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ALLELOPATHIC IMPACT OF TWO WEEDS, *CHENOPODIUM MURALE* AND *MALVA PARVIFLORA* ON GROWTH AND PHOTOSYNTHESIS OF BARLEY (*HORDEUM VULGARE* L.)

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Abstract

The allelopathic impact of aqueous extract of 2 weeds *Malva parviflora* and *Chenopodium murale* on growth and photosynthesis of barley was investigated. The barley plants were sown in plastic pots containing a compost:sand mixture and placed in growth chambers. Plants were treated with the following weed aqueous extract concentrations; 0%, 25%, 50%, 75% and 100%. Different responses of plants to the two weeds were observed. Plant heights, number of leaves, number of tillers, root dry weight were not affected by *M. parviflora*. A clear effect of *C. murale* was recorded on the growth parameters; plant height, number of leaves and number of tillers, root fresh and dry weight. *M. parviflora* affected leaf growth, measured as leaf fresh weight (LFW), leaf dry weight (LDW) and leaf area. All the growth parameters measured were more adversely affected by C. *murale* than by *M. parviflora*. Significant effect at 25% and 50% and highly significant effect at 75% and 100% were recorded. The only concentration of *M. parviflora* that affected chlorophyll content was 100%, while the effect of *C. murale* was significant at 50% and highly significant at 75% and 100%. Photosynthesis was also inhibited by the two weeds, with more effect being with *C. murale* compared with *M. parviflora*

Introduction

One of the main problems that agricultural production faces is weeds that interfere with crop growth and production. These weeds compete with plant species for water, light, nutrients and space. The weeds produce chemical compounds called allelochemicals. Barley (*Hordeum vulgare* L.) has been considered to be among the competitive crops against weeds (Dhima & Eleftherohorious, 2005; Dhima *et al*., 2010). Rice (1984) defined allelopathy as the beneficial or inhibitory effects of one plant on another, by releasing allelochemicals. Weeds can adapt to a wide range of environments and compete with barley growth, resulting in its reduced growth and productivity (Burleigh *et al*., 1988). Among weeds that affect barley growth, the nettle leaf goose foot, *Chenopodium murale* and the common mallow (*Malva parviflora*). *C. murale* is an annual erect plant, with high dispersal rates, due to high number of seeds produced by plants that can reach up to 24,000 seeds/plant (Holm *et al*., 1997; Guertin, 2003). *C. murale* affects vegetation through its adaptability to various environments and by growing in a wide range of soil types (Holm *et al*., 1997; Guertin, 2003). Common mallow (*Malva parviflora*) weed has deterrent effects on a number of plant species, including barley crop. It has been reported that *Malva* weed species affect many plant species by reducing the germination rates and seedling growth (Qasem, 1992; Zahedi & Ansari, 2011).

A number of studies have reported that morphological and physiological traits were used to investigate the allelopathic effects on barley crop (Didon, 2002; Dhima *et al*., 2010). It has been found that seed germination of barely was inhibited by the effects of weed extracts (Kadioglu *et al*., 2005). For example, Qasem (1993) found that *C. murale* reduced the germination rates and seedling growth of wheat and barley, and the results showed that barley was more sensitive to allelochemicals than wheat, and root growth was affected more than shoot growth. In another study, Qasem (1992) reported that the germination rates and seedling growth of barley were inhibited via the effects of the aqueous extract of roots, leaves and shoots (Qasem, 1993). In a recent study, Shahrokhi *et al*., (2011) found that the weed *Amaranthus retroflexus* L. had a high allelopathic impacts on germination and growth of barley crop cultivars. Early stage growth parameters of barley have been found to be important characters that determine crop-weed competitiveness (Jönsson *et al*., 1994). To investigate the allelopathic effects of weeds on germination and growth of seedlings of plant species, some recent studies have used the rain leachates from roots and shoots and root exudates (Hussain & Ilahi, 2011; Iram *et al*., 2011).

The objectives of the present study were to investigate the allelopathic effects of aqueous extract of two weed species, *Malva parviflora* and *Chenopodium murale* on growth, chlorophyll content and photosynthetic capacity of barley.

Materials and Methods

Plant materials and aqueous extracts: Two weeds, *Malva parviflora* and *Chenopodium murale*, were used in this study. Shoots of the weeds were collected from a local field farm at Al-Khlil area in Al-Madinah Al-Munawarah, KSA, just prior to ripening. The shoots were oven dried at 45°C for 72 hours, ground using a household blender and sieved through 1mm sieve. The aqueous extracts were prepared by diluting 200g of the residue powder in 2000ml distilled water in 4 litre capacity glass jars (Wu *et al*., 2007). The mixtures were filtered through 4 layers cheesecloth. The filtrate was centrifuged at 4000 rpm for 30 min at 10°C. The supernatant was considered as full strength (100%) solution. The following extract concentrations were prepared: 25%, 50%, 75% and 100%. Barley plants were grown in growth chambers (JS Research Inc., Korea, Model JSGC-960C, 972 L capacity), with temperature maintained at 25/20 ± 2°C day/night, respectively and light intensity of 400 µmol photons m^{-2} s^{-1}. A complete randomized block design was applied for plant treatments, with three replicates for each treatment. After 4 weeks of treatments, plants were harvested to measure growth

parameters and physiological activities. The following parameters were measured: (1) plant height, (2) number of leaves, (3) number of tillers, (4) fresh and dry weight of shoots and roots, (5) leaf area, (6) chlorophyll content, and (7) photosynthetic rates. The different plant parts, leaves, stems and roots, were oven-dried at 75^{o}C for 72 h to estimate dry weight.

Leaf area: A portable leaf area meter LI-3000C (LICOR Inc., Lincoln, NE, USA) was used to measure leaf area. Harvested leaves were oven-dried at 75 ^{o}C for 48 h to estimate leaf dry weight.

Photosynthesis rates: Intact young leaves were used to measure the photosynthetic rates, using a LICOR Infra Red Gas Analyser IRGA, LI-6400 XT (LICOR Inc., Lincoln, NE, USA). The fourth fully expanded leaves were used to measure photosynthetic rates. The leaf cuvette temperature was maintained at 25^{o}C ± 2^{o}C. The light response curves were carried out at ambient CO_2 concentrations (300-350 µmol). Photosynthesis measurements were estimated on six-week old barley plants. The red-blue LED light source attached to the sensor head was applied to the leaves. Leaves were illuminated with the following light intensities (µmol quanta m^{-2} s^{-1}); 0, 50, 100, 500, 750, 1000 and 1500 µmol m^{-2} s^{-1}.

Chlorophyll content determination: Using a hand-held chlorophyll content meter (CCM-200, Opti-Sciences, USA), leaf chlorophyll content was determined. The chlorophyll was measured three times for each leaf at different spots.

Statistical analysis: Analysis of variance (ANOVA) was used to determine if means of data are significantly different, using the SPSS statistical program version 14.0 (SPSS Inc., Chicago, Illinois).

Results

Plant height and number of leaves: The results showed that barley plant growth was affected differently by the weed types and aqueous extract concentrations (Fig. 1A). *M. parviflora* aqueous extract did not affect the number of leaves, while the *C. murale* weed extract resulted in a significant inhibition ($p<0.01$) at all concentrations, except the 25% concentration that caused no effect (Fig. 1B). The number of tillers did not respond to *M. parviflora* extract, while *C. murale* extract caused an inhibition to number of tillers. It was also found that the number of tillers decreased as the aqueous extract concentrations increased. The decrease in number of tillers was significant ($p<0.05$) at 50% and highly significant ($p<0.01$) at both highest concentrations, 75% and 100% (Fig. 1C).

Root growth: Root fresh and dry weights were not affected in plants treated with M. *parviflora* extract (Fig. 2A). But the root growth was severely reduced by the application of *C. murale* extract. The inhibition in root fresh weight was highly significant ($p<0.001$) at all ranges of the extract concentrations. The inhibition in root dry weight was also significant ($p<0.05$) at 25%, 50% and 75% and highly significant ($p<0.001$) at 100% concentration (Fig. 2A and 2B).

Leaf growth: Leaf growth was the most affected trait by aqueous extract of the 2 weeds, estimated as fresh and dry weight (Fig. 3A and 3B) and leaf area (Fig. 4A). No difference was detected between *M. parviflora* and *C. murale* extracts on leaf fresh and dry weight. The lowest concentration, 25%, did not cause any effect on leaf fresh weight. A significant reduction ($p<0.05$) was found at 50% and 75% concentrations and high significant inhibition ($p<0.01$) was measured at 100% concentration in plants treated with the *C. murale* extract (Fig. 3A and 3B).

Leaf area and chlorophyll content: Similar effect on leaf area was recorded under the treatments of plants by the two weed extracts. A significant ($p<0.05$) inhibition was found at 25% and 50% and highly significant effect ($p<0.01$) at the 2 highest levels of extract, 75% and 100% (Fig. 4A). The chlorophyll content in plant leaves treated with *M. parviflora* aqueous extract was not affected, except at the highest level of extract, 100%, which resulted in a significant inhibition ($p<0.05$) (Fig. 4B). A promising effect on chlorophyll content was recorded in plants treated with *C. murale* extract. The lowest concentration (25%) did not affect the chlorophyll content, while the 50% caused a significant reduction ($p<0.05$) and the 2 highest concentrations, 75% and 100%, caused a highly significant reduction ($p<0.001$) as shown in Fig. 4B.

Total plant fresh and dry weight: Total plant fresh weight was negatively affected by the aqueous extract of the 2 weed species. A significant effect of *M. parviflora* ($p<0.05$) under 25% concentration and highly significant effect ($p<0.01$) at the other concentrations, 50%, 75% and 100%, were recorded (Fig. 5A). The *C. murale* extract at all concentrations resulted in a significant inhibition ($p<0.05$) in total fresh weight (Fig. 5A). A similar effect of the 2 weed species extract on total dry weight was also recorded (Fig. 5B).

Photosynthesis: Fig. 6 represents the effect of *C. murale* on photosynthetic rates of barley *(Hordeum vulgare)* expressed as µmol quanta m^{-2} $s^{-1.}$ The graph indicates that photosynthetic rates of barley increased gradually with increase in photosynthetic active radiation. Different concentrations of the *C. murale* extract negatively affected the rates of photosynthesis. The maximum photosynthetic rates of barely were obtained at 50% of the aqueous extract concentration of *C. murale*, while the lowest rates obtained at the highest concentrations of the aqueous extract of *C. murale.* Moreover, there was a significant difference between all treatments of *C. murale* compared with the control, except at 25% aqueous extract concentration. As Fig. 6 shows, there is a significant response of barely to the high concentrations (75% and 100%) of the aqueous extract of *C. murale*. The highest aqueous extract concentration (100%) of *C. murale* caused about 32% inhibition in photosynthetic rates of barley compared with the control plants. The photosynthetic rates of barely plants treated with 75% extract had similar patterns of variation, with decreases of approximately 30% compared with that of the control plants. On the contrary, the 50% concentration of aqueous extract of *C. murale* resulted in an increase in photosynthetic rates of barely plants by about 20% as compared with that of the control plants.

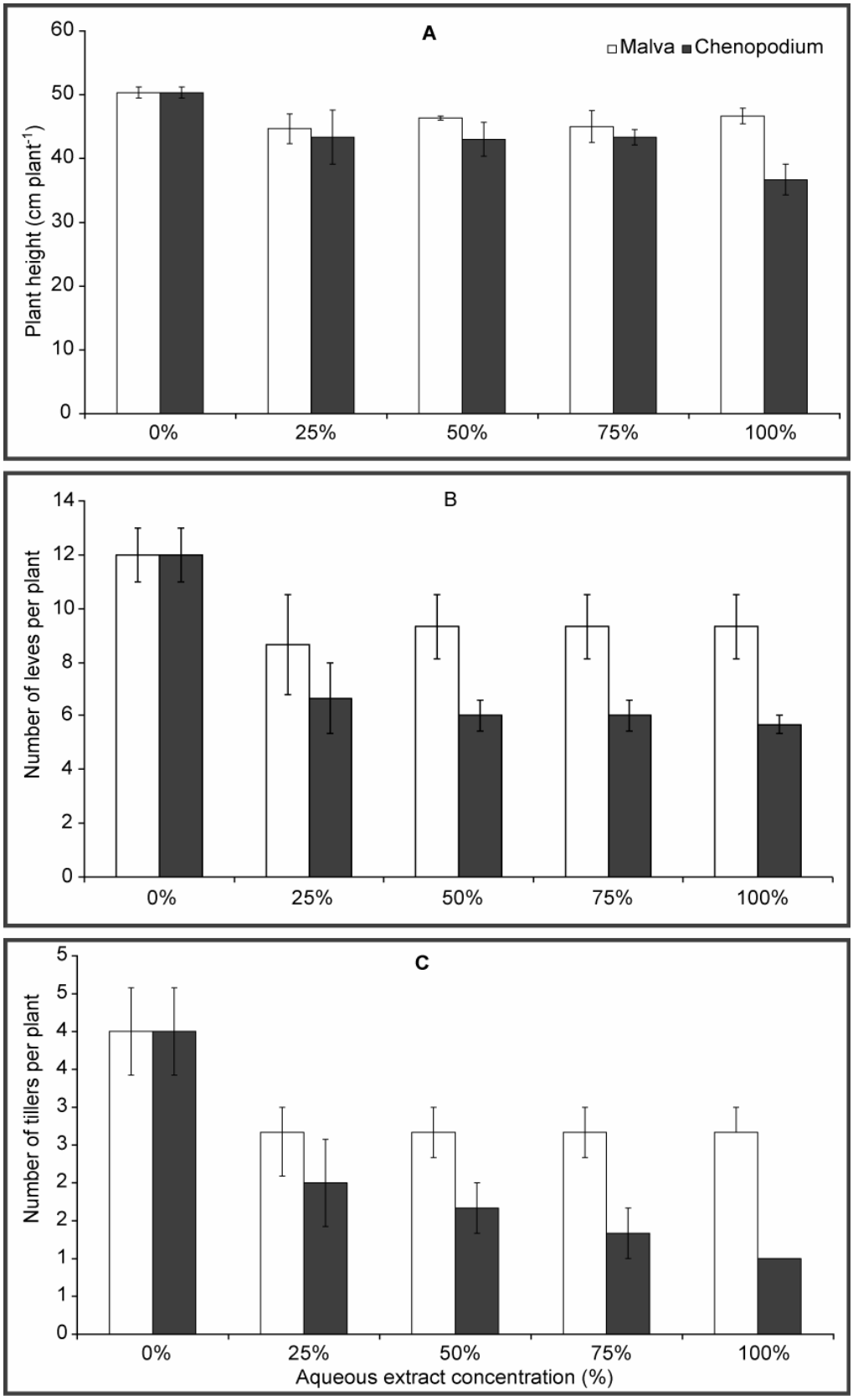

Fig. 1. Effect of aqueous extract of two weeds, *Malva parviflora* and *Chenopodium murale* on (A): plant height, (B): number of leaves and (C) number of tillers of barley (*Hordeum vulgare*).

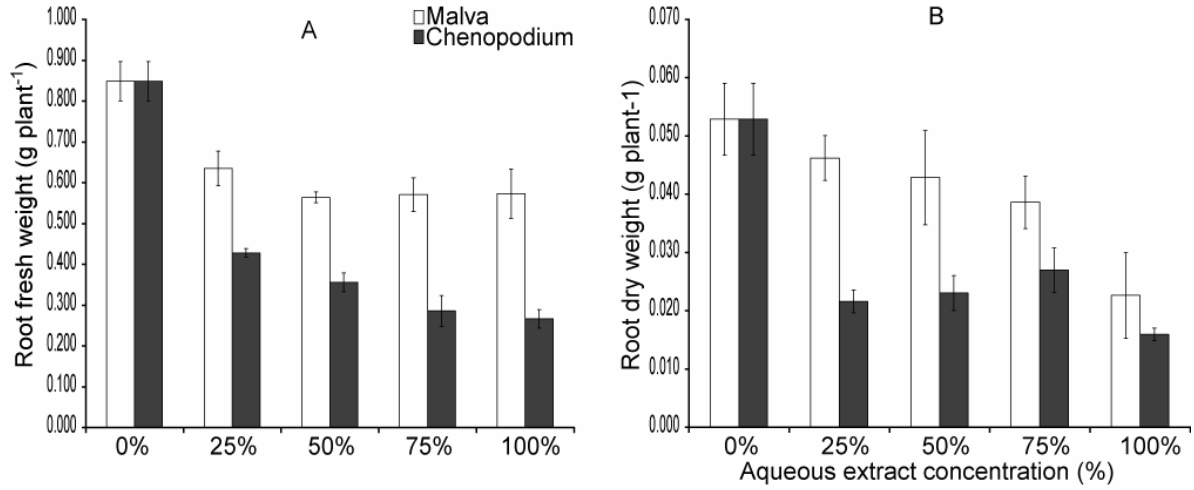

Fig. 2. Effect of aqueous extract of two weeds, *Malva parviflora* and *Chenopodium murale* on (A): root fresh weight and (B): root dry weight of barley (*Hordeum vulgare*).

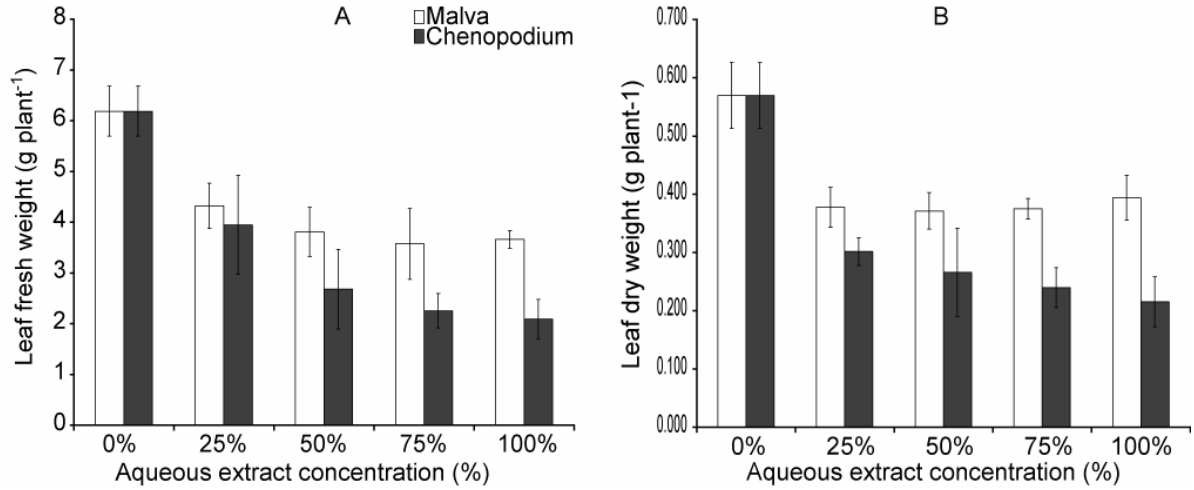

Fig. 3. Effect of aqueous extract of two weeds, *Malva parviflora* and *Chenopodium murale* on (A): leaf fresh weight and (B): leaf dry weight of barley (*Hordeum vulgare*).

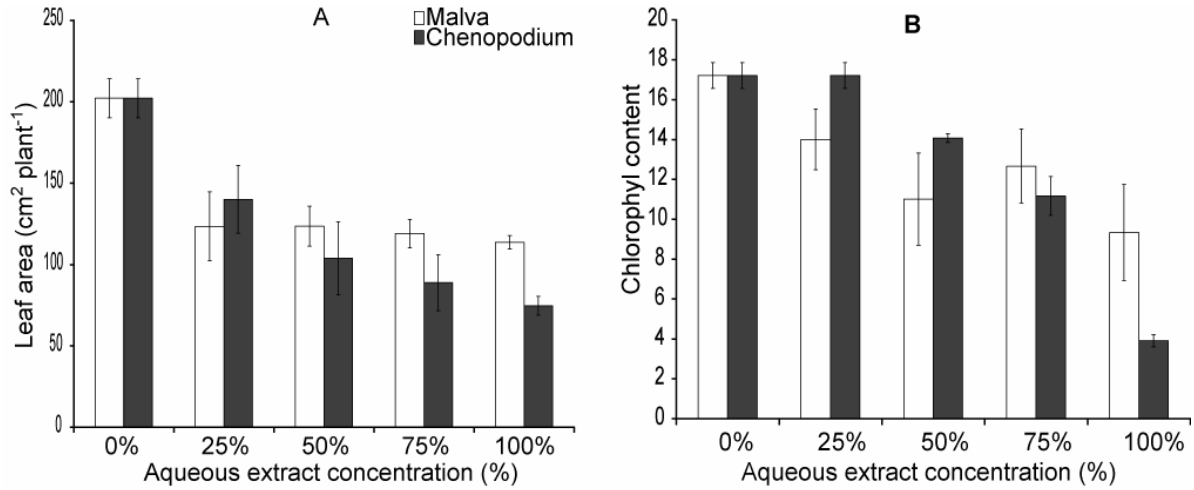

Fig. 4. Effect of aqueous extract of two weeds; *Malva parviflora* and *Chenopodium murale* on (A): leaf area and (B): chlorophyll content (chlorophyll index) of barley (*Hordeum vulgare*).

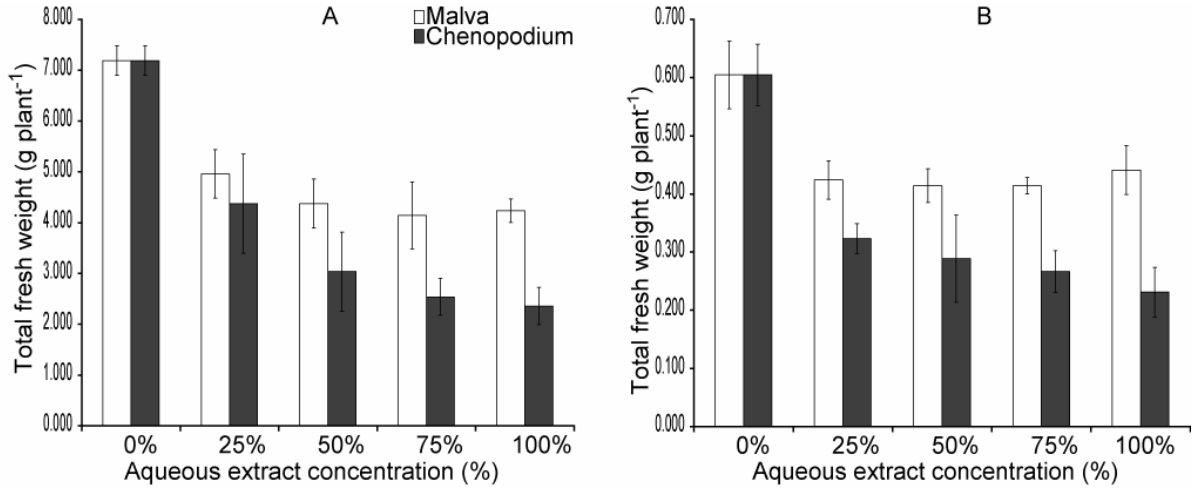

Fig. 5. Effect of aqueous extract of two weeds; *Malva parviflora* and *Chenopodium murale* on (A): Total fresh weight and (B): Total dry weight of barley (*Hordeum vulgare*).

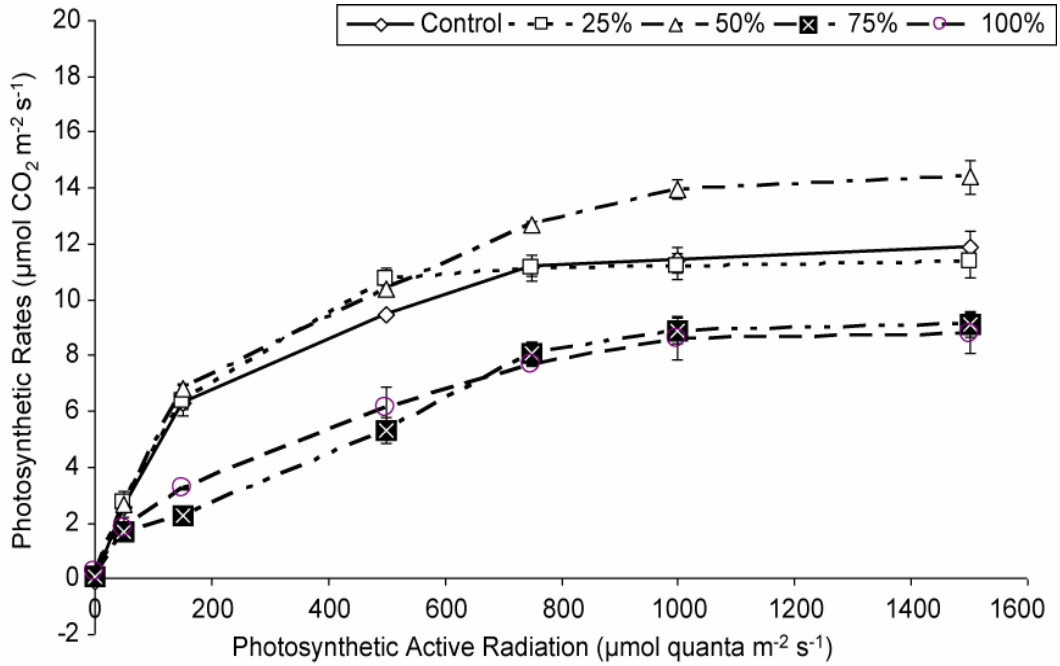

Fig. 6. Allelopathic effect of *Chenopodium murale* on photosynthesis rates of barley (*Hordeum vulgare*).

Figure 7 depicts the allelopathic effect of different concentrations of aqueous extract of the common mallow (*M. parviflora*) on photosynthetic rates of barley. Markedly, photosynthetic active radiation of barely increased with increase in photosynthetic rates of barley. The photosynthetic rates of the control plants were 12 µmol quanta m^{-2} s^{-1}, and the application of the aqueous extract at 25%, 50% and 75% levels resulted in an increase in photosynthetic rates of barley compared with the control (0%). *M. parviflora* aqueous extract enhanced the photosynthetic rates by about 42% and 33% at the 50% and 75% aqueous extract concentrations, respectively. On the other hand, the highest concentration (100%) of aqueous extract of the common mallow (*M. parviflora*) caused a significant reduction in photosynthetic rates of barley in comparison with the control plants (0% extracts), which means that the highest concentration of aqueous extract of common mallow (*M. parviflora*) (100%) reduced barley photosynthetic rates by nearly 50% compared with the control. The greatest photosynthesis rates of barley were observed at the 50% concentration of *M. parviflora*. Furthermore, there were significant difference between all treatments of *M. parviflora* compared with the control, except the 25% aqueous extract concentration. A sharp and significant decrease in photosynthesis rates were observed at 100% under the highest light intensity; 1500 µmol quanta m^{-2} s^{-1}.

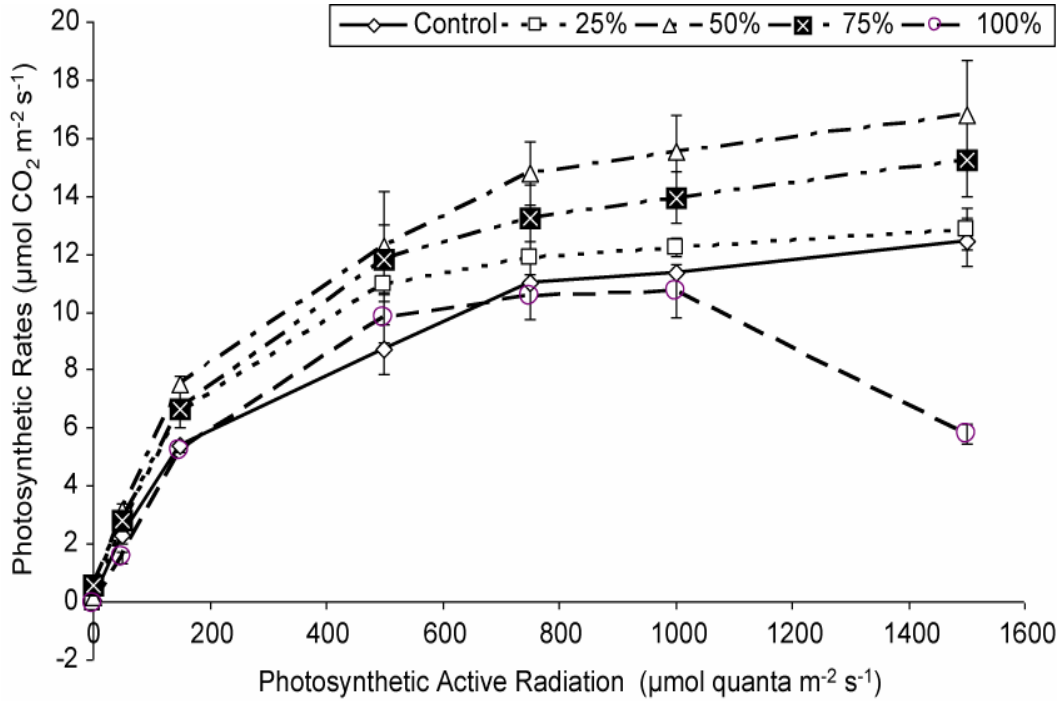

Fig. 7. Allelopathic effect of *Malva parviflora* on photosynthesis rates of barley (*Hordeum vulgare*).

Figure 8 represents the difference between the effects of the two weeds on photosynthetic efficiency. The photosynthetic rates were evaluated as the average values for each treatment for the light intensities from 0 – 1500 µmol quanta m^{-2} s^{-1}. It is clear from the results of this study that the aqueous extract of the weed *C. murale* significantly inhibited the photosynthetic rates in plants, compared with plants treated with *M. parviflora* aqueous extract weed (Figs. 6, 7 and 8). The inhibition in photosynthesis started at 50% concentration and this inhibition increased as the aqueous extract concentrations increased to 75% and 100%.

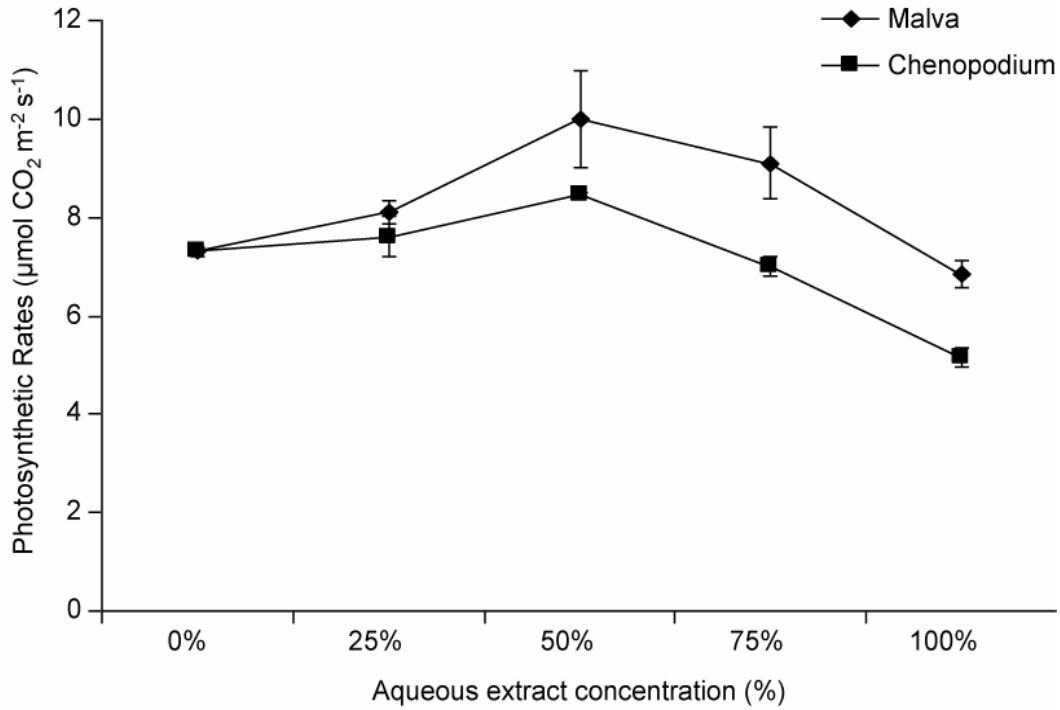

Fig. 8. Effect of aqueous extract of two weeds; *Malva parviflora* and *Chenopodium murale*, on photosynthesis rates of barley (*Hordeum vulgare*).

Discussion

The results of the present study showed that the aqueous extract of the 2 weed species, *M. parviflora* and *C. murale* differed in their effects on different growth parameters and photosynthetic rates of barley (*H. vulgare* L.) plants. The *Malva parviflora*, did not significantly affect the plant height, number of leaves, number of tillers, fresh weight and dry weight of roots (Figs. 6 and 7), and also the chlorophyll content (Fig. 4). In contrast to *M. parviflora*, the *C. murale* affected most of the growth parameters and physiological traits appraised. These effects are in agreement with many studies; which reported that plant growth, measured by plant height, was retarded by the effect of allelochemicals (Yang *et al*., 2002), and this phenomenon was also described earlier by Rice (1984). The effect of the 2 weed species extracts on leaf fresh weight, leaf dry weight, total fresh weight and total dry weight and leaf area were significant. Many reports confirmed that a number of weeds species inhibited barely growth through competition for nitrogen concentration or root space (Maataoui *et al*., 2005). Other investigations reported that the weed aqueous extract of *C. murale* suppressed shoot length, shoot biomass, total root length, number of roots and root biomass (El-Khatib *et al*., 2004; Shafique *et al*., 2011). The results presented in the present study indicated that most of the growth traits inhibited with increase in concentrations of the aqueous extracts and in particular for *C. murale*. The decline in plant growth might have been due to the inhibition in carbohydrate and protein contents that cause an increase in proline accumulation, which is considered as an indicator of plant stress (El-Khatib *et al*., 2004; Batish *et al*., 2007a). The results obtained from this study also demonstrated that *C. murale* possesses stronger allelopathic effects compared with that of *M. parviflora* on growth and photosynthesis of barley (*H. vulgare* L.).

Chlorophyll content was severely reduced by *C. murale* extract (Fig. 4B). It has been reported that in rice seedlings, the chlorophyll content was inhibited due allelopathic phenoloics, and the inhibition of chlorophyll accumulation is related to the phenolic concentrations (Yang *et al*., 2002). The inhibition of chlorophyll accumulation by allelochemicals might be due to the inhibition of chlorophyll biosynthesis or stimulation of chlorophyll degradation or both processes (Yang *et al*., 2002). The findings of this study on the effect of the weed *M. parviflora* on chlorophyll levels are in line with the findings of Benyas *et al*., (2010). They reported that shoot aqueous extract of the weed *Xanthium strumarium* L. did not affect chlorophyll content of lentil plants (*Lens culinaris* Medic.). The results of the current study showed that the reduction in chlorophyll levels is associated with the inhibition in photosynthetic rates (Figs. 4B, 6 and 7). This is in full agreement with many investigations which reported that any decrease in chlorophyll content would adversely affect the photosynthetic capacity (Patterson, 1981; Zhou and Yu, 2006).

The results presented in Figs. 6 and 7, show that there were significant differences between the effect of aqueous extract of *C. murale* and *M. parviflora* at different concentrations on photosynthetic rates. The photosynthetic rates of barely were reduced by increasing the concentrations of *C. murale* and *M. Parviflora* extracts. This might be explained by the fact that these weeds have allelopathic properties that led to negative impact on barley crop as shown previously for growth parameters (Figs. 1-5). *C. murale* and *M. parviflora*, which are classified as one of the nutrient accumulator weeds, compete with barely by limiting the availability of some growth resources, e.g., water, light and nutrients (Qasem, 1992; Qasem, 1995). As mentioned earlier, photosynthetic rates of barely decreased significantly with increase in concentration of *C. murale* extract compared with the control plants. These results can be explained as a result of allelochemicals that contained in the aqueous extract of *C. murale*. These allelochemicals inhibit the Photosystem II transfer electron reaction (Zhou & Yu, 2006), and this explanation can be reinforced by the findings of Batish *et al*. (2007b) who reported that *C. murale* extract have large amount of a number of allelochemicals including; ferulic acid, vanillic acid, *p*-coumaric acid and benzoic acid, and these phytotoxic phenolics affect the overall plant growth and physiology.

Furthermore, these results can be explained by the fact that allelochemicals inhibit the stomatal opening and CO_2 uptake that lead to reduction in photosynthetic rates (Zhou & Yu, 2006). This explanation can be enhanced by the results of Daizy *et al*., (2006) who reported a reduction in total chlorophyll content of chickpea and pea plants via action of *C. murale* extract. Similarly, Majeed *et al*., (2012) reported a reduction in photosynthesis of wheat (*T. aestivum* L.) by the action of different concentrations of *C. album* extracts due to competitive action which lowered the mineral and water uptake. The effects of common mallow (*M. parviflora*) aqueous extract on photosynthetic rates of barely are significantly different at different treatments of aqueous extracts, which can be explained by the inhibitory role of allelochemical compounds already reported in *M. parviflora* extract (Zahedi & Ansari, 2011). These allelochemical materials caused a reduction in photosynthetic rates via competitive action with crop plants (Qasem, 1992). As mentioned earlier, the allelochemicals reduced photosynthetic rates by the inhibition of uptake of several nutrients that are essential in photosynthetic process, such as N, water and CO_2 (Zhou & Yu, 2006). This reduction led to obstruction of the three major processes of photosynthesis (Hussain & Reigosa, 2011): (1) stomatal control of CO_2 supply, (2) thylakoid electron transport and (3) the carbon reduction cycle. Hussain and Reigosa (2011) also reported that allelochemicals inhibit the efficiency of Photosystem II photochemistry in the dark-adapted state in some C_3 plant species. The causes of the non-significant effect of *M. parviflora* on some growth traits might be the different controlling mechanisms of these traits that make plants tolerant to the action of these allelochemical compounds (Benyas *et al*., 2010). On the other hand, *C. murale* weed showed deleterious impact on growth parameters and photosynthetic rates of barley plants. This might be due to some toxic allelochemicals that can be released from the weeds which seriously affect the growth of barely seedlings (Alam & Shaikh (2007).

In conclusion, the findings reported in the present investigation indicate that the growth of barley crop, *H. vulgare*, was severely affected by the two weeds through the effects of allelochemical compounds released by the vegetative parts of weeds. The impact of the weeds, *M. parviflora* and *C. murale* extracts, differed from one weed to another. For example, the weed *C. murale* was highly aggressive, as it affected almost all growth and physiological traits compared with *M. parviflora*.

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