Pak. J. Bot., 48(3): 1197-1202, 2016.

ALLELOPATHIC SORGHUM WATER EXTRACT HELPS TO IMPROVE YIELD OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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Abstract

Allelopathy provides eco-friendly environment in managing weeds by reducing the use of synthetic herbicides that cause environmental pollution and herbicide resistance problems. Therefore, weeds have been controlling by plant derived organic compounds as an alternative of inorganic herbicides since the last two decades. In this study, sorghum aqueous extracts were applied individually as well as accumulatively with reduced levels of Dual Gold® (S-Metolachlor) as foliar sprays in sunflower at 50, 70 and 90 DAS. For comparison, standard level of S-Metolachlor was also applied as foliar sprays along with weedy check. The highest reduction of total weed density (93.7%) was recorded by three sprays of sorghum aqueous extracts at rate of 15 L/ha mixed with 1/3rd S-Metolachlor at 1.6 L/ha as foliar applications. This reduction rate was statistically similar to one that was obtained by standard level of S-Metolachlor (1.6 L/ha). The highest achene yield was achieved by applying three foliar sprays of aqueous sorghum extracts along with reduced doses of S-Metolachlor, which was almost similar to full recommended dose of S-Metolachlor. These findings demonstrate that allelopathy offers environment friendly and economical opportunity for weed control in sunflower reducing the dependence and cost of herbicides.

Key words: Allelopathy; Dual Gold®; Sorghum aqueous extracts; Sunflower; Yield improvement.

Introduction

Sunflower (*Helianthus annuus* L.) is a member of family Compositae and it is considered as one of the most important species of genus Helianthus. It has become popular among masses due to its high edible oil contents and wide range of adaptability (Nasreen *et al.,* 2015). Its dehulled seed on dry weight basis contains 40-50% oil and 20.4-40% protein contents (Boriollo *et al.,* 2014). But its average yield is low due to various biotic and abiotic factors. The most devastating factor for its yield is weeds competition. From literature survey, it has been found that sunflower yield is reduced by 58-81% due to weeds stress (Daugovish *et al.*, 2003; Simic *et al.*, 2011). Weeds are known as major biotic threats to crops because they compete with crop plants with respect to nutrients, water as well as light, and ultimately reduce crop yield and quality. These are more competitive by possessing high genetic variations and physiological plasticity. Sometimes, weeds as poisonous plants and allergens pose human health hazards (Reddy, 2015).

Herbicide usage is the only answer to tackle weed problem. The over-reliance of herbicides resulted in environmental pollution, weed transfer and development of weed resistance against herbicides. The toxic herbicides are continuously polluting the surface and ground water for livestock as well as human consumptions, while their residues released from plants as well as from soil move in nutrition cycle, and ultimately become perilous for descendants (Judith *et al.*, 2001). The development of herbicide tolerant crops through modern biotechnology using nonselective herbicides could tackle the weed shift problem, but it is being resulted in the development of problematic super weeds (Singh *et al.,* 2015). Hitherto, transgenic plant production is a vast debatable topic which requires punctilious observations, experimentations and conclusions for final decisions either its approval or refusal (Vats, 2015). Therefore, there is exigency to develop natural ecological phenomena for controlling weeds. Allelopathy is an alternate solution of problematic weeds and it reduces herbicide resistance development in weed ecospecies. The synergism between allelopathic extracts of various crops and low quantities of herbicides are as efficient in controlling weeds as recommended levels of herbicides (Jabran *et al.,* 2010; Farooq *et al.,* 2011; Dastagir & Hussain, 2015).

In our previous research study, we reported that reduced doses of S- Metolachlor fortified with allelopathic sorghum water extract limited weed density, weed fresh biomass as well as weed dry biomass (Khan *et al.,* 2009). Here, our aim is to conduct the detailed study of the synergistic effect of allelopathic sorghum water extracts and reduced levels of S- Metolachlor to improve the yield of sunflower. We believe that this is first report about sunflower crop improvement by allelopathy.

Materials and Methods

The present study was carried out at Gomal University, D.I.Khan, Pakistan to investigate the possibilities of sorghum allelochemicals alone or in combination with low quantities of Dual Gold® (S-Metolachlor) for managing weeds in sunflower. Seven treatments were tested as shown in Table 1. Seeds of sunflower hybrid “64-A93” were obtained from Pakistan

Oil Seed Development Board, D.I. Khan and were sown on a well-prepared seedbed in furrows 70 cm apart by hand dibbler. There were four rows in each plot and distance between plants was kept at 30 cm by thinning. In this study, 7 kg/ha seed was used in net sub plot size of 2.8 m x 4 m. Fertilizer was applied @ 70-35 kg/ha N-P. At sowing time, all P fertilizer was used, while half nitrogenous fertilizer was used at sowing and half at 1st irrigation by broadcast method. Sorghum aqueous extract was prepared by following the method devised by Cheema *et al.* (2003). At maturity stage, sorghum was harvested, dried under shade and hewed into small bits approximately 2 cm using fodder cutter. These small fragments of sorghum were drenched in water in 1:10 (w/v) for one day. Subsequently, this extract was passed through sieves and heated at 100^{o}C to reduce volume of extract by twenty times. The prepared extract was stored at 25^{o}C for further use.

Agronomic and yield characteristics of sunflower: Number of plants in 1m^2 was counted at maturity stage from each experimental plot. Height of ten randomly chosen plants was calculated in centimeter by a meter rod at maturity stage from each observational trial and then average was recorded. For measuring head diameter, ten plants were selected in an unsystematic manner from each innovative plot and then their head diameter was taken using measuring tape. Similarly, stem diameter of 10 plants from each sub plot was measured using measuring tape, then average was used for statistical analysis. From each observational plot, heads of ten plants were harvested and achenes were separated from heads and then number of achene per head was counted and then average was calculated. One thousand grains were collected from each treatment separately. The counted grains were weighed separately and then 1000-grain weight was computed for each plot.

After sun drying, the heads of all the plants in each observational plot were beaten by hands and yield was calculated per plot basis and changed into tones per hectare by using the following formula:

$$\text{Yield per hectare} = \frac{\text{Plot yield x Area of one hectare}}{\text{Plot size}}$$

Table 1. Different level of sorghum water extracts and Dual Gold® that were tested individually as well as accumulatively in the present study.

Treatments	Various levels of sorghum water extracts and Dual Gold®
T_1	Control (Weedy check)
T_2	Sorgaab @ 15 L/ha (Used as Early Post Emergence 15-20 DAS)
T_3	Sorgaab @ 15 L/ha + Dual Gold® @ 1.6 L/ha (1/3rd of recommended dose) (Used as Pre-Emergence)
T_4	Sorgaab @ 15 L/ha (1/2 dose) (Used as Early Post Emergence)
T_5	Dual Gold® (1/2 of recommended dose of 1.6 L/ha) (Used as Pre-Emergence)
T_6	Dual Gold® (1/3rd of recommended dose) (Used as Early Post Emergence)
T_7	Dual Gold® (Full recommended dose i.e. 1.6 L/ha) (Used as Pre-Emergence)

Sorghum extracts @ 15 L/ha each homogenized with Dual Gold® @ 1.6 L/ha were sprayed as pre-emergence and early post emergence with the help of sprayer (Knapsack-Hand Sprayer) having flat fan nozzle

Three samples from an area of 1 square meter were reaped at maturity stage and knotted into little clumps that were weighed to measure biological yield and subsequently it was changed into hectare. The harvest index was the ratio of achene produce to total biomass in percentage and was measured as follows:

$$\text{Harvest index} = \frac{\text{Achene yield}}{\text{Biological yield}} \times 100$$

Statistical analysis: The experimental layout was done by RCBD and data was analyzed by ANOVA techniques (Steel & Torrie, 1984). The statistical means were compared by LSD test at 5% significance level. The analyses were performed with the help of computer “MSTATC” software package (Bricker, 1991).

Results and Discussion

Synergistic effect of sorghum aqueous extracts and low levels of Dual Gold® improves agronomic characteristics of sunflower: Data in Fig. 1A showed that allelopathic sorghum extracts did not significantly improve the plant population of sunflower. During this experiment taller plants (148.8 cm) were recorded by T_3 and it was followed by T_7 which showed 148.3 cm plant height (Fig. 1B). The productive potential of a sunflower plant is often reflected by the size of its head. The data in Fig. 1C revealed highly significant effect of all the treatments on head diameter. Maximum head diameter (19.75 cm) was noticed in T_3 and it was logically similar to T_7 which produced the head diameter of 19.38 cm. Minimum head diameter (11.88 cm) was observed in case of control. The size of stem in sunflower is positively correlated with the size of head. The data representing stem diameter was influenced significantly by different treatments (Fig. 2A). The treatment T_3 gave maximum stem diameter (2.49 cm) which is not significantly different from treatment T_7 which gave 2.49 cm stem diameter. The minimum stem diameter (1.85 cm) was noticed in control. It was due to more weeds competition with sunflower.

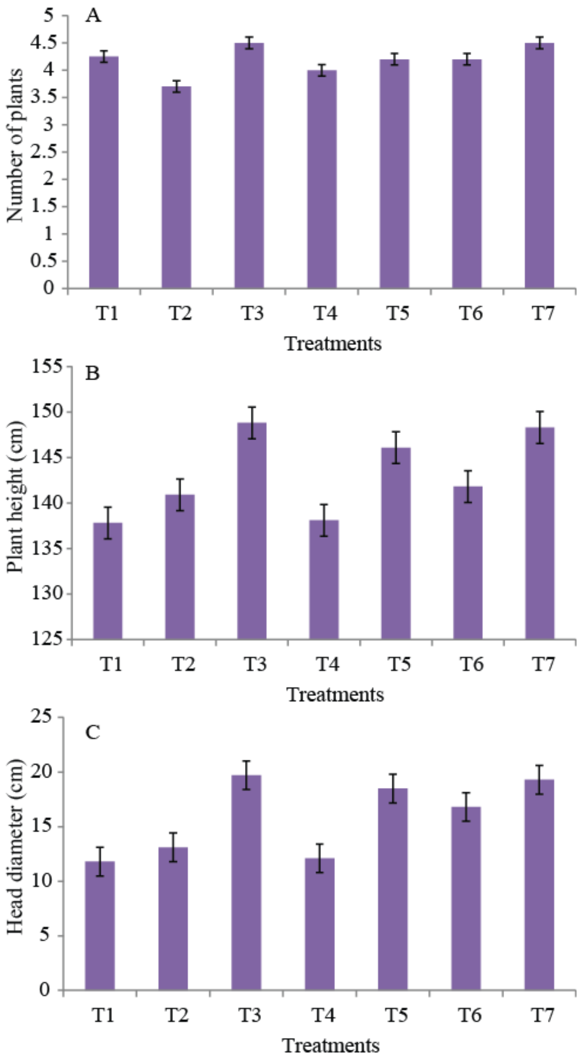

Fig. 1. (A) Synergistic effect of sorghum water extract and reduced levels of Dual Gold® on number of plants of sunflower (B) Synergistic effect of sorghum water extract and reduced levels of Dual Gold® on plant height of sunflower (C) Synergistic effect of sorghum water extract and reduced levels of Dual Gold® on head diameter of sunflower. Vertical bars depict the standard error of the means (n = 3).

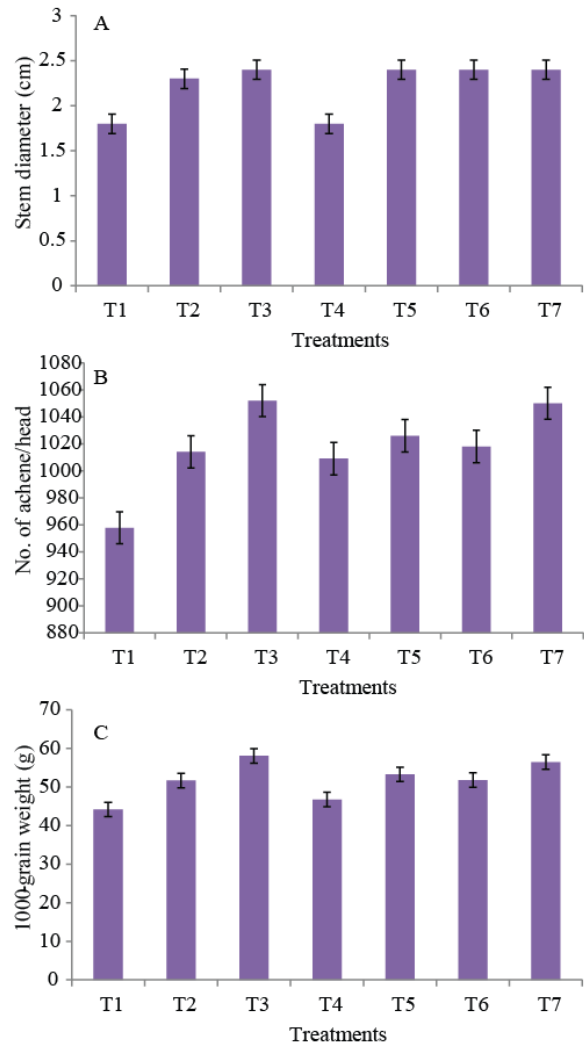

Fig. 2 (A) Synergistic effect of sorghum water extract and reduced levels of Dual Gold® on stem diameter of sunflower (B) Synergistic effect of sorghum water extract and reduced levels of Dual Gold® on number of achene/head of sunflower (C) Synergistic effect of sorghum water extract and reduced levels of Dual Gold® on 1000-grain weight of sunflower. Vertical bars depict the standard error of the means (n = 3).

The growth contributing attributes of sunflower were significantly enhanced by all the treatments tested except plant population which showed non-significant effect by all the combinations relative to control. Plant height is greatly affected by genetic as well as environmental factors including nutrients, light, space and water stress. More plant height indicates efficient vegetative growth, while less plant height specifies under growth of crop plants. In both cases poor achene formation and low yield are resulted which signify the optimum plant height for more achene yield. During this study, more head diameter, more stem diameter and taller plants were recorded applying sorghum aqueous extracts (15 L/ha) and 1/3rd S-Metolachlor (1.6 L/ha). During this study, head diameter, stem diameter and plant height increment were due to less weed density and less weed dry biomass which provided more nutrients, light, growing space and plenty of water limiting the weed competition by allelochemicals. Our findings were inconsistent with the previous research report by Awan *et al.* (2012) who studied the efficacy of three crops aqueous extracts namely brassica, sorghum and sunflower on weed management in wheat and stated that concentrated aqueous extracts of these crops inhibited plant height of wheat relative to weedy check. This research group reported that the highest plant height reduction was noted when homogenous mixture of concentrated sorghum aqueous extracts and concentrated sunflower aqueous extracts was used (6 + 6 L/ha) at 30 and 60 DAS. Similarly, Batish *et al.* (2006); Shahid *et al.* (2006) conveyed that various allelopathic aqueous extracts had suppressive effects on plant height of wheat. This discrepancy might be due to differences in crop, types and concentrations of aqueous extracts, synergistic behaviour of phytotoxins and herbicide used.

The phytochemicals released from the plants are the alternate source to reduce sunflower weed competition. The negative interaction between two or more crop species for their existence and dominance within a restricted space is called competition (Asaduzzaman *et al.,* 2014). Allelopathy is distinct from the negative interaction and also eco-friendly because toxic chemicals are released from the donor plant and have detrimental effect on the germination, growth and development of other undesirable weed plant (Arif *et al.,* 2015; Mengal *et al.,* 2015). During this study, it was found that mutual effect of sorghum extracts and lower doses of S-Metolachlor significantly reduced TWD and density of two competitive weeds namely *Chenopodium album* and *Coronopus didymus* relative to weedy check at 50, 70 and 90 DAS in sunflower. Recommended dose of S-Metolachlor gave statistically similar results to experimental treatment where sorghum water extract and one third dose of S-Metolachlor was applied. The synergism between allelopathic sorghum extract at rat of 15 L/ha and one third recommended dose of S-Metolachlor (at 1.6 L/ha) limited TWD by 87-90.6%, while only S-Metolachlor (1.6 L/ha) yielded 89.4-93.7% reduction of TWD in sunflower at 50, 70 and 90 DAS. The same trend was noticed in *Chenopodium album* and *Coronopus didymus* reduction. Based on this data, we posited that sorghum aqueous extracts along with low levels of S-Metolachlor could reduce the herbicide dose up to 67%. Our findings were in accordance with the previous research study in which it was reported that water extracts of sorghum crop, sunflower crop and mulberry crop combined with low levels of iodo + mesosulfuron controlled weeds in equal ratio with the full recommended dose of the herbicide in wheat (Khaliq *et al.,* 2012). Similarly, another research team asserted that allelopathy was a naturally occurring phenomenon that might be used for weed management in field crops. In this report, it was emphasized that allelopathic plant extracts along with half standard dose of herbicide could manage weeds effectively and might reduce the herbicide resistance in weed ecobiotypes (Farooq *et al.,* 2011).

In this study, the highest inhibition of total weeds fresh weight (95.8-97.5%), *Chenopodium album* fresh weight (97-100%) and *Coronopus didymus* fresh weight (68.2-89.2%) was achieved by allelopathic extracts of sorghum (15 L/ha) amalgamated with S-Metolachlor (1/3rd dose) at 1.6 L/ha at 70+90 DAS. It was almost similar to the inhibition that was obtained on the full recommended dose of S-Metolachlor. A similar nature of study was conducted by Hussain *et al.,* (2014) in which they investigated the effect of allelopathic crop extracts on the reduction of weeds in wheat and predicated that the highest reduction of weed fresh weight (62%) was recorded by accumulative effect of sorghum+sunflower water extracts and Atlantis 3.6 WG at rate of 10.8 g/ha a.i. They also concluded that full dose of Atlantis 3.6 WG (14.4 g/ha) reduced total weed fresh weight by 52%, which was rationally similar to sorghum + sunflower extracts+50-75% rate of the herbicide. But in our study, higher fresh weed biomass inhibition was recorded. These differences between our findings and the previous report might be due to disparity of crops, type and concentrations of allelopathic crop aqueous extracts and the herbicides used. Awan *et al*. (2012) scrutinized the effectiveness of brassica crop + sorghum crop + sunflower crop aqueous extracts to control weeds present in wheat field under rainfed conditions and stated the role of these allelopathic aqueous extracts in the reduction of weed fresh biomass. In their study they found that sorghum crop, sunflower crop, brassica crop water extracts (4+4+4 L/ha) gave the highest reduction of fresh weed biomass among all treatments tested at 30 and 60 DAS. Our findings were also confirmatory with the earlier reports by Cheema *et al*. (2003) who noticed reduced fresh weight of weeds applying sorghum extracts as foliar sprays along with reduced levels of herbicides at different DAS in wheat crop. In another research study, mutual effect of sorghum+sunflower aqueous extracts at rate of 15 L/ha enriched with synthetic herbicide (pendimethalin; 0.6 kg/ha a.i.) produced similar control of weed fresh biomass like that of recommended dose of the herbicide (Jabran *et al.,* 2010).

Synergistic effect of sorghum aqueous extracts and low levels of Dual Gold® improves yield characteristics of sunflower: The data showed significant effects of all the treatments on number of achene/head (Fig. 2B). The highest number of achene/head (1052) was achieved by T_3. It was statistically equal with treatment T_7 which gave (1050) number of achene/head. The data regarding 1000 - achene weight is presented in Fig. 2C. The highest 1000 - achene weight (58.1 g) was obtained by T_3 followed by T_7 which produced 56.51 g 1000 - achene weight. The treatment combinations (T_2, T_5 and T_6) produced 51.72, 53.33 and 51.81 g 1000 - achene weight, respectively (Fig. 2C). Achene yield was the aggregate effect of no. of plants/unit area, size of head, number of achene/head and weight of 1000-achene produced under specific environmental conditions. The data revealed that maximum achene yield (2.68 t/ha) was given by T_3. It was followed by T_7 which gave 2.47 t/ha achene yield, while other treatments including T_5 and T_6 yielded 2.32 and 2.29 t/ha achene yield, respectively. The treatments T_2 and T_4 gave 2.28 and 2.275 t/ha achene yield, respectively (Fig. 3A). The highest biomass (7.47 t/ha) was produced by T_3 and it was followed by T_7 which produced 7.43 t/ha biomass (Fig. 3B). The minimum biological yield (7.03 t/ha) was recorded by T_4. The similar trend of results was found for harvest index, where the highest harvest index (35.85) was obtained by T_3 followed by T_7. The treatments such as Dual Gold® (1.6 L/ha, half dose), Dual Gold® (1.6 L/ha, one third dose), Sorgaab @ 15 L/ha, full dose and Sorgaab @ 15 L/ha, 1/2 dose produced 31.61, 31.57, 31.93 and 32.37 harvest index, respectively (Fig. 3C).

Achene yield contributing characteristics of sunflower were improved significantly by all the treatments with respect to weedy check (control). The highest no. of achene/head, weight of 1000 achenes, achene yield, biological yield and harvest index were obtained when sorghum aqueous extracts (15 L/ha) homogenized with S-Metolachlor reduced levels at 1.6 L/ha was used as two foliar applications at 70 and 90 DAS. In current scenario, Mushtaq *et al*. (2010) ascertained that maximum 1000-grain weight in wheat was due to less weeds competition and hence more nutrients as well as more water availability to wheat plants to improve overall growth and ultimately 1000-grain weight. During our study, harvest index was also increased due to optimum weed control and plenty of nutrients attributed to less competition of sunflower with weeds (Marwat *et al.,* 2005). The results of present study lead us to suggest that there is exigency to fully understand the functions and involvement of allelochemicals in the crop plant responses to weed stress to bridge the gap in the existing knowledge.

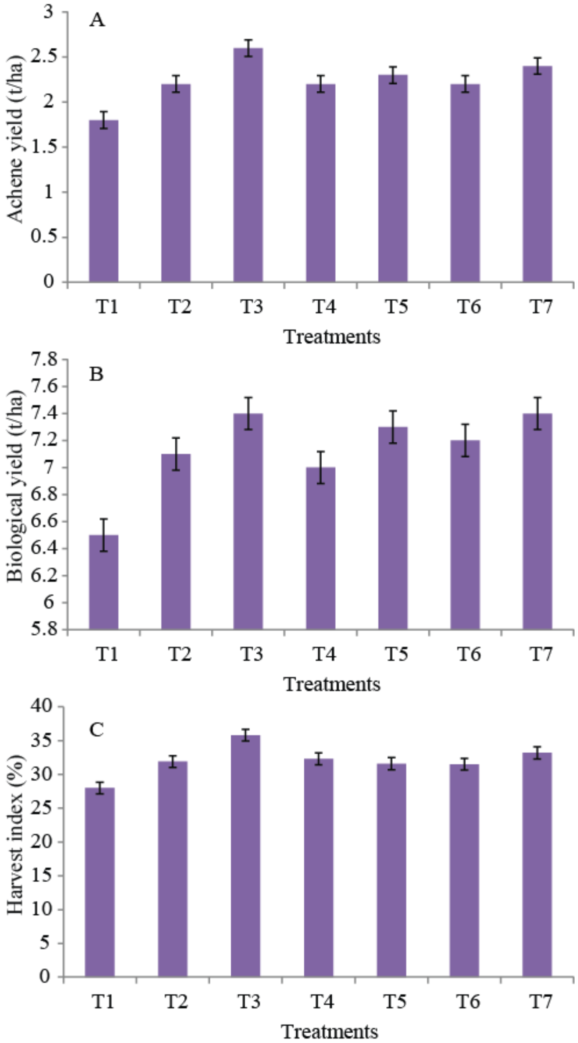

Fig. 3 (A) Synergistic effect of sorghum water extract and reduced levels of Dual Gold® on achene yield of sunflower (B) Synergistic effect of sorghum water extract and reduced levels of Dual Gold® on biological yield of sunflower (C) Synergistic effect of sorghum water extract and reduced levels of Dual Gold® on harvest index of sunflower. Vertical bars depict the standard error of the means (n = 3).

This study showed the reduction of total weeds dry weight by various treatments applied at different time periods. The aqueous extracts of sorghum at rate of 15 L/ha reduced weed dry weight but reduction rate was lower as compared to full dose of S-Metolachlor as well as synergistic effect of sorghum aqueous extract and one third dose of S-Metolachlor. Our findings were consistent with the recent report by Arif *et al*. (2015) who examined organic allelopathic crop aqueous extracts in managing weeds of wheat and argued that 76-89% weed dry weight reduction was recorded when recommended herbicide (idosulfuron + mesosulfuron) were applied, while 48-58% inhibition of weed dry weight was found when combined sorghum crop, brassica crop and sunflower crop aqueous extracts (each at 20 L/ha) were used as two foliar sprays at 25 and 40 DAS. Similarly, researchers revealed the inhibition of weed dry biomass by 41% that improved yield up to 17% relative to control in wheat. They also pointed out that *Chenopodium album* dry weight was reduced applying sorghum allelopathic extracts (pre-emergence; 25 and 35 DAS) indicating that the herbicide use was reduced by 75% integrating sorghum and sunflower aqueous extracts in wheat (Naseem *et al.,* 2009; 2010). Our findings were coincided with another field study by Elahi *et al.* (2011) who scrutinized the influence of sorghum crop, sunflower crop, brassica crop and rice crop aqueous extracts (each at 12 L/ha) homogenized with low levels of phenoxaprop-p-ethyl (at 287 g/ha) and isoproturon at rate of 333 g/ha and concluded that 92-93% inhibition of weed dry biomass was obtained at 70 DAS that improved grain yield up to 22.79%. From this study, they also posited that alleopathy was economical, eco-friendly and high grain yield and marginal return producer in wheat. In our study, the inhibition of weed dry weight by two sprays (70+90 DAS) of sorghum aqueous extracts at 15 L/ha might be attributed to the availability of phytochemicals in aqueous extracts that limited the weeds growth. In an earlier research report, it has been documented that 12 types of phytotoxins namely gallic acid, syringic acid, *p*-coumaric acid, ferulic acid, *m*-coumaric acid, benzoic acid, sorgoleone, protocateuic acid, phydroxybenzaldehyde, *p*-hydroxybenzoic acid, caffeic acids and vanillic acid were found in sorghum (Cheema *et al.,* 2009). These allelochemicals reduced the density and dry biomass of *Coronopus didymus, Anagallis arvensis, Rumex dentatus, Chenopodium album, Convolvulus arvensis, Phalaris minor* and *Cyperus rotundus* (Ahmad *et al.,* 1991). Coherence with our findings, it has been predicated in another proposition by Arif *et al.* (2015) that physico-metabolic processes of weeds were adversely affected by allelochemicals due to their phytotoxic nature and complementary actions resulting in growth inhibition of weeds. It has also been documented that in an aqueous mixture, the compounds might substitute one another due to their high biological exchange rate resulting in the potency increment to each other (Gerig & Blum, 1991). It might be the possible reason for maximum inhibition of weeds by the application of sorghum aqueous extracts fortified with reduced levels of S-Metolachlor during this study. It has been reported that the high concentration of allelochemicals interfered with weeds cell division, hormone production, mineral uptake and transport, membrane permeability, stomatal conductance, photosynthesis, respiration, protein metabolism and plant water relations which caused rapid growth reduction of weeds (Farooq *et al.,* 2013; Arif *et al.*, 2015).

Conclusion

Herbicides pose serious threats to environment as well as human beings. The mutual use of sorghum allelopathy and lower rates of herbicides could reduce usage of herbicide. In this study, lower doses of Dual Gold® (1/2 & 1/3rd of recommended one) combined with allelopathic sorghum water extract (15 L/ha) were compared with standard level of Dual Gold® (1.6 L/ha). The highest sunflower yield (2.68 t/ha) was obtained by the application of sorghum extracts amalgamated with 1/3rd level of Dual Gold® that demonstrated 45% more than that of control. While full recommended dose of Dual Gold® @ 1.6 L/ha gave 2.47 t/ha achene yield which was 34% increase in achene yield over weedy check. Conclusively, combination of different crop water extracts especially sorghum with one third dose of Dual Gold® might be applied for suppressing common weeds of sunflower including *Chenopodium album* and *Coronopus didymus* in order to reduce the usage of herbicides to about 67% of recommended one. It could also be suggested that studies of this nature might be continued using different crop water extracts in various proportions with herbicides in sunflower as well as other economically important crops. This study could decrease the cost of production and strengthen efforts for protecting the environment on sustainable basis.

Acknowledgments

I would like to thank Department of Agronomy/Botany Gomal University, D.I. Khan for funding, designing and implantation of the experimental works.

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(Received for publication 9 March 2015)