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ORIGINAL ARTICLE

Evaluation of the allelopathic effect of wheat and redroot pigweed on growth indices and antioxidant system activity in intercropping

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Abstract

Allelopathy refers to the beneficial and detrimental effects of one plant on another plant in both crops and weeds through the production of secondary compounds. In order to evaluate the allelopathic effects of wheat (*Triticum aestivum* L.) as a crop and redroot pigweed (*Amaranthus retroflexus* L.) as a common weed worldwide on each other in intercropping, these plants were cultivated under controlled conditions at Tabriz University laboratory. The ratios of wheat to redroot pigweed were, 100 : 0 and vice versa as a control, 75 : 25, 50 : 50, and 25 : 75. The results showed that at the ratio of 25 : 75 (wheat : redroot pigweed), the fresh and dry weight of roots and shoot length of wheat decreased significantly compared to the control. The fresh and dry weight of wheat shoots showed a significant decrease at different ratios compared to the control. Shoot peroxidase (POD), root superoxide dismutase (SOD), and root and shoot catalase (CAT) activities in redroot pigweed increased in all intercropping ratios compared to the control. POD activity in wheat roots was higher at all ratios than in the control. Furthermore, the ratio of 75 : 25 (wheat : redroot pigweed) led to increased activity of POD enzymes and malonedialdehyde (MDA) content in wheat shoots. Moreover, roots of redroot pigweed showed increased activity of ascorbate peroxidase (APX) and SOD enzymes and MDA content. With increased density of redroot pigweed, the soluble sugar content of wheat roots reduced significantly. However, the content of insoluble sugar and total protein increased. Root exudate compounds such as terpenoids, phenolic compounds, fatty alcohol, steroids, fatty acids, and alkanes were identified using gas chromatography/mass spectrometry (GC/MS). The findings showed that the roots were more exposed to oxidative stress due to direct contact with allelochemical compounds. Our results support the hypothesis that increasing the density can reduce the toxicity of allelochemical compounds and that increasing the activity of the antioxidant system will improve plant growth under allelochemical stress.

Keywords: allelochemical, density, growth parameters, malonedialdehyde, reactive oxygen species

Introduction

Allelopathy is considered to be an important factor in weed management and crop rotation (Mushtaq and Siddiqui 2018). It is the result of the production of biologically active molecules (allelochemicals) by plants or their residues, fungi, viruses, and microorganisms that have an inhibitory or stimulating effect on biological and agricultural systems directly or after deformation in the environment (Farooq *et al.* 2011; Iqbal and Fry 2012). Allelochemicals are secondary metabolites that fall into various chemical groups, including phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, lignins, tannins, fatty acids, and non-

protein amino acids. These compounds alone or in combination with each other and environmental stress factors have inhibitory effects on plants (Bhatla 2018). Flavonoids are one of the first allelochemical groups that inhibit mitochondrial oxygen uptake and reduce the production of adenosine triphosphate (ATP) in mitochondria, as well as negatively affecting photosynthesis (Effiong *et al.* 2022). Alteration of enzyme function, inhibition of protein synthesis, and inactivation of plant hormones are inhibitory mechanisms of phenolic allelochemicals (Batish *et al.* 2006; Li *et al.* 2010). Benzoxazolin-2 (3H) -one (BOA) and cinnamic acid (CA) inhibit growth, leaf water relations, PSII photochemistry, non-photochemical fluorescence extinction and heat energy dissipation (Hussain and Reigosa 2011).

Allelochemicals are released from different parts of the plant such as leaves, roots, flowers, and seeds during different growing seasons. Also, degradation of plants can produce residues, or release volatiles and impact crops. Generally, exposure of sensitive plants to allelochemicals leads to lower seed germination and reduced growth rate (Hussain and Reigosa 2011; Srivasava *et al.* 2017).

Crops have allelopathic effects on other crops and weeds. Weeds also have allelopathic effects on crops and other weeds. The interaction between crops and weeds is direct or indirect. Releasing an allelochemical from one species in a growing medium can have an inhibitory or stimulating effect on germination, development, reproduction, biosynthesis of photosynthetic pigments and growth of another plant (Bachheti *et al.* 2020). Redroot pigweed is one of the most important weeds in the world, and its allelopathic effects have been fully documentated. Aldehydes, alkaloids, saponins, flavonoids, phenolic acids, chlorogenic acid, carvacrol and benzoic acid are among the allelopathic compounds of redroot pigweed (Shahrokhi *et al.* 2011; Ma *et al.* 2015; Bakhshayeshan-Agdam *et al.* 2021). Allelopathic effects of redroot pigweed on crops such as wheat (Shahrokhi *et al.* 2012), corn (Konstantinović *et al.* 2014), cotton (Ma *et al.* 2015), and tomatoes (Qasem 2018) have been reported.

Live wheat plants and their dead residues have different allelochemical compounds. Some allelochemical compounds of wheat plants are hydroxamic acids and their derivatives, phenolic compounds, flavonoids and short-chain fatty acids. The chemical profile of wheat and its allopathic activity are determined by weeds (Aslam *et al.* 2017; Hussain *et al.* 2022).

In previous studies, Zheng *et al.* (2007) showed that wheat had allelopathic effects on germination and growth of weed plants such as *Digitaria sanguinalis*, *Poa annua* L., *Amaranthus retroflexus* L., *Echinochloa crus-galli* L., and *Avena fatua* L. Studies have shown that an aqueous extract of wheat has a negative effect on seedling germination, growth and the antioxidant system of wild oat (Mahmood *et al.* 2013).

Given the importance of wheat in ensuring world food security and the role of weeds in reducing wheat yield, the cultivation of wheat cultivars with allelopathic potential can be used as a method to control weeds or reduce their effects in wheat fields (Jabran 2017). Plant density is an important factor in increasing the crop yield. For example, proper planting density and pattern determine which light penetrates into the plant community and play an influential role in increasing production (Postma *et al.* 2021). The growth and production of most plants depend on density. Due to the fact that in allelopathic effects, the effect of toxins released into the environment decreases with increasing plant density, so it can be expected that with increasing crop density weed growth and production in cultivation systems will decrease. The aim of this study was to investigate the effects of intercropping of redroot pigweed and wheat plants at different density ratios on physiological processes, including growth characteristics, photosynthetic pigment content, as well as enzymatic and non-enzymatic antioxidant systems of both wheat and redroot pigweed.

Materials and Methods

Plant culture and harvesting

The seeds of wheat (*Triticum aestivum* L. "Pishgam") and redroot pigweed (*Amaranthus retroflexus* L.) were supplied by the Agricultural and Natural Resources Research Center of East Azerbaijan (Tabriz, Iran). Appropriate numbers of seeds were selected based on their vigor and uniformity, disinfected using 5% (v/v) sodium-hypochlorite solution for 5 min and sufficiently washed using sterile distilled water. The seeds of redroot pigweed were planted at a depth of 1 cm in pots with 500 ml sterilized soil and peat moss (Tables 1 and 2) and kept in darkness to germinate. After 3 days, the pots were transferred to controlled conditions (25–30°C, 16 h /8 h light/dark photoperiod and relative humidity of 60%). The water content of the pots was adjusted to 100% field capacity every day using sterile distilled water. After 14 days, 25 and 50% of Hoagland solution was used. Twenty days after the redroot pigweed had grown significantly, the weak plants were removed and a fixed number of plants were left for each ratio. The wheat seeds were sterilized in 5% sodium hypochlorite for 5 min, then, rinsed with distilled water three times. Wheat seeds with ratios of 100 : 0, 75 : 25, 50 : 50, and 25 : 75 (wheat : redroot pigweed) were sown in pots containing redroot pigweed using three replications. The ratio of 100 : 0 (redroot pigweed : wheat) was used as the control for redroot

Table 1. Analysis of soil physical-chemical properties

pH	EC [ds · m^{-1}]	Sand [%]	Silt [%]	Clay [%]	K [mg · kg^{-1}]	P [mg · kg^{-1}]	N [%]
8.1	0.60	42	32	26	181	10.2	0.04

Table 2. Chemical properties of peat moss

pH	N-NO_3 [mg · l^{-1}]	N-NH_4 [mg · l^{-1}]	MgO [mg · l^{-1}]	P_2O_5 [mg · l^{-1}]	SO_3 [mg · l^{-1}]	CaO [mg · l^{-1}]
6.8	0.57	32.78	2	10	4	0.54

pigweed. The plants were harvested after 67 days for the measurements.

Assay of growth parameters

The plants were harvested 67 days after planting and shoot height and root length of wheat and redroot pigweed were measured. The samples were sufficiently washed with water, immediately dried on a paper towel, and transferred to 70°C after determining the fresh weight. The dry weight of samples was measured after 72 h.

Measurement of photosynthetic pigments content

Photosynthetic pigments content (chlorophyll a, b, total chlorophyll, and total carotenoids) was determined according to the method of Lichtenthaler (1987). Briefly, 0.1 g of fresh leaf samples was homogenized with 5 ml of acetone using a mortar and pestle on an ice bath. Homogenizes were filtered using number 42 Whatman filter paper and the absorbance of extracts was recorded at 645, 663, and 470 nm by Spectrophotometer (Analytic Jena, Specol1500, Germany).

Measurement of total protein content and antioxidant enzyme assays

Using a mortar and pestle 0.1 g of samples was homogenized with ice-cold phosphate – buffered solution (PBS, 50 mM, pH = 7). Homogenizes were centrifuged at 10,000 g for 10 min at 4°C. The supernatants were used immediately for determination of the total soluble protein content by the method of Bradford (1976) as well as the activities of superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT).

SOD activity was assayed by determination of nitro-blue-tetrazolium (NBT) photoreduction inhibition by extracts (Winterbourn *et al.* 1976). The reaction mixture (3 ml) contained 2.7 ml sodium phosphate solution (1 M, pH = 7.8), 100 µl NBT (1.5 mM), sodium cyanide (NaCN, 0.3 mM), ethylenediaminetetraacetic acid EDTA) (1 M), 50 µl of riboflavin (0.12 mM) and 50 µl of enzyme extract. The mixtures were illuminated at a light intensity of 5000 µmol · m^{-2} · s^{-1} for 12 min and the absorbance of the solutions was recorded at 560 nm. The amount of the enzyme causing 50% protection of NBT photoreduction was considered as one unit, and SOD activity expressed as U · mg^{-1} protein.

The activity of POD was determined by recording the increase in absorbance at 470 nm during polymerization of guaiacol to tetraguaiacol for 3 min (Chance and Maehly 1955). The reaction mixture (1 ml) included 300 µl of guaiacol (4 mM), 350 µl of phosphate buffer (10 mM, pH = 7), 300 µl of hydrogen peroxide (H_2O_2) (50 mM) and 50 µl of enzyme extract. The reaction was initiated by adding H_2O_2 to the reaction mixture and POD specific activity was calculated using the extinction coefficient of 26.6 mM^{-1}cm^{-1} for guaiacol. One unit of POD activity was considered as the enzyme amount capable of oxidizing 1µM guaiacol to tetraguaiacol per minute and POD activity expressed as U · mg^{-1} protein.

CAT activity was assayed according to the methods of Chance and Maehly (1955). The reaction mixture contained 2.5 ml potassium phosphate buffer (50 mM, pH = 7), 1 ml of H_2O_2 (10 mM) and 500 µl of enzyme extract. The absorbance was measured at 240 nm by following the decomposition of H_2O_2 for 3 min and CAT specific activity (expressed as U · mg^{-1} protein) was calculated using the extinction coefficient of 27 mM^{-1} cm^{-1} for H_2O_2 and one unit of enzyme activity was considered as the amount of enzyme necessary for the reduction of 1 µM H_2O_2 per minute.

APX activity was evaluated by monitoring the absorbance decline at 290 nm for 3 min (Boominathan and Doran 2002). The reaction mixture (1 ml) contained 300 µl of potassium phosphate buffer (50 mM, pH = 7), 200 µl of bovine serum albumin (BSA, 50 mM), 50 of EDTA 0.2 mM), 200 µl of ascorbic acid (0.5 mM), 50 µl of enzyme extract and 50 µl of H_2O_2 (0.1 M). The reaction was initiated by adding 50 µl of H_2O_2 (250 mM), and APX specific activity (U · mg^{-1} protein) was calculated using the extinction coefficient of 2.8 mM^{-1} cm^{-1} for ascorbic acid. One unit of enzyme activity was considered as the amount of enzyme necessary for the reduction of 1 µM ascorbic acid per min.

Soluble and insoluble sugars

The soluble and insoluble sugar content was determined by the phenol-sulfuric acid method (Kochert *et l.* 1978). Briefly, 5 ml of ethanol (70%) was

added to 50 mg of dry powdered sample and refrigerated for 1 week. The samples were centrifuged at 10,000 g for 15 min at room temperature. Finally, supernatant and sediment were separated and used for determination of soluble and insoluble sugar content, respectively. For determination of soluble sugar, distilled water was added to 0.5 ml of the extract until it reached 2 ml. Then, 1 ml of 5% phenol and 5 ml of concentrated sulfuric acid were added to each sample, respectively. The resulting mixture was kept at room temperature for 30 minutes after stirring. To determine insoluble sugar, 20 ml of distilled water was added to the centrifuged precipitate after drying and pulverization. The samples were placed in a warm water bath for 15 min. Then they were filtered with filter paper and distilled water was added until it reached a volume of 20 ml. One mL of 5% phenol and 5 ml of sulfuric acid were added to 2 ml of the samples. The samples were kept at room temperature for 30 min. The absorption was recorded at 485 nm, and glucose was used for the preparation of the standard curve. Results were expressed as mg · g^{-1} dry weight (DW).

Measurement of malondialdehyde content (MDA)

Malondialdehyde (MDA) content was measured by a method described by Boominathan and Doran (2002). First, 0.1 g of samples was homogenized with 0.1% (w/v) trichloroacetic acid (TCA, Merck, Germany) and centrifuged for 5 min at 10,000 g. Then, 0.5 ml of the supernatants was mixed with 2 ml of 20% TCA containing 0.5% of 2-thiobarbituric acid (Merck, Germany) and heated in hot water (95°C) for 30 min. Mixtures were immediately transferred to an ice bath and centrifuged at 10,000 g for 15 min. Finally, the absorbance of the supernatants was recorded at 532 nm and MDA concentration was calculated according to a standard curve prepared using 3,1,1,3-tetraethoxy propane (0-100 nM) and expressed as µmol · g^{-1} fresh weight (FW).

Measurement of hydrogen peroxide (H_2O_2)

Fresh samples of 0.1 g were homogenized in 5 ml of TCA 0.1% (w/v). The homogeneity was centrifuged at 10,000 g for 15 min at 4°C. Then, 0.5 ml of the supernatant, 0.5 ml of potassium phosphate buffer (10 mM, pH = 7) and 1 ml of potassium iodide (KI) (1M) were mixed at 4°C for 10 min and kept at room temperature (25°C) for 15 min. The absorbance of each sample was recorded at 390 nm. A calibration curve was provided using H_2O_2 standard solutions in TCA 0.1%, and H_2O_2 content was expressed as µmol · g^{-1} FW (Harinasut *et al.* 2003).

GC-MS analysis

Ten g of soil samples in 100 ml of ethanol was placed on a shaker. The sample was shaken every hour for the first 16 h, and then it was set aside and again shaken after 5 h. This process was repeated three times. Then it was filtered through Whatman 42 filter paper. The extract was collected and freeze dried. The final residue thus obtained was subjected to GC-MS analysis (Gopinath *et al.* 2013). One ml of extract was injected to GC-MS (Agilent 6890 model) with a HP 5MS capillary column with a length of 30 m and an internal diameter of 0.25 mm, which was connected to HP 5989A and operated in an ionization mode at 70 eV. The temperature program started at 60°C and after 1 minute reached 290°C at 15°C · min^{-1} and remained at the final temperature for 10 min. The resulting chromatograms were analyzed using NIST and PubChem software to identify the desired compounds (Lee *et al.* 1979).

Statistical analysis

All data were analyzed by using SPSS 16 (Statistical Product and Service Solutions, International Business Machines Corporation (IBM), New York, U.S.A) software and Duncan's multiple range tests were used for mean comparisons ($p < 0.05$). SPSS software was used to calculate the correlation coefficient (Pearson) between characteristics. The graphs were prepared by using Microsoft Excel 2013 software. The results in all graphs and tables were presented as average values of three replications ± standard deviation (SD).

Results

Growth indices

The fresh and dry weight of roots and shoot length of wheat had no significant differences after 67 days of treatment. Only at the ratio of 25 : 75 (wheat : redroot pigweed), root fresh weight (39%), root dry weight (17%) and shoot length (24%) of wheat showed significantly decreased, while wheat root length increased by 35.54% compared to the control. The fresh and dry weight of wheat shoots at different ratios showed a significant decrease. The highest decrease in the fresh weight (61%) and dry weight (68%) was related to the ratio of 25 : 75 compared to the control (Table 3).

Compared to the control the shoot length of redroot pigweed showed a significant increase (17%) when the ratio of wheat and redroot pigweed was equal. With increased density of wheat, a significant decrease was observed in the root length of redroot pigweed. The root fresh and dry weight of redroot pigweed increased significantly at the ratio of 75 : 25 and

Table 3. The effect of different ratios of intercropping on the growth parameters of wheat and redroot pigweed roots and shoots

	Root length [cm]	Shoot length [cm]	Root FW [mg]	Root DW [mg]	Shoot FW [mg]	**S**hoot DW [mg]
Ratios of intercropping			Wheat			
100 W : 0 R	15.25 b ± 1.06	24.94 a ± 0.55	129.66 a ± 2.18	10.55 a ± 0.50	311.1 a ± 6.24	39.44 a ± 2.22
75 W : 25 R	14.58 b ± 0.716	23.51 a ± 0.23	131.88 a ± 2.45	10.47 a ± 0.74	254.106 b ± 2.50	37.21 a ± 0.38
50 W : 50 R	15.88 b ± 1.019	23.73 a ± 0.89	128.22 a ± 0.69	10.98 a ± 0.32	197.77 c ± 14.06	31.77 b ± 2.04
25 W : 75 R	20.67 a ± 1.72	19.00 b ± 1.00	79.33 b ± 4.04	8.77 b ± 0.69	99.543 d ± 9.67	15.55 c ± 1.67
			Redroot pigweed			
100 R : 0 W	12.70 a ± 0.86	15.88 b ± 0.66	81.33 c ± 0.67	11.33 c ± 0.33	700.86 bc ± 59.87	168.99 b ± 6.63
75 R : 25 W	12.92 a ± 0.80	16.29 b ± 0.74	110.11 a ± 1.84	20.33 b ± 1.76	761.00 ab ± 44.00	176.11 b ± 7.16
50 R : 50 W	12.14 a ± 0.79	18.59 a ± 0.93	100.77 b ± 2.17	28.77 a ± 1.39	810.44 a ± 19.84	196.66 a ± 3.05
25 R : 75 W	9.38 b ± 0.47	16.52 b ± 0.50	64.66 d ± 3.51	12.76 c ± 0.68	660.00 c ± 36.00	176.33 b ± 7.09

Different letters above the bars indicate significant differences ($p < 0.05$). Dw – dry weight, FW – fresh weight, W – wheat and R – redroot pigweed

50 : 50 (redroot pigweed : wheat). At the ratio of 25 : 75, root fresh weight of redroot pigweed decreased by 20% compared to the control (Table 3).

Content of photosynthetic pigments

There were no significant changes in wheat chlorophyll (a, b and total) content by intercropping with redroot pigweed except at the density ratio of 75 : 25 (wheat : redroot pigweed) when chlorophyll showed a significant decrease (17%). Wheat carotenoid content showed an increase (29%) at the equal ratio of cultivation with redroot pigweed and a decrease (27%) at the highest ratio of redroot pigweed (25 : 75) compared to its absence (Fig. 1A).

A significant increase was observed in chlorophyll a and b, total chlorophyll and carotenoids of redroot pigweed at the ratio of 75 : 25 (redroot pigweed : wheat) (41, 60, 45, and 135%, respectively) as well as at the ratio of 50 : 50 (58, 118, 92, and 46%, respectively). A decrease was observed for chlorophyll a and total chlorophyll at the highest ratio of wheat, compared to the control (Fig. 1B).

Protein content

There were significant changes in protein content of the studied plants. The protein content of wheat shoots showed a significant increase (23%) at 50 : 50 and a significant decrease (64%) at the ratio of 25 : 75 (wheat : redroot pigweed). The protein content of wheat roots increased significantly with increased density of redroot pigweed. The highest (74% higher than that of the control) wheat root total protein was obtained from the ratio of 25 : 75. In the redroot pigweed plants, changes in protein levels at different densities varied

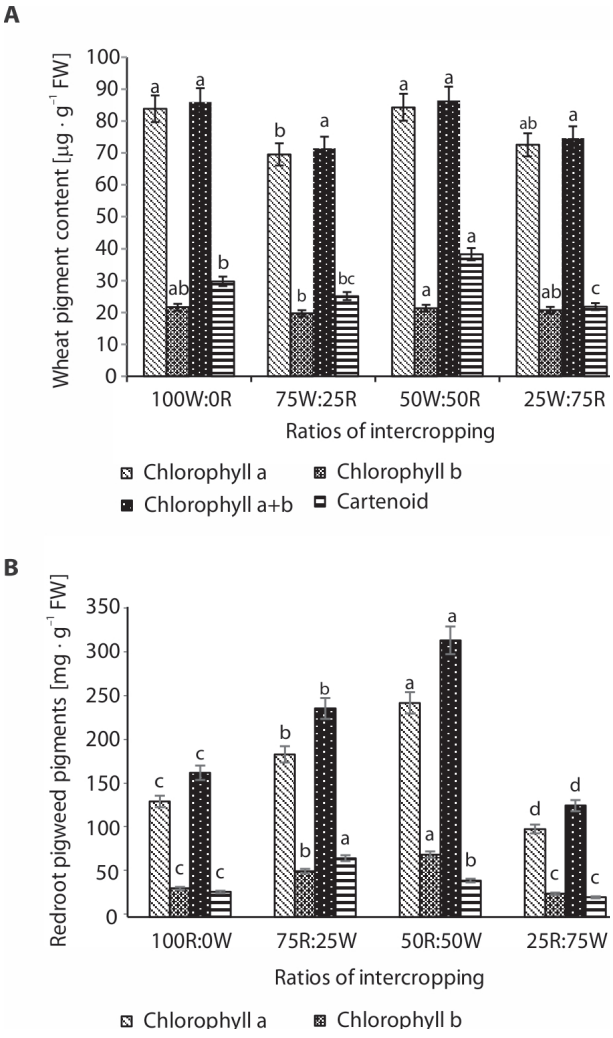

Fig. 1. The effect of different ratios of intercropping on photosynthetic pigment content of *Triticum auestivum* L. cv. Pishgam – A and *Amaranthus retroflexus* L. – B. The data represent the mean of three replications and error bars indicate SD. Different letters above the columns indicate significant differences ($p < 0.05$). W – wheat, R – redroot pigweed

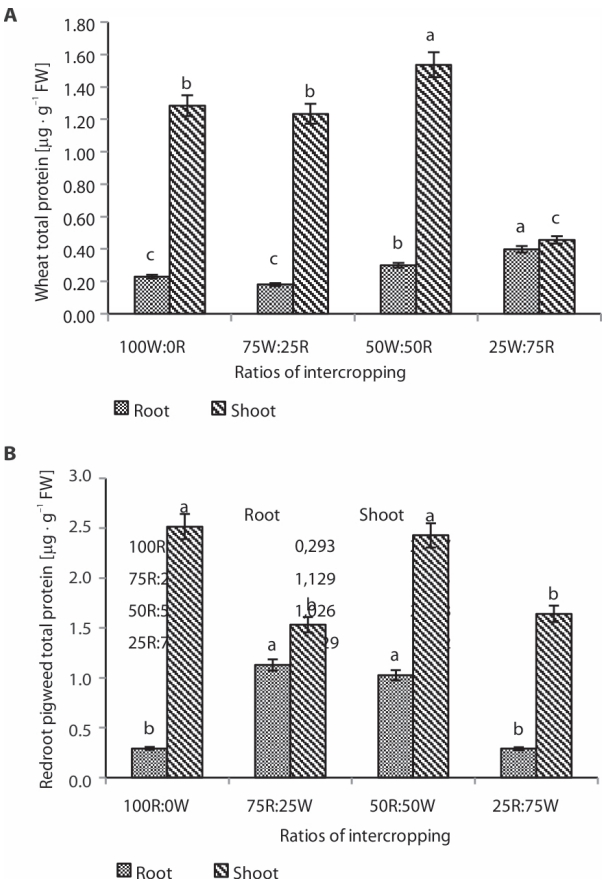

Fig. 2. The effect of different ratios of intercropping on total protein content of *Triticum auestivum* L. cv. Pishgam – A and *Amaranthus retroflexus* L. – B. The data represent the mean of three replications and error bars indicate SD. Different letters above the columns indicate significant differences ($p < 0.05$). W – wheat, R – redroot pigweed

in both roots and shoots. Redroot pigweed shoots showed 39 and 42% reduction in protein content at the ratios of 75 : 25 (redroot pigweed : wheat) and 25 : 75, respectively. On the other hand, the protein content of redroot pigweed roots increased at the ratios of 75 : 25 (285%) and 50 : 50 (248%), in comparison to the control (Fig. 2A, B).

Soluble and insoluble sugar content

Intercropping had a significant effect on the soluble sugar content of wheat and redroot pigweed. When the density of redroot pigweed was increased, soluble sugar content was significantly decreased in the roots (at the ratios of 25 : 75 and 50 : 50, wheat : redroot pigweed) and shoots of wheat compared to the control (Fig. 3A).

The soluble sugar content of redroot pigweed shoots significantly decreased at the ratios of 75 : 25 (redroot pigweed : wheat) and 50 : 50 compared to the control. In contrast, with increasing the density of wheat, the soluble sugar content of redroot pigweed root showed an increasing trend. The highest increase was observed at the ratio of 25 : 75 compared to the control (Fig. 3B).

Intercropping with redroot pigweed significantly increased the insoluble sugar content of wheat at the ratios of 75 : 25 (wheat : redroot pigweed) or 25 : 75 in roots, while it was lower in shoots than in the control at the same ratios. The shoot insoluble sugar content of wheat was significantly was greater at the ratio of 50 : 50, than in the control (Fig. 3C). The insoluble sugar content of redroot pigweed roots showed a significant increase at all intercropping ratios. In the redroot pigweed plants, the shoot insoluble sugar content showed a significant decrease (53%) only at the ratio of 50 : 50 (Fig. 3D).

Oxidative damage indices (MDA and H_2O_2)

The MDA and H_2O_2 content of wheat roots and shoots was affected by intercropping ratios with redroot pigweed. In wheat roots, the highest level of MDA was observed at 25 : 75 (wheat : redroot pigweed, 49%) and 50 : 50 (52%), compared to the control. In wheat shoots, MDA content increased at 75 : 25 (134%) and 50 : 50 (141%), compared to the control (Fig. 4A).

The MDA content of redroot pigweed roots and shoots was greater at all intercropping ratios than in the control. The highest level of MDA was observed in redroot pigweed roots (209%) at the ratio of 25 : 75 (redroot pigweed : wheat) (Fig. 4B).

The H_2O_2 content of wheat roots decreased at 75 : 25 (wheat : redroot pigweed 14%) and increased at 25 : 75 (41%). The H_2O_2 content of wheat shoots increased at 50 : 50 (81%) and decreased at 25 : 75 (34%) ($p < 0.05$) (Fig. 4C).

The H_2O_2 content of redroot pigweed roots increased at 75 : 25 (redroot pigweed : wheat 12%) and decreased at 50 : 50 (16 %) and 25 : 75 (16%), compared to the control, and the H_2O_2 content of the shoots increased at all intercropping ratios compared to the control (Fig. 4D).

Activity of antioxidant enzymes

The analysis of variance of the data showed that the activity of the enzymes, CAT, POD, APX and SOD in the roots and shoots of wheat and redroot pigweed was significantly influenced by intercropping.

CAT activity was significantly increased in wheat roots at 75 : 25 (wheat : redroot pigweed), compared to the control. In wheat shoots, CAT activity decreased at the ratios of 75 : 25 and 50 : 50, and increased at 25 : 75, compared to the control ($p < 0.05$) (Table 4).

There was a significant increase in the activity of catalase in redroot pigweed roots and shoots in all intercropping ratios compared to the control (Table 4).

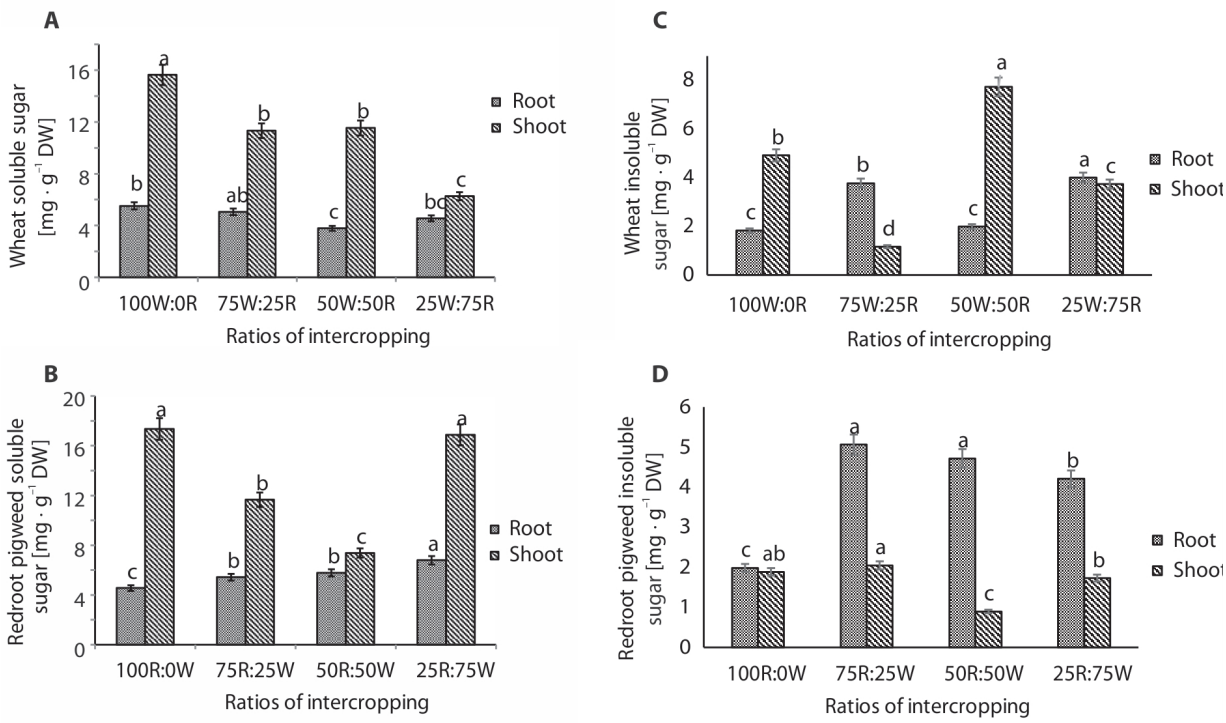

Fig. 3. The effect of different ratios of intercropping on soluble sugar content of *Triticum auestivum* L. cv. Pishgam – A, sugar content of *Amaranthus retroflexus* L. – B, insoluble sugar content of *Triticum auestivum* L. cv. Pishgam – C, insoluble sugar content of *Amaranthus retroflexus* L. – D. The data represent the mean of three replications and error bars indicate SD. Different letters above the columns indicate significant differences ($p < 0.05$). W – wheat, R – redroot pigweed

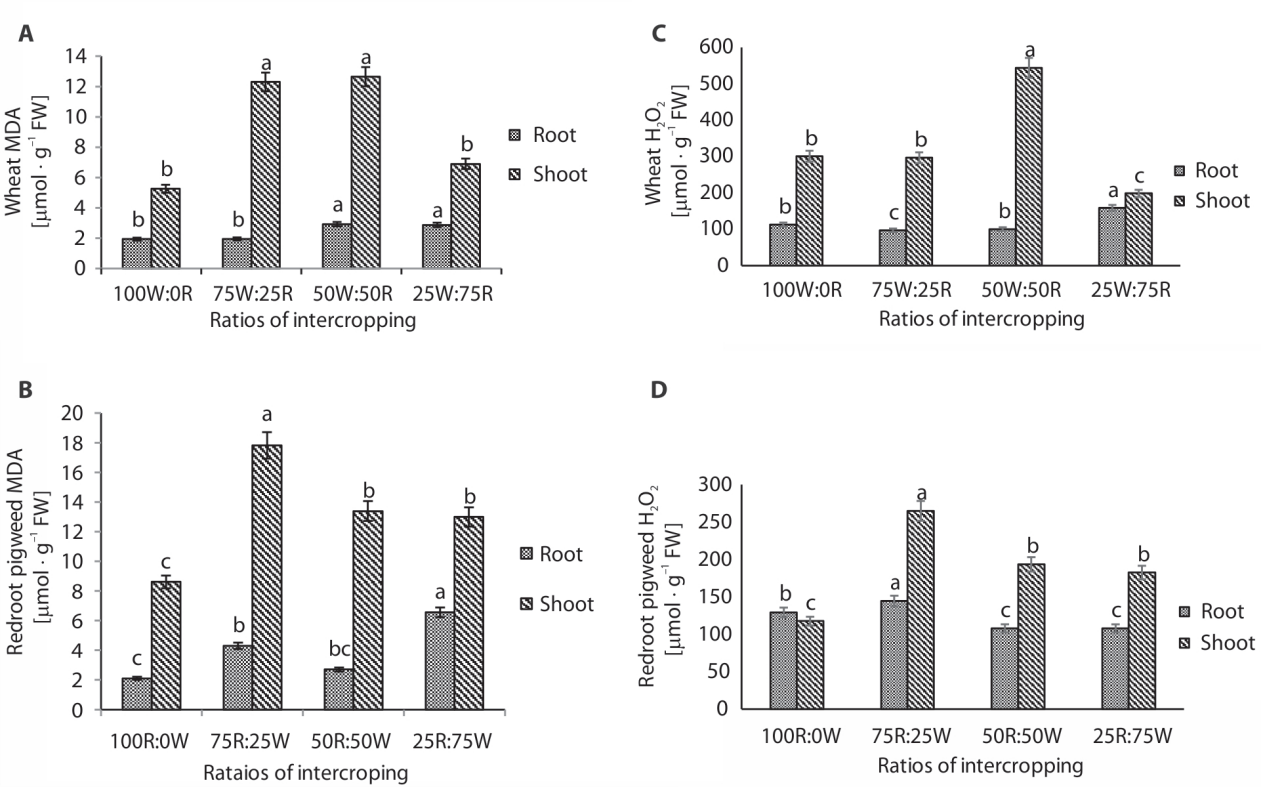

Fig. 4. The effect of different ratios of intercropping on MDA content of *Triticum auestivum* L. cv. Pishgam – A, MDA content of *Amaranthus retroflexus* L. – B, H_2O_2 content of *Triticum auestivum* L. cv. Pishgam – C, H_2O_2 content of *Amaranthus retroflexus* L. – D. The data represent the mean of three replications and error bars indicate SD. Different letters above the columns indicate significant differences ($p < 0.05$). W – wheat, R – redroot pigweed

The POD activity in wheat roots was significantly increased at all cultivation ratios compared to the control. In addition, in wheat shoots, the POD activity was higher than the control at 50 : 50 and 75 : 25 (wheat : redroot pigweed). There was no significant difference in shoot POD activity between these two intercropping ratios (Table 4). The POD activity in redroot pigweed roots at 25 : 75 (redroot pigweed : wheat) was significantly increased compared to the control. The POD activity in redroot pigweed shoots increased in all intercropping ratios compared to the control. The highest level of POD activity in redroot pigweed shoots was observed in the highest wheat density ($p < 0.05$) (Table 4).

When compared to the control, wheat roots exhibited higher APX activity at the ratio of 50 : 50. In wheat shoots, there was a significant decrease at 50 : 50 and a significant increase at 25 : 75 (wheat : redroot pigweed) compared to the control ($p < 0.05$) (Table 4).

The APX enzyme activity in redroot pigweed roots and shoots showed a significant increase at 75 : 25 (redroot pigweed : wheat) (Table 4).

According to Table 4, in wheat roots, SOD activity was greater at 75 : 25 (wheat : redroot pigweed) while it was smaller at 50 : 50 and 25 : 75 than in the control. The highest SOD activity in the shoots of wheat was related to the ratio of 25 : 75 compared to the control ($p < 0.05$). The activity of the SOD enzyme in redroot pigweed roots increased at all ratios compared to the control, In the shoot, this increase was observed at the ratios of 75 : 25 (redroot pigweed : wheat) and 25 : 75 (Table 4).

Correlation analysis

Correlation analysis between the MDA content and the POD enzyme activity of wheat showed a significant positive correlation coefficient in roots and shoots between the enzyme activity and the MDA content. A significant negative correlation coefficient was observed between the MDA content and the SOD enzyme activity of roots, as well as the shoot MDA content and the CAT and APX enzyme activities. The correlation analysis between the root H_2O_2 content and the root SOD activity as well as between H_2O_2 and CAT, APX, and SOD enzyme activities of wheat shoots showed a significant negative correlation coefficient (Table 5).

According to Table 6, there was a significant positive correlation between the MDA content and activity of SOD, POD, and CAT enzymes in the redroot pigweed roots as well as between the MDA content and the activity of APX and CAT in the shoots. There was a significant positive correlation coefficient between the H_2O_2 content and the APX enzyme activity of roots and shoots as well as between the H_2O_2 content and CAT enzyme activity and MDA content of redroot pigweed shoots.

Identification of root exudate compounds at different ratios of redroot pigweed

GC-MS analysis identified different groups of compounds such as terpenoids, phenolic compounds, fatty alcohol, steroids, fatty acids, alkanes at the ratios of 100 : 0, 75 : 25, 50 : 50 and 25 : 75 (redroot pigweed : wheat). This compound exudates from the plants' roots to the soil (Tables 7–10). Alkanes and fatty alcohols were detected in the control, and terpenes, esters, fatty acids, and other compounds were detected in the presence of wheat. Exudates were different in the nature and structure of the compounds, time of secretion from the root, and time of manifestation in the GC-MS. Wheat caused the release of terpenes and their esters from the roots of the redroot pigweed, as the main allelochemicals.

Table 4. The effect of the concentrations of different ratios of intercropping on antioxidant enzymes activity (U · mg $^{-1}$ protein) in the shoots and roots of wheat and redroot pigweed

Ratios of intercropping	Wheat Root				Wheat Shoot			
	CAT	POD	APX	SOD	CAT	POD	APX	SOD
100 W : 0 R	0.06 b ± 0.01	5.34 b ± 0.46	3.2 b ± 1.23	313.87 b ± 20.26	0.074 b ± 0.01	0.79 b ± 0.98	3.81 b ± 0.77	54.41 bc ± 9.01
75 W :25 R	0.2 a ± 0.07	9.37 a ± 2.68	5.8 b ± 2.19	416.42 a ± 47.35	0.04 c ± 0.00	7.41 a ± 1.96	2.16 bc ± 0.48	58.21 b ± 3.23
50 W : 50 R	0.06 b ± 0.01	11.00 a ± 1.56	15.15 a ± 2.48	233.87c ± 43.41	0.03 c ± 0.00	6.03 a ± 0.48	1.53 c ± 0.04	45.34 c ± 4.64
25 W : 75 R	0.02 b ± 0.00	11.11 a ± 0.468	4.86 b ± 1.04	183.55c ± 22.23	0.16 a ± 0.02	1.34 b ± 0.36	5.44 a ± 0.45	159.20 a ± 3.94
	Redroot pigweed root				Redroot pigweed shoot			
100 R : 0 W	0.015 c ± 0.00	1.03 b ± 0.14	2.00 b ± 0.81	43.32 c ± 4.08	0.01 b ± 0.00	0.13 c ± 0.01	0.92 b ± 0.46	28.50 b ± 2.30
75 R : 25 W	0.03 ab ± 0.00	0.20 b ± 0.02	4.14 a ± 0.99	64.70 b ± 8.35	0.02 a ± 0.00	0.80 b ± 0.14	5.52 a ± 1.31	39.40 a ± 0.78
50 R : 50 W	0.023 b ± 0.00	0.86 b ± 0.08	1.70 b ± 0.22	70.58 b ± 3.50	0.02 a ± 0.00	0.52 bc ± 0.06	0.87 b ± 0.03	26.50 b ± 0.39
25 R : 75 W	0.034 a ± 0.00	8.32 a ± 1.89	1.45 b ± 0.39	233.55 a ± 19.45	0.02 a ± 0.00	1.62 a ± 0.52	0.78 b ± 0.12	42.00 a ± 3.56

Different letters above the bars indicate significant differences (p <0.05), W – wheat and R – redroot pigweed, SOD – superoxide dismutase, APX – ascorbate peroxidase, POD – peroxidase, CAT – catalase

Table 5. Statistical analysis for correlation between the activity of antioxidant enzyme and malonedialdehyde (MDA) content in the shoot and root of wheat plants

	Shoot CAT	Root CAT	Shoot POD	Root POD	Shoot APX	Root APX	Shoot SOD	Root SOD	Shoot H2O2	Root H2O2	Shoot MDA	Root MDA
Root MDA	0.241 ns	–0.497 ns	–0.032 ns	0.724**	0.199 ns	0.45 ns	0.358 ns	–0.8**	0.328 ns	0.377 ns	0.224 ns	1
Shoot MDA	–0.633**	0.570 ns	0.914**	0.445 ns	–0.598*	0.59*	–0.421 ns	0.253 ns	0.552 ns	–0.55 ns	1	
Root H2O2	0.928**	–0.564 ns	–0.632*	0.244 ns	0.880**	–0.347 ns	0.936**	–0.669*	–0.60*	1		
Shoot H2O2	–0.661*	–0.024 ns	0.482 ns	0.276 ns	–0.745**	0.780**	–0.654*	–0.114 ns	1			
Root SOD	–0.594*	0.771**	0.426 ns	–0.481 ns	–0.474 ns	–0.242 ns	–0.532 ns	1				
Shoot SOD	0.940**	–0.407 ns	–0.483 ns	0.326 ns	0.817**	–0.340 ns	1					
Root APX	–0.444 ns	–0.08 ns	0.497 ns	0.405 ns	–0.588*	1						
Shoot APX	0.858**	–0.427 ns	–0.665*	0.004 ns	1							
Root POD	0.189 ns	–0.112 ns	0.351 ns	1								
Shoot POD	–0.683*	0.766**	1									
Root CAT	–0.541 ns	1										
Shoot CAT	1											

Notes: *correlation is significant at 0.05 levels, **correlation is significant at 0.01 levels, ns – correlation is not significant

Table 6. Statistical analysis for correlation between the activity of antioxidant enzyme and malonedialdehyde (MDA) content in the shoot and root of redroot pigweed plants

	Shoot CAT	Root CAT	Shoot POD	Root POD	Shoot APX	Root APX	Shoot SOD	Root SOD	Shoot H_2O_2	Root H_2O_2	Shoot MDA	Root MDA
Root MDA	0.372 ns	0.775**	0.846**	0.775**	0.170 ns	0.028 ns	0.834**	0.828**	0.413 ns	–0.182 ns	0.376 ns	1
Shoot MDA	0.64*	0.733**	0.366 ns	–0.119 ns	0.746**	0.693*	0.507 ns	0.06 ns	0.913**	0.315 ns	1	
Root H_2O_2	–0.13 ns	–0.210 ns	–0.378 ns	–0.489 ns	0.745**	0.7**	0.203 ns	–0.512 ns	0.168 ns	1		
Shoot H_2O_2	0.691*	0.739**	0.351 ns	–0.101 ns	0.653*	0.609*	0.421 ns	0.073 ns	1			
Root SOD	0.638*	0.073 ns	0.868**	0.968**	–0.310 ns	–0.380 ns	0.665**	1				
Shoot SOD	0.251 ns	0.688*	0.684*	0.608*	0.420 ns	0.307 ns	1					
Root APX	0.121 ns	0.168 ns	–0.105 ns	–0.426 ns	0.861**	1						
Shoot APX	0.284 ns	0.330 ns	0.039 ns	–0.411 ns	1							
Root POD	0.069 ns	0.488 ns	0.740**	1								
Shoot POD	0.4 ns	0.841**	1									
Root CAT	0.614**	1										
Shoot CAT	1											

Notes: **correlation is significant at 0.01 levels, *correlation is significant at 0.05 levels, ns – correlation is not significant

Table 7. Root exudate identified at the ratio of 100 : 0 (redroot pigweed : wheat)

Compound	Retention time	Retention index
Heptatriacontane, 17,21-dimethyl-	12.21	805.91
Cyclotriacontane	14.45	971.53
Dotriacontane, 1,32-dibromo-	15.04	1015.15
1-Eicosanol, 2-hexadecyl-	16.04	1089.09
2-Tetradecanol	20.25	1400.36
5-Methyl-Z-5-docosene	24.36	1704.25
9-Tricosene, (Z)-	25.75	1807.02
Triacontane	26.16	1837.33
11-Methylnonacosane	28.55	2014.04
Octacosanol	31.38	2223.29
Pentacosane	31.57	2237.33
1-Eicosene	32.6	2313.49
geyrine diacetate	34.02	2418.48

Table 8. Root exudate identified at the ratio of 75 : 25 (redroot pigweed : wheat)

Compound	Retention time	Retention index
Dodecyl ether	8.33	519.03
Linalool	9.77	625.50
Borneol	10.87	706.83
Terpinen-4-ol	11.13	726.06
Dotriacontane	12.66	839.18
Linalyl acetate	12.73	844.36
Butyric acid, linalyl ester	12.81	850.27
Geraniol	13.68	914.60
Triacontane	24.72	1730.86
Eicosene; Cetyl ethylene	25.61	1796.67
1-Eicosanol, 2-hexadecyl-	28.16	1985.21
4,8,12-Trimethyldotriacontane	32.48	2304.62
Eicosane	33	2343.06
11-Methylnonacosane	34.79	2475.41
Hexanedioic acid, dioctyl ester	35.2	2505.73
1-Bromo-11-iodoundecane	36.57	2607.02
3-Methylheneicosane	36.68	2615.15
Pentacosane	38.79	2771.16
2-Ethylacridine	39.52	2825.13
Thymol, TMS derivative	41.18	2947.87

Table 9. Root exudate identified at the ratio of 50 : 50 (redroot pigweed : wheat)

Compound	Retention time	Retention index
Linalool	8.54	534.56
Brneol	10	642.51
Terpinen-4-ol	10.22	658.78
Linalyl Acetate	12.06	794.82
Geranyl propanoate	13.07	869.50
Octadecane, 1-iodo-	38.04	2715.71
Heneicosane, 3-methyl-	38.41	2743.06
2,4-Dimethoxy-3-isopropylbenzoic chloride	41.68	2984.84
Bis(trimethylsiloxy)methylsilane	44.54	3196.30

Table 10. Root exudate identified at the ratio of 25 : 75 (redroot pigweed : wheat)

Compound	Retention time	Retention index
Oxalic acid	5.69	323.84
Linalool;	8	494.63
Linalyl acetate	11.62	762.29
Linalyl propionate	11.67	765.98
β-Myrcene	12.67	839.92
Heneicosane, 3-methyl-	37.37	2666.17
11-Methylnonacosane	37.57	2680.96
Cyclobarbital	40.7	2912.38
Thymol, TMS derivative	40.26	2879.85
2-Ethylacridine	41.01	2935.30
Megestrol Acetate	42.42	3039.55

Discussion

Plant-plant interactions through chemical compounds are important allelopathic mechanisms that are created by some plant species by producing and releasing organic compounds by washing the root secretions of volatile substances and decomposing the created materials. From one plant to a recipient plant allelopathy has beneficial or harmful effects. Plant root exudates are a dynamic source of allelochemicals that, in sufficient concentrations, affect plant growth and development (Akter and Islam 2019; Ding *et al.* 2019). As mentioned in the results, with the presence of redroot pigweed at the ratio of 25 : 75 (wheat : redroot pigweed), the fresh and dry weight of roots and shoots and shoot length of wheat decreased significantly compared to the control. An increase in the shoot length and fresh and dry weight of shoots and roots of redroot pigweed were observed at different intercropping ratios. At the ratio of 25 : 75 (redroot pigweed : wheat), the fresh weight of redroot pigweed roots showed a 20% decrease compared to the control. Similar results showed the allelopathic effect of weeds on germination, root and shoot length, and fresh and dry weight of wheat roots and

shoots (Pouresmaeil and Motafakkerazad 2018; Akter and Islam 2019; Shinde and Salve 2019).

Allelopathic substances in plants reduce the dry matter of crops such as wheat, maize, and soybean (Tian *et al.* 2022). Recently, Gfeller *et al.* (2018) revealed that buckwheat (*Fagopyrum esculentum*) prevents growth of redroot pigweed (*Amaranthus retroflexus*), goosefoot (*Chenopodium album* L) and barnyard grass (*Echinochloa crus-galli* L) through allelopathic compounds. Variation in the expression of allelopathy depends on the different nature of allelochemical compounds and their concentrations, light, temperature, water deficiency, soil specifications, time, space, and the target plant (Scavo *et al.* 2018; Uesugi *et al.* 2019). It can be said that allelochemicals from root exudates passed into the soil, and thereby, caused reduced growth by reducing the absorption of water and nutrients into the root of the target plant and reducing photosynthesis (Gfeller *et al.* 2018). Carotenoids have a structural role and protect the photosynthetic system, contribute to the stability of the light-collecting proteins and also protect against free radicals (Demmig-Adams *et al.* 2020). In this study, the level of carotenoids in wheat at 50 : 50 and in redroot pigweed at 75 : 25 (redroot pigweed : wheat) and 50 : 50 was increased compared to the control, which could preserve the chlorophyll pigment. Chlorophylls and carotenoids are the main pigments in plant photosynthesis, and their functionality and content are essential for absorbing and directing light to photosystems (Prasad *et al.* 2004). In the present study, wheat chlorophyll did not show a significant change in different intercropping ratios with redroot pigweed and had a significant decrease only at 75 : 25 (wheat : redroot pigweed). However, chlorophyll a and b and total chlorophyll content of redroot pigweed showed a significant increase at 75 : 25 (redroot pigweed : wheat) and 50 : 50. Photosynthetic pigments have different changes in the presence of allelochemical compounds. In the study done by Siyar *et al.* (2019), chlorophyll content in different varieties of wheat showed different responses to weeds. Wheat Pirsabaq and Serin cultivars increased chlorophyll and the Atahabib cultivar decreased chlorophyll content compared to the extract of three weeds (*Avena fatua* L., *Melilotus officinalis* L., and *Polypogon hissaricus)*. Furthermore, different concentrations of an aqueous extract of *Datura metal* L. leaves had stimulatory and inhibitory effects on the chlorophyll content of *Parthenium hysterophorus* L. (Ramachandran and Venkataraman 2016). The difference in the allelopathic effect on the chlorophyll content can be due to stimulatory or inhibitory effects and reduced nutrient uptake. Chlorophyll depletion is probably related to damage to the photosynthetic system (Ibrahim *et al.* 2013; Siyar *et al.* 2019).

The sensitivity of plant protein to different concentrations of allelochemicals varies. The protein level of wheat shoots showed a 23% increase at 50 : 50, and a 64% decrease at 25 : 75 (wheat : redroot pigweed) compared to the control. The redroot pigweed shoots in the presence of wheat had a 39 and 42% reduction in protein content at the ratios of 75 : 25 (redroot pigweed : wheat) and 25 : 75, respectively, compared to the control. The allelopathic effects of weed extract on mung bean and rice protein content revealed that, with increasing the concentration of an aqueous extract of weed *Neanotis lancifolia* (Hook. f.), the protein levels of mung bean and rice decreased (Torawane and Mokat 2021). The protein content of *Parthenium hysterophorus* L. decreased together with the concentration of the aqueous extract of *Datura metel* L. leaves (Ramachandran and Venkataraman 2016). It was shown that phenolic allelochemicals were able to decrease the protein content through the inhibition of protein biosynthesis, or proteolysis (Singh *et al.* 2008), or oxidative adjustment (Fazeli *et al.* 2007). The reduction in protein content can be due to inhibition of the synthesis process, disruption in the translation pathway or activation of other protective mechanisms against stress caused by allelochemical compounds (Knox 2010; Jali *et al.* 2021). In the intercropping of wheat and redroot pigweed, an increase in the protein content was observed at different intercropping ratios in wheat and redroot pigweed roots. Changes in the expression, accumulation, and synthesis of proteins in response to environmental stress factors are important mechanisms of plants to protect cellular metabolism and to adapt (Horn *et al.* 2007). Increasing the protein content of roots is probably due to increased synthesis because of the existence of phenolic compounds, decreased decomposition or stability of root proteins as osmolytes to maintain cellular metabolism under stress caused by allelochemical compounds (Singh and Thapar 2003; Fangue-Yapseu *et al.* 2021).

Carbohydrate metabolism is affected by stress stimuli or environmental factors. The mechanism in the plant life cycle suggests that the allocation of carbon resources between different organs reflects the source-sink transition (Roitsch 1999). In this study, there was a significant change in the carbohydrate levels of roots and shoots of plants in intercropping. The soluble sugar content of wheat and redroot pigweed roots and wheat shoots showed a significant decrease, but the soluble sugar content of the redroot pigweed shoots showed a significant increase at different intercropping ratios. A decrease in the soluble sugar content of the root tissues may be due to the poor activity of the sink caused by the reduced availability of the substrate. It can be said that different stress factors with phloem dysfunction in transport lead to a decrease in the supply of soluble sugar from source to sink (Morsy *et al.* 2007). Stress factors accelerate the imbalance of sugar distribution and transport in different parts (Arbona *et al.*

2005). Increased soluble sugar may express its role as an osmotic protector and stabilization of cell membranes in response to stress caused by allelochemical compounds. Our results are consistent with similar findings in which growth was influenced by the treatment with allelochemicals and metabolic stress was imposed through carbohydrate metabolism (Bernat *et al.* 2004; Algandaby and Salama 2018; Ding *et al.* 2019). Insoluble sugar is a significant form of carbohydrate storage. In this study, insoluble sugar accumulated in wheat roots more than in its shoots. Decreased insoluble sugar in shoots may be associated with increased soluble sugar in the shoots.

Oxidative stress is one of the states indicating the activity of allelochemicals. These compounds induce biotic stress known as allelochemical stress (Cruz-Ortega *et al.* 2007). Like other biological agents, allelochemicals target specific physiological processes so that they disrupt membrane permeability, iron uptake, inhibition of the electron transfer chain, photosynthesis and respiration, cell division, altered enzyme activity, and altered balance between antioxidant defenses and reactive oxygen species (ROS) levels (Gniazdowska and Bogatek 2005). Oxidative stress caused by allelopathy is due to an imbalance of the metabolic system in overproduction and accumulation of ROS, peroxidation of membrane lipids, and damage to cell membrane structure. Allelopathic compounds induce oxidative stress through the production of ROS in plants, shown by the accumulation of MDA (Zhang *et al.* 2011; Staszek *et al.* 2021). Increased MDA content in roots and shoots of wheat and redroot pigweed indicates the allelopathic effects of these plants on each other. Based on the results, with increasing the density of redroot pigweed, the MDA content of roots and shoots of wheat increased. On the other hand, the MDA content of roots and shoots of redroot pigweed in the presence of wheat and with increased density showed a significant increase. Previous reports have shown that MDA and/or H_2O_2 levels increased in response to allelochemical stress such as oxidative stress in lettuce (Ladhari *et al.* 2020) and cucumber when intercropped with garlic (Ding *et al.* 2019). Elevated levels of MDA and H_2O_2 suggest that the secretion of allelopathic compounds from the root induces oxidative stress, cell membrane disruption, lipid peroxidation, loss of cell integrity, and ROS formation (Batish *et al.* 2006; Singh *et al.* 2006; Zhang *et al.* 2012;).

ROS is highly reactive and toxic in plants and thus causes potential damage to proteins, lipids, carbohydrates, and DNA under oxidative stress (Desikan *et al.* 2005). To protect against oxidative damage, plants have an enzymatic antioxidant defense system with very high efficiency for scavenging ROS (Gill and Tuteja 2010; Talukdar 2013). Catalase is an important enzyme of the defense system in ROS detoxification and can be induced in various plant species. This enzyme prevents the accumulation of H_2O_2 in cells and makes plants resistant to oxidative stress (Li and Jin 2010; Liang *et al.* 2003). The APX enzyme is most prone to H_2O_2 because even at low concentrations, it scavenges the produced H_2O_2 that is not removed during catalase activity (Tuzet *et al.* 2019). POD plays a critical role in the production and decomposition of hydrogen peroxide, which shows its antioxidant ability as an important factor in the defense system of plants against various stress factors (Cipollini 1998). SOD converts superoxide anion to hydrogen peroxide, which is then converted to the harmless H_2O molecule in the reaction catalyzed by CAT (Foyer and Noctor 2005).

In this study, intercropping had a stimulating effect on the antioxidant system of wheat and redroot pigweed. The results showed that the activity of enzymes of wheat and redroot pigweed (CAT, POD, APX, SOD) increased at different intercropping ratios. Allelochemicals in low concentrations affect receptor plants and stimulate them to improve the ability of their antioxidant enzyme activity to resist oxidative stress (Ming *et al.* 2020). Previous research has confirmed the effect of allelopathic compounds on the enzymatic activity of CAT, SOD, APX, and POD in various plants, including *Vigna unguiculata* L., tomatoes, rice seedlings, *Cucumis sativus* L., and cucumber (Zuo *et al.* 2012; Ahmad *et al.* 2013; Siddique and Ismail 2013; Oyeniyi *et al.* 2016; Ribeiro *et al.* 2017).

There was a positive correlation between H_2O_2 and APX in roots ($r^2 = 0.7$) and shoots ($r^2 = 0.653$) as well as between H_2O_2 and MDA in shoots ($r^2 = 0.913$) of redroot pigweed, which indicates an increase in H_2O_2 due to lipid peroxidation. Furthermore, there was a positive correlation between MDA content and SOD, CAT, and POD activities ($r^2 = 0.775$, $r^2 = 0.775$, and $r^2 = 0.828$) in the root as well as between MDA content and APX and CAT activities ($r^2 = 0.640$ and $r^2 = 0.746$) in the shoot of redroot pigweed, showing increased activity of CAT, POD, and APX enzymes against oxidative stress. There was a significant negative correlation between the H_2O_2 content and SOD ($r^2 = -0.669$) in wheat roots as well as between the H_2O_2 content and activities of CAT ($r^2 = -0.661$), APX ($r^2 = -0.745$), and SOD ($r^2 = -0.654$) in the shoots. Increasing the activities of CAT, APX, and SOD decreased the H_2O_2 content. Moreover, there was a significant negative correlation between MDA and SOD in wheat roots ($r^2 = -0.8$) as well as between MDA and CAT ($r^2 = -0.663$) and APX ($r^2 = -0.598$) in the shoots. Increasing the MDA content induces oxidative stress (Salehi-Lisar and Deljoo 2015), the role of enzymes in ROS detoxification, and increases plant resistance. The results of this study are consistent with the findings of the study done by Ding *et al.* (2019). Plants increase the activity of their antioxidant enzyme defense

system to reduce the damage caused by ROS when exposed to stress.

Based on GC-MS analysis results, the allelopathic compounds were characterized and identified from the soil samples. Some of these compounds were characterized in previous studies (Bakhshayeshan-Agdam, *et al.* 2019, 2021). It was reported that some of these compounds such as Thymol, Triacontane, 1-Bromo-11iodoundecane, Silane, [[4-[1,2 bis[(trimethylsilyl) o xy]ethyl]-1,Eicosane (Bakhshayeshan-Agdam *et al.* 2019), Linalool, Myrcene, Borneol, Camphor, 4-Terpineol (Zheljazkov *et al.* 2021) have allelopathic activity in wheat, barley, and cucumber. This study showed the effect of allelochemical compounds on plant growth and physiology. Since researchers use different methods to isolate and identify plant compounds, various allelochemical compounds have been reported.

Conclusions

The present findings revealed that in the intercropping of wheat and redroot pigweed, a high density of redroot pigweed reduced the growth and increased the activity of the enzymatic antioxidant system of wheat. However, if wheat is at a higher density than redroot pigweed, the function of the enzymatic antioxidant system will improve wheat growth. Redroot pigweed was also reciprocally affected by intercropping, and its growth was limited in high wheat density. Allelochemical compounds induced oxidative stress, which was demonstrated by the accumulation of MDA and H_2O_2. The increased activity of enzymes (CAT, POD, APX, and SOD) in wheat roots and shoots in the presence of redroot pigweed showed that the toxicity of redroot pigweed allelochemicals activated the antioxidant system. However, the activity of enzymes in wheat roots by increasing the density of redroot pigweed probably decreased because of damage to the enzymatic antioxidant system. High density of wheat in the intercropping with redroot pigweed increased the activity of antioxidant enzymes in the roots and shoots of redroot pigweed. The high accumulation of total protein and insoluble sugar in the roots of both plants showed that the roots were more impacted by oxidative stress because of direct contact with allelochemical compounds. Most of the compounds detected in soil extract are known as allelochemical, however further studies are needed to investigate the role of other compounds. It can be stated that in intercropping, increasing the density of wheat reduces the effect of toxins released into the environment. Therefore, wheat can be grown in areas where redroot pigweed is present. Utilizing this (cultivation) advantage is important for maintaining biodiversity and sustainable agricultural development. It is suggested that future research should focus on the effect of intercropping with various weeds on growth and physiological responses of wheat. The results can be used to improve agriculture and reduce the use of herbicides in wheat cultivation. To confirm these results, more research is needed under field conditions.

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