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Winter wheat (*Triticum aestivum* L.) allelopathy responses to soil moisture and phosphorus stresses

S.H. Mahmoodi, R. Hamidi*

Crop Production and Plant Breeding Department, College of Agriculture, Shiraz University, Shiraz, Iran

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Abstract

Field, greenhouse, and laboratory experiments were conducted to evaluate the impact of soil moisture and phosphorus on allelopathic potential of wheat residues, and to study the effect of wheat extract concentrations on the germination and growth of wild mustard (*Sinapis arvrnsis* L.). The field experimental design was split-plot with 4 replications. The main factor was three moisture levels including 100, 200, and 300 mm crop evapotranspiration and the sub factor was five phosphorus fertilizer levels of 0, 50, 100, 150, and 200 kg/ha. The results showed that under soil moisture and phosphorous stresses, the inhibitory effects of wheat residues on *Sinapis arvrnsis* seed germination and other growth parameters had an obvious increase. The weed seed germination peaked (62%) at extract that prepared from plants that received 100 kg P/ha (F3) and the highest amount of water (W1). A significant decrease in mustard seed germination percentage was recorded with increasing extract concentration. At all soil moisture levels the severe reduction of the weed shoot dry weight was obtained from no fertilized plots indicated the wheat plant produced the highest amounts of allelochemicals. The extract that made from wheat plant which received the lowest amounts of moisture markedly inhibited the weed plant height in a concentration-dependent manner. In all soil moisture levels, wheat plant that received 150 kg P/ha produced leachates that exerted the lowest inhibitory effects on wild mustard 1000-seeds weight. Results showed that utilizing the naturally occurring chemicals may play an important role in controlling weeds in sustainable agriculture system.

*Corresponding Author: R. Hamidi ✉ hamidi@shirazu.ac.ir

Introduction

Soil moisture stress is the main limiting factor influencing the growth, development, and yield of crop plants (Wilhite and Glantz, 1985). Allelopathic regulation is one of the most significant responses by which the plants can change their growth and developmental phenotype to deal with an arid environment through long ecological adaptation (Shao *et al*., 2008). Allelochemicals are secondary metabolites with multiple functions because they protect the plant against a variety of unpredictable biotic and abiotic environmental stresses (Izhaki, 2002). Abiotic stresses play a key role in accumulation and transportation of allelochemicals in recipient plants (Mwaja *et al*., 1995). Concentration of allelochemicals in the donor plant can be influenced by environmental conditions such as temperature, light, soil moisture or nutrient status (Reigosa *et al.*, 2002; Petterson, 1995).

The effect of some abiotic stressing conditions on secondary metabolite production is well known since long time. Zuo *et al.* (2010) reported that soil water deficit would induce the production and accumulation of more allelochemicals in wheat (*Triticum aestivum* L.) by passive transport of energy cost. Kong *et al.* (2004) showed that in an adverse environment with water deficit and fertilizer shortage, allelopathic potential in *Ageratum conyzoides* was very strong. Tang *et al.* (1995) observed that *Tagetes erecta* under water stress could be induced to exude a higher concentration of phenolics compared with the control as normal water.

Recently, utilization of the allelopathic potential of crop plants for weed control, instead of herbicide application, has been given great emphasis, because it can help reduce the risk of environmental toxicity (Chou, 1999). Some of the major agronomic crops produce allelochemicals which can affect weed growth or influence growth of the next crop (Einhellig, 1996). Sunflower (*Helianthus annuus*) (Azania *et al*., 2003), sorghum (*Sorghum bicolor*) (Alsaadawi and Dayan, 2009), rye (*Secale cereale*) (Teasdale *et al*., 2008), wheat (Ma, 2005; Wu *et al*., 2001; Zuo *et al*., 2005), barley (*Hordeum vulgare*) (Kremer and Ben-Hammouda, 2009), oats (*Avena sativa*) (Kato-Noguchi *et a*l., 1994), and rice (*Oryza sativa* L.) (Kong *et al*., 2004) are perhaps the better documented examples of both living biomass and residue allelopathy, albeit a number of other crops could be cited. According to some reports, leaves are the largest source of allelochemicals, so, extracting from the leaves is one of the most common methods of extracting allelochemicals materials from plant organs. (Hamidi *et al*., 2008). Wild mustard (*Sinapis arvensis* L.) is one of the most widespread cruciferous weeds in cultivated land in Iran a dominant troublesome weed in most wheat fields in Fars province (Baghestani and Zand, 2003), and reduces economic returns to wheat growers through yield losses associated with competition for soil moisture, nutrients, and light (Dhima and Eleftherohorines, 2005). Wheat is well known from high allelopathic potential (Wu *et al*., 2001; Ma, 2005) and its use in the form of mulch, in low chemical input or sustainable agriculture, as an alternative strategy for weed control is under evaluation. Despite of it, data available in literature on wheat allelopathic activity mostly refers to many weed species other than wild mustard. The present study was undertaken in order to investigate the influence of soil moisture and phosphorus levels on the allelopathic potential of wheat plant residues through wild mustard characteristics responses.

Materials and methods

Field, greenhouse, and laboratory experiments were carried out on the Experimental Station of College of Agriculture, Shiraz University at Bajgah located 1810 meters above the mean sea level with a longitude of 25° 32′ E and latitude of 29° 36′ N. The soil was a Daneshkadeh clay loam (fine, mixed, mesic, Calcixerochrept, Xerochrept) composed of approximately 16% sand, 62% silt 22% clay, with 1.5% organic matter, 0.054% total nitrogen, 1.8 mg/kg available P, 200 mg/kg exchangeable K_2O, and a pH of 7.5.

Planting of wheat seed

In the fall before planting, the field was plowed with a moldboard plow to depth of 30 cm and then disked. The wheat cultivar "Shiraz" was sown by grain driller to a depth of 3-5 cm in the field. The seeding rate was 180 kg/ha in rows spaced 20 cm apart. Nitrogen fertilizer (Urea) was applied at 250 kg/ha in two wheat growth stages (1/3 pre-plant and 2/3 at jointing stage).

The experimental design was split-plot with 4 replications. The main factor was three moisture levels including 100 (W1), 200 (W2), and 300 (W3) mm of crop evapotranspiration that measured by evaporation pan, and the sub factor was five phosphorus (P) fertilizer (Triple superphosphate) levels of 0 (F1), 50 (F2), 100 (F3), 150 (F4), and 200 (F5) kg/ha. After maturity, wheat plants were harvested at the soil surface from 1 m^{-2} of the middle 4 rows of each subplot.

Preparation of extracts

Wheat plant residues were chopped by hand into 1-cm long pieces and then oven-dried at 48° C for 48 h (Inderjit and Dakshini, 1995). Extracts were prepared by soaking appropriate amounts of chopped plant materials (3, 6, and 12 g) in 1000 ml distilled water for 24 h at room temperature. The containers were shaken at intervals and after 24 h, the extracts were collected and filtered through 2 layers of Whatman # 2 filter paper and stored in cool temperature (5° C) until experiments were conducted.

Seed bioassay

Germination tests were conducted for each of extracts. Fifty surface sterilized (with 50% ethanol for 2 minutes) seeds of wild mustard (*Sinapis arvensis* L.), were germinated in sterilized 9-cm Petri dishes contained 2 Whatman # 2 filter paper layers moistened with 5 ml of the appropriate extract or with distilled water (control treatment) at constant temperature of 25 ±1° C in germinator. Three ml of each appropriate extract or distilled water (control) were added to each Petri dish after 3 days to prevent drying. After 7 days, seed germination percentage was measured and averaged for each replicate within each treatment. Germination was considered to occur when radicle length was 3 mm or longer. Polyethylene glycole (PEG) was not used in this study because the extract solution concentrations did not exceed 50 milliosmoles (about -0.11 Mpa) (Bell, 1974). Germination bioassays was conducted in a completely randomized design (CRD) with 4 replications. Homogeneity of variances was tested and those data not normally distributed were $\log_{10}$ transformed and retransformed data presented in the results. Data were analyzed by analysis of variance procedure and differences between means were subjected to Duncan`s new multiple range test at the p=0.05 level. In all trait measurments, there were no significant differences between control and the lowest extract concentration (3 g/L), so, we omitted the control data.

Greenhouse experiment

Greenhouse experiment was conducted under 16 h photoperiod, air temperatures of 25/15 OC (day/night), a relative humidity of 50 to 60% and a light flux density of 400 µ moles $m^{-2}s^{-1}$. Mature and non dormant (Goudey *et al.*, 1986) seeds of wild mustard were collected from the experiment Station Farm, College of Agriculture, Shiraz University located in Bajgah 18 km north of Shiraz, Iran. The potting soil was silty clay loam having a pH of 7.2, 1.5% organic matter, and a total N content of 0.07%. Soil was passed through a 5-mm sieve, mixed throughly with the well decomposed cow manure in ratio of 50:50. Three kg_s of soil was placed in each 25-cm diameter uniform plastic pot with draining holes. All pots had draining trays to prevent loss of leachates. Twenty seeds of wild mustard were placed on the soil surface and covered with 150 g of dry soil to provide an appropriate and uniform planting depth. The pots were moistened with appropriate extract concentrations throughout the experiment. All nutritional demanded for wild mustard was supplied. Immediately after emergence, seedlings were thinned to 10 plants per

pot. Measured variables were the weed plant height, shoot dry weight, 1000-seeds weight, and biological yield. Experiment was conducted in a completely randomized design (CRD) with four replications. Data were analyzed by analysis of variance procedure and differences between means were subjected to Duncan`s new multiple range test at the p=0.05 level.

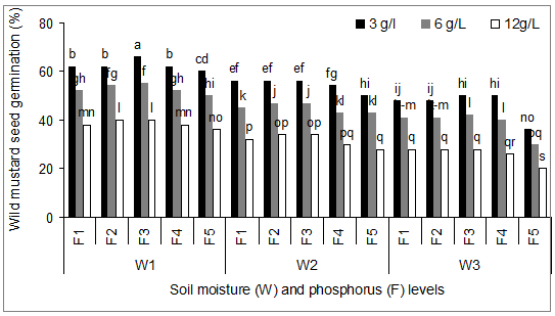

Fig. 1. Wild mustard seed germination percentage influenced by different extract concentrations of wheat plant straw that received various soil moisture (W) and phosphorus (F) levels.

Results and discussion

Weed seed germination percentage

The interaction effect of soil moisture levels and soil P amounts was significant ($p<0.05$) on all wild mustard characteristics. Significant ($p<0.05$) and gradually decrease in wild mustard seed germination percentage was recorded with the progression water and P stress treatments at all extract concentrations. (Fig.1). Weed seed germination peaked (62%) at extract that prepared from plants that received 100 kg P/ha (F3) and the highest amount of water (W1). Extracts that made from plants that received the maximum P (200 kg/ha), exerted the most inhibitory effects on weed seed germination percentage and this effect was markedly obvious at the lowest soil moisture treatment (W3). Plants that were grown in 0 and 50 kg P/ha plots (F1 and F2) and soil moisture levels of W1, W2 and W3, produced extracts did not significantly reduce mustard seed germination. A significant decrease in mustard seed germination percentage was recorded with increasing extract concentration. This result is in agreement with results reported by other researchers. Wang *et al.* (2008) suggested that enhancement of rice allelopathic potential in the suppression of the target weeds under K deficiency might be attributed to the up-regulation of the key enzymes involved in phenolic metabolism, which leaded to the activation of phenolic metabolism, and increased phenolic allelochemicals, consequently inhibited growth of barnyardgrass (*Echinocloa crus-galli*).

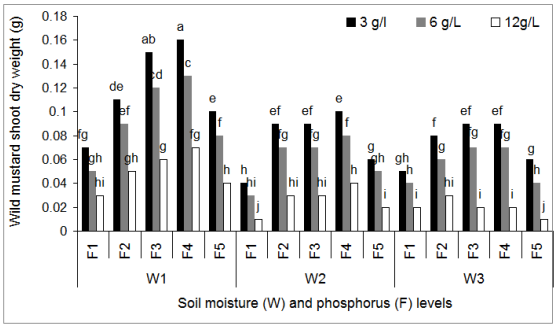

Fig. 2. Wild mustard shoot dry weight influenced by different extract concentrations of wheat plant straw that received various soil moisture (W) and phosphorus (F) levels.

Weed shoot dry weight

Inhibitory status of each extract was differently influenced by water and P levels. Under zero P (F1) and the highest water stress treatment (W3), allelopathic effects of wheat plant residues were recorded. Average wild mustard shoot dry weight was 0.07, 0.05, and 0.03 at extract concentration of 3, 6, and 12 g/L, respectively. Conversely, the lowest allelopathic potential of wheat plant residues was obtained from the 150 kg P/ha and no water stress conditions (Fig. 2). At all soil moisture and P treatments, as the extract concentration was increased, the allelopathic potential of wheat plant residues followed an increasing trend. At all soil moisture levels the severe reduction of weed shoot dry weight was obtained from no fertilized plots indicated the wheat plant produced the highest amounts of allelochemicals. Several studies have been shown that the amount of allelochemicals produced and released by plants, is in correlated to environmental stresses (Rose *et al.*, 1995; Agarwal, 1998; Kong *et al.*, 2002). The most inhibitory effects of wheat plant residues were obtained from the lowest amounts of water and P. In fact, water and

nutrition stresses caused significant alterations in the crop physiological processes (Stanciu and Neacsu, 2008) including photosynthetic pigments and enzymes that cause the production of new secondary metabolites named as allelochemicals (Bagavathy and Xavier, 2007).

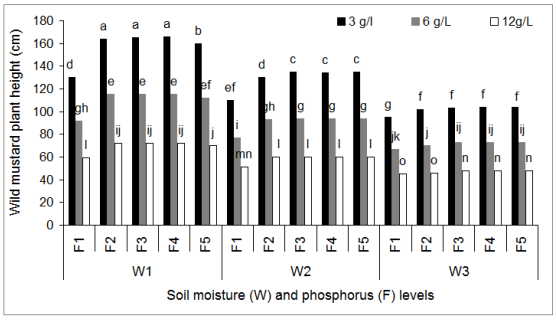

Fig. 3. Wild mustard plant height influenced by different extract concentrations of wheat plant straw that received various soil moisture (W) and phosphorus (F) levels.

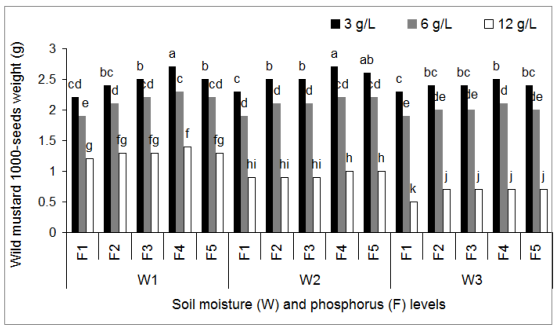

Fig. 4. Wild mustard 1000-seeds weight influenced by different extract concentrations of wheat plant straw that received various soil moisture (W) and phosphorus (F) levels.

Weed plant height

Wheat plant residues allelopathy had stressful impact on wild mustard plant height. The data that illustrated on the Figure 3 show that both nutritional defficiency (F1) and soil moisture stress (W3) affect negatively compared to fertilized and well watered plots. At all soil moisture levels, the wheat plant that received P amounts of 50 (F1), 100 (F2), and 150 (F3) kg/ha produced extracts that exerted the same effects on the weed plant height. Wheat plants that received the lowest level of water (W3) produced extracts that exerted the highest inhibitory effects on wild mustard plant height at all fertilized plots, however, there were not significant differences between fertilized plots in each extract concentration (Fig. 3). Wild mustard plant height was not affected by extract at 3 g/L concentration with compare to control. The extracts (6 and 12 g/L) evaluated for their phytotoxicity on wild mustard plant height exhibited various degree of inhibition of weed plant height. The extract that prepared from wheat plant that received the lowest amounts of moisture markedly inhibited the weed plant height in a concentration-dependent manner (Fig. 3). Wild mustard height decreased as the concentration of the extract increased and the greatest inhibition was observed at the 12 g/L concentration. With respect to the control, application of extract solution at concentrations of 6 and 12 g/L decreased the plant height of *S. arvensis* by 13.6 and 32%, respectively. The maximum height recorded in plants treated with water (control) and extract concentration of 3 g/L were 166 and 165 cm, respectively. The results of previous studies showed that a number of phytotoxic substances suspected of causing allelopathic effects have been identified in wheat plant parts (Neves and Gaspar, 1990; Wu *et al*., 2001; Nakano *et al*., 2006). Spruell (1984) screened 286 wheat accessions for allelopathic potential in the United States of America. Root and shoot exudated of each accession inhibited root and shoot growth of *Bromus japonicus* and *Chenopodium album*. Among *Triticum* species, *T. aestivum* was strongly allelopathic while, *T. boeoticum* and *T. dicoccoides* were weakly allelopathic (Zuo *et al*., 2005).

Weed 1000-seeds weight

When wild mustard plants were grown on soil which moistened with two concentrations (6 and 12 g/L) of wheat plant residue extracts, the weed 1000-seeds weight was decreased particularly when the wheat pant did not received any P level (Fig. 4). Wheat plants that were grown in unfertilized plots, produced extracts that exerted the most inhibitory effects on weed 1000-seeds weight. The inhibitory activity was stronger as the concentration of wheat straw leachate was increased. This result indicate that allelochemical(s) inhibiting the weed 1000-

seeds weight, are leached from the wheat straw into the water (Fig. 4). In all soil moisture levels, wheat plant that received 150 kg P/ha produced leachates that exerted the lowest inhibitory effects on wild mustard 1000-seeds weight. In general and in comparison with other weed traits, weed 1000-seeds weight response to extracts of wheat straw was the lower than the other ones. Weed growth and seed production reduction with response to wheat residue extracts have been reported by many researchers (Lodhi *et al.*, 1987; Neves *et al.*, 1990; Nakano *et al.*, 2006). Several types of allelochemicals are induced in plants by various biotic and abiotic stresses (Dixon and Paiva, 1995). In a study, Huaqin *et al.* (2006) reported that in phosphorus defficiency stress, the inhibitory effect of rice (*Oryza sativa* L.) residues on *Echinocloa crus-galli* L. root growth had an obvious increase. The results of this study showed that under P deficincy, the allelopathic potential of rice enhanced through two pathways, i.e., to increase weed peroxydase and Indole acetic acid oxydase activities to slow down its growth rate and to decrease the nitrate reductase activity to effect its nitrogen uptake.

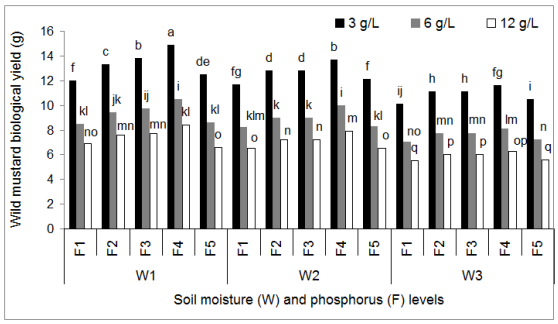

Fig. 5. Wild mustard biological yield influenced by different extract concentrations of wheat plant straw that received various soil moisture (W) and phosphorus (F) levels.

Weed biological yield

Wheat plant straw leachates caused a markedly reduction in weed biological yield, particularly in concentrations of 6 and 12 g/L. Data in Figure 5 showed that in soil moisture and P deficiency conditions, wheat plants produced more allelochemical(s) that could inhibited the biological yield of wild mustard. Weed biological yield was recorded 12, 11.7, and 10.1 g as influenced by wheat plant residue extract concentration of 3 g/L and unfertilized conditions at W1, W2, and W3, respectively (Fig 5). This weed trait was recorded 6.9, 6.5, and 5.5 g as affected by residue extract concentration of 12 g/L at the some soil moisture levels. On unfertilized plots, weed biological yield was decreased by 18, 18, and 28% at W1, W2, and W3, respectively, as compared to the highes amounts of P (F5). Wheat plants that received the lowest soil moisture level (W3) and did not received P, produced the highest amounts of allelochemical(s) that exerted the highest inhibitory effects on weed biological yield (Fig. 5).

Results of our study showed that the decrease in the amount of wild mustard traits was more pronounced in plants treated with high extract concentrations (6 and 12 g/L). In addition, soil moisture and P stresses negatively affected on wheat plant growth and produced plant residues with high allelopathic potential. These results are in accord with results of other studies reported that wheat straw has phytotoxicty effects on many weeds seed germination and growth (Liebl and Worsham, 1983). The three main categories of allelochemicals identified in wheat are phenolic acids, hydroxamic acids, and fatty acids (Wu *et al.*, 2001). Of the allelochemicals in wheat, *p*-hydroxybenzoic, vanilic, *p*-coumaric, syringic, and ferulic acids are most frequently reported (Lodhi *et al.*, 1987; Wu, 2001) Allelochemicals caused a decrease in photosynthetic pigments (Bagavathy and Xavier, 2007) and inhibit the activity of protoporphyrogen IX and 4-hydroxyphenylpyruvate dioxygenase or phytoen desaturase, the key enzymes in chlorophyll and carotenoide biosynthesis, respectively (Romagni *et al.*, 2004).

Conclusion

Many sustainable agriculture farmers are unconsciously receiving benefits of allelopathy when they plant crops no-till into certain cover crops or straw residues. Utilizing this naturally

occurring chemical warfare among plants may play an important role in controlling weed in crops in the future (Chou, 1999). Results of our study showed that abiotic stress conditions such as water and nutritional deficiencies could be helpful in weed control through planting crops with highly allelopathic potential. With regard to global warming and changes in pattern of precipitations, crop plants experience water stress under allelopathic conditions and this may be interesting to find out the new biological ways to control of many weed species.

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