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Article

Phytotoxic Effect of Aqueous Extracts of Different Plant Parts of Milkweed on Weeds and Growth and Yield of Wheat

Efeito Fitotoxico de Extratos Aquosos de Diversas Partes Vegetais da Bombardeira em Plantas Daninhas e no Crescimento e Rendimento de Trigo

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ABSTRACT - The present research work was undertaken to find out the phytotoxic effects of different concentrations of aqueous extracts of leaves, stems and root of milkweed (*Calotropis procera*) in the Agronomic Research Area of Department of Agronomy, Faculty of Agriculture, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa (KPK), Pakistan during 2013-14 and 2014-15. The treatments consisted of different concentrations of aqueous leaf, stem and root extracts (10, 20, 30 and 40%) of milkweed, which were compared with tap water (control). The results showed that the reduction of all studied parameters, including weeds, growth and yield of wheat was proportional to the concentration of aqueous extracts of milkweed in use. The level of inhibition was concentration-dependent as inhibition increased with an increase in the concentration of aqueous extracts of all three plant parts of milkweed. Therefore, application of maximum concentration (40%) of aqueous leaf, stem and root extracts correspondingly reduced all the studied parameters. On the basis of the findings, it is concluded that the *Calotropis procera* should be removed near the cultivating wheat fields because it contains some phytotoxic substances, which may be leached out by the roots and cause serious losses to the growers and crop species.

Keywords: Phytotoxicity, *Calotropis procera*, aqueous extracts, weed density, growth, yield, wheat.

*RESUMO - O presente estudo foi desenvolvido com o objetivo de investigar os efeitos fitotóxicos de diferentes concentrações de extratos aquosos de folhas, caules e raízes da bombardeira (**Calotropis procera**) na Área de Pesquisa Agronômica do Departamento de Agronomia, Faculdade de Agricultura, Universidade de Gomal, Dera Ismail Khan, Khyber Pakhtunkhwa (KPK), Paquistão, durante os períodos de 2013-14 e 2014-15. Os tratamentos consistiram de diferentes concentrações de extratos aquosos de folhas, caule e raízes (10, 20, 30 e 40%) da bombardeira, que foram comparadas com água encanada (controle). Os resultados mostraram que a redução de todos os parâmetros estudados, incluindo ervas daninhas e crescimento e rendimento do trigo, foi proporcional à concentração dos extratos aquosos de bombardeira utilizados. O nível de inibição foi dependente da concentração, pois a inibição aumentou com o aumento na concentração de extratos aquosos de todas as três partes vegetais da bombardeira. Portanto, a aplicação da concentração máxima (40%) de extratos aquosos de folhas, caule e raiz reduziu proporcionalmente todos os parâmetros estudados. Com base nos resultados obtidos, conclui-se que **Calotropis procera** deve ser removida perto dos campos de cultivo por conter algumas substâncias fitotóxicas que podem ser lixiviadas pelas raízes e causar sérios prejuízos aos produtores e às espécies em cultivo.*

Palavras-chave: Fitotoxicidade, *Calotropis procera*, extratos aquosos, densidade de plantas daninhas, crescimento, rendimento, trigo.

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INTRODUCTION

Wheat (*Triticum aestivum*) is the staple food crop in Pakistan. It accounts for 12.5% to price crops in agriculture and 2.7% of gross domestic products (GDP) of the country (Aslam et al., 2013). It is annually cultivated in an area of 9.04 million hectares with total production of 25.3 million tons in Pakistan (Pakistan, 2014). The government has given high priority to wheat over the last few decades; however, there is a big difference between actual (2.5-3 ton ha^{-1}) and potential yield (6-8 ton ha^{-1}) of existing wheat varieties.

Average wheat yield is low in Pakistan because of a number of biotic and abiotic factors, but weed-crop competition appears to be the major yield-deteriorating factor (Qureshi et al., 2001; Ullah et al., 2013). The main cause of low yield in wheat is weed infestation. It reduces yield by 25-30% in Pakistan (Willis, 2004) Weeds compete with crop plants for CO_2, O_2, light and nutrients and some intervene with crop growth by discharging phytotoxic compounds in the rhizosphere (Hussain et al., 2007).

Certain plants may have the capacity to obstruct germination and development of other plants by discharging virulent extracts called allelopathic chemicals (Taiwo and Makinde, 2005). These allelochemicals interfere with other plants and affect growth and yield of crops (Alam and Islam, 2002). The growth of other plants may be delayed by phytotoxic plants that produce toxic chemicals present in leaves, stems, fruits rhizomes and seed of plants (Zeng et al., 2008); sometimes they are present in one or two of such parts from where the toxic substance gets released into the soil through decomposition, root exudation, leaching and volatilization of plant residues (Fujii et al., 2004). It is, however, a complex mode of interaction between plants, accomplished through the release of chemical substances into the environment (Bais et al., 2003).

Scientists have developed farming techniques which bring socio-economic sustainability to crop production and protection (Machado, 2007). Integrated Weed Management (IWM) is one of those techniques whereby allelopathy can be used as an eco-friendly tool in weed management (Dangwal et al., 2010). Angiosperm plants have vigorous type of important toxic substances used for weed control (Han et al., 2008). Many of the crops are said to have toxic viability and a great deal of effort has been made to avail them for weed management (Travlos et al., 2007).

Milkweed (*Calotropis procera*) is a xerophytic perennial shrub or small tree. It is an evergreen plant of the Asclepiadaceae family and native to subtropical Africa, Asia and common in the Middle East (Grace, 2006). It is famous with different names based on its habitat such as Mudarfibre, Swallow-wort, Calotropis and Sodom apple in English; in Arabic it is called Usher, Kisher and Dead Sea Plant and its local name is Aak (Orwa et al., 2009). It is a drought-resistant plant and can grow in highly salt-affected areas and found in sandy soils/arid/semi-arid regions in the world (Kareem et al., 2008). Milkweed is considered to be one of the dominant plants amongst the natural flora in Pakistan (Qureshi et al., 2009).

Large quantities of secondary metabolites (alkaloids, calotropis and glycosides) are present in milkweed (El-Khawaga et al., 2013). A number of secondary metabolites have been isolated from this plant which include many sterols, flavonoids (Heneidak et al., 2006) and triterpens (Chundattu, 2012) and cardiac glycosides (Hanna et al., 2002). It has the potential of dominating its natural habitat because of its phytotoxic substances which enable it to compete with other species and inhibit the growth and yield of crops (Al-Zahrani and Al-Robai, 2007). In Dera Ismail Khan District, of the eight genera and species of Asclepiadaceae family, milkweed (*Calotropis procera*) is an important invasive shrub affecting fruits, vegetables and crop productivity, and it poses a possible threat to cropping system in the area. This is because *C. procera* can potentially dominate its natural habitat as a result of the presence of allelochemicals which enable it to compete with other species and inhibit the growth and yield of crops (Al-Zahrani and Al-Robai, 2007). However, previous studies haver shown that little research has been carried out to evaluate its alleopathic and phytotoxic effects in many plants including wheat (Kayode, 2004; Samreen et al., 2009). The present research was, therefore, designed to investigate the phytotoxic potential of different aqueous extracts of leaves, stem and root of *Calotropis procera* on weeds, growth and yield of wheat.

MATERIALS AND METHODS

The experiment was laid out in a randomized complete block design with four replications. The land was prepared by proper ploughings in order to make fine seed bed for sowing. Wheat crop was sown during the first week of November by manual seed drill. Net plot size was kept at 1.5m x 4.5 m. Each plot consisted of 6 rows with 5 m length and 25 cm distance between the rows. A recommended seed rate i.e. 100 kg ha^{-1} of approved wheat variety Gomal-8 was used in this trial. Fertilizers including Urea, Di-ammonium Phosphate and Potassium Sulphate were used to apply 150:120:90 NPK kg ha^{-1} in each treatment. At sowing, half dose of nitrogen and full doses of phosphorous and potassium were applied while the remaining nitrogen dose was top dressed with first irrigation.

Collection of plant materials and preparation of aqueous extracts

Fresh leaves, stem and root of milkweed were collected from fields around the experimental site of Faculty of Agriculture, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan. These were then shed dried, crushed and ground. The ground leaves, stem and roots of *C. procera* were weighed and immersed in tap water at different ratios at room temperature for 72 h. The water extract was collected in bottles and tagged by filtering through 10- and 60-mesh sieves. Tap water was used as control treatment.

The detail of treatments are given below:

T_0 =Tap water (control)

T_1 =10% concentration of leaf aqueous extract of *C. procera*

T_2 =20% concentration of leaf aqueous extract of *C. procera*

T_3 =30% concentration of leaf aqueous extract of *C. procera*

T_4 =40% concentration of leaf aqueous extract of *C. procera*

T_5 =10% concentration of shoot aqueous extract of *C. procera*

T_6 =20% concentration of shoot aqueous extract of *C. procera*

T_7 =30% concentration of shoot aqueous extract of *C. procera*

T_8 =40% concentration of shoot aqueous extract of *C. procera*

T_9 =10% concentration of root aqueous extract of *C. procera*

T_{10} = 20% concentration of root aqueous extract of *C. procera*

T_{11} = 30% concentration of root aqueous extract of *C. procera*

T_{12} = 40% concentration of root aqueous extract of *C. procera*

Aqueous extracts of leaf, stem and roots of *C. procera* were applied at 30 and 60 days after sowing (DAS).

The following parameters were recorded during the course of experimentation.

Weeds parameters

Weed density (m^{-2})

Total number of weeds (m^{-2}) was taken randomly in each sub-plot at 30 and 60 DAS.

Fresh weed biomass (g m^{-2})

Weeds in 1 m^2 area were taken randomly in each sub-plot at 30 and 60 DAS and their weight was measured with the help of an electronic balance.

Dry weed biomass (g m^{-2})

Fresh weeds were oven-dried at 72° for 72 hours and then their dry weight was measured.

Leaf area index

Leaf area index is the ratio of leaf area to ground area. It was calculated at 49 and 98 DAS by using the following formula:

$$Leaf\ area\ index\ (LAI) = \frac{Area\ of\ green\ leaves\ per\ plant}{Area\ occupied\ by\ plant}$$

Crop growth rate (g m^{-2} day^{-1})

Crop growth rate from boot to maturity stage (before and after anthesis) was determined by using the following formula:

$$Crop\ growth\ rate\ (g\ day^{-1}\ m^{-2}) = \frac{(W2 - W1)}{(T2 - T1)}$$

where W2 = final weight; W1 = initial weight; T1 and T2 are the time intervals in days

Net assimilation rate (g g^{-1} day^{-1})

Net assimilation rate is the net gain in dry weight (dry matter) of a plant per unit leaf area per unit time. It was calculated by using the following formula:

$$NAR = \frac{(W2 - W1)(\log LA2 - \log LA1)}{(T2 - T1)(\log LA2 - \log LA1)}$$

Chlorophyll content (µg cm^2)

Chlorophyll content was recorded in 10 randomly selected wheat plants at 7, 9, 11, 13 and 15 weeks after sowing (WAS). A SPAD meter was used for measuring chlorophyll content.

Number of tillers (m^{-2})

The number of tillers was counted randomly in each sub-plot at harvest using a $1m^2$ quadrate.

Spike length (cm)

Ten spikes were selected from each sub-plot and then length was measured, averaged and recorded.

Number of grains (spike^{-1})

Ten ear heads were randomly selected from each sub-plot. These ears were threshed and cleaned to record number of grains per spike.

1,000 grain weight (g)

One thousand grains were counted from a seed lot in each sub-plot and their weight was recorded.

Grain yield (kg ha^{-1})

From each sub-plot, an area of 1 m^2 was harvested and threshed. The grains harvested were sun dried for 2-3 days and then their weight was recorded and converted into kg ha^{-1} by using the following formula:

Grain yield (kg ha^{-1}) = grain yield m^{-2} (kg) x 10000

Harvest index (%)

Harvest index was calculated by using the following formula::

$$H.I = \frac{Economic\ yield}{Biological\ yield} \times 100$$

Grain protein content (%)

Grain samples were analyzed for nitrogen content by the Modified Micro Kjeldahl method (Piper, 1966). Then, protein content in the grains of the individual treatment was calculated by multiplying the nitrogen content (%) in the grains by the factor 6.25. It was expressed in percentage.

Statistical analysis

Analysis of variance (Steel et al., 1997) was used for recording and statistically analyzing the data, whereas means were separated through the least significance difference test by the MSTATC computer software (MSTATC, 1991).

RESULTS AND DISCUSSION

Weed density (30 and 60 DAS)

The data shown in Table 1 revealed that the application of aqueous extracts of leaves, stem and root of milkweed had no significant effect on weed density at 30 days after sowing while there was a significant and comparable reduction in weeds by applying these aqueous extracts

Table 1 - Phytotoxic effect of milkweed (*Calotropis procera*) on weed density, fresh weed biomass and dry weed biomass of wheat in 2013-14 and 2014-15

Treatment	Weed density m^{-2}				Fresh weed biomass (g m^{-2})				Dry weed biomass (g m^{-2})			
	2013-14		2014-15		2013-14		2014-15		2013-14		2014-15	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
T_0 = Tap water	20.00^{NS}	57.50 a	17.00^{NS}	50.50 a	8.96^{NS}	24.33 a	6.715	19.58 a	3.81^{NS}	9.73 a	2.94^{NS}	8.10 a
T_1 = 100 g leaves	20.75	50.50 cd	17.75	43.00 cd	8.96	23.06 b	6.712	18.31 b	4.12	9.22 b	3.25	7.59 b
T_2 = 200 g leaves	19.25	47.25 ef	16.2	39.75 ef	8.50	21.57 c	6.255	16.82de	3.97	8.62 c	3.15	6.99 de
T_3 = 300 g leaves	21.50	45.75 fg	18.5	37.00 hi	8.61	20.04 de	6.362	14.92 f	3.80	8.01 de	2.93	6.23 f
T_4 = 400 g leaves	19.25	39.50 i	16.25	32.00 k	8.73	18.48 f	6.482	13.73 g	4.19	7.39 f	3.37	5.76 g
T_5 = 100 g stem	19.25	51.75 bc	16.35	44.25 bc	7.69	22.60 b	5.44	17.85bc	3.89	9.04 b	3.12	7.40 bc
T_6 = 200 g stem	20.75	48.75 de	17.75	41.25 de	7.56	22.59 b	5.312	17.84bc	4.07	9.03 b	3.18	7.45 bc
T_7 = 300 g stem	20.00	45.25 g	17.05	37.75 gh	8.03	20.73 cd	5.782	15.98 e	4.15	8.29 cd	3.35	6.72 e
T_8 = 400 g stem	16.25	42.00 h	13.25	34.50 j	7.43	19.35 ef	5.187	16.05 e	3.92	7.74 ef	3.13	6.68 e
T_9 = 100 g roots	19.50	52.75 b	16.5	45.25 b	7.95	23.38 b	5.707	18.63ab	4.10	9.35 b	3.23	7.71 ab
T_{10} = 200 g roots	20.75	50.50 cd	17.75	43.0 cd	7.24	22.78 b	4.99	18.45 b	3.95	9.11 b	3.16	7.65 b
T_{11} = 300 g roots	18.25	46.50 fg	15.5	39.0 fg	8.57	21.60 c	6.325	17.66 bcd	4.05	8.64 c	3.09	7.33bcd
T_{12} = 400 g roots	17.75	42.25 h	14.75	35.75 ij	8.28	19.67 e	6.03	17.06 cd	4.01	7.86 e	3.21	7.09 cd
$LSD_{0.05}$	- - -	1.759	- - -	1.98	- - -	0.903	- - -	o.978	- - -	0.361	- - -	0.391

Means followed by different letter(s) in a column are statistically significant at 5% level of probability.

at 60 days after sowing as compared to control in the 2013-14 and 2014-15 periods. Minimum weed density (39.50, 42.00 and 42.25 m^{-2}) was recorded with maximum concentration (40%) of aqueous leaves, stem and root extracts respectively as compared with the control, which had maximum weed density (57.50 m^{-2}) at 60 days after sowing during the first cropping year. During the second cropping season, there was significantly lower weed density (32.00 m^{-2}) with the application of maximum concentration (40%) of aqueous leaf extract over the control (tap water) at 60 DAS. Likewise, the highest level (40%) of aqueous stems and root extracts recorded minimum weed density (34.50 and 35.75 m^{-2}) at 60 DAS compared with the control.

Fresh weed biomass (30 and 60 DAS)

Data shown in Table 1 revealed that fresh weed biomass was non-significantly affected by various aqueous extracts of milkweed at 30 days after sowing. However, the application of different concentrations significantly reduced fresh weed biomass at 60 DAS for two consecutive years. In the first cropping season, minimum fresh weed biomass (18.48, 19.35 and 19.67 g m^{-2}) was noted by applying maximum concentration (40%) of aqueous leaves, stem and root extracts, respectively, while fresh weed biomass was maximum (24.33 g m^{-2}) in the control treatment at 60 DAS.

Dry weed biomass (30 and 60 DAS)

The data presented in Table 1 showed that there were non-significant differences in dry weed biomass at 30 DAS during both cropping seasons. All the same, these were significantly shortened by the application of different concentrations of aqueous leaves, stems and roots extract of milkweed at 60 DAS in the 2013-14 and 2014-15 periods. By applying a higher concentration (40%) of aqueous leaves extract, there was maximum reduction in dry weed biomass (7.39 and 5.76 g m^{-2}) as compared with the untreated control, which had maximum dry weed biomass (9.73 and 8.10 g m^{-2}) at 60 days after sowing throughout both cropping years. Similarly, the highest concentration (40%) of aqueous stems and root extracts correspondingly reduced dry weed biomass (7.74 and 6.68 g m^{-2} and 7.86 and 7.09 g m^{-2}, respectively) at 60 days after sowing compared with the control, where no extract was used.

Chlorophyll Content (µg cm^2)

The data shown in Table 2 indicated that chlorophyll content was significantly reduced with increased concentrations of aqueous leaves, stems and root extract of milkweed when compared with tap water in the experimental years. Throughout 2013-14, maximum chlorophyll content

Table 2 - Phytotoxic effect of milkweed (*Calotropis procera*) on chloropyll content and leaf area index (49 and 98 DAS) of wheat in 2013-14 and 2014-15

Treatment	Chloropyll Content (µg cm^2)					Chloropyll Content (µg cm^2)					Leaf area index			
	2013-14					2014-15					2013-14	2014-15	2013-14	2014-15
	7 WAS	9 WAS	11 WAS	13 WAS	15 WAS	7 WAS	9 WAS	11 WAS	13 WAS	15 WAS	(49 DAS)	(49 DAS)	(98 DAS)	(98 DAS)
T_0 = Tap water	36.40 a	42.27 a	51.45 a	56.47 a	51.42 a	38.12 a	43.87 a	52.52 a	57.65 a	52.50a	0.384 a	0.386 a	2.68 a	2.705 a
T_1 = 100 g leaves	34.70 bc	38.75 c	49.10 d	55.42 bc	50.12 bc	36.42 bc	40.35 c	46.89 c	56.60 bc	51.20bc	0.374 ab	0.346 b	2.61 ab	2.426 b
T_2 = 200 g leaves	34.20 cd	38.65 c	48.05 e	54.90 cd	49.05 de	35.92 cd	40.25 c	45.29 e	56.08 cd	50.12de	0.340 c	0.320 c	2.38 c	2.245 c
T_3 = 300 g leaves	33.60 ef	38.47 cd	47.02 f	53.75 e	47.87 f	35.32 ef	40.07 cd	44.39 f	54.93 e	48.95f	0.313 d	0.287 d	2.19 d	2.011 d
T_4 = 400 g leaves	31.27 j	35.35 h	45.32 g	51.82 g	45.60 g	32.99 j	36.95 h	43.44 h	53.00 g	46.67g	0.285 e	0.264 e	1.99 e	1.854 e
T_5 = 100 g stem	33.12 fg	37.60 e	49.77 bc	55.67 b	50.82 ab	34.84 fg	39.20 e	47.29 c	56.85 b	51.90ab	0.368 ab	0.376 a	2.58 ab	2.635 a
T_6 = 200 g stem	32.82 gh	36.30 f	48.22 e	53.97 e	49.67 cd	34.54 gh	37.90 f	46.29 d	55.15 e	50.75cd	0.335 c	0.345 b	2.34 c	2.419 b
T_7 = 300 g stem	32.65 ghi	36.72 f	47.12 f	52.82 f	48.55 ef	34.37 ghi	38.32 f	45.14 e	54.00 f	49.62ef	0.316 d	0.320 c	2.21 d	2.243 c
T_8 = 400 g stem	32.15 i	35.67 gh	45.82 g	52.70 f	46.25 g	33.87 i	37.27 gh	43.64 gh	53.88 f	47.32g	0.287 e	0.291 d	2.00 e	2.037 d
T_9 = 100 g roots	35.02 b	39.75 b	50.25 b	55.52 b	50.67 b	36.74 b	41.35 b	47.92 b	56.70 b	51.75b	0.363 b	0.380 a	2.54 b	2.662 a
T_{10} = 200 g roots	33.85 de	38.85 c	49.52 cd	54.62 d	49.70 cd	35.57 de	40.4 5c	47.12 c	55.80 d	50.77cd	0.330 cd	0.344 b	2.31 cd	2.413 b
T_{11} = 300 g roots	33.12 fg	37.92 de	48.15 e	53.45 e	48.25 f	34.84 fg	39.52 de	46.04 d	54.63 e	49.32f	0.313 d	0.315 c	2.19 d	2.205 c
T_{12} = 400 g roots	32.45 hi	36.17 fg	46.85 f	52.80 f	46.32 g	34.17 hi	37.77 fg	44.17 fg	53.98 f	47.40g	0.290 e	0.295 d	2.03 e	2.109 d
$LSD_{0.05}$	0.562	0.561	0.671	0.573	0.743	0.562	0.561	0.581	0.573	0.743	0.018	0.0172	0.129	0.1207

Means followed by different letter(s) in a column are statistically significant at 5% level of probability.

(36.40, 42.27, 51.45, 56.47 and 51.42 µg cm^2) was recorded at 7, 9, 11, 13 and 15 weeks after sowing (WAS), respectively, in the tap water treatment. A corresponding decrease in chlorophyll content was noted with increased aqueous extract of all three plant parts of milkweed. A higher concentration (40%) of the aqueous leaf extract resulted in minimum chlorophyll content (31.27, 35.35, 45.32, 51.82 and 45.60 µg cm^2) at all time intervals. Similarly, chlorophyll content (32.15, 35.67, 45.82, 52.70 and 46.25 µg cm^2) was significantly reduced by applying maximum concentration (40%) of the aqueous stem extract over the control. A similar trend (32.45, 36.17, 46.85, 52.80 and 46.32 µg cm^2) was noted at all time intervals by applying a higher concentration (40%) of the aqueous root extract when compared with the control. During the next cropping season, minimum chlorophyll content (32.99, 36.95, 43.44, 53.00 and 46.67) was recorded by using the highest concentration (400 g) of aqueous leaf extract as compared with the untreated control, which had maximum chlorophyll content (38.12, 43.87, 52.52, 57.65 and 52.50) at 7, 9, 11, 13 and 15 weeks after sowing (WAS) respectively. Similarly, chlorophyll content was subsequently reduced by increasing the concentration of the aqueous stem and root extract. The highest concentration (400 g) of the aqueous stem and root extracts produced minimum chlorophyll content as compared to the untreated control.

Leaf area index (m^{-2}) at 49 DAS

Leaf area index refers to the efficiency of the photosynthesis process. It is the ratio of total leaf area to the ground cover, which increases to maximum after crop emergence (Reddy, 2004). The data shown in Table 2 indicated that a different concentration of aqueous extracts of milkweed had a significant effect on leaf area index (LAI) at 49 days after sowing in the 2013-14 and 2014-15 periods. Minimum leaf area index (0.285 and 0.264 m^{-2}) was noted with the highest concentration (40%) of aqueous leaf extract followed by LAI (0.287, 0.291 and 0.290, 0.295 m^{-2}) recorded with the maximum concentration (40%) of aqueous stem and root extracts, respectively, as compared with the control, where no extract was used.

Leaf area index (m^{-2}) at 98 DAS

The data shown in Table 2 revealed that aqueous extract of leaves, stems and root of milkweed negatively affected leaf area index at 98 DAS. Maximum leaf area index (3.892 and 3.897 m^{-2}) was noted in the control, where no extract was used. Among different concentrations applied, the aqueous leaves extract (40%) showed maximum reduction in leaf area index (3.158 and 2.952 m^{-2}) in both years. There was also a similar trend with increased concentration of aqueous stems and root extract, which significantly reduced leaf area index. However, the minimum leaf area index (3.160, 3.200 and 3.168, 3.128 m^{-2}) was recorded with the maximum (40%) concentration of stem and root extracts, respectively, in both experimental years.

Crop growth rate (g m^{-2} day^{-1})

One of the important physiological traits of plants is crop growth rate, which is determined by temperature, radiation, cultivar usage, water and food supply. The data shown in Table 3 revealed that use of aqueous leaf, stem and root extracts significantly reduced crop growth rate (CGR) as compared with the control in 2013-14 and 2014-15. Increasing the absorption of aqueous extracts correspondingly decreased CGR. Among various treatments, the application of maximum level (40%) of aqueous leaf extracts considerably reduced crop growth rate (21.54 and 23.21 g m^2 day^{-1}) for two consecutive years. Similarly, applying maximum concentration (40%) of aqueous stem and root extracts reduced CGR (21.93, 26.28 and 23.02, 25.26 g m^{-2} day^{-1}, respectively) compared with the untreated control (27.76 and 30.27 g m^{-2} day^{-1}) in two cropping seasons.

Net assimilation rate (g $g^{-1}$$day^{-1}$)

Net assimilation rate (NAR) refers to a plant's capacity to increase dry weight in terms of the area of its assimilatory surface. It stands for the photosynthetic efficiency in the overall sense

Table 3 - Phytotoxic effect of milkweed (*Calotropis procera*) on crop growth rate, net assimilation rate, number of tillers, spike length, number of grains (spike^{-1}) and 1,000 grain weight of wheat in 2013-14 and 2014-15

Treatment	Crop growth rate (g m^{-2} day^{-1})		Net assimilation rate (g g^{-1}day^{-1})		Number of tillers (m^{-2})		Spike length (cm)		Number of grains (spike^{-1})		1000-grain weight (g)	
	2013-14	2013-14	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
T_0 = Tap water	27.76 a	27.76 a	3.72 a	4.02 a	402.00 a	420.7 a	11.12 a	11.26 a	69.25 a	70.26 a	43.45 a	43.86 a
T_1 = 100 g leaves	25.76 b	25.76 b	3.15 b	3.66 b	386.25 b	404.7 de	10.67 b	10.65 cd	65.75 bc	67.05 bc	42.72 bc	42.81 de
T_2 = 200 g leaves	24.97 bc	24.97 bc	3.00 bc	2.92 h	382.25cde	404.2 de	10.48 bc	10.63 de	64.00 cd	63.78 ef	42.55 bcd	42.66 ef
T_3 = 300 g leaves	24.42 cd	24.42 cd	2.87 cde	2.67 i	379.50 ef	393.2 h	10.33 c	10.23 ef	63.25 de	62.96 fg	42.32 cde	42.36 g
T_4 = 400 g leaves	21.54 g	21.54 g	2.42 h	2.41 j	372.75 h	387.7 i	9.42 e	9.62 g	56.00 i	58.75 h	41.65 f	41.99 h
T_5 = 100 g stem	24.40 cd	24.40 cd	3.03 bc	3.48 c	384.00bcd	412.7 b	10.10 d	10.87 bc	67.25 ab	68.53 ab	42.85 b	42.86 cd
T_6 = 200 g stem	24.27 cde	24.27 cde	2.96 c	3.31d e	383.0 bcd	406.7 cd	10.07 d	10.82 bc	63.25 de	66.51 cd	42.67 bc	42.79 ef
T_7 = 300 g stem	23.41 ef	23.41 ef	2.74 def	3.10 fg	381.00 de	402.5 ef	9.94 d	10.58 de	61.50 efg	64.23 ef	42.10 def	42.61 fg
T_8 = 400 g stem	21.93 g	21.93 g	2.53 gh	2.96 gh	376.00 gh	397.7 g	9.57 e	9.80 g	58.25 hi	61.26 g	41.75 f	42.51 fg
T_9 = 100 g roots	23.77 def	23.77 def	2.96 c	3.15 ef	384.75 bc	410.2 bc	10.37 c	10.99 ab	64.00 cd	67.26 bc	42.72 bc	43.19 b
T_{10} = 200 g roots	23.32 ef	23.32 ef	2.93 cd	3.46 cd	381.00 de	408.0 cd	10.08 d	10.98 bc	62.50 def	65.25 de	42.75 bc	43.11 bc
T_{11} = 300 g roots	24.07 cde	24.07 cde	2.69 efg	3.24 ef	379.25 efg	406.2 de	9.91 d	10.36 de	60.75 fg	63.77 ef	42.30 cde	42.69 ef
T_{12} = 400 g roots	23.02 f	23.02 f	2.60 fgh	2.84 hi	376.75 fg	399.0 fg	9.58 e	9.84 fg	59.25 gh	61.77 g	42.07 ef	42.54 fg
$LSD_{0.05}$	0.974	0.974	0.1878	0.174	3.465	4.0063	0.229	0.4137	2.279	1.7872	0.450	0.2893

Means followed by different letter(s) in a column are statistically significant at 5% level of probability.

and it is related to relative growth rate (Reddy, 2004). Different elements influence NAR, such as temperature, light, CO_2 water, leaf age, mineral elements, chlorophyll and genotype (Reddy, 2004). The data presented in Table 3 indicated that net assimilation rate was significantly reduced with increasing concentration of aqueous leaf, stem and root extracts of milkweed when compared with the control. Significantly lower net assimilation rate (2.42 and 2.41 g g^{-1} day^{-1}) was recorded with the application of a higher concentration (40%) of aqueous leaf extract over the control, which showed a higher net assimilation rate (3.72 and 4.02 g g^{-1} day^{-1}) in both cropping seasons. Similarly, a higher concentration (40%) of aqueous stems and roots extract recorded the minimum net assimilation rate (2.53 and 2.60 g g $^{-1}$ day^{-1}) was noted during the first and second year respectively.

Number of tillers (m^{-2})

Tillering in cereal crops is one of the most important yield contributing parameters, and it is influenced by genotype, environment and plant nutrition. Data on number of tillers per unit area are shown in Table 3, which revealed significant variations among treatments in 2013-14 and 2014-15. The data further exhibited that the number of tillers decreased with an increase in extract concentration of leaf, stem and root extracts of milkweed as compared with the untreated control. The maximum number of tillers (402.00 and 420.7 m ^{2}) was recorded in the control, while the highest concentration (40%) of aqueous leaf extract considerably reduced the number of tillers (372.75 and 387.7 m^{-2}) in both cropping seasons. There was a similar reduction in the number of tillers (376.00, 397.7 and 376.75, 399.0 m^{-2}) by applying a higher concentration (400) of aqueous stem and root extract, respectively, in 2013-14 and 2014-15.

Spike length (cm)

Data on spike length are shown in Table 4, which indicated that the application of different concentrations of aqueous leaves, stem and root extracts of milkweed had a significant effect on spike length. In the 2013-14 period, spike length was maximum (11.12 cm) in the control, where no extract was used while the application of 40% aqueous leaf extract of milkweed recorded minimum spike length (9.42 cm). Similarly, the use of 40% aqueous stem and root extracts reduced spike length (9.57 and 9.58 cm), respectively, as compared with the control. The data further revealed that minimum spike length (9.62 cm) was attained when maximum level (40%) of aqueous leaf extract was used. A similar trend was noted with the same concentration (40%) of aqueous extracts of the stem and root of milkweed, which produced spike length of 9.80 and 9.84 cm, respectively, as compared with the control, which had longer spikes of 11.26 cm in 2014-15.

Table 4 - Phytotoxic effect of milkweed (*Calotropis procera*) on grain yield, harvest index and grain protein content of wheat in 2013-14 and 2014-15

Treatment	Grain yield (kg ha^{-1})		Harvest index (%)		Grain protein content (%)	
	2013-14	2013-14	2013-14	2014-15	2013-14	2014-15
T_0 = Tap water	43.45 a	43.45 a	27.34 j	27.63 h	15.08 a	15.24 a
T_1 = 100 g leaves	42.72 bc	42.72 bc	27.52 hij	28.11 g	14.41 bc	14.75 a
T_2 = 200 g leaves	42.55 bcd	42.55 bcd	27.80 fgh	28.49f g	13.49 d	13.77 b
T_3 = 300 g leaves	42.32 cde	42.32 cde	27.91 efg	29.41 b	12.50 ef	12.88 d
T_4 = 400 g leaves	41.65 f	41.65 f	28.85 a	30.48 a	11.38 g	11.90 f
T_5 = 100 g stem	42.85 b	42.85 b	27.95 efg	28.29 fg	14.72 ab	14.91 a
T_6 = 200 g stem	42.67 bc	42.67 bc	28.44 bcd	28.52 fg	13.92 cd	13.71b c
T_7 = 300 g stem	42.10 def	42.10 def	28.20 cde	28.93 cd	12.86 e	12.95 d
T_8 = 400 g stem	41.75 f	41.75 f	28.76 ab	29.20 bc	12.19 f	12.29 ef
T_9 = 100 g roots	42.72 bc	42.72 bc	27.43 ij	28.54 ef	14.69 ab	15.07 a
T_{10} = 200 g roots	42.75 bc	42.75 bc	27.71 ghi	28.85 de	13.64 d	13.86 b
T_{11} = 300 g roots	42.30 cde	42.30 cde	28.15 def	29.07 bc	12.59 ef	13.20 cd
T_{12} = 400 g roots	42.07 ef	42.07 ef	28.55 abc	29.42 b	12.38 ef	12.75 de
$LSD_{0.05}$	0.450	0.450	0.3664	0.4299	0.6151	0.5167

Means followed by different letter(s) in a column are statistically significant at 5% level of probability.

Number of grains (spike^{-1})

Number of grains is an important yield contributing factor which plays a pivotal role in yield improvement. The data given in Table 4 revealed that the application of different concentrations of aqueous extracts of leaves, stem and root significantly reduced grain per spike as compared with the control for two cropping seasons. The maximum number of grains (69.25 and 70.26 spike^{-1}) was counted in the control, where no extract was used for two consecutive years. Among different concentrations, the leaf extract (40%) produced minimum number of grains (56.00 and 58.75 spike^{-1}). Similarly, the 40% concentration of aqueous stem and root extracts also resulted in a lower number of grains ((58.25, 61.26 and 59.25, 61.77 spike^{-1}) in the two years of experimentation as compared with the control.

1,000 grains weight (g)

One of the important yield-determining components is grain weight. It is a genetic character which is less influenced by environmental factors; sometimes, however, the different nutritional behavior affects grain weight substantially. The results showed that the aqueous extract of leaves, stems and root of milkweed negatively affected grain weight (Table 4). All these treatments showed a significant reduction in grain weight in both years as compared with the control. Maximum grain weight (43.45 and 43.86 g) was found in the control, where no extract was used. Among the different concentrations applied, the aqueous leaf extract (40%) showed maximum reduction in grain weight (41.65 and 41.99 g) in both the years. There was also a similar trend with increasing concentration of aqueous stem and root extracts, which significantly reduced grain weight. However, minimum grain weight (41.75, 42.51 and 42.07, 42.54 g) was recorded with maximum (40%) concentration of stem and root extracts, respectively, in both years of experiments.

Grain yield (kg ha^{-1})

The most integrative trait of a genotype is its grain yield (Araus et al., 2001). Grain yield is the interplay of different components such as spike per unit area, grains spike and thousand grain weight. However, grain weight exerts an influence on grain yield, but its effect is lower as compared with spike and grains per spike. The data presented in Table 4 revealed a significant

reduction in grain yield (3870 and 3927.5 kg ha^{-1}) with the highest concentration (40%) of aqueous leaf extract of milkweed than the untreated control, which produced grain yield of 4120 and 4165.0 kg ha^{-1} in the two cropping seasons. A similar trend was noted by applying the maximum level (40%) of stem and root extracts of milkweed, which produced grain yield of 3887.5, 3895 and 3992.5, 4010.0 kg ha^{-1} in both cropping seasons, respectively.

Harvest index (%)

Harvest index is the ratio between grain and biological yield, expressed in percentage. The data shown in Table 4 revealed that harvest index was significantly affected by using aqueous leaf, stem and root extracts of milkweed. All the study treatments showed corresponding increases in harvest index over the control. Among various treatments, maximum harvest index (28.85 and 30.48%) was recorded by applying 40% leaf extract of milkweed in both years of experimentation. Significantly lower harvest index (27.34 and 27.63%) was noted in the control, where no extract was used. There was a similar trend in the second experimental year.

Grain protein content (%)

The data shown in Table 4 revealed a significant variation in grain protein content by applying various aqueous extract concentrations of different plant parts of milkweed. Significantly lower grain protein contents (11.38 and 11.90%) were recorded by applying a higher concentration (40%) of the aqueous leaf extract over the control (15.08 and 15.24%) in both cropping seasons. Similarly, the highest concentration (40%) of the aqueous stem and root extract recorded minimum grain protein contents (12.19, 12.38 and 12.29, 12.75% than the untreated control in both experimental years.

Numerous allelochemicals are resealed by many weeds into the environment, which are known to be phytotoxic in nature. These chemicals are secondary metabolites and are alleopathic (Farooq et al., 2011). In the present work, the effect of different concentrations of aqueous leaf, stem and root extracts of milkweed indicated a corresponding reduction in weed density with the concentration of the extract applied. The decrease in weed density was higher at 60 days after sowing than early plant growth stages (30 DAS). Maximum weed density was noted in the control (tap water) because no extract was applied in this treatment. Minimum weed density was recorded by using a higher concentration (40%) of aqueous extract of milkweed. At early stages of plant growth, the phytotoxic effect of milkweed is usually dominating. It also causes inhibition of growth and development of weeds and crops (Farooq et al., 2008; Jabran et al., 2010). It contains some allelochemicals that can be used as a potential source for natural herbicides (Hirai, 2003; Cheema et al., 2004; Macias et al., 2007; Norton et al., 2008; Jabran et al., 2008, 2010; Razzaq et al., 2010, 2012). Similarly, the aqueous extract of milkweed has been reported to have a retarding effect on germination and growth of weed species, especially when treated with increasing concentrations (Gulzar et al., 2014).

Milkweed aqueous extracts were found to be phytotoxic and to decrease the fresh and dry weed biomass compared with the control. The maximum fresh and dry weed biomass obtained in the control was due to the fact that no extract was applied in this treatment, and throughout the crop season all the germinated weeds remained unchecked whereas fresh and dry weed biomasses were subsequently decreased by increasing the concentration of the aqueous leaf, stem and root extracts. It was probably due to reason that milkweed contains tannins, flavonoids, glycosides, steroids, saponins and cardiac glycosides, which are important allelopathic sources to reduce weed biomass (Umar and Mustapha, 2014). Samreen et al. (2009) reported that aqueous leaf extract of milkweed inhibits germination and seedling growth and reduces fresh and dry biomass.

In the study, leaf area index (LAI) and leaf area duration (LAD) have shown a direct correlation with aqueous extract concentration because the minimum LAI was recorded with the highest concentration (40%) of aqueous extract of three plant parts of milkweed at 49 and 98 days after sowing, as compared with the control. Allelochemicals such as coumarin, flvonoids, resins, phenols and many alkaloids present in milkweed have been reported to inhibit cell division, changes in phytohormones and their balance, water uptake and germination of seeds (Chon et al., 2002).

Ghasemi et al. (2012) also reported that the milkweed aqueous leaf extract has allelopathic properties including germination inhibition and yield reduction. The aqueous extracts of leaves, stem and root of milkweed have resulted in retardation of crop growth rate (CGR) and net assimilation rate (NAR). The maximum reduction in CGR and NAR were recorded at the highest concentration (40%) of aqueous extracts of three plant parts of *C. procera*. Among these, the aqueous leaf extract had the most reduction followed by maximum concentration of aqueous stem and root extracts of milkweed, respectively, as compared with the control. Manzoor et al. (2013) reported that an aqueous leaf extract of milkweed has had suppressing effects on lentil because at the highest leaf extract concentration, it showed significantly reduced growth in all physical growth parameters. The result also confirmed that phytotixcity is a concentration dependent phenomenon (Valthiyanathan et al., 2014). Allelochemicals retarded the various physiological and metabolic functions of plants such as photosynthesis, nutrient and water uptake, DNA synthesis and respiration (Einhelling, 2002). In the present study, phytotoxicity of milkweed was also investigated for growth, yield and quality parameters including number of tillers, spike length, number of grains per spike, 1,000 grain weight, grain yield, harvest index and grain protein content of wheat. Different applied concentrations of aqueous leaf extract significantly reduced all these growth and yield parameters. Other concentrations of aqueous stem and root extracts of milkweed also retarded growth and yield of wheat as compared with the control. This is because milkweed releases different types of water soluble phytotoxins in soil and in the surrounding environment, hence the growth of different crops is inhibited (Kadioglue et al., 2005; Singh et al., 2005; Batish et al., 2007). The release of toxic compounds to the environment, from leaching, litter decomposition, root exudation, or direct volatilization, negatively affects growth and yield of other crop species (Djurdjevic et al., 2004). These findings are also consistent with previous results (Al-Zahrani and Al-Robai, 2007; Tanveer et al., 2010; Chandra and Mali, 2012; Pukclai and Kawashty, 2012). The study revealed that the inhibition of growth and yield parameters was concentration dependent, i.e. inhibition increased with an increase in concentration of the aqueous extracts of three plant parts of milkweed and vice-versa.

This study showed that milkweed had inhibitory effects on various weeds grown in a wheat field. The presence of phytotoxic chemicals in milkweed also suppressed growth and yield of wheat. Therefore, wheat should not be planted close to milkweed. It can, however, be used as bio-herbicide to control weeds in wheat crops.

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