Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 3, No. 12, p. 85-91, 2013
http://www.innspub.net

RESEARCH PAPER | OPEN ACCESS

Allelopathic potential of littleseed canary grass (*Phalaris minor* Retz.) on seedling growth of barley (*Hordeum vulgare* L.)

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Article published on December 14, 2013

Key words: *Activated charcoal, allelopathy, barley, bioassay, canary grass, root exudates.*

Abstract

In order to assess the allelopathic effects of littleseed canary grass (*Phalaris minor* Retz.) against barley (*Hordeum vulgare* L.) the root exudates bioassays were conducted using the equal-compartment-agar method. The experiments were conducted in laboratory of weed ecology of Faculty of Agriculture, University of Tabriz, Tabriz Iran in 2012. Allelopathic effects of canary grass at different growing times (3, 6, 9, 12 and 15 days) were investigated on barley seedling growth. Results indicated that the co-growth of canary grass and barley induced barley root and shoot dry matter reduction. Canary grass growth for 9 and 12 days had the most inhibitive effect on barley shoot and root dry matter, respectively. To validate the allelopathic effect between canary grass and barley, activated charcoal was added to growth medium (2 % v/v) with different densities of canary grass seeds including 0, 5, 10, 15, 20, 25, 50 and 75 plants per beaker. Increasing canary grass density beyond 25 and 75 plants per beaker had no significant further effect on barley root and shoot dry matter, respectively. Thus the inhibition effect of canary grass was directed through root exudates, being greater on barley roots than on shoots growth. The allelopathic effect of canary grass root exudates was evaluated on four barley cultivars, including Karun, Afzal, Valfajr and Zarjou that were differed significantly. The greatest allelopathic impact was noticed on root growth of cv. Zarjou and shoot growth of cv. Karun.

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Introduction

Little seed canary grass (*Phalaris minor* Retz.) is an important weed of winter crops such as barley in Iran which affect their growth and yield (Shimi and Termeh, 2004). Little seed canary grass is a native of Mediterranean Europe and Hawaii (Arnold and de Wet, 1993). It is one of the most widespread grassy weeds in cultivated land in Greece, Mediterranean countries, India, Pakistan and Iran (Shad and Siddiqui, 1996). Other weed seeds may be destroyed by rainy season flooding under cultivation but canary grass remains unaffected due to an impermeable seed coat. Dhaliwal *et al*., (1997) observed that a density of 60-70 plants m^{-2} of canary grass reduced wheat yield by 10%. Dhima and Eleftherohorinos (2003) found that season long competition of 400 canary grass plants m^{-2} reduced wheat grain yield by 48%. Many herbicides have been used to control canary grass including fenoxaprop-p-ethyl, diclofop-methyl, tralkoxydim and triazine (Marwat *et al*., 2005). Furthermore, the evolution of resistance to several herbicides including atrazine, fenoxaprop-p and triazine has been reported (Malik and Singh, 1995).

Allelopathy is a process whereby plants provide themselves with a competitive advantage by putting phytotoxins into the near environment (Pratley, 1996). Allelopathy occurs by the release of chemicals from one plant species affecting other species in its vicinity, usually to their detriment (Rice, 1984). Root exudates represent one of the largest direct inputs of plant chemicals into the rhizosphere environment, and therefore root exudates also likely represent the largest source of allelochemical inputs into the soil environment (Bertin *et al.* 2003). Om *et al*., (2002) observed that broad-leaved weeds of wheat such as *Chenopodium album, Convolvulus arvensis, Cirsium arvense* and *Rumex acetosella* inhibited the germination of canary grass. Khan *et al*., (2001) found that residues from harvested crops of sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*) showed phytotoxic effect on germination and dry matter production of canary grass. Jabran *et al*., (2010) reported that mulberry (*Morus alba* L.), barnyard grass [*Echinochloa crusgalli* (L.) Beauv.], and winter cherry [*Withania somnifera* (L.)] extracts resulted in a complete inhibition of canary grass.

There are a few studies about the allelopathic effects of canary grass ob barley. Therefore, we used the 'equal-compartment-agar method' (ECAM), has been developed for the assessment of wheat seedling allelopathy on ryegrass (Wu *et al*., 2000) to evaluate the allelopathic effect of canary grass on barley cultivars by determining the relative tolerance of cultivars to allelochemicals released by canary grass. The objectives of this study were to evaluate the chemical interaction of root exudates between canary grass and barley seedlings and to compare four barley cultivars challenged by canary grass.

Materials and methods

Barley cultivars for testing

In this study, three experiments were conducted in laboratory of weed ecology of Faculty of Agriculture, University of Tabriz, Tabriz Iran during 2012. Test barley cultivars including Karun, Afzal, Valfajr and Zarjou were obtained from the Iranian winter cereals collection. Canary grass seeds were provided from the wheat field of Eeast-Azarbayjan, Iran in 2011. Barley and canary grass seeds were surface sterilised by soaking in 2.5% sodium hypochlorite solution for 15 min. followed by 5 rinses in sterilised distilled water. The surface-sterilised seeds of barley and canary grass were incubated in light at 25°C for 48 and 72 h, respectively, to germinate. Germinated seeds were used for bioassay experiments.

Experiment 1: The effects of canary grass growing time

The ECAM (Wu *et al*., 2000) was used to evaluate the allelopathic potential of canary grass against barley. Twelve pre-germinated canary grass seeds were uniformly selected and sown on the aseptic agar surface with the embryo upwards, in 3 rows (1cm apart) on one-half of a glass beaker (7.5 cm. diameter and 12 cm. depth, 500 ml) prefilled with 30 ml of 0.3% water agar. The beakers were sealed with parafilm and kept in a controlled growth cabinet [light/dark 13 h/11 h and 24°C /12°C.] The fluorescent light intensity in the cabinet was 3.21 ± 0.16 × 10^3 lux. In co-growth experiment, after the growth of canary grass seedlings for 3, 6, 9, 12 and 15 days, 12 pre-germinated seeds of barley were transplanted on the other half of the agar surface in 3 rows. A piece of pre-autoclaved white paperboard was inserted across the centre and down the middle of the beaker with the lower edge of the paperboard kept 1 cm above the agar surface. The entire beaker was thus divided into two equal compartments, to separately grow the barley and canary grass seedlings. Competition above the agar surface between barley and canary grass was thus avoided by confining plants within their own compartment. After barley sown, the beakers were again wrapped with parafilm and placed back in the growth cabinet for continuous growth of further 10 days. The growth of barley alone was used as the control. In canary grass-cut experiment, the canary grass was grown 3, 6, 9, 12 and 15 days, and then removed from the beakers. Thereafter 12 pre-germinated barley seeds were transplanted into the beakers and grown for 10 days alone without canary grass. Barley seedlings without canary grass pre-treatment were used as control.

Experiment 2: The effects of activated charcoal and canary grass density

To determine the allelopathic effect between the canary grass and barley, 2% (v/v) activated charcoal (0.140 g/beaker) was added to the agar in the beakers. Activated charcoal absorbs organic compounds such as allelochemicals and therefore, the allelopathic effect if present will be reduced where activated charcoal is added to water agar medium. Different densities of canary grass seeds used were 0, 5, 10, 15, 20, 25, 50 and 75 plants per beaker. Also the germinated seeds of canary grass were transplanted into the beakers at the same mentioned densities without adding activated charcoal to the agar in the beakers as no charcoal treatment. All germinated canary grass seeds (transplanted at different densities in the beakers with and without adding activated charcoal) incubated for 9 days under the conditions previously mentioned. After incubation for 9 days, 12 pre-germinated barley seeds were added to all the beakers and incubated for another 10 days.

Experiment 3: Allelopathic effects of canary grass on different barley cultivars

The four test cultivars of barley were pre-germinated and transplanted into beakers that had previously grown canary grass for 9 days at densities of 0, 5, 10, 15, 20 and 25 plants per beaker.

Experimental design and measurements

The experiments were arranged in a randomised complete block design with three replicates. After 10 days of co-growth of canary grass with barley in the growth cabinet, the barley seedlings root and shoot were separated and dried (in the oven at 80 C for 48 h). Finally dry matters of barley root and shoot were measured.

Statistical analysis

Experimental data were subjected to analysis of variance using SAS (version 6.2) and Minitab (version 13) and the treatment means were tested separately using standard error. The data that were used in ANOVA met the assumptions such as normality and homogeneity of variance and did not require any transformation.

Results and discussion

Experiment 1: Canary grass growing time

In canary grass-cut experiment, when canary grass grew up to 3 days, no significant effect on barley root dry matter was observed. But when it grew for 9 days, after the removal of canary grass from the agar medium, the barley root dry matter was significantly reduced due to the chemicals existed in the agar (Fig. 1). Increasing the canary grass growing time beyond 15 days had no significant effect on barley root dry matter. Thus it can be concluded that the allelopathic activity of canary grass was time-related and maximum inhibition was 9 days of its growth. In co-growth experiment, root exudates of canary grass significantly reduced the barley root dry matter and the reduction was greater than when the canary grass was removed before barley transplanting. The canary grass growth for 12 days caused maximum inhibition in barley root growth. Increasing canary grass growing time up to 15 days had no further significant inhibition effects. In canary grass-cut experiment, canary grass root exudates significantly reduced the barley shoot dry matter and growth time of 9 and 12 days had the lowset shoot dry matter (Fig. 2). In co-growth experiment, increasing the canary grass growing time up to 9 days reduced the barley shoot dry matter. Increasing the canary grass growing time more than 9 days significantly reduced its inhibitory effect. By increasing the canary grass growing time beyond 6 days, the inhibition effect of canary grass on barley shoot dry matter decreased.

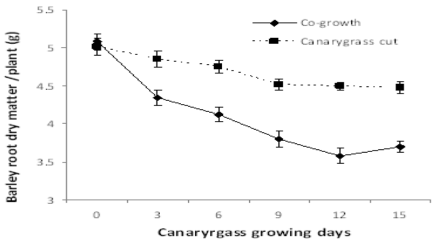

Fig. 1. Effects of canary grass growing days on barley root dry matter in co-growth and canary grass removal experiment. Bars indicate the standard error of observations for each treatment.

Results indicated that when canary grass grew for 12 days, it had the greatest inhibition effects on barley root growth and increasing the growing time beyond that had no further significant inhibitory activity effect. This is probably due to canary grass root exudates degradation, thereby the inhibition effects of canary grass decreased. Namdari *et al.*, (2012) found that the greatest inhibition effect on common bean (*Phaseolus vulgaris* L.) root and shoot growth observed when redroot pigweed (*Amaranthus retroflexus* L.) seedlings grew for 6 days in agar. Huang *et al.* (2003) also reported that concentration of allelopathic compounds was greatest between 6 and 8 growing days and after that their concentration declined. One explanation could be associated with the limited half-life of these compounds in agar medium. The decline in allelopathic compounds toward the end of experimental period could be due to desorption by the growing canary grass plants as observed in wheat (Kobayashi *et al.* 1996) and *Agropyron repens* (Friebe *et al.* 1995). Amini *et al.*, (2009) reported that annual ryegrass (*Lolium rigidum* L.) growth for 6-8 days in agar medium was the most inhibitive to wheat (*Triticum aestivum* L.) root growth.

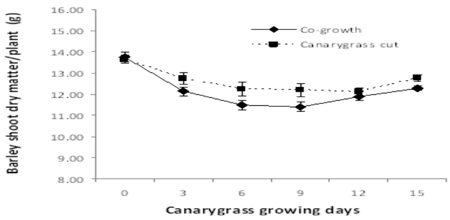

Fig. 2. Effects of canary grass growing days on barley shoot dry matter in co-growth and canary grass removal experiment. Bars indicate the standard error of observations for each treatment.

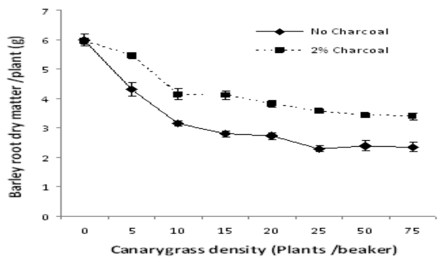

Fig. 3. Effects of activated charcoal and canary grass density on barley root dry matter. Bars indicate the standard error of observations for each treatment.

The canary grass effect on barley growth in the co-growth experiment was greater than in canary grass removal experiment. The presence of canary grass in growth medium intensifies its inhibitory effects on barley root and shoot. The effect of canary grass root exudates on barley root growth was greater than its effect on wheat shoot growth and is consistent with previous studies (Mandal, 2001). Root exudates characterize the largest direct inputs of plant chemicals into the rhizophore environment (Bertin *et al.* 2003) and in the present study it is found that canary grass root exudates had more inhibitory effect on barley root than its shoot. As root growth is a critical trait in plant establishment, the canary grass has the potential allelopathy to prevent the growth and development of barley plants.

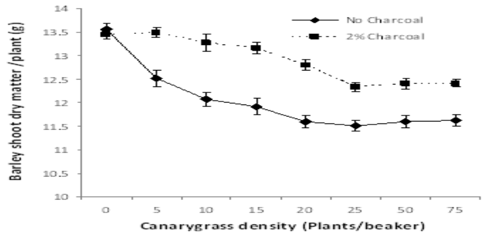

Fig. 4. Effects of activated charcoal and canary grass density on barley shoot dry matter. Bars indicate the standard error of observations for each treatment.

Experiment 2. Activated charcoal

In the absence of activated charcoal, canary grass reduced the barley root and shoot dry matter and this effect amplified significantly with increasing canary grass density up to 25 plants per beaker. Increasing canary grass density beyond 25 had no effect on barley root dry matter (Fig. 3 and 4). Addition of activated charcoal to the growth medium substantially decreased the inhibitory effect of canary grass on barley root (Fig. 3) and shoot dry matter (Fig. 4). Increasing the barley root and shoot dry matter in presence of activated charcoal in the growth medium indicated that the root exudates of canary grass contain the allelopathic compounds. Numerous researchers have used activated charcoal to absorb allelopathic compounds and to demonstrate the presence of allelopathic activity (Inderjit and Callaway, 2003; Kulmatiski and Beard, 2006; Amini *et al.*, 2012).

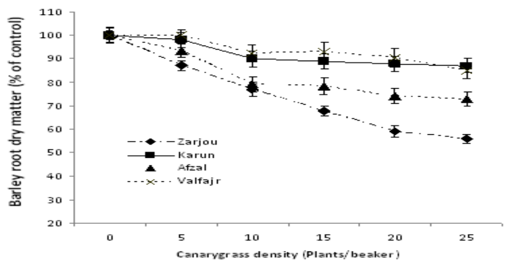

Fig. 5. Effects of canary grass densities on root dry matter of different barley cultivars. Bars indicate the standard error of observations for each treatment.

Experiment 3. Canary grass effects on barley cultivars

Canary grass inhibited the barley root growth and among the barley cultivars, there were different responses to canary grass root exudates. The increasing canary grass density up to 25 plants/beaker reduced the barley root dry matter of all test cultivars (Fig. 5). The responses of cv. karun and Valfajr were similar and these cultivars were the most tolerant to the allelopathic effects of canary grass. The canary grass had the most inhibition effect on root dry matter of cv. Zarjou. The canary grass root exudates reduced the barley shoot dry matter, but the magnitude of reduction was a lesser amount of the root dry matter (Fig. 6). The cv. Karun and Afzal had the highest and lowest reduction% in shoot dry matter at high densities of canary grass. Canary grass root exudates decreased the barley root dry matter up to 40%, while the shoot dry matter was reduced up to 15% at a canary grass density of 25 plants per beaker.

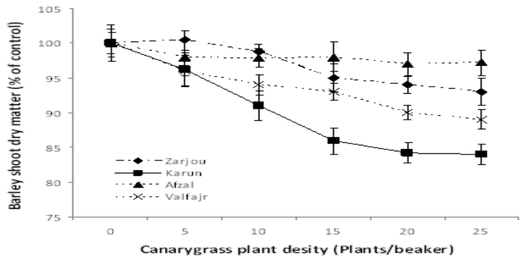

Fig. 6. Effects of canary grass densities on shoot dry matter of different barley cultivars. Bars indicate the standard error of observations for each treatment.

We found that the barley cultivars had different response to root exudates of canary grass and the cv. Zarjou showed the maximum reduction in root dry matter. These results indicate that barley cultivars differed in response to the allelopathic challenge of canary grass root exudates and this diversity should assist in the selection of barley cultivars that can grow in the presence of canary grass root exudates. Amini *et al.*, (2011) also observed that annual ryegrass inhibited the wheat root and shoot growth and there were different responses among the wheat cultivars to annual ryegrass root exudates. The common bean (*Phaseolus vulgaris* L.) cultivars showed different responses to allelopathic effect of common amaranth (*Amaranthus retroflexus* L.) (Amini *et al.*, 2012). Potentially, such barley cultivars could reduce the requirement for herbicide applications. Previous studies have indicated that allelopathy can be used in integrated weed management (Pratley 1996; Narwal *et al.* 1997; An *et al.* (2007). Given the increasing public concern about the use of synthetic herbicides, there is great need for new approaches to weed management (Bertin *et al.* 2007). Therefore, the identification of crop cultivars with high tolerance to weed allelopathy may contribute to the development of effective and friendly environmental weed management systems.

Acknowledgement

Special thanks go to Prof. James Pratley from Charles Sturt University, Wagga Wagga, Australia for their continued support and encouragement.

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