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Allelopathic effect of aqueous extracts of three weed species on the growth and leaf chlorophyll content of bread wheat

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ABSTRACT

The purpose of this study was to evaluate the allelopathic effect of weeds (*Avena fatua*, *Melilotus officinalis* and *Polypogon hissaricus*) on germination, growth, dry biomass and chlorophyll concentration of three cultivars of wheat (Ata Habib, Pirsabaq and Serin). In germination test, different concentrations of aqueous extracts (5, 10 and 15 g/l) of the three weeds significantly reduced percent germination; however, 15 g/l extract of *M. officinalis* resulted in complete failure of germination of cultivar Pirsabaq. In pot culture, root and shoot length, chlorophyll concentration and seedling dry biomass of the three wheat varieties showed differential responses to different weeds. Aqueous extract at 15 g/l of *A. fatua* increased root and shoot length and dry biomass of cultivar Pirsabaq; however, these parameters were significantly retarded in other two wheat cultivars by extract of weeds. Moisture content of the cultivars did not show any response to allelopathic stress of the weeds. In contrast, chlorophyll concentration in Pirsabaq and Serin was significantly increased by aqueous extract of all the weeds but reduced it in cultivar Ata Habib by 50%. In general, Ata Habib was found to be the most sensitive cultivar to the imposed allelopathic stress. The phytotoxic potential of three weeds was found in the order of *A. fatua* > *M. officinalis* > *P. hissaricus*.

1. Introduction

Wheat (*Triticum aestivum* L.) in the grass family (Poaceae) is an important agronomic crop widely cultivated for grains which are used for domestic food purposes and in the preparation of many other processed materials [1]. After maize and rice, wheat is ranked the third most widely produced grain crops in the world which has a significant impact on meeting global food loads and dietary requirements. Global wheat production during 2016/17 was recorded as 753.9 million metric tons from approximately 250 million hectares of land (https://www.statista.com). Currently, Pakistan has been listed as the 9th largest wheat producing country of the world, with an output of 25.6 million metric tons [2]. Still, wheat production in Pakistan is not at par with the growing population's needs.

One of the several challenges associated with the lower yields and production of wheat and other crops is weed infestation. Weed infestation account for 35% yield losses of wheat, 28% losses of vegetables and 29% losses of rice [3].

Drastic effects of weeds on germination and growth wheat and other crops are due to their competiveness with cultivated crops for resources and allelopathic potentials. Through allelopathy, weeds may cause significant effects on the growth and germination capacity of other crops. Weeds may possess diverse allelochemicals, which can interfere with other plants in a number of ways by either retarding or enhancing the germination and growth of receiving plants [1,4].

In earlier works, reduced germination, radicle and plumule length, dry biomass of wheat and in maize were caused by aqueous extracts of sunflower [4], stimulated growth of wheat seedling at lower concentration of *Chenopodium album* and sugarcane [1,5], drastic effects on germination and growth of wheat by extracts of *Melilotus alba* and *Centaurea maculosa* [6]. *Avena fatua*, *Melilotus officinalis* and *Polypogon hissaricus* are commonly occurring weeds of wheat in many countries of the world, including Pakistan. In order to determine, the allelopathic potential of these weeds against seed germination, growth, dry biomass and chlorophyll concentration of three wheat cultivars namely Ata Habib, Pirsabaq and Serin, this study was undertaken.

2. Materials and methods

Whole plant specimens of three weeds viz. *Melilotus officinalis*, *Avena fatua* and *Polypogon hissaricus* were collected from the vicinity of Khyber Pakhtunkhwa Agricultural University and cultivated fields in

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Peshawar. Weeds were identified at the herbarium of the department of Weed Science, The University of Agriculture Peshawar. Seeds of three varieties (Pirsabaq-2015, Atahabib-2010 and Serin) were obtained from Pirsabaq Research station, Nowshera.

2.1. Extract preparation

The collected weed samples were washed to remove the dust and other impurities, and were identified in the herbarium of the department of Weed Science, The University of Agriculture Peshawar. The plant materials were dried in an oven at 70 °C for 48 h. Then the dried material were ground in a grinder and passed through a 40 mesh screen. Different concentration, e.g., 5, 10, 15 g/l aqueous extracts were obtained by dissolving the appropriate amount of dried powders of each weed species in distilled water.

2.2. Laboratory assay

Seeds of three cultivars of wheat and were placed on twice folded Whatman #1 filter paper as seed beds in petri dishes. Each petri dish was provided with 10 ml of different aqueous extract concentration (5, 10 and 15 g/l) of three weeds. Each petri-dish was replicated five times each containing 10 seeds. Distilled water was used as control. The dishes were incubated at 25oC in a Completely Randomized (CR) Design.

2.2.1. Pot experiment

Pot experiment was performed at the net house of Department of Weed Science, Agriculture University Peshawar during October 2016. Five seeds of each of three cultivars of wheat were grown in plastic pots of equal size containing loamy soil. Each pot was provided with 500 ml of 5, 10 and 15 g/l aqueous extract concentration of three weeds. Provision of aqueous extract to pots was done twice a week. Same amount of tap water were provided to control seeds. The pot experiment was designed in complete randomized block design, each pot with five replications. Germination percentage was determined in lab bioassay only while root and shoot length, seedling dry biomass, moisture content of seedling and leaf chlorophyll concentration (SPAD-502 Chlorophyll meter, Japan) were determined in pot experiment. Results were statistically tested through analysis of variance (ANOVA) while LSD test was performed to measure significant effects of the treatments.

3. Results

3.1. Germination

Water treated control seeds showed 100% germination in all varieties of wheat. Significant differences were observed for germination of three varieties of wheat under the effects of different extract concentrations of *Avena fatua*, *Melilotus officinalis and Polypogon hissaricus*; however, complete failure of germination was recorded in Pirsabaq at 15 g/l extract of *M. officinalis* followed by cultivar Ata Habib where only 20% germination occurred at the same amount of extract by that weed (Table 1). Cultivar Serin was the least effected wheat variety by allelopathic extracts of *P. hissaricus* while Ata Habib and Pirsabaq showed differential responses to the applied extracts of all the weeds. In general, higher concentration of the three weed species were more growth inhibitory than lower extract concentration.

Table 1
Effect of different extract concentration of *M. officinalis*, *A. fatua* and *P. hissaricus* on the germination of three wheat varieties.

Extract concentration	Cultivars			Concentration means
	Pirsabaq	Serin	Ata habib	
Melilotus officinalis				
Control	100	100	100	100a
5 g/l	40	80	80	66.6b
10 g/l	30	60	50	46.6c
15 g/l	0	50	20	23.3d
Cultivars means	42.5c	72.5a	62.5b	
Avena fatua				
5 g/l	70	100	100	90ab
10 g/l	80	90	70	80b
15 g/l	40	60	50	50c
Cultivars means	72.5b	87.5a	80a	
Polypogon hissaricus				
5 g/l	70	95	77	80.6b
10 g/l	40	90	70	66.6cd
15 g/l	40	70	60	56.6d
Cultivars means	62.5b	88.75a	76.75c	

3.2. Root length (RL)

In control pots, root length (RL) for variety Pirsabaq was recorded as 14.5 cm, Serin 19.8 cm and Ata Habib 16.9 cm. 10 g/l extract concentration of each weed species imparted differential effects on the RL of three varieties of wheat. Compared to control, extract of *M. officinalis* had no effect on Pirsabaq where slightly lower, but the insignificant RL (12.9 cm) was observed; however, in Serin, RL was reduced significantly to 13.8 cm against 19.8 cm of control. Ata Habib did not show any response to *M. officinalis*. Extracts of *Avena fatua* promoted RL of cultivar Pirsabaq to 17.1 cm when compared to control 14.5 cm. Significantly lowered RL (12.7 cm) was observed in the variety Serin while the weed extract had no effect on this parameter of Ata Habib. Extract of *Polypogon hissaricus* stimulated RL of Pirsabaq to 16.9 cm, reduced it to 14.5 cm in Serin. Slight decrease was observed in Ata Habib where the studied attribute under the influence of extract of *P. hissaricus* was 15.3 cm when compared to control 16.9 cm (Fig. 1).

3.3. Shoot length (SL)

Compared to control, allelopathic effects of the three weeds were significant on shoot length (SL) of test cultivars. Three varieties showed an almost similar tendency in terms of SL in control treatment where they ranged from 14 to 18.2 cm. Extract of *M. officinalis* significantly declined shoot length in Pirsabaq where it was recorded as 10.4 cm when compared to control 14 cm. Almost 50% reduction in SL (9.8 cm) was found in Serin when results were compared with control 18.2 cm. In variety Ata Habib, compared to control (14.3 cm), *M. officinalis* resulted in lower SL of 11.7 cm. Under the respective treatment of aqueous extracts of *Avena fatua*, cultivar Pirsabaq showed almost similar SL of 14.9 cm but Serin had significantly lowered 9.8 cm length while Ata Habib also showed decline SL of 11.7 cm. Extract of *P. hissaricus* had no significant influence on cultivars Pirsabaq and Ata Habib where SLs were recorded as 14.5 and 14 cm respectively. Significantly lowered shoot length 12.6 cm was caused by the extract in Serin which was much lower than control 18.2 cm (Fig. 2).

3.4. Moisture content (MC)

Under control conditions, maximum moisture content (MC) was recorded for variety Pirsabaq (61.1%) followed by Serin (58.7%) while lower MC (49.8%) was obtained in Ata Habib. Extract of *M. officinalis* slightly reduced MC of variety Pirsabaq (53.3%) but increased in Serin (60%) and Ata Habib (65%). Extract of *A. fatua* had slight inhibitory effect on Pirsabaq and Serin but stimulated MC of Ata Habib where they were recorded as 50, 56.2 and 60% respectively. Similarly, extract of *P. hissaricus* had both stimulatory as well inhibitory effects on MC of the three cultivars. In variety Pirsabaq, MC was reduced to 56.6% and in Serin to 53.8% against control 61.1 and 58.7% respectively. However, in

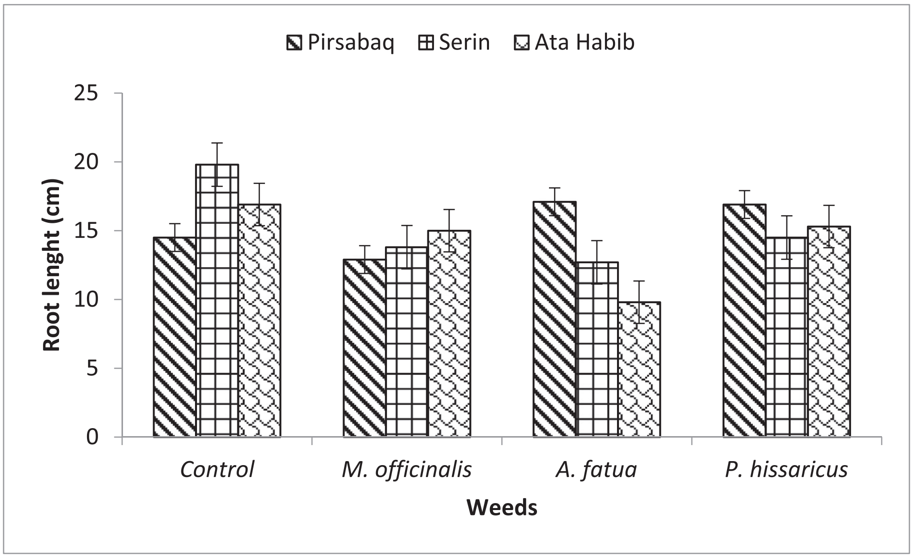

Fig. 1. Effect of 15 g/l aqueous extracts of three weeds on root length of three wheat cultivars.

variety Ata Habib, MC was increased to 63.6% when compared to 49.8% in control (Fig. 3).

3.5. Dry biomass (DBM)

Results indicated that dry biomass (DBM) of seedling did not vary significantly in cultivar Pirsabaq under the applied allelopathic extracts of three weeds and results were almost similar to control. DMB in Pirsabaq was slightly stimulated by *A. fatua* and *P. hissaricus* which revealed 0.1 and 0.8 g respectively as compared to control (0.07 g). In Serin, maximum DBM (0.18 g) was recorded in control followed by significant decline at extract treatment of *P. hissaricus* (0.06 g), *A. fatua* (0.07 g) and *M. officinalis* (0.08 g). Cultivar Ata Habib was the most severely influenced by extract of the weeds. Compared to control (0.16 g), allelopathic extract of *M. officinalis* caused decline in DBM to 0.06 g while the other two weeds reduced the studied parameter to 0.04 g respectively (Fig. 4).

3.6. Chlorophyll concentration

In control pots, highest chlorophyll concentration (23 μg cm^{-2}) was recorded in variety Ata Habib followed by Serin (11 μg cm^{-2}) while lowest values (5.5 μg cm^{-2}) were observed in Pirsabaq. Extract of *M. officinalis* significantly increased leaf chlorophyll concentration in varieties Pirsabaq and Serin to 20 and 17.2 μg cm^{-2}respectively when compared to control treatments (Pirsabaq, 5.5 μg cm-2 and Serin, 11 μg cm^{-2}). However, chlorophyll concentration decreased to 16.5 μg cm^{-2} from 23 μg cm^{-2} in variety Ata Habib. Like *M. officinalis*, the other two weeds had a stimulatory effect on chlorophyll in varieties Pirsabaq and Serin but had a negative influence in variety Ata Habib. *A. fatua* increased leaf chlorophyll from 5.5 to 9.5 μg cm^{-2} in cultivar Pirsabaq and from 11 μg cm^{-2} to 17.2 μg cm^{-2} in cultivar Serin but declined the parameter under study from 23 to 12.7 μg cm^{-2} in cultivar Ata Habib. *P. hissaricus* also showed a similar tendency to *A. fatua*. It slightly increased leaf chlorophyll in Pirsabaq from 5.5 to 5.7 μg cm^{-2}

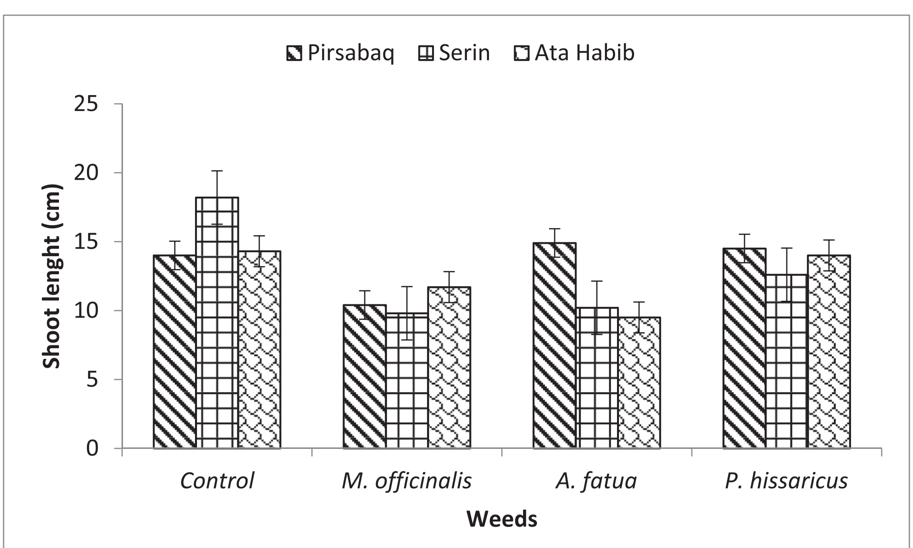

Fig. 2. Effect of 15 g/l aqueous extracts of three weeds on shoot length of three wheat cultivars.

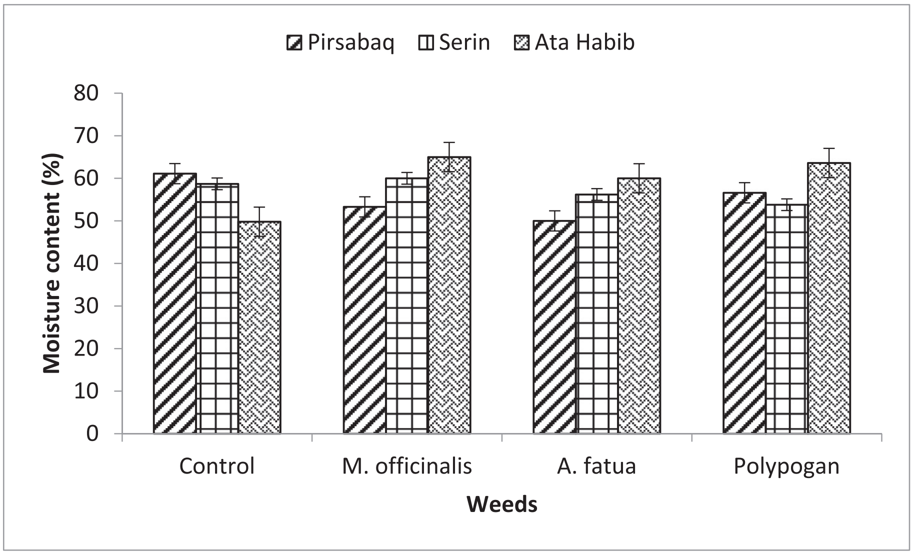

Fig. 3. Effect of 15 g/l aqueous extracts of three weeds on moisture content (%) of three wheat cultivars.

and in the variety Serin from 11 to 14 μg cm^{-2} but reduced the studied parameter in Ata Habib from 23 to 16 μg cm^{-2} (Fig. 5).

4. Discussion

Reduced germination in three varieties of wheat as a result of the allelopathic aqueous extracts of three weeds indicated that they possess allelochemicals which exhibited phytoinhibitory effects on the test crops. Cultivar Ata Habib was found to be the most sensitive variety to the applied allelopathic stress than the other two cultivars which might be due to different genomic characteristics of the cultivars. Moreover, higher concentration (15 g/l) caused maximum inhibition in germination of wheat. The present results are in agreement with studies conducted by [7–10], who reported that aqueous leaf, stem and root extracts of different concentration of *Prosposis juliflora*, Sunflower, *Euphorbia helioscopia* and *A. fatua* caused lower germination percentage of wheat, lentil and chickpea. Reduced germination in wheat (*Triticum durum*) was also recorded by [11] as a result of aqueous leaf extracts of *Euphorbia hierosolymitana* which support the result of present study. Reduced germination in wheat due to allelopathic stress of different weeds might be due to different abnormalities in metabolic activities, imbibition potential of seeds, death of some of the dividing cells and embryonic abnormalities [12–14].

Root and shoot length in this study decreased differently when aqueous extracts of three different weeds were applied; however, the effect was different in different cultivars. These results are in close agreement with findings of [15] who revealed that aqueous extracts of different plant parts of *Parthenium hysterophorus* had strong inhibitory effects on germination, root and stem length of four wheat cultivars. However, the results disagree with studies of [16] who revealed that different concentration of aqueous extracts of *Jatropha curcas* caused increase in root and stem length of maize (*Zea mays*). The results are also in contradiction with finding of [17] which reported that aqueous leaf, stem, root and flower extracts of *Parthenium hysterophorus* promoted seedling growth of *Eragrostis tef*. It may be inferred that reduced length of root and shoot of wheat in this experiment at the applied allelopathic extracts of different weeds might be due to the presence of phytotoxic compounds in those weeds species which were water

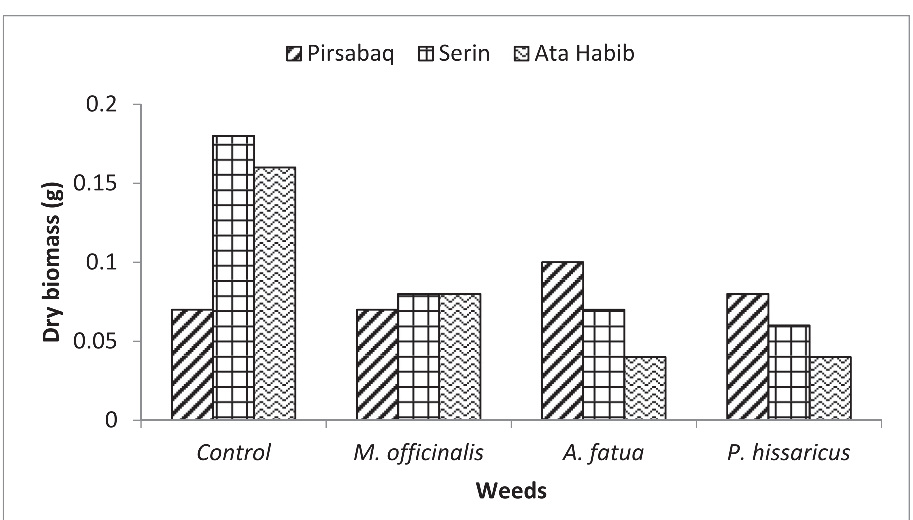

Fig. 4. Effect of 15 g/l aqueous extracts of three weeds on dry biomass of three wheat cultivars.

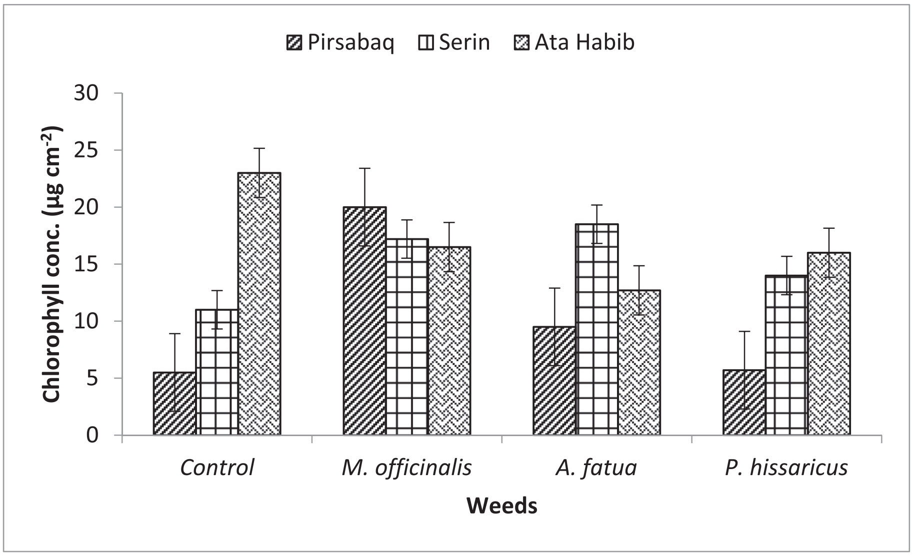

Fig. 5. Effect of 15 g/l aqueous extracts of three weeds on chlorophyll concentration of three wheat cultivars.

soluble and exhibited inhibitory effects on the studied parameters. Since roots are sensitive to any chemical changes in their surroundings, they may respond more quickly. Reduced length in root and shoots might be due to reduced cell division and abnormalities in growth hormones [18]. Allelopathic stress may also cause changes in pH, changes in osmotic capacities of seedling, cell injury, changes in membrane permeability, reduced mineral uptake and water absorption potency of radicle which could result in decreased stem growth [19–22].

Dry biomass of wheat seedling was lowered by extract treatment of different weeds. This reduced biomass might be due to lower photosynthetic activity in seedling. Moreover, lower root and shoot length could have contributed to lower fresh and dry weight of shoot and root. These results are in close conformity with the study of [9] who documented reduced weight of wheat and other crops to allelopathic stress of *Euphorbia heliscopia*. Similarly, in other studies, significantly reduced dry biomass of *Parthenium hysterophorus* in response to 50 and 100% concentrated aqueous extracts of sunflower, rice and sorghum has been established [23]. [24] have also shown that aqueous leaf and stem extract of bindweed (*Convolvulus arvensis*) exhibited negative effects on fresh weight and root/shoot length of maize at higher extract concentration. [25] found that aqueous extracts of *Eclipta alba* at different concentration (0.5–4%) decreased root and shoot length and dry biomass of rice.

Results of this study revealed that moisture content of wheat seedling of three cultivars was not affected extract of the weeds. These results are in disagreement with [26] who observed reduced moisture content, fresh and dry weight of wheat seedling to the allelopathic aqueous extracts of sunflower. This might be due to different cultivars and nature of allelopathic substances used in their study.

Chlorophyll concentration in different wheat varieties showed both increasing and decreasing tendency towards allelopathic stress of three weeds. In previous studies, a reduced concentration in leaf chlorophyll *a* contents, seedling growth of lentil was observed when aqueous shoot extracts (0.5–2%) of *Xanthium strumarium* were applied [27]. They attributed reduced chlorophyll concentration of lentil to cellular damage and biosynthetic abnormalities in photosystem triggered by allelochemicals. Aqueous extracts of *Tithonia diversifolia* decreased root and shoot length, chlorophyll *a*, *b* and total chlorophylls but enhanced fresh and dry weight and leaf area of *Zea mays* [28]. On the other hand, [29,30] revealed that chlorophyll contents of wheat varieties (Chakwal 97 and Margala 99) increased with root allelochemicals of sunflower which is in accordance with result of this study. [31] observed significantly reduced chlorophyll contents of maize at 100% aqueous extract of *Malva parviflora* and *Chenopodium murale*. [31] showed that aqueous extracts of maize (3–10%) significantly increased chlorophyll *b* but non-significantly decreased chlorophyll *a* of wheat. It is possible that certain allelochemicals (depending on their type and concentration) may contribute to enhance or reduced synthesis of chlorophyll pigments, their maturation and proper functioning. Thus, different response observed in chlorophyll concentration in this study may be attributed to stimulatory or inhibitory effects, reduced mineral uptake and deformities in chlorophyll regulatory system. [32] argued that certain allelochemicals impart negative effects on the quantum efficiency of photosystem 2. [33] suggested that allelopathic agents cause retardation in net assimilate of photosynthesis, while increase the peroxidase activity in roots. [34] recorded reduced chlorophyll contents of wheat and other crops as a result of 50% concentrated aqueous extracts of *Calotropis procera* and they attributed the reduction pigment contents to reduced synthesis and degradation of necessary molecules for chlorophylls.

Avena fatua contains different allelochemicals such as syringic acid, tricin, acacetin, syringoside, and diosmetin which have been documented with inhibitory potential on seedling growth of wheat [35]. Similarly, *Melilotus* species particularly *Melilotus messanensis* possess lupanetriterpenes, steroids and phenolic compounds which have known allelopathic activities against germination and growth of *Triticum aestivum* and some other crops [37]. [36] isolated coumarin and identified it as a major allelochemical in *Melilotus officinalis* with strong inhibitory effects against different weeds such as *Chenopodium album*, Poa and others. Inderjit and Dakshini suggested the presence of phenolic compounds in *Polypogonmon speliensis* which suppressed germination and growth of cluster bean and radish. [38] summarized their results and stated that allelochemicals released from *Cardaria draba* and *Alhagi maurorum* had reduced the uptake of macro and micronutrients by wheat. Thus, reduced uptake of necessary nutrients may correspond to decreased germination, seedling growth, fresh and dry biomass and chlorophyll concentration because some macro and micronutrients are required in proper amounts for normal germination, growth and physiological processes of plants. Under stress condition imposed by allelochemicals or other factors, proline contents and its allied enzymes may show either increase or decrease which will eventually bring about changes in osmotic potentials, photosynthesis and stomata conductance [39]. Depending on the changes in these processes, plants may show either positive or negative responses to the allelochemicals stress.

5. Conclusions

From the results of this study, it is concluded that germination percentage of three varieties of wheat varied under different concentration of aqueous extracts of three weeds. Compared to control, germination % of cultivar Pirsabaq was greatly reduced by 15 g/l aqueous extract of *Melilotus officinalis*. Similarly, in pot experiment, root length, shoot length, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight were significantly reduced in all the three cultivars of wheat by all aqueous extract of three weeds. However, maximum inhibition in these parameters was observed in Ata Habib variety under the allelopathic stress of *A. fatua*. Serin variety was the second most severely affected wheat cultivar while the weeds were recorded in their phytoinhibitory effects as *A. fatua* > *M. officinalis* > *P. hissaricus*. Moisture contents of the wheat varieties were neither affected by allelopathic extracts of weeds nor by the type of cultivars. On the other hand, chlorophyll concentration was increased in Pirsabaq and Serin by extract of *M. officinalis*, *A. fatua* and *P. hissaricus* but was significantly decreased in variety Ata Habib under the respective weeds extract. Thus, Ata Habib was the most sensitive while Pirsabaq and Serin varieties were comparatively tolerant to the allelopathic stress. Although all the weed species were found allelopathic and phytotoxic; however, *A. fatua* and *P. hissaricus* resulted in maximum growth inhibition than *M. officinalis* except germination percentage where it was more phytotoxic than the other two weeds.

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