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Table: 1, Figs : 7

New bioassay method to find the allelopathic potential of wheat cultivars on rye (*Secale cereale* L.) seedlings

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ABSTRACT

This experiment was done to develop a new simple and valid method for allelopathy studies and facilitating the use of image analysis in such research. A special transparent growth box containing black fabric enclosed inside was used to provide the seedbed to allow diffusion of allelochemichals from each side to opposite side. The allelopathy potential of 9-wheat cultivars was evaluated against rye (*Secale cereale* L.) to examine the suitability of this method. Images were captured every 12 h to evaluate the effects of wheat cultivars on rye seedling growth over time. The rye seedling growth was significantly affected by allelopathy of different wheat cultivars. The root surface area and spread (root architecture) of rye was decreased by more allelopathic cultivars in the growing medium. There was a clear correlation between the root spread of rye and seedling growth, suggesting the potential of root spread as an important parameter of allelopathic activity. Root spread measurement could be precisely done by using one photograph taken from the expanded roots. This method provided a consistent and reliable tool for analyzing the changes in root architecture of allelochemical receiver plants during the exposure to allelopathic neighbours.

Key words: Allelopathy, bioassay, rye, image analysis, wheat cultivars.

INTRODUCTION

Weed control technology has developed from hand weeding, or control by simple tillage, to the more expensive chemical control (7). In modern agriculture, chemicals are frequently used for weed control. However, environmental and economical costs and weed resistance to herbicides have lead to the public pressure to minimum the use of herbicides, hence, there is increasing interest in organic farming systems (8, 13). Allelopathy is a natural and environment-friendly mechanism which may provide weed control and thereby increase the crop yields (18). In-depth understanding of the allelopathic interference in cropping systems can assist in developing new environmentally safe biological control strategies for sustainable agriculture (12). Selection of crop varieties based on their allelopathic weed-suppressing capability suggested as the applicable strategies to use allelopathy (23). Crop varieties with high allelopathic potential suppresses the weeds by natural bioactive allelochemicals in their vicinity, thereby reducing dependence upon synthetic herbicides (25). For example, some prospective allelopathic rice lines highly suppressed the barnyardgrass (*Echinochloa crus-galli* L.) shoot growth (9).

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Wheat (*Triticum aestivum* L.) is a staple food for billions of people (21) and the most important winter crop in Iran. Allelopathic capability of wheat varieties reduces the weeds population below the threshold level thus minimising the herbicide use (1). Wheat root exudates inhibits the root growth of perennial ryegrass (*Lolium perenne* L.) by 50 % (1). Wheat accessions differed significantly in their seedling allelopathy, with 10% to 91% inhibition in the root growth of ryegrass (24). Wheat accessions varied in their allelopathic activity in the fields, some accessions inhibited the weed growth up to 75 % (19).

Laboratory bioassay is the first step to investigate the allelopathy activity of crop varieties (5). A number of bioassays are available to evaluate the crop allelopathic potential, the selection of method depends on the research objective, bioassay species, the availability of analytical instruments etc (25). However, convenience and reliability of a screening method are the most important factors in allelopathy research. Additionally, screening bioassays must be inexpensive, rapid and easy to operate, have broad application to numerous target species, be reproducible and statistically valid, and require a limited time and space (25).

Aqueous extract bioassays, which are conducted in Petri-dishes and seedling screening bioassays with the "equal compartment agar method"(ECAM), are two bioassay tests widely used in laboratory screening bioassays (24). Despite many advantages of these methods, there are also criticisms. For example, the release of certain salts, amino acids and nitrogen compounds, all may not be released under natural circumstances (3,25), inconsistent results due to non-uniform wetting of growth medium(17,25) were mentioned as strong criticism of extract bioassays in Petri-dishes. ECAM bioassays were developed to overcome these problems. In this method, pre-germinated donor seeds are sown on an agar surface in one-half of a glass pre-filled with agar. After the growth of donor seedlings for seven days, pre-germinated seeds of receiver-weed species are sown on the other half of the agar surface. After 10 days growth of weed with crop seedlings in the growth cabinet, both wheat and weed seedlings are harvested to measure the growth parameters, such as root and shoot length (25). Using this method, measurement of root length can be tedious. Preparation of agar and also rinsing the roots for further measurement is time consuming, and can be costly especially when a large number of varieties are tested.

Recent improvements in digital camera technology have made it easy to get large numbers of high-quality image sets detailing the dynamics of root growth (6). The use of image analysis systems, has facilitated rapid root length, spread and surface area measurements (11,16). To make this method applicable in allelopathy research, high resolution photographs of growth of donor and receiver plants which should be taken. Therefore, we tried to introduce a new simple, cheaper, quick and valid method for use in both allelopathy field and image analysis.

MATERIALS AND METHODS

This study was conducted in 2012 at Zanjan University, Iran. Nine most popular Iranian wheat cultivars (Azar2, Falat, Shahriar, Son 60, Zagross, Gohar, Maroon, Sardari and Nicknezhad) were studied for allelopathic capability to control rye. The rye, a troublesome weed in crop fields was chosen as a test species. Experimental design was a completely randomized one with four replications.

Pre-germinating the seeds before planting in growth box

Wheat and rye seeds were surface sterilized with 6% sodium hypochlorite for 5 min, rinsed thoroughly with distilled water. For pre-germination, seeds were uniformly placed in a sterilized system consisting of 2-layers of filter paper (No. 2; Whatman International, Maidstone, UK) in a Petri dish moistened with 1.5 mL distilled water and allowed to germinate at 25°C in incubator for 24 h.

Preparation of growth box

Two pieces of glass measuring 15 cm by 21 cm with a tap washer (5 mm thickness) between them were attached together using aquarium glue. The space provided by washer advocated for placing the seedbed. A piece of cloth measuring 13×18 cm was used as seedbed. This allowed the allelochemicals movement along the seedbed, thus allelochemicals exuded from the seeds could reach/travel to distant neighbouring species. Required moisture was provided by injecting the distilled water from bottom of glass box.

Twenty mL distilled water was added to each growth boxes. Metal staples were inserted manually with paper stapler were used to fix the wheat and rye seeds on the cloth. Ten pre-germinated seeds of each wheat cultivars were uniformly placed between staple and cloth manually on the one side of the cloth surface with the embryo up. Similarly one pre-germinated seed of rye was also placed on the other side of the cloth as shown in Fig 1. After the punching of receiver and donor plant seeds, cloth was transferred to the glass box and placed in the space between the glasses with a long needle. The growth boxes were wrapped with a piece of aluminium foil and placed in a controlled growth cabinet at 25°C for 10 days. The growth of rye without the wheat plants was treated as control.

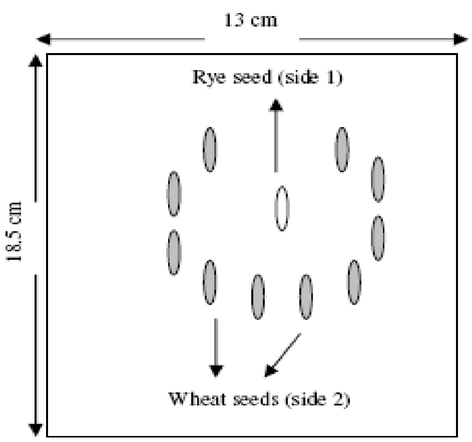

Figure 1. Position of pre-germinated seeds of donor (10 wheat seeds on one side of sheet) and receiver sheets.

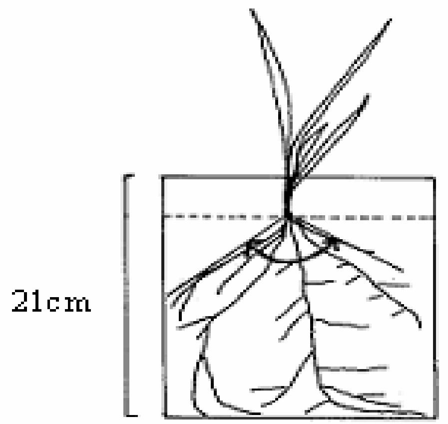

Figure 3. Spread of root system of rye (one rye seed on other side of sheet) plants on the sheets.

Figure 2. Position of pre-germinated seeds of donor wheat and receiver plants rye on the fabric sheets.

Image Analysis

Seedlings photography was started from the start of germination for 10 days. Images of root system were captured every 12 h using a high-resolution (10 Megapixel) digital camera (Canon S95). Data on Root and shoot length over time, angle (the spread of root system) and surface area of rye roots were collected using UTHSCS A Image Tool. The spread of rye root system was shown in Fig. 3 (14). After 10 days, the rye seedlings were harvested, shoots and roots were separated, dried for 24 h at 70°C and dry weights were determined.

Statistical analyses: The relationships between rye root and shoot growth grown adjacent to wheat cultivars by time were described using nonlinear regression. Root and shoot lengths (y, cm) were fitted to sigmoidal-logistic model:

$$y = \frac{a}{\left(1 + (\exp(\frac{-(x - x_0)}{b})\right)}$$

Where, a: Maximum root or shoot length, x_0 : Time to 50% final root length and b : Rate of root or shoot growth.

Nonlinear regression analyses were conducted using Sigma Plot 11.0 (22). Data on rye root dry weight, spread and surface area were subjected to ANOVA using PROC GLM procedure of SAS, version 9.0 (20). Means were separated with the SE.

RESULTS AND DISCUSSION

Seedling growth

The logistic functions described the cumulative growth of roots and shoots against time. However, the parameter estimated showed the allelopathic potential of wheat cultivars in suppressing the rye growth (Table 1). Rye roots were more sensitive to adjacent wheat plants than shoots. Cultivar Azar2 was most inhibitory to rye roots and shoots. The final root and shoot lengths of rye were 0.87 cm and 5.98 cm, respectively, when grown adjacent to Azar2 cultivar (Figs 4 and 5). In contrast, cultivars Maroon and Nicknezhad were least inhibitory to rye root growth, hence, the rye shoots were longest when grown with these cultivars (Figs 4 and 5). The wheat cultivars differed in allelopathic capability (25). Different allelopathic potential also existed in rice cultivars (10). Weed-suppressing wheat cultivars can be used to naturally suppress the weeds (2). In this study, Azar2 cultivar proved most inhibitory (94%) to roots elongation of rye seedlings. Hence, such cultivars with high allelopathic potential can be used to decrease the germination and lower the establishment of rye especially in areas of severe infestation of rye in wheat crop.

Table 1. Parameter estimates for the logistic regression describing the relationship between the root and shoot lengths of rye as a function of time in response to allelopathic effects of different wheat cultivars.

Wheat variety	Seedling part	Parameters			R^2
		A	b	X_0	
Check	Root	15.85 (0.19)[a]	34.84 (1.35)	112.02 (1.63)	0.99
	Shoot	16.94 (0.15)	27.46 (0.83)	134.17 (1.02)	0.99
Azar2	Root	0.87 (0.004)	12.91 (0.55)	51.91 (0.60)	0.99
	Shoot	5.98 (0.12)	43.05 (1.75)	149.63 (2.68)	0.99
Zagross	Root	8.25 (0.49)	53.37 (6.34)	128.86 (9.20)	0.97
	Shoot	11.06 (0.23)	34.80 (1.97)	134.19 (2.56)	0.99
Gohar	Root	9.25 (0.07)	19.93 (0.93)	83.41 (1.06)	0.99
	Shoot	7.31 (0.08)	22.66 (1.21)	116.06 (1.43)	0.99
Shahriar	Root	6.67 (0.04)	21.68 (0.74)	74.12 (0.82)	0.99
	Shoot	8.47 (0.06)	26.06 (0.75)	109.85 (0.88)	0.99
Son 60	Root	9.06 (0.39)	44.09 (5.16)	112.26 (6.39)	0.97
	Shoot	11.64 (0.13)	33.67 (1.01)	140.49 (1.34)	0.99
Maroon	Root	12.94 (0.30)	38.57 (2.69)	112.95 (3.28)	0.99
	Shoot	11.71 (0.143)	33.41 (1.12)	139.67 (1.47)	0.99
Falat	Root	5.90 (0.04)	17.15(0.86)	65.12 (0.97)	0.99
	Shoot	9.99 (0.06)	28.20 (0.62)	124.73 (0.75)	0.99
Nicknezhad	Root	12.80 (0.19)	32.15 (1.77)	109.42 (2.09)	0.99
	Shoot	10.08 (0.07)	30.53 (0.65)	137.76 (0.83)	0.99
Sardari	Root	10.80 (0.37)	45.56 (3.75)	124.06 (5.02)	0.99
	Shoot	7.09 (0.11)	33.80 (1.51)	128.81 (1.91)	0.99

[a] Figures in parentheses are standard error

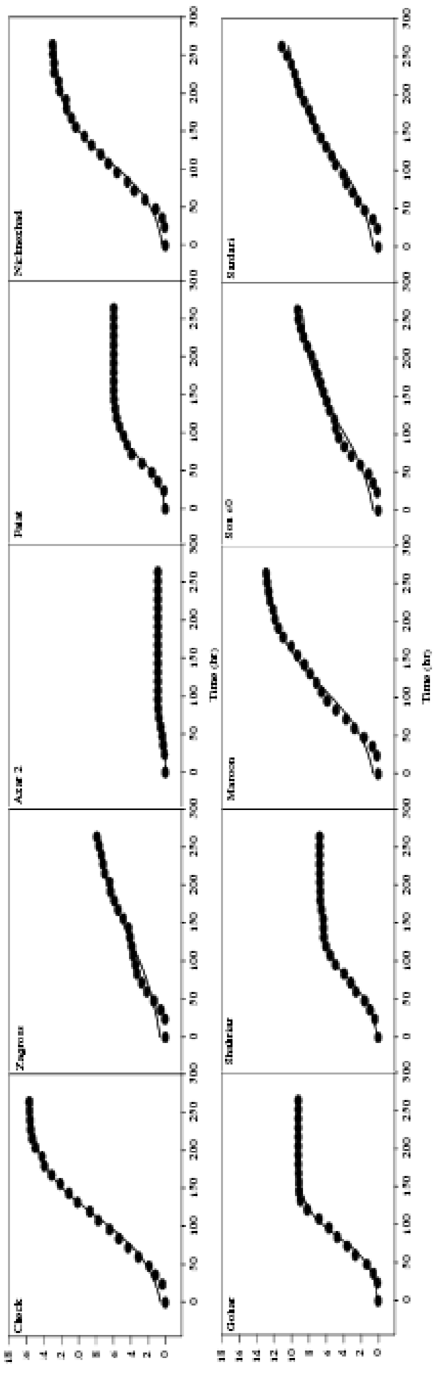

Figure 4. Allelopathic effects of 9- wheat cultivars on the root elongation of rye seedlings.

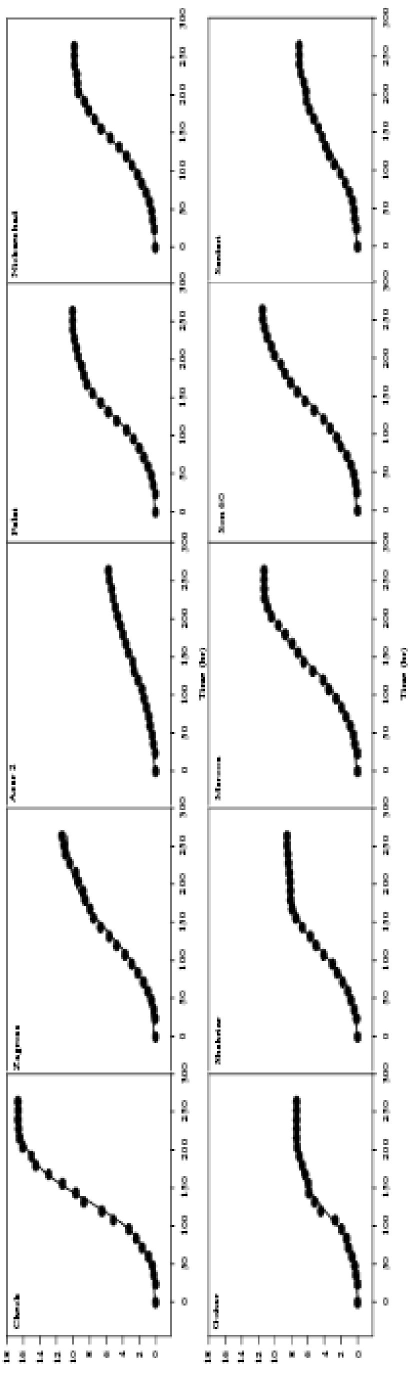

Figure 5. Allelopathic effects of 9- wheat cultivars on the root elongation of rye seedlings.

Less Allelopathic cultivar 'Maroon' **More Allelopathic cultivar 'Azar 2'**

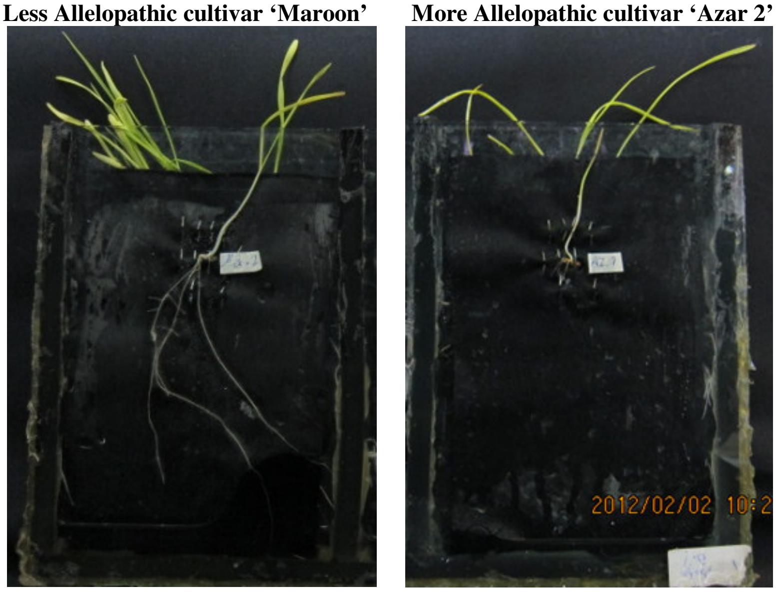

Figure 6. The effects of more allelopathic wheat cultivar 'Azar2' and less allelopathic cultivar 'Maroon' on the seedling growth of rye at the end of experiment.

Root and shoot dry weight

The neighbouring wheat plants reduced the root and shoot dry weights of rye plants up to 93 % and 68 %, respectively. However, cultivars differed in allelopathic capability. The rye root dry weight was drastically reduced (93 %) by Azar 2 cultivar (Fig. 7).

In contrast, Maroon, Zagross and Nicknezhad cultivars had the least inhibitory effect on root dry weight of rye; however, they also decreased the dry weight of rye seedlings significantly (Fig. 7). Based on the decrease in the average dry weight of rye seedlings, Azar2, Gohar and Sardari were determined the most allelopathic cultivars. On the contrary, Maroon showed the least allelopathic effect (Fig. 7).

Surface area and spread of rye root system

The rye root surface area and root distribution were significantly reduced when wheat cultivars were present in the growing medium. Rye root surface area in absence of wheat cultivars was 2.5 cm^2. Co-growth of Azar 2 variety with rye inhibited the root surface area of rye by 92% than control. In contrast, Maroon and Nicknezhad varietes stimulated the rye root surface area by 21 % and 8%, respectively. However, no statistically significant differences were found between the respective cultivars and control in root surface area (Fig. 7). It was suggested that some allelopathic compounds of wheat stimulates the germination of *Orobanche minor* with some variability in cultivars (4).

The spread of rye root was greatly decreased when the allelopathic inhibitory effects were increased. Azar 2 variety was most inhibitory to root spread, inhibiting rye root by 69 %, however, Maroon and Nicknezhad varietis were least inhibitory (Fig. 7).

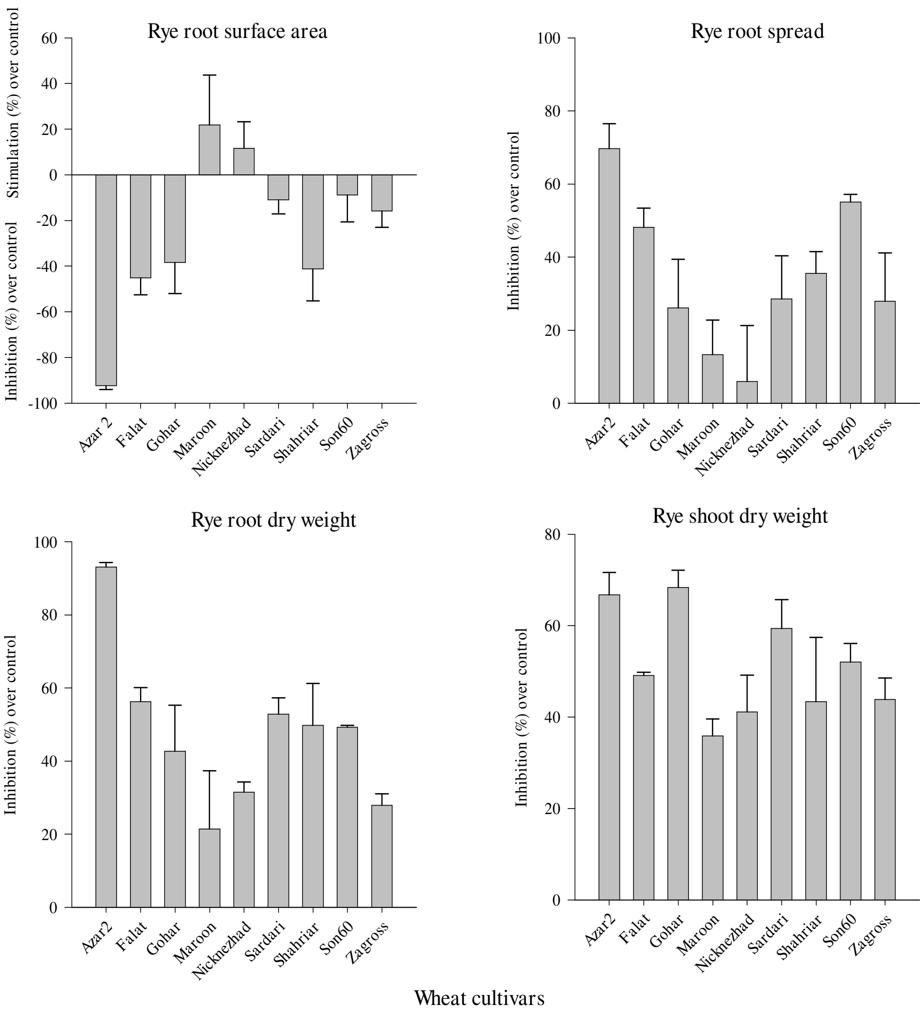

Figure 7. Effects of wheat cultivars on rye root surface areas and root spread, root and shoot dry weight.

Implementation for allelopathy study

The root system plays an important role in plant adaptation to edaphic limitations and boiotic and abiotic stimuli (26). The measurement of radicle elongation and root dry weight are commonly used to determine allelopathic activity (25). However, roots of tested species may adhere to the filter paper and damaged when detached, resulting in errors in the measurement of root length or dry matter (24). Because roots can actively direct their growth toward regions of higher nutrient availability and stress-free environment, it is conceivable that root architecture and curvature might be affected in different ways by nutrient availability, stress environment (27) and allelochemical presence. Results showed that the spread of receiver plant roots (as indicator for root architecture and curvature) was significantly affected by donor plant and there was clear correlation between spread and length of root, suggesting that spread of root as an important determinant of allelopathic activity. This measurement was made using a photo that simply taken at the end of experiment. Simplicity, accuracy and quickness are the major factors of this method to evaluate and analyse allelopathic interactions between neighbouring germinating seeds.

Allelochemical slow diffusive movement through the cloth piece between neighbouring seedlings makes the conditions more similar to a natural environment. With this method, the inhibitory effect of the donor species on the root and shoot growth of the receiver species can be closely monitored over time. Allelochemicals effect only on neighbouring plants placed at a very close distance. Thus, the distance between donor and receiver seedlings is considered in allelopathic possibility. The growth box introduced in present study provides manipulation of the distance between neighbouring plants to estimate the critical distance for receiving the allelochemical. It also provides collecting information on the dynamic of root spread or surface area, with closely monitoring the growth. This method has a potential to apply in other biological studies such as analysis of root curvatures and elongation rates in roots growth studies.

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