}$) were planted on January 25th, 2002 and January 10th, 2003. Fertilizer was applied prior to planting at a rate of 15-15-15, 300 kg $ha^{-1}$ NPK and later top dressed with 400 $kgNha^{-1}$ as ammonium nitrate to ensure vigorous growth. *Brassicaceae* crops were harvested when the roots reached economical harvest maturity except rape seed which was harvested at harvest maturity of seeds. At the end of May harvest were completed and plant residues on the soil surface were incorporated into soil by plowing followed by disc-harrowing twice. After incorporation of crop residue, the plots were sprinkler irrigated when needed. The effect of crop residues on johnsongrass was determined as ratio in percentage comparing to non-treated check plots (0 no effect, 100 total control) at 7, 15, 30 and 60 days after residue incorporation (DARI).

### Effect of plant extracts

Effects of extracts of 6 *Brassicaceae* crops on re-growth of johnson-grass rhizomes were studied under laboratory conditions.

### Rhizome sources

Johnsongrass rhizomes were collected from a naturally infested field. Rhizomes were washed with tap water and disinfected in 45% bleach at 15 min, then rinsed with distilled water. Then they were cut in to 2-3 cm pieces each containing a bud.

### Crop sources

*Brassicaceae* species were grown at the same field described above in 2002 in an individual plot of 15 $m^2$ (3 m x 5 m). Fertilizer was applied prior to planting at a rate of 15 - 15 - 15, 300 $kgha^{-1}$ NPK and later top dressed with 400 $kgNha^{-1}$ as ammonium nitrate to ensure vigorous growth.

### Extract preparation

On May 2002, 1.0 $m^2$ area in each plot was cut when the plants

**Table 1.** Suppression rate of *Brassicaceae* species on the infestation of johnsongrass in field conditions 30 and 60 DARI.

| Species | Johnsongrass control (%) | | | |
|---|---|---|---|---|
| | 30 days later after incorporation | | 60 days later after incorporation | |
| | 2002 | 2003 | 2002 | 2003 |
| Black radish | 93.3a* | 76.7a | 63.0abc | 58.0ab |
| Rapeseed | 90.0a | 66.7a | 73.7a | 63.0a |
| Little radish | 83.3ab | 68.3a | 68.3ab | 63.3a |
| Round white radish | 83.3ab | 71.7a | 68.3ab | 58.0ab |
| Turnip | 73.3bc | 53.3ab | 52.7bc | 53.0ab |
| Garden radish | 63.3c | 38.3b | 47.3c | 37.0b |
| Johnsongrass coverage (%) | 80 | 85 | 95 | 95 |

*Means within a column followed by the same letter do not differ according to Duncan's multiple range test at $P \leq 0.05$.

were in the flowering stage. Above ground parts of plants were shade dried at room conditions for 3 weeks and ground in a Wiley mill through a 40 mesh screen. The powders were stored at 4°C until needed.

3 concentrations of extracts were prepared; 2, 4 and 8 g crop powder in100 $ml^{-1}$ (w/v) distilled water for 24 h at room conditions. The solutions were filtered through a double layer of cheese cloth followed by a Whatman no. 1 filter paper. The extracts were kept in a refrigerator at 4°C until further use.

#### Rhizome bioassays

Plastic containers with 600 g sterilized quartz sand (10 x 20 x 5 cm) were used for rhizome bioassays. 20 rhizomes were placed into 2.5 cm depth of sand. Each container received 80 ml of water extract of a *Brassicaceae* species at the rates 2, 4 or 8 g 100 $ml^{-1}$ (w/v). Sterile distilled water was used as a check. Containers kept in a growth chamber with 30°C temperature and having 14/10 h day/night scheme. The experimental design was complete randomized design (CRD) in a split plot design with 3 replications. *Brassicaceae* species were main plots and extract rates were split plots. Experiment was repeated twice. Sprouted johnsongrass rhizomes were counted after 21 days from the beginning of experiments.

#### Statistical analysis

Because of low johnsongrass coverage at $7^{th}$ and $15^{th}$ DARI, only data of the $30^{th}$ and $60^{th}$ DARI from the field experiment were subjected to analysis of variance using the general linear models procedure in the statistical analysis system (SAS Institute, 1997). Transformation of data did not improve ANOVA. Due to significant difference between years for $30^{th}$ DARI, data were analyzed separately for each year. Means of parameters were compared by using Duncan test at 95% level of probability.

Data from laboratory experiment were subjected to analysis of variance using the general linear models procedure in the statistical analysis system (SAS Institute, 1997). When transformation did not improve ANOVA then raw data were used. Two experiments were pooled when there was no difference between them. Means were compared by Duncan test at 95% level of probability.

#### Determination of isothiocyanates

Isothiocyanates of radishes, rapeseed and turnip were analyzed using Shimadzu, LC-10AT VP HPLC (High performance liquid chromatography) with SPD-M20A prominence DAD (Diode Array Detector) in Mustafa Kemal University Science Applied and Research Center Laboratories (MKUFAM). The methods and analysis procedure was modified from Petersen et al. (2001). The column was a H5ODS-12318 (5 µm, Hichrom). The mobile phase was a 25% acetonitrile and 75% of 10 mmol phosphate buffer (pH=2.4) and detected at 275 nm.

Electrical conductivity (EC) and pH of radishes, rapeseed and turnip extracts were measured using a combined meter (Hanna Instruments model HI 255).

## RESULTS

### Effect of plant residues

Johnsongrass coverage in check plots was 3, 20, 80 and 95% in 2002 and 5, 20, 85 and 95% in 2003 at 7, 15, 30 and 60 DARI, respectively. At 30 DARI, black radish and rapeseed gave the highest suppression in 2002 with over 90% effect (Table 1). Effect of species on johnsongrass did not exceed 72% in 2003 and 4 of them (black radish, rapeseed, little radish, and round white radish) showed similar effect. Garden radish controlled johnsongrass the least in both years.

Johnsongrass suppression at 60 DARI was not significantly different between years, but results presented separately for each year follow the same pattern as 30 DARI. Suppression order of species followed the same pattern as 30 DARI. However, black radish was not as good as in 60 DARI as it was in 30 DARI (Table 1). Highest johnsongrass suppression was obtained from rapeseed in 2002 and little radish in 2003. Garden radish controlled johnsongrass the least in both years in 60 DARI too.

### Effect of plant extracts

All rhizomes in check containers sprouted. There was significant difference among species (Table 2) regardless extract rates applied. Check was different from all bras-

**Table 2.** The effect of shoot powder extract of *Brassicaceae* species on the regrowth of johnsongrass rhizome and the pH, EC, Isothiocyanate benzyl and Isothiocyanate allyl contents of extracts

| Species | The number of sprouted johnsongrass rhizome | Specifications of extracts | | | |
|---|---|---|---|---|---|
| | | pH | EC ($S\ m^{-1}$) | Isothiocyanate benzyl (mg/L) | Isothiocyanate allyl (mg/L) |
| Black radish | 9.1d | 6.6 | 1780 | 9.6 | 23.1 |
| Rapeseed | 9.7d | 6.8 | 1769 | 9.6 | 22.7 |
| Turnip | 10.4cd | 6.6 | 2274 | 9.4 | 22.7 |
| Round white radish | 10.5cd | 6.6 | 1891 | 9.3 | 18.3 |
| Garden radish | 11.7bc | 7.4 | 1546 | 9.3 | 14.5 |
| Little radish | 13.0b | 7.1 | 1352 | 9.3 | 17.5 |
| Check | 20.0a* | N/A** | N/A | N/A | N/A |

*Means within a column followed by the same letter do not differ according to Duncan's multiple range test at $P \leq 0.05$.
**Not applicable.

sica treatments. The highest johnsongrass sprouting occurred at little radish extract, which was 13 out of 20 rhizome parts and followed by garden radish. Sprouted rhizome parts were least with black radish and rapeseed.

## Determination of isothiocyanates

The extract pH of the *Brassicaceae* species was between 6.6 and 7.4 (Table 2). The extracts of garden radish and little radish were more alkaline than the others. Turnip and round white radish extracts had higher EC, while garden radish and little radish had the lowest EC (Table 2). Among tested *Brassicaceae* species, round white radish, little radish and garden radish had the lowest (9.3 mg/l) isothiocyanate benzyl, whereas black radish and rapeseed had the highest (9.6 mg/l) isothiocyanate benzyl content. Black radish had the highest isothiocyanate allyl content (23.1 mg/l), followed by rapeseed and turnip (22.7 mg/l), while garden radish had the lowest (14.5 mg/l) (Table 2).

## DISCUSSION

Six *Brassicaceae* crops were investigated under laboratory and field conditions for allelopathic potential of their residues to control johnsongrass. In both conditions all species suppressed johnsongrass and followed similar pattern. Johnsongrass infestation level gradually increased in check plots as expected, which reached 95% coverage at 60 DARI in both years. However, lower johnsongrass suppression was obtained in 2003 (Table 1). This might be explained with the difference of environmental and climatic factors between growing seasons. For example, in the first five months of the year, which match with Brassica growing seasons in the experiments, total rain was 314 mm in 2002 and 481 mm in 2003. In fact climatic conditions, sulphur and nitrogen concentrations, growing season and water supply affect glucosinolate concentration in brassica species (Rosa et al., 1997; Falk et al., 2007; Zhang et al., 2008).

It was found that the allelopathic effect was reduced at 60$^{th}$ DARI in comparison to 30$^{th}$ DARI. It might result from new emergence of johnsongrass seeds as well as decrease of suppression. At 30 DARI, black radish and rapeseed gave the highest suppression in 2002, but, four of them (black radish, rapeseed, little radish, and round white radish) showed similar effect in 2003 (Table 1). At 60 DARI, leading species were rapeseed in 2002 and little radish and rapeseed in 2003. But, similar to 30 DARI, the first four species were the same. Garden radish was the least effective on johnsongrass sprouting in both years, which is already studied, recommended and implemented species for johnsongrass control in cotton and some other crops in Turkey (Koseli, 1991; Uludag and Uremis, 2000).

The results of the laboratory experiments were parallel to those of field experiments (Tables 1 and 2). Black radish suppressed the best johnsongrass sprouting and followed by rapeseed (Table 2). Turnip was better than field experiments. Little radish affected johnsongrass suppression the least. It is clear that brassica extracts affect the sprouting johnsongrass and support that johnsongrass suppression in the field is due to brassica residues.

The extract pH was higher for garden radish and little radish which gave the least johnsongrass suppression in extract application in the laboratory. Electrical conductivity (EC) did not follow any pattern regarding to effect of extracts on johnsongrass. The effect of pH and EC remained unexplained in this study. However, higher level of suppression of johnsongrass suppression by black radish might have resulted from higher contents of isothiocyanates, especially isothiocyanate allyl because amount of isothiocyanate benzyl was similar among all extracts studied (Table 2). Glucosinolates play a key role for weed suppression as they can be converted to the corresponding ITCs by the enzyme myrosinase (Petersen et al., 2001). Furthermore, isothiocyanate rates were higher in extracts which suppressed johnsongrass sprouting more.

Variation in plant glucosinolate content among crops can be attributed to genetic, environmental and husbandry factors (Fenwick et al., 1983; Milford and Evans, 1991).

The suppression of johnsongrass lasted over 60 DARIs. The longer suppression duration can make *Brassicaceae* crops more useful in cropping system. After mixing the *Brassicaceae* species at the end of the May, johnsongrass will be under control until the end of the July in which summer crops such as corn, cotton or soybean as a second crop can reach a level that can compete with johnsongrass. Ghosheh et al. (1996) found that the critical period for johnsongrass control was between 3 and 6.5 weeks after corn emergence to avoid 5% yield losses. It is clear from the current experiment and earlier critical period data that suppression duration and the end of the critical period for johnsongrass competition matched.

Most of the researches on the allelopathic potential of *Brassica* species have focused on green manure and cover crops (Boydston and Hang, 1995; Al-Khatib et al., 1997; Krishnan et al., 1998). In practice, growing *Brassicaceae* species as green manure or cover crops brings additional cost to the growers. Also additional herbicide application was suggested to achieve weed control at an acceptable level (Boydston and Hang, 1995; Krishnan et al., 1998). However, the integration of *Brassicaceae* crops into cropping systems could suppress weeds, subsequently decreasing seedbank for the next crop and brings extra incomes to the growers. The current study, in which *Brassicaceae* crops were harvested first and then residues were incorporated soil, shows that *Brassicaceae* crops can be used as a rotational crop in regions where climate give opportunity to grow second crop. Furthermore, control of johnsongrass under heavy infestations with herbicide requires several applications with proper timing to ensure effectiveness. However, integration of *Brassicaceae* species into crop rotation could eliminate herbicides or reduce the amount in the control of johnsongrass.

In conclusion, it has been shown that *Brassicaceae* crops, which are grown in Turkey, can be used as rotational crops in cropping systems to reduce johnsongrass infestation. However, there are differences in the control of johnsongrass with *Brassicaceae* species. Currently, the use of garden radish gave the least control in our experiments but farmers have satisfied results with it. If farmers prefer black radish and rapeseed they can ensure higher suppression of johnsongrass. However, the effect of brassicas should be studied on crops that are will be sown. Higher amount of isothiocyanates (isothiocyanate benzyl, isothiocyanate allyl) in black radish extract and lower amount of isothiocyanates in garden radish extract were determined. Parallel results for johnsongrass suppression and amount of isothiocyanates show that allelopathy play roles in johnsongrass suppression by brassica species. The effect of *Brassicaceae* species on other common weed species and crops in the same cropping system needs to be researched as well as the mechanism and of allelopathy.

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