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Exploring the Allelopathic Potential of Plant Extracts for Weed Suppression and Productivity in Wheat (*Triticum aestivum* L.)

Ali Raza Gurmani[1] · Sami Ullah Khan[2] · Tariq Mehmood[2] · Waseem Ahmed[3] · Mazhar Rafique[1]

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Abstract

Weeds infestation poses huge threat to agricultural crop production systems and their management in modern agriculture. It is imperative to reduce yield losses and ensure food security. In order to minimize herbicide application, a study was carried out to evaluate the allelopathic potential of leaves extracts tested on weed suppression in wheat crop under rainfed conditions. Leaves extracts of *Moringa oleifera, Parthenium hystorophorus* and *Cannabis sativa* alone and in different combinations along with a control (distilled water) were sprayed to explore the allelopathic potential for weed management in wheat-maize cropping system. Foliar application of moringa + parthenium + cannabis leaves extract was the best treatment to reduce the number of leaves, leaf length and shoot length of all tested weed species. Leaf chlorophyll content and photosynthetic rate of weed plants was significantly reduced under exogenously application of leaf extracts. Various phenolic compounds were also detected in parthenium, cannabis and moringa leaf extracts. Maximum phenolic compounds were found in parthenium followed by cannabis and moringa leaf extracts. The combination of leaf extracts of *Moringa oleifera* (MLE) with *Parthenium hysterophorus* (PLE) and *Cannabis sativa* (CLE) (water extract at 3%) was significantly impactful to suppress weeds in wheat and achieve higher wheat growth.

Keywords Allelopathy · Cannabis · Plant leaf extract · Weed management · Wheat

✉ Sami Ullah Khan
samiullah@uoh.edu.pk

✉ Mazhar Rafique
mazharrafiq88@gmail.com

[1] Department of Soil and Climate Sciences, The University of Haripur, 22620 Haripur, Pakistan

[2] Department of Agronomy, The University of Haripur, 22620 Haripur, Pakistan

[3] Department of Horticulture, The University of Haripur, 22620 Haripur, Pakistan

Untersuchung des allelopathischen Potenzials von Pflanzenextrakten zur Unkrautbekämpfung und Produktivitätssteigerung bei Weizen (*Triticum aestivum* L.)

Zusammenfassung

Unkrautbefall stellt eine enorme Bedrohung für die landwirtschaftlichen Pflanzenproduktionssysteme und deren Management in der modernen Landwirtschaft dar. Es ist unerlässlich, Ertragsverluste zu verringern und die Ernährungssicherheit zu gewährleisten. Um den Herbizideinsatz zu minimieren, wurde eine Studie zur Bewertung des allelopathischen Potenzials von Blattextrakten durchgeführt, die zur Unkrautunterdrückung in Weizenkulturen bei Regenfeldanbau getestet wurden. Blattextrakte von *Moringa oleifera, Parthenium hystorophorus* und *Cannabis sativa* wurden einzeln und in verschiedenen Kombinationen zusammen mit einer Kontrolle (destilliertes Wasser) versprüht, um das allelopathische Potenzial für die Unkrautbekämpfung im Weizen-Maisanbau zu untersuchen. Die Behandlung mit Moringa- + Parthenium- + Cannabisblattextrakt war die beste Behandlung, um die Anzahl der Blätter, die Blattlänge und die Sprosslänge aller getesteten Unkrautarten zu reduzieren. Der Blattchlorophyllgehalt und die Photosyntheserate der Unkrautpflanzen wurde unter exogener Applikation der Blattextrakte signifikant reduziert. Verschiedene phenolische Verbindungen wurden in Parthenium-, Cannabis- und Moringa-Blattextrakten nachgewiesen. Das Maximum an phenolischen Verbindungen wurde in Partheniumextrakten gefunden, gefolgt von Cannabis- und Moringa-Blattextrakten. Die Kombination von Blattextrakten von *Moringa oleifera* (MLE) mit *Parthenium hysterophorus* (PLE) und Cannabis sativa (CLE) (3%iger wässriger Extrakt) war signifikant wirksam, um Unkräuter in Weizen zu unterdrücken und ein höheres Weizenwachstum zu erzielen.

Schlüsselwörter Allelopathie · Cannabis · Pflanzenblattextrakt · Unkrautbekämpfung · Weizen

Introduction

Biotic and abiotic factors have key importance in modern agriculture because they limit crop productivity. In order to sustain high productivity of crops, efforts to minimize biotic and abiotic factors are important. Abiotic factors include extreme temperature, moisture limitation, heat stress, nutrients supply and climate change while in biotic stress, insects, pests and weeds play a key role. All of these factors reduce crop yield and plant productivity rigorously (Mammadov et al. 2018; Suzuki et al. 2014). Weeds have detrimental effects on plant growth and severely damage plant productivity potential. Weeds compete with plants for available resources required for plant growth such as nutrients, water, light and space, subsequently crops compromise on yield (Zimdahl 2018). Wheat (*Triticum aestivum* L.) is an important cereal crop and more than one third of world's population consume wheat as staple food. Weed infestation is one of the main causes of wheat yield reduction in Pakistan, which reduces crop yield up to 36% (Ali et al. 2017; Muhammad Ashiq and Ahmad 2006).

Weeds are ubiquitous to most crops and reduce plant yield, consequently increase food production and make it cost effective (Ali et al. 2017). A huge group of weeds is present in crops and limits their productivity. Weed plants compete in certain environment and different species of weeds are involved with varying competitive ability (Guglielmini et al. 2017). It is difficult to estimate the loss of crop yield due to specific species of weeds, but the collective contribution of all weeds is estimated and considered much realistic. Weed control and management is an important characteristic of crop production in agricultural systems. Various hazardous synthetic herbicides are extensively applied in various crop species to control weed density in a short time period (Harrington and Ghanizadeh 2017; Tang et al. 2010). Although, weeds are effectively controlled through chemicals, but indiscriminate use of hazardous chemical herbicides built up weed resistance to various herbicides and created serious environmental pollution. However, allelopathy is an eco-friendly and sustainable solution against weed suppression and can be used in different ways such as use of water extracts and use of leaf extracts from allelopathic crops (Jmii et al. 2020; Mushtaq et al. 2020). The allelopathic effects may appear at both stages of seed germination as well as on the entire growth cycle of plant, with restrictive effects on photosynthesis. The secondary metabolites with allelopathic action belong to various groups of chemicals such as phenols, flavonoids, terpenoids, glucosynolates, benzoxaquinones, and cyanogenic compounds (Alshahrani and Suansa 2020; Oliveira et al. 2016). Keeping in view, the present study was designed to assess and compare the performance of plant extracts taken from *Moringa oleifera, Parthenium hystorophorus* and *Cannabis sativa* on weed suppression in wheat under wheat-maize cropping system for rainfed condition of Pakistan. This is the first study where the allelopathic effects of *Moringa oleifera* leaves extracts has been used alone and in combination with other potential allelopathic weed extracts to suppress weeds in wheat.

Materials and Methods

The experiment was carried out in pots in a plastic tunnel at Government Fruit Nursery Farm (34.01618° N, 72.91095° E), Agriculture Extension Department, District Haripur, Khyber Pakhtunkhwa, Pakistan. Wheat cultivar Pirsabak-2005 was obtained from Cereal Crop Research Institute (CCRI), Pirsabak, Nowshera, Pakistan while seed of weeds were obtained from Weed Research Laboratory, University of Agriculture, Peshawar, Pakistan. The experiment was laid out in a completely randomized design (CRD) with four replications. Before taking soil for pot experiment, soil samples of nearby field were collected from Ap horizon with the help of auger from 0–30 cm (0–15 and 16–30 cm) depth at different locations of experimental site. In total, eight subsamples were taken per hectare in a diagonal pattern and one composite sample of 500 g was obtained. Composite samples were air dried, ground and passed through 2 mm sieve to remove clods and materials other than soil. The physicochemical characteristics of soil were estimated by hydrometer method (Ryan and Rashid 2006). Soil texture was determined by hydrometer method (Gee and Bauder 1986) using a soil texture triangle. Soil pH and electrical conductivity (EC) determination were performed using 50 g air dried soil (<2 mm) in a 100 mL glass beaker, 50 mL deionized water (DI) was added to make saturated soil paste. Soil pH and EC were measured in a 1:1 soil water suspension (HANNA HI 9814 digital pH/EC/TDS meter, Japan). Soil organic matter was measured by taking 1 g air dried soil in a beaker and 10 mL 1 N potassium dichromate solution was added through a pipette, then 20 mL concentrated H_2SO_4 was added. Mixture was allowed to cool, and 10–15 drops of diphenylamine indicator was added. After stirring on magnetic stirrer, titration was done using 0.5 M ferrous ammonium sulfate until violet blue color changed to green. Simultaneously, blanks were prepared using same reagents, and without soil samples. Percentage organic matter was measured in soil using a formula described in soil and plant analysis manual (Ryan and Rashid 2006). Phosphorus and potassium (AB-DTPA extractable) were estimated by procedure described by Soltanpour and Schwab (1977) using spectrophotometer and flame photometer. In total, 5 g air-dry soil was weighed, added 100 mL 0.5 M sodium bicarbonate solution, and shaken for 30 min. Solution was filtered through a Whatman No. 40 filter paper and 10 mL clear filtrate was acidified with 5 N sulfuric acid to maintain pH 5.0. Exactly, 40-mL deionized water and 8 mL of reagent were added. A standard curve was prepared through pipetting 2 mL of each standard (1–5 ppm). Absorbance of blank, standards, and samples was measured at 882 nm wavelength using T-80+ UV/Vis Spectrophotometer (UK). Potassium was determined using 5 g air-dried soil, adding 33 mL ammonium acetate solution. The solution was put on shaker for 5 min and centrifuged until supernatant liquid became clear. Furthermore, filtrate was obtained, and process was repeated twice. Absorbance of soil sample extracts was measured on a PFP7 Flame Photometer (Jenway, UK) at 767-nm wavelength. The Kjeldhal method was used to calculate total nitrogen in soil (Bremner and Mulvaney 1996). About 1 g air-dried soil was taken in a digestion tube. Then 5.0–5.5 g catalyst mixture, a few pumice boiling granules, 15 mL concentrated sulfuric acid were added and swirled carefully. A glass funnel was placed in the neck of the tube and placed in rack and left overnight. Later, the test tube rack was placed in a block-digester; temperature was increased slowly to about 370 °C. When H_2SO_4 condensed to half-way up the tube neck and the solution cleared, it was heated for about 3 h. The test tubes rack was moved out of the block-digester and placed on a rack holder until cooled to room temperature. Then 15 mL deionized (DI) water was added to tubes, allowed to cool, and brought to volume with DI water. Each batch of samples for digestion contained one reagent blank (no soil), and one chemical standard (no soil, 1 mL of the stock solution). Then distillation was done and percent nitrogen in soil was calculated. Soil was silt loam with pH 7.1, EC 0.29 dS m^{-1}, organic matter 0.98%, nitrogen 0.051%, phosphorus 8.5 ppm, potassium 123 ppm and moisture 19%. The treatments were

- T1 = Control (distilled water spray),
- T2 = *Moringa oleifera* leaves extract (MLE),
- T3 = *Parthenium hystorophorus* leaves extract (PLE),
- T4 = *Cannabis sativa* leaves extract (CLE),
- T5 = *Moringa oleifera* + *Parthenium hystorophorus* leaves extract (MLE + PLE),
- T6 = *Moringa oleifera* + *Cannabis sativa* leaves extract (MLE + CLE),
- T7 = *Parthenium hystorophorus* + *Cannabis sativa* leaves extract (PLE + CLE) and
- T8 = *Moringa oleifera* + *Parthenium hystorophorus* + *Cannabis sativa* leaves extract (MLE + PLE + CLE).

Leaf extraction was performed according to Price et al. (2008) briefly by grinding young leaves with a drop of water (1 L/10 kg fresh material) in a locally fabricated extraction machine. The extracts from all treatments were applied at 3% alone, in various combinations and distilled water spray was used as control.

Crop husbandry

Wheat and weed seeds were sown in soil filled earthen pots (20 cm × 45 cm) and kept under plastic tunnel conditions. In each pot, 3 seeds of wheat and 3 seeds of weed species were sown. Before sowing, fungicide (Benomyl) at 2 g/kg of seeds were applied to both wheat and weed seeds. Recom-

mended fertilizer dose of nitrogen (N), and phosphorus (P) at the rate of 100 and 90 kg ha^{-1} were applied. Half dose of N was applied as basal dose while remaining was applied at knee stage. Plant extract spray (1:10) was applied on 10–15 days after crop and weeds seedling emergence as early post emergence through knapsack hand sprayer with T-jet nozzle. The first, second and third spray were done after 25, 45 and 65 days of sowing. Data were recorded at 15 days interval in each spray. After each spray, data regarding number of leaves, leaves length, shoot length of each tested weed species and wheat plant data was counted and recorded in centimeters (cm). The length was measured from point where shoot joins root. The fresh shoot and root weight were recorded in milligram (mg) and after that dried in an oven at 70 ºC to attain constant dry weight.

Determination of leaf chlorophyll concentration

Total chlorophyll concentration was determined (Arnon 1949). Fully expanded leaf from shoot apex of each weed specie was sampled (0.5 g) and grounded in 20 ml of 80% (v/v) ice cold acetone by mortar and pestle. The absorbance of homogenate was measured at 645 and 663 nm using UV/Vis Spectrophotometer (T-80+, UK) and total chlorophyll content were calculated:

$$Totalchlorophyll = \frac{20.2\,(D_{645}) + 8.2\,(D_{663})\,x\,V}{1000x\,W}$$

where:

D is optical density reading of sample extract at specific wavelength
V is volume of acetone chlorophyll extract
W is fresh weight of tissue extract (g)

Leaf photosynthesis

Gaseous exchange was measured on fully expanded new leaves of weeds using Portable Photosynthesis System (CID BioScience, Inc. USA) under 21% O_2, 1500 mol m^{-2} s^{-1} light intensity and leaf chamber temperature as 28 °C.

Phenolic compounds

The collected leaves of moringa, parthenium and cannabis were dried and ground. The samples were soaked in methanol for phenolics extraction. These methanol extracts of moringa, parthenium and cannabis leaves were sequentially partitioned against ethyl acetate, (1:1 v/v). The filter extracts were evaporated under vacuum and phenolic compounds were measured using HPLC. Dried extracts were liquefied in methanol (1 µg/mL) and filtered using a poly filter (pore size, 0.45 µm). Before analysis via HPLC, respective samples were diluted 2-folds with methanol. Futecs model NS-4000 HPLC (Daejeon, Korea) was used for analysis of phenolic compounds such as 4-hydroxy benzoic acid, P-hydroxy benzoic acid, vanillic acid, gallic acid, gentesic acid, 4-venyle phenols, 4-hydroxy phenyl acetic acid, B-resorcylic acid, P-coumaric acid and ethyl hematommate were recorded using HPLC (Nour et al. 2012). The analysis was supervised at 280 nm and carried out using a C18 column (250 mm × 4.6 mm, 5 µm; R Stech, Daejeon, Korea). The mobile phase contained 1% aq. acetic acid solution (Solvent A) and acetonitrile (Solvent B), the flow rate was kept at 0.7 ml/min, column was thermostatically controlled at 28 °C and injection volume was adjusted at 20 µl. A gradient elution was executed by varying proportion of solvent B to solvent A. The gradient elution was changed from 10% to 40% B in a linear fashion for duration of 28 min, from 40 to 60% B in 39 min, from 60 to 90% B in 50 min. The mobile phase composition back to initial condition (solvent B: solvent A: 10: 90) in 55 min and allowed to run for another 10 min, before injection of another sample. Total analysis time per sample was 65 min. The HPLC chromatograms were detected using a photo diode array UV detector at three different wavelengths (272, 280 and 310 nm) according to absorption maxima of analyzed compounds. Each compound was identified by its retention time and spiking with standards under same conditions. The quantification of sample was done by measurement of integrated peak area and content was calculated using calibration curve through plotting peak area against concentration of respective standard sample. All samples were run in triplicate.

Statistical analysis

The mean values for each treatment were recorded and subjected to the Analysis of Variance (ANOVA), using Statistix-8.1 (Robert et al. 1997). Means of various treatments were compared using Tukey's HSD test at 5% probability level.

Results and Discussion

This study indicated that all leaf extracts significantly affected weed dynamics and growth of wheat. High inhibitory effects on different wheat weeds were observed where MLE + PLE + CLE extract was sprayed as compared to MLE, MLE + PLE, MLE + CLE and PLE + CLE extract in case of number of leaves, leaves length and shoot length of different weeds species of wheat (Table 1). Maximum improvement in number of leaves, leaves length and shoot length of wheat was recorded in plant with

Table 1 Effect of foliar spray of various plant extracts on number of leaves, leaf length, shoot length and grain yield of wheat

Treatment	Number of leaves			Leaf length (cm)			Shoot length (cm)			Grain yield
	1st FS	2nd FS	3rd FS	1st FS	2nd FS	3rd FS	1st FS	2nd FS	3rd FS	
Control	12.12 c	8.17 d	12.12 c	6.55 bc	9.62 cde	12.85 c	14.20 d	27.20 e	40.87 d	2.76 f
MLE	14.05 b	10.05 c	14.05 b	9.05 a	10.8 bc	15.35 b	16.92 c	30.42 bc	46.15 b	3.26 d
PLE	9.475 d	7.37 d	9.475 d	6.45 c	8.37 e	9.675 d	13.67 d	25.25 f	36.52 e	3.13 d
CLE	9.175 d	7.25 d	9.175 d	6.67 c	8.75 e	9.125 d	14.00 d	24.62 f	35.07 f	2.99 e
MLE + PLE	14.20 b	11.2 b	14.20 b	8.22 ab	11.5 b	14.97 b	17.00 b	31.82 b	44.00 c	3.44 e
MLE + CLE	13.22 bc	8.02 d	13.22 bc	8.25 ab	10.15 cd	13.87 c	17.57 c	29.32 cd	43.02 c	3.18 d
PLE + CLE	12.52 c	7.65 d	12.52 c	7.12 bc	9.12 de	13.50 c	16.60 c	28.40 de	41.00 d	3.60 b
MLE + PLE + CLE	15.60 a	12.90 a	15.60 a	9.42 a	14.82 a	17.67a	25.05 a	35.82 a	51.07 a	3.76 a
HSD at 0.05 P	1.11	1.04	1.11	1.29	1.35	1.06	1.21	1.68	1.28	0.137

FS Foliar spray, *MLE Moringa oleifera* leaf extract, *PLE Parthenium hystorophorus* leaf extract, *CLE Cannabis sativa* leaf extract, $n = 3$

Table 2 Effects of foliar sprays of various plant extracts on number of leaves of weed plants

Treatments	*Avena fatua*	*Carthamus oxyacantha*	*Chenopodium album*	*Convolvulus arvensis*	*Euphorbia helioscopia*	*Fumaria indica*	*Phalaris minor*	*Sonchus oleraceus*
Control	6.54 ± 0.8	6.67 ± 0.9	7.35 ± 0.7	9.46 ± 1.1	10.81 ± 2.0	8.35 ± 1.3	7.17 ± 0.5	6.00 ± 1.2
MLE	4.97 ± 0.6	4.39 ± 0.9	4.36 ± 0.7	5.62 ± 1.4	6.09 ± 1.7	4.94 ± 0.9	3.92 ± 0.5	3.80 ± 1.1
PLE	6.04 ± 0.9	6.09 ± 1.3	6.41 ± 0.6	8.48 ± 1.4	9.92 ± 2.1	7.29 ± 1.3	6.08 ± 0.8	5.44 ± 1.4
CLE	6.05 ± 0.7	5.91 ± 0.2	6.65 ± 0.8	8.61 ± 1.5	9.87 ± 2.3	7.46 ± 1.0	6.27 ± 0.7	5.44 ± 1.4
MLE + PLE	4.39 ± 0.5	3.91 ± 0.7	3.50 ± 0.5	4.18 ± 1.2	4.77 ± 1.5	3.70 ± 1.1	4.37 ± 0.6	3.19 ± 0.8
MLE + CLE	4.19 ± 0.6	4.05 ± 0.8	3.33 ± 0.6	4.01 ± 1.3	4.60 ± 1.4	3.90 ± 1.1	4.23 ± 0.7	3.13 ± 0.8
PLE + CLE	5.16 ± 0.9	4.81 ± 1.1	5.05 ± 0.9	5.29 ± 1.3	6.05 ± 1.5	5.10 ± 1.3	4.78 ± 1.2	4.14 ± 0.7
MLE + PLE + CLE	4.13 ± 0.8	3.08 ± 0.6	2.79 ± 0.2	2.79 ± 0.5	2.91 ± 0.6	2.54 ± 0.5	3.51 ± 0.5	2.51 ± 0.5
Mean	5.18	4.86	4.93	6.06	6.88	5.41	5.04	4.21

FS Foliar spray, *MLE Moringa oleifera* leaf extract, *PLE Parthenium hystorophorus* leaf extract, *CLE Cannabis sativa* leaf extract, $n = 3$

Table 3 Effects of foliar sprays of various plant extracts on leaf length of weed plants

Treatments	*Avena fatua*	*Carthamus oxyacantha*	*Chenopodium album*	*Convolvulus arvensis*	*Euphorbia helioscopia*	*Fumaria indica*	*Phalaris minor*	*Sonchus oleraceus*
Control	56.2 ± 4.0	7.58 ± 1.4	7.29 ± 1.2	7.42 ± 1.7	7.62 ± 2.0	8.94 ± 1.8	7.68 ± 1.3	6.99±
MLE	44.2 ± 3.1	4.38 ± 0.7	4.62 ± 0.7	4.51 ± 1.1	4.35 ± 1.4	5.18 ± 1.0	5.25 ± 0.7	4.02±
PLE	38.0 ± 2.9	7.06 ± 1.3	6.54 ± 1.1	5.78 ± 1.6	6.68 ± 1.8	8.17 ± 1.5	6.86 ± 1.3	6.27±
CLE	42.0 ± 2.5	6.73 ± 1.1	6.36 ± 0.9	5.69 ± 1.6	6.41 ± 1.6	8.06 ± 1.7	6.87 ± 1.3	6.18±
MLE + PLE	34.0 ± 2.7	3.65 ± 0.5	3.73 ± 0.4	4.00 ± 1.0	3.37 ± 1.0	4.16 ± 1.0	5.52±–0.4	3.41±
MLE + CLE	31.0 ± 1.8	3.68 ± 0.4	3.75 ± 0.4	3.94 ± 0.9	3.42 ± 1.1	3.91 ± 1.0	4.84 ± 0.5	3.34±
PLE + CLE	27.0 ± 1.8	4.91 ± 0.7	5.18 ± 0.8	4.66 ± 0.9	4.48 ± 1.1	5.32 ± 1.5	5.37 ± 0.9	4.30±
MLE + PLE + CLE	25.0 ± 0.97	2.76 ± 0.4	3.00 ± 0.1	3.15 ± 0.8	2.38 ± 0.5	2.85 ± 0.4	4.08 ± 0.2	2.81±
Mean	6.68	5.09	5.06	4.89	4.84	5.82	5.81	4.67

FS Foliar spray, *MLE Moringa oleifera* leaf extract, *PLE Parthenium hystorophorus* leaf extract, *CLE Cannabis sativa* leaf extract, $n = 3$

MLE + PLE + CLE extract as compared to MLE, PLE and CLE application only. These beneficial effects of leaves extract may be attributed to presence of diverse allelochemicals in extracts which may adversely affect weed growth and hence favored wheat growth. Sole application of PLE and CLE extract significantly stimulated weeds growth and produced high number of leaves (Table 2), improved leaf length (Table 3) and shoot length (Table 4). Thus, it is likely that allelochemicals present in PLE and CLE have both beneficial and harmful effects on growth of tested weed species. In an earlier study, aqueous extracts of leaves markedly repressed the seed germination of sorghum with application of *Parthenium hysterophorus* (Murthy et al. 1995). The allelopathic activity of CLE might be due to its aggressive-

Table 4 Effects of foliar sprays of various plant extracts on shoot length of weed plants

Treatments	*Avena fatua*	*Carthamus oxyacantha*	*Chenopodium album*	*Convolvulus arvensis*	*Euphorbia helioscopia*	*Fumaria indica*	*Phalaris minor*	*Sonchus oleraceus*
Control	13.51 ± 2.2	12.06 ± 1.8	10.68 ± 2.2	13.47 ± 2.9	11.69 ± 3.0	12.32 ± 2.3	13.73 ± 2.5	9.73 ± 2.2
MLE	9.94 ± 1.4	7.15 ± 1.9	7.24 ± 1.5	9.31 ± 1.6	7.93 ± 2.8	8.55 ± 2.2	8.77 ± 1.7	5.41 ± 1.7
PLE	12.95 ± 2.3	10.53 ± 2.2	10.02 ± 2.0	12.02 ± 3.6	10.97 ± 2.8	11.31 ± 2.1	13.29 ± 2.6	8.26 ± 1.7
CLE	12.95 ± 2.0	10.54 ± 2.2	9.93 ± 2.1	11.53 ± 3.3	11.15 ± 3.1	11.32 ± 2.0	13.17 ± 2.3	8.64 ± 2.1
MLE + PLE	8.46 ± 0.7	6.30 ± 1.1	5.86 ± 0.7	6.12 ± 1.9	5.92 ± 2.4	6.10 ± 1.4	7.68 ± 1.1	4.31 ± 1.1
MLE + CLE	7.99 ± 1.1	5.59 ± 1.3	5.62 ± 0.7	6.04 ± 1.8	5.80 ± 2.5	6.12 ± 1.3	7.69 ± 1.1	4.27 ± 1.1
PLE + CLE	9.54 ± 1.4	7.25 ± 1.4	7.25 ± 1.0	7.49 ± 2.4	7.38 ± 2.6	7.61 ± 1.7	8.97 ± 1.5	5.35 ± 1.3
MLE + PLE + CLE	6.29 ± 1.2	4.31 ± 1.1	4.96 ± 0.2	3.07 ± 0.6	3.06 ± 0.7	3.61 ± 0.8	6.50 ± 0.6	3.21 ± 0.6
Mean	10.20	7.97	7.70	8.63	7.99	8.37	9.98	6.15

FS Foliar spray, *MLE Moringa oleifera* leaf extract, *PLE Parthenium hystorophorus* leaf extract, *CLE Cannabis sativa* leaf extract, $n=3$

Table 5 Effects of foliar sprays of various plant extracts on total leaf chlorophyll content (mg g^{-1} FW) of weed plants

Treatments	*Avena fatua*	*Carthamus oxyacantha*	*Chenopodium album*	*Convolvulus arvensis*	*Euphorbia helioscopia*	*Fumaria indica*	*Phalaris minor*	*Sonchus oleraceus*
Control	46.3 ± 0.4	56.6 ± 0.9	59.6.±0.7	63.4 ± 1.1	48.9 ± 1.5	63.3 ± 1.3	67.5 ± 0.6	66.0 ± 1.8
MLE	32.9 ± 0.9	44.3 ± 0.9	43.3 ± 0.8	51.6 ± 1.5	31.2 ± 1.3	54.0 ± 0.6	53.0 ± 0.4	53.8 ± 1.6
PLE	34.0 ± 0.7	42.0 ± 1.6	46.0 ± 0.7	48.8 ± 1.9	28.4 ± 1.0	48.8 ± 1.8	50.4 ± 0.8	50.4 ± 1.6
CLE	36.4 ± 0.7	41.7 ± 0.5	38.5 ± 0.7	43.8 ± 1.7	25.8 ± 1.5	43.9 ± 1.5	48.5 ± 0.8	47.9 ± 1.2
MLE + PLE	24.8 ± 0.6	30.2 ± 0.7	27.3 ± 0.6	30.2 ± 1.6	14.5 ± 1.2	23.8 ± 1.4	36.3 ± 0.6	34.5 ± 0.6
MLE + CLE	23.2 ± 0.6	28.4 ± 0.9	26.5 ± 0.8	31.5 ± 1.6	17.9 ± 1.7	26.3 ± 1.2	33.9 ± 0.7	29.6 ± 0.9
PLE + CLE	26.3 ± 0.8	25.5 ± 1.3	23.5 ± 0.9	28.4 ± 1.8	15.9 ± 1.2	24.5 ± 1.5	31.9 ± 1.2	30.8 ± 0.7
MLE + PLE + CLE	14.4 ± 0.9	15.0 ± 0.8	13.6 ± 0.8	15.7 ± 0.9	7.5 ± 0.3	11.3 ± 0.7	15.4 ± 0.3	14.4 ± 0.6
Mean	29.7	35.4	34.7	39.1	23.7	36.9	42.1	40.9

FS Foliar spray, *MLE Moringa oleifera* leaf extract, *PLE Parthenium hystorophorus* leaf extract, *CLE Cannabis sativa* leaf extract, $n=3$

Table 6 Effects of foliar sprays of various plant extracts on photosynthetic rate (μmol m^{-2} s^{-1}) of leaves of weed plants

Treatments	*Avena fatua*	*Carthamus oxyacantha*	*Chenopodium album*	*Convolvulus arvensis*	*Euphorbia helioscopia*	*Fumaria indica*	*Phalaris minor*	*Sonchus oleraceus*
Control	63.5 ± 2.4	53.6 ± 0.7	46.0 ± 1.7	59.4 ± 1.1	69.9 ± 1.0	72.3 ± 1.3	66.1 ± 0.5	58.7 ± 1.0
MLE	48.0 ± 1.6	44.0 ± 0.9	36.9 ± 0.9	46.6 ± 1.8	51.0 ± 1.4	61.9 ± 0.9	52.9 ± 1.5	45.8 ± 1.2
PLE	42.0 ± 2.2	40.0 ± 1.6	33.5 ± 0.6	44.4 ± 1.7	47.4 ± 1.4	55.2 ± 1.3	45.0 ± 0.6	42.4 ± 1.5
CLE	37.0 ± 2.4	33.0 ± 0.2	31.9 ± 1.5	41.6 ± 1.4	39.8 ± 1.8	47.4 ± 1.5	41.8 ± 1.0	38.7 ± 1.7
MLE + PLE	31.3 ± 1.5	26.5 ± 0.9	28.7 ± 0.8	31.0 ± 1.6	30.4.±1.5	25.6 ± 1.3	32.0 ± 0.6	23.5 ± 1.4
MLE + CLE	34.1 ± 1.6	24.0 ± 0.8	23.7 ± 0.9	27.0 ± 1.7	28.6 ± 1.4	20.7 ± 1.5	27.9 ± 0.8	26.0 ± 1.0
PLE + CLE	28.0 ± 0.9	24.8 ± 1.2	24.6 ± 1.5	24.6 ± 1.3	23.0 ± 1.2	23.8 ± 1.0	28.8 ± 1.5	22.1 ± 0.9
MLE + PLE + CLE	19.2 ± 0.8	18.0 ± 1.6	18.3 ± 0.8	17.2 ± 0.8	14.9 ± 1.3	16.4 ± 0.5	15.9 ± 0.9	14.9 ± 0.5
Mean	38.4	32.9	30.4	36.4	38.1	40.4	38.8	34.0

FS Foliar spray, *MLE Moringa oleifera* leaf extract, *PLE Parthenium hystorophorus* leaf extract, *CLE Cannabis sativa* leaf extract, $n=3$

ness and allelopathic effects on neighboring plants (Bárdi 2002). Possible reason of reduction in number of leaves, leaves length and shoot length of different weeds species by foliar spray of mixture of MLE + PLE + CLE is likely due to more inhibitory action of allelochemicals, either by creating physiological drought, prevention of cell division and elongation, or by reduction of stimulatory growth similar to findings of Al-Wakeel et al. (2007). Wheat usually undergoes stress through competition with weeds for sunlight, space, water and nutrients (Iqbal et al. 2014). Further, parthenium contains various phenolic compounds such as caffeic, vanillic, ferulic, chlorogenic and anisic acid and moringa leaves contain 4-monoacetyl-4-(R-Lrhamnopyranosyloxy)-benzylglucosinolate, 3-caffeoylquinic acid,

Table 7 HPLC quantification of phenolic acids (mg/100 g DW) identified in leaves of moringa, parthenium and cannabis

Phenolic compounds	Moringa leaves (mg/g)	Parthenium leaves (mg/g)	Cannabis leaves (mg/g)
4-hydroxy benzoic acid	0.56	1.09	2.1
P-hydroxy benzoic acid	0.67	1.56	1.32
Vanillic acid	1.23	2.56	ND
Gallic acid	1.34	1.67	1.45
Gentesic acid	ND	1.10	ND
4-Venyle phenols	ND	ND	ND
4-hydroxy phenyl acetic acid	ND	0.23	1.10
β-resorcylic acid	ND	0.34	2.34
P-coumaric acid	1.12	1.64	1.10
Ethyl hematommate	1.13	1.34	1.39

$n = 3$, ND: not detected

5-caffeoylquinic acid and three monoacetyl isomers of this glucosinolate while cannabis contains alkaloid, flavonoid, saponin, tannin, phenol, glycoside and essential oil (Sharma and Devkota 2014). Phenolic allelochemicals enter plants through the plant cell membrane and alter activity and function of certain enzymes. Similarly, they affect plant respiration rate and reduce oxygen absorption capacity of plants and chlorophyll content and as a result, plants show lower photosynthesis rate which is observed in lower number of leaves, leaves length and shoot growth. In recent research, leaf chlorophyll content and photosynthetic rate of weed plants reduced significantly under exogenously sprayed MLE, PLE, CLE, MLE + PLE, MLE + CLE, PLE + CLE and MLE + PLE + CLE as compared to control (untreated plants). The maximum decease in leaf chlorophyll content and photosynthetic rate was found when combinations of MLE + PLE, MLE + CLE, PLE + CLE and MLE + PLE + CLE were applied (Tables 5 and 6). The concomitant decrease in physiological traits was highest when all three plant extracts were combined. Some phenolic allelochemicals can reduce or inactivate physiological activity of plant hormones, which may then inhibit the normal physiological process of plants. In an earlier study, shoot and root extract of CLE significantly reduced shoot and seminal root length of *Lactuca sativa* L. (Mahmoodzadeh et al. 2015). Similarly, Akhtar et al. (2014) reported that CLE showed strong inhibitory effects on radicle and plumule growth of lettuce. Parthenin is known to have specific inhibitory effects on various weed species. Parthenium has more allelopathic effect in leaves extract (Tefera 2002) and significantly reduced germination and growth seedlings in various crop species and weeds (Demissie et al. 2013). The adverse effects of foliar leachates of parthenium against *Ageratum conyzoides* and *Avena fatua* are well documented (Batish et al. 2002; Belz et al. 2007). Parthenium leaf extracts at 4–5% significantly reduced germination as well as root and shoot growth of *Phalaris minor* (Shafique et al. 2013). In the current study, HPLC analysis of plant leaves extracts showed more phenolic compounds in PLE followed by MLE and CLE (Table 7). The PLE extract contained 4-hydroxy benzoic acid, P-hydroxy benzoic acid, Vanillic acid (2.56 mg/g leaf fresh weight), Gallic acid, Gentesic acid, 4-hydroxy phenyle acetic acid, β-resorcylic acid, P-coumaric acid and ethyle hematommate. The CLE extract contained all phenolics (except vanillic acid, Gentesic acid and 4-venyle phenols), while MLE extract contained all phenolics except Gentesic acid, 4-venyle phenols, 4-hydroxy phenyle acetic acid and β-resorcylic acid. The improvement in wheat growth by combined use of leaf extracts of PLE, MLE and CLE might be due to enhanced cell division, cell elongation, root size and branching which enhanced water uptake, nutrient absorption, and photosynthesis (Cheema et al. 2013).

Interestingly, the application of allelopathic extracts alone was not as effective as the mixture of various leaves extracts. Earlier findings by Khan et al. (2015) have confirmed that phytotoxicity of different allelopathic extracts increased when they were mixed together. This is likely due to more pronounced synergistic effects of one allelopathic plant when used combined with other allelopathic plant. Earlier research findings revealed that allelochemical compounds when present in proper combination and concentration can cause more phytotoxicity in locality (Khaliq et al. 2011). Our results are in line with findings of different studies that mixture of compounds/allelochemicals reduced weeds more than a single compound/allelochemical (Koul and Walia 2009). Combination of two or more allelopathic aqueous extracts acted synergistically and caused more phytotoxic effects on weeds (Mushtaq et al. 2010). Mixture of sorghum + sunflower + eucalyptus water extracts caused more than 70% weed suppression than sole sorghum water extract (Cheema et al. 2003). Jamil et al. (2009) reported that mixture of sorghum and sunflower aqueous extracts was inhibitory to *Avena fatua* and canary grass than sorghum aqueous extract alone. It indicates that the efficacy of a plant extract can be enhanced by mixing it with other allelopathic plant water extracts. Similarly, Awan et al. (2012) reported the allelopathic effect of water extracts of sorghum, sunflower and brassica on wheat field for reduction of weeds. They found that the highest weed density and biomass suppression was recorded where mixture of sorghum + sunflower + brassica extract was sprayed as compared to sole foliar spray sorghum, sunflower and brassica. Plant water extracts sprayed alone

and combined with each other suppressed weeds growth and hence augmented wheat growth. Extract made from the mixture of MLE + PLE + CLE was more effective in suppressing growth of wheat weeds than sole use of MLE, PLE or CLE extracts. Belz et al. (2007) also found pronounced synergistic effects of PLE extract when used with other plants. These growth suppressing effects of PLE, MLE and CLE were augmented due to greater concentration of allelochemicals present in their leaves. Plant extract treatments significantly affected the grain yield in wheat (Table 1). Highest grain yield (3.76 t ha^{-1}) was obtained where mixture of MLE + PLE + CLE was applied followed by PLE + CLE application (3.60 t ha^{-1}) which was at par with sole application of MLE and PLE. The highest increase in grain yield (26.60%) with respect to control was observed in MLE + PLE + CLE applied plants followed by PLE + CLE (23.34%) and MLE + PLE (19.77%). Similar results were described by Cheema et al. (2003) in which sorghum water extracts 21% at 1:10 w/v applied twice at 30 and 60 days after sowing increased wheat grain yield by 21% with decrease in weed density and dry weight by 44 and 49% over control, respectively. The results of present research suggest an accumulative phytotoxic effect of PLE, CLE and MLE extracts in terms of weed control in wheat. This has been manifested in the form of harmful effects of these allelochemicals on weed plant's light harvesting apparatus and decreasing overall photosynthetic capacity which lead to suppressive effect on weeds. On the other hand, wheat plants exploited these conditions in the form of better growth and overall better yield at the end. The combination of allelopathic plant water extracts each at 18 L ha^{-1} can be used as an effective and environment friendly weed management strategy in modern agriculture with increasing wheat grain yield.

Conclusion

Wheat plants were evaluated for plant growth and yield enhancement with foliar application of three plants leaf extracts. They were applied on wheat plants and different weeds. In conclusion, the combination of *Moringa oleifera* (MLE) with *Parthenium hysterophorus* (PLE) and *Cannabis sativa* (CLE) water extract at 3% is the best mixture to suppress weeds in wheat and achieve higher wheat growth. Number of leaves, leaves length and shoot length were significantly improved. There is a dire need to confirm these results in wheat under field conditions with the possibility of developing an effective natural herbicide to achieve satisfactory and environment-friendly weed control. The combination of allelopathic plant water extracts each at 18 L ha^{-1} can be used as an effective and environment friendly weed management strategy in modern agriculture with increasing wheat grain yield.

Conflict of interest A.R. Gurmani, S.U. Khan, T. Mehmood, W. Ahmed and M. Rafique declare that they have no competing interests.

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Mazhar Rafique PhD, is working as Assistant Professor with experience on Plant Sciences and Applied Soil Sciences. He has published 11 high impact factor papers and 6 book chapters. He is interested in working for application of this research knowledge in product development.