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Research Article

Allelopathic Effects of Avena Fatua L. on Wheat Cultivars of District Kohat, KPK, Pakistan

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ABSTRACT

Avena. fatua L commonly called wild oat has been increasing enormously both in irrigated and rain fed regions of Pakistan and in other parts of the world as well. It is annual grassy weed and difficult to eliminate due to their seeds shattering before crop maturation. The study aims at evaluating its damages in selected wheat varieties (KT-2000 and LOCAL) that are cultivated in Kohat region of Khyber Pakhtunkhwa (KP) Pakistan. Several crops have been screened for their antibacterial activities to use them as antimicrobial agents such as Cabbage (Brassica oleracea L.) and Garlic (Allium sativum L.) Among crops wheat is an important constituent of the traditional diet all around the world so we also need to investigate the antibacterial activities of wheat. Antibacterial studies of weed induced wheat varieties were also carried out on two bacterial strains i.e. Escherichia coli and Shigella sonnei by using agar well diffusion method. Wheat seedlings treated with different concentrations of aqueous extracts of weeds (25%, 50%, 75% and 100%) was compared with control seedlings. The increasing concentrations of weeds resulted in significant decreases in the root and shoot lengths of wheat varieties along with their fresh and dry biomasses. Chlorophyll a and b, carotenoids' content and total soluble proteins (TSP) were also negatively affected depending on weeds' concentrations. Similarly, the medicinal potency of wheat seedlings was affected adversely specially at higher concentration of weeds' treatments. we can conclude A. fatua adversely affected the morphological, physiological and biochemical characteristics of wheat seedlings However, on the other side, the concentration of soluble sugars was significantly and positively influenced by the application of weed extracts' treatments, which shows accumulation of sugars in both roots and shoots of the seedlings of both wheat varieties. Soil profile of both wheat varieties was insignificantly altered as all the treatments showed no major differences as compared to control [1,2].

Keywords: Allelochemicals; Herbicides; Weeds management; Antibacterial potential, Biochemical; Weeds soil profile.

INTRODUCTION

Allelopathy is a term to mean all the biochemical interactions (stimulatory and inhibitory) among plants (M. J. Reigosa, et al. 2006). Biochemical interactions between plants (allelopathy) are a product of the actions of a various group of compounds that are synthesized by plants and microorganisms. These compounds are called allelochemicals. Most plants have both inhibitory and stimulatory allelopathic effects on the associated plant species in terms of germination, growth, development, and phytochemicals. More specifically, weeds are among those plant species, which can influence crops including wheat through the production of certain allelochemicals.

A. fatua L. commonly called wild oat has been increasing enormously both in irrigated and rain fed regions of Pakistan and in other parts of the world as well. It is annual grassy weed and difficult to eliminate due to their seeds shattering before crop maturation. Their seeds are shed and remain in soil, where

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they can stay dormant up to several years, before they germinate (M U. S. Walia et al., 1998). A. fatua and Rumex crispus L. were found to have the highest density having a value of (75.96) and (67.87), respectively as compared to other weeds species found among the wheat fields of Kohat (M. A. Khan et al., 2013).

Pakistan is the 4th largest producer of wheat in Asia and stands 11th in the world among wheat producing countries (Space and Upper Atmosphere Research Commission 2016). However, wheat production is declining every year and weeds are considered to be a major reason of low yield across the world. Similarly in Pakistan wheat production is 30-35% lower than the potential yield for which weeds are one of important but less noticed constrain (S. B. Gul et al., 2012).

Similarly, crops are a chief source of antimicrobial agents due to the secondary metabolites such as, flavonoides, alkaloids, terpenoids and tannins that are present in these crop plants (F. A. Einhellig 1985). Several crops have been screened for their antibacterial activities to use them as antimicrobial agents such as Cabbage (Brassica oleracea L.) and Garlic (Allium sativum L.) Among crops wheat is an important constituent of the traditional diet all around the world so we also need to investigate the antibacterial activities of wheat.

Two wheat cultivars “KT-2000” and “LOCAL” are commonly cultivated in the Kohat region of Pakistan (S. G. Khan, BARS Kohat, 2017). Avena fatua (wild oat) is the most abundant weed species found in these wheat (Triticum aestivum L.) fields (R. Ahmad, A. S. Shaikh 2003). Weeds have the potential to alter the biochemical, phytochemical and antibacterial properties of plants species growing in its vicinity (J. J. Muzik 1970). However, such information is very scarce related to the above mentioned weed species and wheat cultivars of Kohat region. It is hypothesized that A. fatua may induce changes in the biochemical and antibacterial potential of T. aestivum.

It is hypothesized that A. fatua may induce changes in the biochemical and antibacterial potential of T. aestivum. Hence, this study aims to assess the morphological physiological and biochemical profile as well as in-vitro antibacterial activities of allopathically exposed wheat varieties. The outcomes of this study would have significant contribution towards weeds' eradication, higher grain yield, and increasing medicinal potency of wheat crop [3,4].

MATERIALS AND METHODS

Collection and Sterilization of seeds

The seeds of two wheat cultivars “LOCAL” and “KT-2000” were procured from Barani Agricultural Research Station Jarma, Kohat Khyber Pakhtunkhwa, Pakistan. Vigorous seeds of (Wheat cv. “LOCAL” and “KT-2000”) were sterilized using 70% (v/v) ethanol up to 5 minutes and rinsed 2 to 3 times by distilled water. These sterilized seeds were placed inside the incubator at 30-35oC for 24 hours.

Preparation of Weed Extracts

A. fatua plants were collected from the wheat fields of district Kohat (Khyber Pakhtunkhwa) and the plants were identified by the taxonomist at the Department of Botany, Kohat University of Science and Technology. Plants of A. fatua L. were air-dried in shade and then cut into small pieces. To prepare extracts 100 g plant material of A. fatua L. was soaked in 1000 ml (1 liter) distilled water for up to 24 hours and standard solution was obtained. Four different concentrations were prepared by using the standard solution i.e. (100%). The different concentrations were labeled as 25%, 50%, 75% and 100%. Control 0% was treated with distilled water.

Germination and Growth Conditions

For experimentation sterilized Petri dishes were used. About 200 g of sterilized soil was added to each petri dish. There were three replicates for every treatment (each replica having 10 seeds). Each petri plate was treated with suitable solution of about 10 ml daily. All replicates were placed in dark growth chambers by maintaining the temperatures at 28 ± 02 0C for two days until germination. Petri plates were placed in Light conditions at 3rd day, with relative humidity of 60 %. Germination was observed on daily basis. The emergence of radical of a seed was considered to be germinated [5].

Roots and Shoots Length Measurements

Wheat seedlings were harvested after 10 days of growth, and their lengths measured after being separated in shoots and roots. for measurement of length, Centimeter (cm) was used as standard unit.

Determination of Fresh and Dry Biomass of Shoot and Root

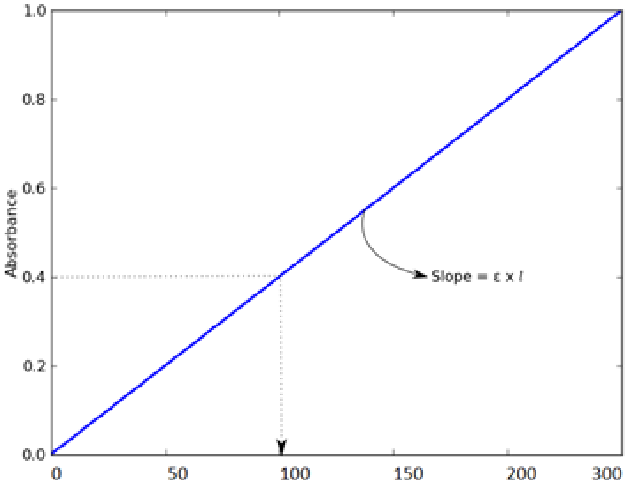
To quantify fresh biomass, roots and shoots were cut into individual part and weighted separately on electronic weight balance in gram (g) unit. For dry biomass, freshly cuted out shoots and roots were placed in an oven up to 72 h at a temperature of 68 oC and then weighted.

Biochemical Assays

Shoots and roots were analyzed for different biochemical parameters such as content of photosynthetic pigments (chlorophyll a, b and total carotenoids) in leaves, total soluble sugars (TSS) and total soluble proteins (TSP) content of seedlings.

Estimation of Photosynthetic pigments

Sample of (25 mg) from dried plant matter was added to a test tube. To deactivate plant acids and to avoid the synthesis of pheophytin, 25 mg of MgO (Magnesium Oxide) was also added to it. After that, methanol (5 ml) was added in each sample. The mixture obtained was homogenized for 2 hours on orbital shaker. This turbid plant solution was moved to a 5 ml Conical-Bottom Glass Centrifuge Tube and subjected to centrifugation of 4000 rpm at room temperature for 5 minutes. Afterwards, the supernatant obtained was shifted to type 1 cuvette (1 cm path length) with the help of a pipette and subjected to absorbance reading in a compact UV-2600 spectrophotometer against a solvent blank for three different wavelengths: 470 nm, 653 nm and 666 nm. For the calculation of chlorophyll "a" chlorophyll "b" and total carotenoids standard methodology of (Lichtenthaler and A. R. Wellburn 1983) was followed using the formula given below [6].



Estimation of Total Soluble Sugars

The total soluble sugars were determined by following the standard methodology as shown (R. Shields and W. Burnett 1960) after slight modifications. Dried sample of shoots and roots (50 mg) was added and blend in mortar and afterwards extracted two times with hot 90% ethanol (3 ml) for about 1 hour at a temperature of 60-70oC in an incubator. This extract was transferred to volumetric flask (25 ml). By further addition of 90% ethanol, the final volume of 25 ml was prepared. Then 1 ml of 5% phenol and 1 ml prepared solution (extract) was added to thick walled test tubes, 5 ml of commercial grade (95-98% H_2SO_4) sulphuric acid was also added and then mixed properly. For the process of exothermic chemical reaction to take place the test tubes were left as such to cool down. Absorbance was taken at 485 nm wavelength. The amount of total soluble sugars was measured against a curve of 1 percent standard glucose solution. The quantity of sugar content was given as mg g-1 DW-1 [7].

Total Soluble Protein (mg g-1 DW) = C x V/VT x W

C=Absorbance value

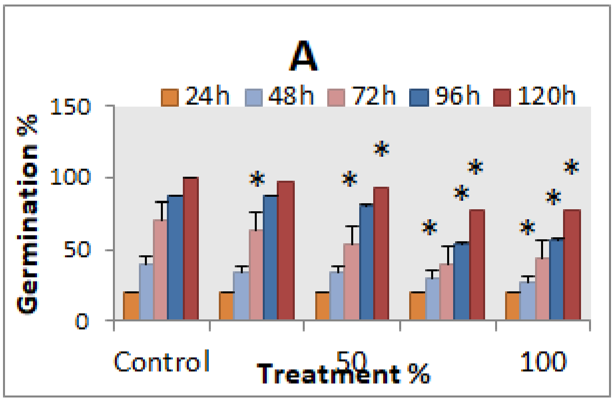
V=Volume of phosphate buffer

VT=Volume of enzyme extract

W=Plant weight

Estimation of Total Soluble Proteins

The amount of Soluble proteins were determined by the standard methodology of (M. M. Bradford 1976). Dry Shoots of (100 mg) were taken and homogenized by using mortar and pestle in (1 ml) phosphate buffer pH 7.0. The homogenized material was subjected to centrifugation for 15 minutes at 4000 rpm. Afterwards 20 µl extract, distilled water (2 ml) and Bradford reagent (0.5 ml) was added to type 1 cuvette (1 cm path length). Absorbance reading was recorded at 595 nm by compact UV-2600 spectrophotometer. Bovine serum albumin (BSA) was used as a standard solution. Finally total soluble proteins were estimated by using the following formula.



Determination of Antibacterial Activities

ATCC strain of E.coli and S. sonnei was obtained from the Department of Microbiology, KUST. Antibacterial potential of wheat extracts was carried out through agar well diffusion method coupled with antibiotic susceptibility discs as described by Kirby et al. (W. M. Kirby et al., 1957).

Determination of Zone of Inhibition (ZOI)

The inoculum was freshly prepared and swabbed all over the surface of the (MHA) Agar plate with the help of sterilized cotton swabs. Five (5 mm) diameter well were bored in culture medium (MHA) using of sterile cork-borer with 5 mm diameter. Petri plates were labeled appropriately and 25 µl of the suspension/solution of wheat grass extract and the equal volume of extracted solvent form antibiotics was added to the wells using micropipette. The plates were kept for some time until the extract diffused properly in the medium. Petri plates were lid closed and were incubated in oven for 24 hours at a temperature 37°C. After 24 hours plates were observed for zone of inhibition. Millimeter was used as unit for measurement of zone of inhibition.

Soil Sampling and Analysis

From each replica of treatments, a total of 42 soil samples were collected. Total Organic matter (OM) and nitrogen (N) content was determined by using the methodology of Hussain (Hussain, A. 2012). For the determination of pH, by using pH meters firstly soil-water extracts were prepared [8].

Statistical Analysis

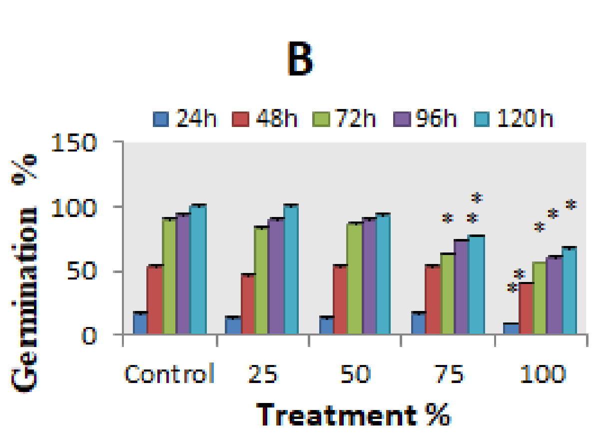
Mean and standard deviation of the three replicates for each parameter were calculated using Microsoft Excel. The difference between the control and treatment was considered to be significant if p value was smaller than 0.05 ($p < 0.05$).

RESULTS

Effect of Weeds on the Germination of Wheat Varieties

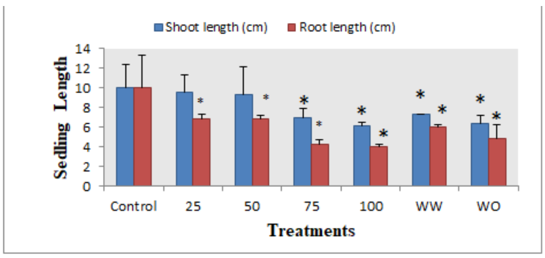
Germination percentage decreased gradually with increasing weed extract concentration from 0-100 in KT-2000 variety. Germination percentage was significantly lowest ($p < 0.05$) at 100% weed's extract concentration (76.6% germination) and 75% weed's extract concentration showed same results (76.6% germination) after 120 hours. Germination percentage was not significantly affected at the lower concentrations i.e. 25% and 50%. Similar results were found at 48 hours, 72 hours and 96 hours (Figure 1).

Figure 1 (A): Germination percentage of kt-2000 (A) and local (B) wheat variety against different weeds concentrations. Values are means of three replicates. * Represents statistically significant results (T-Test at $p < 0.05$) of a particular treatment as compared to its counterpart control.



In Local wheat variety, lowest germination percentage of 66% was observed at 100% weed's extract concentration followed by 76.6% germination recorded at 75% weed's extract concentration after 120 hours. Similar trends were observed in 48, 72 and 96 hours (Figure 2).

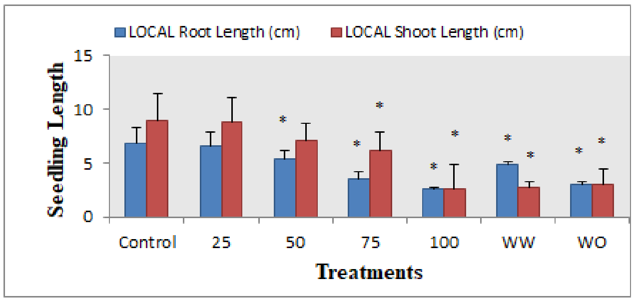
Figure 2: Germination percentage of kt-2000 (A) and local (B) wheat variety against different weeds concentrations. Values are means of three replicates. * Represents statistically significant results (T-Test at $p < 0.05$) of a particular treatment as compared to its counterpart control.



Effect of Weed Extract on Morphological Parameters

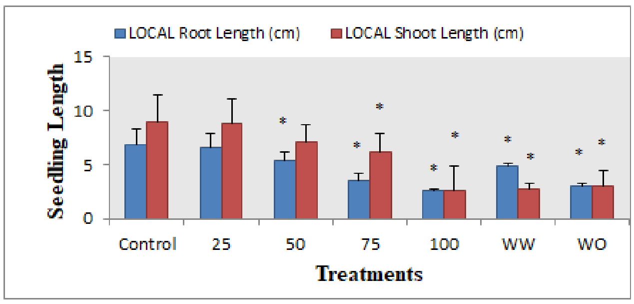
Root and Shoot Length: Root lengths of variety KT-2000 at 25%, 50%, 75% and 100% weed extracts concentrations were recorded as 6.85 cm, 6.78 cm 4.23 cm and 3.96 cm, respectively, which were significantly less than the control (10.01 cm) (Figure 3). On the other hand shoot length of the variety KT-2000 observed in control was 10.033 cm which decreased significantly only at higher doses (75, and 100% weed extract) i.e. 6.97 cm and 6.11 cm respectively. Beside these, the treatments (weeds + weedicide and weedicide only) also show significant reduction in both shoot and root lengths (Figure 3) [9,10].

Figure 3: Seedlings' lengths (R, S) of KT-2000 under Weed Stress. Values are Means of three Replicates. WO Represents Weeds Only While WW Shows Weeds and Weedicides. * Shows Statistically Significant Results (T-Test at $P < 0.05$) of a Particular Treatment as Compared to its Counterpart Control Treatment.



Similarly root length of LOCAL wheat variety shows significantly lower results in all of the treatments as compared to the unchecked control treatment, however at lower concentrations i.e. 25% weeds' extract concentration no significant results were recorded. In LOCAL wheat variety root length reduced from 5.33 cm to 3.55 cm and 2.59 cm at 50%, 75%, and 100% weeds' extract concentration respectively. Shoot length decreased significantly from 8.96 cm in control to 7.1 cm at 50% weeds' extract concentration and 6.2 cm at 75% weeds 'extract concentration. The lowest 2.59 cm was measured for 100% weeds' extract concentration. While treatments (weeds + weedicide and weedicide only) also show significant reduction in both shoot and root lengths as shown in (Figure 4).

Figure 4: Seedlings' lengths (R, S) of LOCAL Variety under Weed Stress Values are Means of three Replicates. WO Represents Weeds Only While WW Shows Weeds and Weedicides. * Shows Statistically Significant Results (T-Test at $P < 0.05$) of a Particular Treatment as Compared to its Counterpart Control Treatment.



Effect of Weed Extracts on the Biomass (Fresh and Dry Weight) of Both Wheat Varieties

For KT-2000 Statistical analysis of the data showed that A. fatua L. exhibited significant effect on wheat's biomass. Results displayed an obvious decreased in the fresh and dry weight of roots and shoots. The data (Table-1) shows that, the minimum (0.78 g) fresh weight and (0.39 g) dry weight was recorded from 100% weeds' extract concentration. At 75% weeds' extract concentration root fresh and dry weight were (0.83 g and 0.42 g) respectively, showing significant decrease.

Table 1: Biomass 'Fresh and Dry' (S, R) of both Wheat Varieties under Weeds' Stress. Values are the means ± S.D of three replicates. WO Represents Weeds Only While WW Shows Weeds and Weedicides. * Shows Statistically Significant Results (T-Test at P < 0.05) of a Particular Treatment as Compared to its Counterpart Control Treatment.

	KT-2000	LOCAL						
Treatments (%)	Shoot (g)		Root (g)		Shoot (g)		Root (g)	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Control	2.09± 0.10	1.07± 0.05	1.75± 0.24	0.84± 0.15	1.01± 0.45	0.75± 0.33	1.11± 0.15	0.53± 0.09
25%	1.89± 0.54*	1.02± 0.26	1.74± 0.57	0.88± 0.31	0.82± 0.45	0.61± 0.33	1.11± 0.36	0.50± 0.11
50%	1.86± 0.26*	0.97± 0.08	1.70± 0.50	0.85± 0.15	0.56± 0.11*	0.41± 0.08*	1.08± 0.32	0.49± 0.04
75%	0.83± 0.15*	0.42± 0.07*	0.69± 0.07*	0.45± 0.15*	0.40± 0.12*	0.29± 0.09*	0.44± 0.04*	0.28± 0.10*
100%	0.78± 0.14*	0.39± 0.09*	0.62± 0.34*	0.34± 0.18*	0.29± 0.11*	0.21± 0.07*	0.41± 0.19*	0.21± 0.11*
WO	0.72± 0.26*	0.38± 0.12*	0.76± 0.19*	0.39± 0.13*	0.33± 0.03*	0.24± 0.02*	0.48± 0.11*	0.24± 0.08*
WW	0.70± 0.12*	0.34± 0.10*	0.70± 0.14*	0.39± 0.02*	0.63± 0.15*	0.46± 0.12*	0.44± 0.09*	0.24± 0.01*

Similarly Root fresh and dry weight was recorded (1.75 g, 0.84 g) in control, showed significant decrease at 75% and 100% weeds' extract concentration i.e. 0.69 g and 0.62 g respectively.

Increasing concentration of treatments also caused decrease in both fresh and dry weight of root and shoot but only at higher concentration across treatments. For shoot, lowest (0.29 g) fresh and (0.21 g) dry weight was observed in 100% weeds' extract concentration while for root the same was (0.41 g) and (0.21 g) while at 75% weeds' extract concentration (0.40 g) shoot fresh (0.29 g) shoot dry (0.44 g) root fresh and (0.28 g) root dry weights were recorded as significant results.

The treatments (weeds + weedicide and weedicide only) also showed adversely significant results as compared to control shown in (Table 1).

Effect of Weed Extract on Biochemical Parameters

Effect of Weeds' Extract on Photosynthetic Pigments (Chlorophyll a, Chlorophyll b and Total Carotenoids): For KT-2000 Outcomes of different concentrations treatments were significant at higher concentration of weeds' extract i.e. 75% and 100%. The amount of chlorophyll a reduced significantly from 20.42 mg/g in control to 15.18 mg/g 75% and 8.22 mg/g 100% weeds' extract concentration respectively. Similarly, the amounts of (chlorophyll b and carotenoids) in control were observed 6.85 mg/g and 7.09 mg/g while lowest was 2.09 and 1.70 mg/g observed in 100% weed extract.

For LOCAL chlorophyll content was not significantly decreased up to 50% weeds' extract concentration but thereafter decrease was more pronounced. Chlorophyll a content decreased approximately from 10.6 mg/g in control to 16.8 mg/g in 50%, 16.6 mg/g in 75% and 16.4 mg/g in 100% weeds' extract concentration. Chlorophyll b was not as sensitive to weeds' stress and decreased significantly at 75% and 100% as compared to Chl a. On the other hand, carotenoids content was most affected at higher concentrations and declined from 7.97 mg/g in control to at 3.32 mg/g in 75% and 1.91 mg/g in 100% weeds' extract concentration.

The concentration of chlorophyll a, b and total carotenoid in treatment WW and WO also showed significant decrease as shown in (Table 2) and (Table 3).

Table 2: Photosynthetic Pigments of Wheat Leaves under Weeds' Stress of Wheat Variety KT-2000. Values are means ± S.D of three replicates. WO Represents Weeds Only While WW Shows Weeds and Weedicides. * Shows Statistically Significant Results (T-Test at P < 0.05) of a Particular Treatment as Compared to its Counterpart Control Treatment.

Treatment (%)	**KT-2000**	**Chlorophyll (mg/g DW)**	
	Chl a	Chl b	Carotenoids
Control	20.4 ± 0.70	6.85± 0.60	7.09 ± 0.14
25%	16.1 ± 0.22*	4.47 ± 0.06*	6.69 ± 0.09
50%	16.1 ± 1.03*	3.56 ± 0.22*	3.44 ± 0.22*
75%	15.1 ± 0.21*	2.85 ± 0.04*	2.54 ± 0.03*
100%	8.22 ± 0.05*	2.09 ± 0.01*	1.70 ± 0.01
WW	10.8 ± 0.07*	3.82 ± 0.02*	5.48 ± 0.03*
WO	8.44 ± 0.05*	2.73 ± 0.01*	2.89 ± 0.02*

Table 3: Photosynthetic Pigments of Wheat leaves under Weed extract stress of Wheat Variety LOCAL. Values are means ± S.D of three replicates. WO Represents Weeds Only While WW Shows Weeds and Weedicides. * Shows Statistically Significant Results (T-Test at P < 0.05) of a Particular Treatment as Compared to its Counterpart Control Treatment.

Treatment (%)	**LOCAL Chlorophyll (mg/g DW)**		
	Chl a	Chl b	Carotenoids

Control	22.8±0.16	10.6±0.07	7.97±0.056
25%	18.1±0.12*	8.98±0.06	6.280.044
50%	16.8±0.11*	8.52±0.06	6.06±0.042*
75%	16.6±0.11*	8.39±0.05*	3.32±0.023*
100%	16.4±0.23*	8.39±0.11*	1.91±0.027*
WW	16.8±0.35*	8.52±0.18*	0.72±0.015*
WO	16.7±0.11*	8.47±0.05*	0.01±0.011*

Effect of Weeds' on Total Soluble Proteins: Results indicate that protein content in seedlings increase as stress of weed extract increases. The lowest value observed in KT-2000 was in control (9.67 mg/g DW), while in LOCAL the lowest value was also recorded in control but 3% more as compared to KT-2000 (12.75 mg/g DW). Significant increase in values of wheat variety KT-2000 were only recorded at higher concentrations of weeds' extract i.e. 75% and 100% weed extract concentrations (17.06 mg/g, and 17.09 mg/g) respectively.

In wheat variety KT-2000 the treatments (weeds + weedicide and weedicide only) showed similar results with its counterpart control i.e. no significant results (Table 4).

Table 4: Total Soluble Protein of Wheat Seedlings (of Both Wheat Varieties) under Weeds' Extract Stress. Values are the means ± S.D of three replicates. WO Represents Weeds Only While WW Shows Weeds and Weedicides. * Shows Statistically Significant Results (T-Test at $P < 0.05$) of a Particular Treatment as Compared to its Counterpart Control Treatment.

	TSP (mg/g DW)	
Treatments (%)	**KT-2000**	**LOCAL**
Control	9.67±0.068	12.75±0.090
25%	10.17±0.07	12.89±0.091
50%	11.81±0.08	12.98±0.091
75%	17.06±0.12*	13.31±0.094*
100%	17.09±0.12*	13.35±0.094*
WW	9.96±0.070	13.28±0.093*
WO	9.57±0.067	13.11±0.092*

Effect of Weeds on Total Soluble Sugar: The amount of total soluble sugar under weed extract stress increased at higher concentration of weeds' extract in both shoots and roots. Shoots of wheat variety KT-2000 shows maximum value (154.56 mg/g DW) at 100 %, in roots the maximum value was also recorded at 100% weeds' extract concentration i.e. (88.65 mg/g DW) as compared to control (20.11 mg/g DW).

IN LOCAL wheat variety Maximum amount of soluble sugar in shoots and roots were detected at 100% weeds' extract concentration i.e. 175.16 and 100.4 mg/g DW respectively (Table 5).

However the results were clearly adverse in treatments (weeds + weedicide and weedicide only) in both varieties.

Table 5: Total Soluble Sugars of Wheat Seedlings (of Both Wheat Varieties) under Weed Extract Stress. Values are means ± S.D of Three Replicates. * Shows Statistically Significant Results (T-Test at $P < 0.05$) of a Particular Treatment as Compared to its Counterpart Control Treatment.

	TSS (mg/g DW)			
Treatment (%)	**KT-2000**		**LOCAL**	
	Shoot	Root	Shoot	Root
Control	117.21±0.82	20.11±0.14	132.83±1.86	22.79±0.32
25%	119.07±0.84	39.48±0.27*	134.94±1.89	44.74±1.27*
50%	124.78±0.88 *	55.67±0.78*	141.41±4.03 *	63.09±0.88*
75%	136.27±1.92 *	73.49±1.03*	154.43±2.17 *	83.28±2.37*
100%				
WW	154.56±11.0 *		175.16±5.00 *	
WO	88.65±6.85*		100.4±1.41*	
	110.14±1.55 *		124.82±1.75 *	
	19.15±0.27*		21.70±0.61*	
	105.16±1.48 *		119.18±3.40 *	
	18.40±0.26*		20.85±0.59*	

Effect of Weeds on Antibacterial Activities: Methanolic extracts of wheatgrass (of both varieties of wheat) in control treatments posed maximum inhibitory effect on the bacterial strains. The bacterial strains S. sonnei visible zones of inhibition was between 22.00 mm in control and 16.00 mm minimum at 100% weeds extract concentration. While results showed that E. coli was more resistant to extract of wheat grass (for both varieties of wheat) amongst the two strains studied. In wheat variety KT-2000 S. sonnei showed maximum inhibition 14.00mm visible zone of inhibition which was significant as compare to its counterpart control i.e. 21.0 mm. visible zone of inhibition. S. sonnei exhibited inhibition for all treatments and being thus more susceptible of the two strains. Detailed results are shown in (Table 6).

Table 6: Soil Profile for Both Wheat Varieties after Harvest of Replicates. Values are The Means ± S.D of Three Replications. * Shows Statistically Significant Results (T-Test at $P < 0.05$) of a Particular Treatment as Compared to its Counterpart Control Treatment.

Treatment % Soil Profile

KT-2000 LOCAL						
	pH	O.M %	N %	pH	O.M %	N %
Control	8.12 ± 0.02	0.62 ± 0.12	0.03 ± 0.006	8.02 ± 0.025	0.90 ± 0.16	0.04 ± 0.005
25%	8.08 ± 0.03	0.86 ± 0.27	0.03 ± 0.006	7.98 ± 0.034	0.93 ± 0.27	0.01 ± 0.014
50%	8.01 ± 0.04	1.03 ± 0.35	0.05 ± 0.014	7.9 ± 0.043	0.94 ± 0.25	0.03 ± 0.012
75%	7.9 ± 0.011	1.2 ± 0.26*	0.06 ± 0.013*	7.78 ± 0.016	0.95 ± 0.25	0.04 ± 0.006
100%	7.8 ± 0.013	1.49 ± 0.32*	0.08 ± 0.017*	7.67 ± 0.019	0.96 ± 0.27	0.06 ± 0.016*
WW	8.16 ± 0.03	0.62 ± 0.26	0.03 ± 0.005	8.15 ± 0.026	0.61 ± 0.17*	0.03 ± 0.011
WO	8.22 ± 0.03	0.87 ± 0.27	0.03 ± 0.005	8.21 ± 0.029	0.86 ± 0.19	0.03 ± 0.011

Effect of Weeds on Soil Profile: Results indicated that in allopathically altered soils were non-significantly different from non-weed associated soils. The only significant increase was observed in O.M matter at 100% weeds' extract in both the varieties from 0.62% in control to 1.49% in, and for Nitrogen content which also increased from 0.3% in control to 0.8% at similar concentration treatments.

DISCUSSION

The allelopathy has gained much attention in last few decades, shown by several reports on the matter [(J. Kamal, 2011), (A. L. Anaya 1999), (Pedrol, N. et al.,) (H. P. Singh 2001 et al)]. The study was conducted to understand allelopathic effects of A. fatua (wild oat) on the morphology, biochemical profile, and antibacterial features of wheat varieties of Kohat region.

The germination was recorded for 96 hours. Initially it was concluded that at first 24 hours there was no significant difference in any of the treatments as compared to control except at 100 in which none germinated. However the results were very clear after 96 hours there were significant difference in the germination percentage of all the seedlings of different concentrations.

It was established that the aqueous extracts of A. fatua inhibit the germination of T. aestivum significantly at higher concentrations of weeds' extract. The degree of inhibition of seed germination was increased with the increase in concentration of treatment. According to Laosinwattanna et al. several of earlier reports have also proposed that same results that the level of inhibition increases with increasing weeds' extract concentrations. The inhibition of germination of the both wheat varieties, i.e. KT-2000 and LOCAL, could be attributed to the impact of released allelochemicals from the weeds' extract because of water-soluble property of allelochemicals they can accumulate inside seeds after their direct contact with bioactive concentrations when they are released in their vicinity. Experiments conducted show that not only wheat but there are other studies showing that plants secondary metabolites such as phenolics alkaloids, terpenoids and others can result in complete or partial inhibition of germination of seeds.

The reduction in morphological parameters (roots' and shoots' length and fresh and dry biomass) may also be due to water-soluble allelochemicals present in the weeds' extracts and their negative impact and toxicity on the measured parameters. Earlier studies also show that toxicity of weeds' extracts gradually increase with the increase of concentration from lower towards higher [(A. R. Putnam 1994), (A. Sinkkonen 2003), (S. L. Peng et al., 2004)]

Aqueous extract of weeds' at all concentrations suppressed the shoots' and roots' length, and reduced the dry and fresh biomass of wheat seedlings as compared to unchecked control. The aqueous extracts of A. fatua L. at 100% concentration resulted in maximum inhibition in seedling growth followed by 75% concentration. While among the two varieties the minimum shoot length (2.59 cm) was observed with 100% concentration in LOCAL wheat variety. This indicates that LOCAL wheat variety was more vulnerable to alleohemicals. However, at 25% concentration results did not statistically differ from control in both varieties. But the results showed that LOCAL wheat variety was more affected as compared to KT-2000. Perhaps allelochemicals of this weed inhibited cell division. Phenolics represent one of the largest groups of allelochemicals and studies have shown that various phenolic compounds inhibit cell division in plants. It is also possible that cell elongation was affected by aqueous extracts of A. fatua. There are a number of studies presenting that accumulation or introduction of plant residues to growth environments of other plants result in inhibition of growth and development.

The amount of chlorophyll of wheat seedlings under weed stress decreased significantly. The amount of chlorophyll a in both wheat varieties reduced significantly at higher concentration of weeds' extract i.e. 75% and 100%. On the other hand the amount of chlorophyll b showed more significant decrease at 75% and 100% weeds' extract concentration as compared to chlorophyll a in both varieties. The reduction in the amount of chlorophyll may be due to the interfering of phenolic compounds with the biosynthesis of porphyrin, which is the precursor to biosynthesis of chlorophyll. A number of studies have established that phenolic acids can inhibit the production of chlorophyll and its allies. Various allelochemicals were reported to interfere with the chlorophyll biosynthesis due to their interference with porphyrin and porphyrin containing compound (F. Guerrero, J. E. Mullet 1986). As it is clear that an artificially developed variety (KT-2000) is not as much vulnerable as a LOCAL (naturally evolved variety).

Through the present study, it was also established that weeds' extracts resulted in greatly increasing the buildup of total soluble sugars in wheat seedlings of both wheat varieties i.e. KT-2000 and LOCAL. The allelochemicals treatment presented a significant rise in the amount of sugar contents, both in shoots and roots. (Ahmadi and Bakicer 2001) described that abscisic acid is involved in regulating of plant osmolytes under water stress conditions. Plants excess expressing of metabolic genes and osmolyte biosynthesis have shown improved stress tolerance. (Mahajan and Tuteja 2005) concluded that under intense water deficient conditions, growth is repressed by greater concentrations of abscisic acid and result in sugar accumulation, while low concentrations of abscisic acid promote growth. Decreased rate of chlorophyll content and photosynthesis may have caused increase in the concentration of sugars due to increased abscisic acid or allelochemicals treatments however this relationship is not very simple. Drought stress tolerance in plants is improved by abscisic acid which is increased by treatment of allelochemicals, which is possible due to the accumulation of osmolytes, for example; sugars. Very few studies have been conducted on the same phenomena but results are quite in agreement with same studies. Sugar accumulation may help overcome water stress and may be concerned with the allelochemcials stress resulting in possible drought.

Applications of extracts of A. fatua plants also caused an increase in the content of protein in both wheat varieties. Nonetheless, the amount of increase was greater in KT-2000 as compared to LOCAL. Once again these results may be due to the fact that abscisic acid has a positive role on protein accumulation similar to sugars. Scientists have reported that synthesis of protein in developing seeds is brought about by abscisic acid (Singh, K. Usha (2003). It is also clear from studies that protein phosphorylation is improved due to moisture stress because of the increased concentration of abscisic acid. Literature represents similar results in other similar studies (I. Iturbe-Ormaetxe, et al., 1998). The results show that the accumulation is actually due to water stress on wheat seedlings but the inhibition of growth and development shows otherwise.

The wide ranges of impacts of weedicides have already been well-known by the amounts and type of their application, health of plants and growth stage of plant. It has been recognized that there are morphological changes and disorders of cell division due to effect of both the weedicides and their allies (M. Jiang and J. Zhang 2004).

Phytochemical components of wheatgrass comprises of carbohydrates, alkaloids saponins, mucilage and gums (S. A. Ashok 2011). We obtained antimicrobial activity of methanolic extract of wheat grass against two strains of food borne bacteria namely E. coli and S. sonnei. The results showed that while exposing the wheat seeds and seedlings to different concentration altered the biochemical profile of seedling and it also degraded the medicinal potency of wheat seedlings significantly. The increase in stress resulted in decreasing in the potential of wheats' methanolic extract to inhibit bacteria. Studies have confirmed that chlorophyll stops growth and development of bacterial strains.

The soil profile was insignificantly transformed the O.M slightly increased while pH and N content reduced somewhat from lower to higher concentration. The results were same for both wheat varieties. Though they were noticeable in LOCAL as compared to KT-2000 wheat variety. This indicate that free phenolic compounds and other secondary metabolites may accumulate in rhizosphere, especially in soils flooded with weeds waste waters, thereby influencing the accumulation and availability of soil nutrients and rates of nutrient cycling, which both ultimately affect plant growth (J. R. Qasem 2007) Along with that the difference in soil profile of varieties also indicate that the resistant varieties that are scientifically developed are different in releasing of phenolics and may also be different in absorbing of the phenolics and other secondary metabolite present in soil. This shows that there is over all difference in interaction of individual varieties that how they interact with soil and thus eventually being more or less affected physiologically, morphologically or biochemically.

From results of our study it is very clear that the herbicides/weedicides possess negative effect on crops in terms of morphology, physiology and biochemistry. The affect was observed in all parameters under study. The decreased value of soluble sugar content after the applications of herbicides/weedicides showed the likelihood of depletion of sugar reserves from roots and shoots may have started after germination. It may be reckoned that major reduction of sugars reserve starts from the 96 hours application or 4rd day foliar application of the weedicides.

CONCLUSION

A. fatua is allelopathicaly active plant species, the aqueous extract obtained from this weed exhibited mostly the negative impression on germination, seedlings lengths seedling biomass and chlorophyll content.

To cope with the A. fatua toxicity, and stresses the wheat variety KT-2000 was much better as compared to LOCAL wheat variety.

Allelopathy can also offer a cost effective and more anticipated method of control than conventional methods, like the use of herbicides or mechanical removal; it can be one of the best weapons for weed control.

Gap Analysis

A plot study may be conducted for better understating's of A. fatua due to the fact that the effect of debris may be different because weed aqueous extract interacts directly with wheat seedlings.

Weedicides effects can be better understood in case of plot experiment because the crop residues have different method of interaction with soil and subsequent crop.

Study can be further advanced by in including parameters such as Gene toxicity, Cell toxicity, Tissue damage, Heavy metals analysis and Molecular studies.

Allelopathic properties of wheat crop can be explored for the probability of using wheat crop residues for the suppression of weeds growth and more precisely development of weed-resistant wheat varieties.

The anatomical changes must be determined by studying the root, shoot growth and reduction in the size and length of vascular bundles such and xylem and phloem cells.

Possible secondary metabolites and allelochemicals need to be considered because they may offer new and inexpensive natural equivalents of synthetic products with better selectivity, reliability and effectiveness to suppress weeds and pests.

Weedicides must furthermore go through toxicity tests to check the safety of non-target crop plants.

A logical development to figure out wheats' medicinal potency is to test the aqueous and methanolic extracts of wheat crop on several bacterial strains of gram positive and gram negative nature.

Weedicides may act as a potential growth promoters as shown by several studies but the nature of such phenomena need to be specified to determine the cause of improving plant development and yield under acute soil water deficiency.

There is a need to understand the complexity behind the increase in sugar and protein content while decrease in all other aspects of plant growth such as photosynthetic pigment content, seedling length and germination is also negatively affected while the sugar and protein content is increases.

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