



Effect of sewage water and plant growth regulators on growth and yield of Mung Bean (*Vigna radiate* L.)

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ABSTRACT

The current scenario of water scarcity and the disposal problem of sewage water leads to its use in agriculture. Despite being rich of organic matter; sewage water may suppress crop growth and yield due to nutrient toxicity. In such case, growth regulators usage can ensure crop productivity under sewage water irrigations. In this study, the effect of exogenously applied growth regulators Moringa (*Moringa oleifera* L.) leaf extract (MLE) and Thiourea (TU) on mung bean plants irrigated with sewage water was evaluated. Field trials were conducted at Bahauddin Zakariya University, Multan, Pakistan, during the spring season of 2016 and 2017, using Randomized Complete Block Design (RCBD) with factorial arrangements and three replications. Treatments included different irrigation water sources (Tube well (TW) as a control, sewage water (SW) and mixed water (TW + SW)) in main plots and exogenously applied plant growth regulators (30 times diluted MLE; TU at rate of 1000 ppm; distilled Water (DW) as control) applied before and after flowering in sub-plots. Results revealed that interaction of mixed water irrigation with foliar application of MLE significantly enhances leaf chlorophyll 'a' and 'b' contents, antioxidant activity and improves plant growth characters like number of branches, leaves, plant height, and number of pods during both years. In comparison to control, MLE interaction with mixed water irrigation significantly improved the yield of mung by producing 45.6% and 4.9% more grain yield, 138% and 13.3% biological yield, and 60.2% and 60.3% heavier thousand grains weight during 2016 and 2017, respectively. MLE exogenous application on sewage water irrigated plants also produced significantly better yield followed by thiourea sprayed on sewage water irrigated plants. It was concluded that sewage water can only be used for crop production after water analysis, and for higher yields under semi-arid conditions, foliar application of MLE followed by TU can be recommended.

INTRODUCTION

Mung bean is considered to be the best source of protein (25%) and also known as the poor man's meat (Imran et al. 2016). In Pakistan it was previously cultivated as a summer crop after the successive monsoon season, but after the introduction of new varieties, it can be grown in the spring season in the south Punjab region to minimize the yield gap. For the year 2015-16 mung bean was cultivated on 146.3 thousand ha producing 98 thousand tons in Pakistan (Govt.-Of-Pakistan 2016) in which 113.14

thousand ha area was cultivated only in Punjab and produced 81.55 thousand tons (Pakistan-development-Statistics 2016). Per unit area yield of pulses is low in Pakistan because of insufficient irrigation. Rising issue of water scarcity puts a significant negative impact on the economy, human life, and environmental quality throughout the world (Singh et al. 2010). In agriculture, limited good quality water for irrigation forcibly increases the use of municipal wastewater (Palese et al. 2009).

In developing countries, the use of sewage water in

agriculture is an old practice (Ullah et al. 2012). Domestic wastewater is considered to be a good source of enriched organic nutrient water supply for farmers (Ghosh et al. 2012) and is used as the cheapest and reliable system of its disposal in agriculture (Faruqui et al. 2004, Perveen et al. 2012). Wastewater contains organic matter and certain essential plant macronutrients (N, P, K, S, Ca, Cu, Zn, and Mn) (Kannan et al. 2005), and have reported yield increase in few crops (Lubello et al. 2004, Nagajyothi et al. 2009, Nath et al. 2009). However, continuous sewage water usage leads to soil and groundwater contamination, phytotoxicity (Hicks and Hird 2000), micronutrient accumulation at higher concentrations and produces undesirable effects on plants (Kocak et al. 2005).

In such situation, exogenous application of plant growth regulators (PGRs) is effective for enhancing plant growth under normal and stressful environment (Bråthe et al. 2002, Li et al. 2016), minimizing the harmful effects of heavy metal in crop and improve shoot growth (Ouzounidou and Ilias 2005). Although plants can produce PGRs endogenously, yet the plant responds better to exogenous applications and stores extra amount of hormones which activates when needed (Yasmeen et al. 2012). Moringa leaf extract (MLE) is a natural PGR and is a rich source of antioxidants, calcium, potassium, iron, (Barciszewski et al. 2000), amino acids, and growth-regulating hormones like zeatin (Basra et al. 2009a, Basra et al. 2009b, Yasmeen et al. 2013), ascorbates, carotenoids, flavonoids and phenols (Iqbal and Bhangar 2006). Zeatin is the cytokinin that promotes plant growth, anti-aging potential (Marcu 2005), cell division, and elongation (Taiz and Zaiger 2006). Exogenous application of MLE has been reported to improve seedling emergence, vigor, and growth in grasses (Nouman et al. 2012a, Nouman et al. 2012b), suppressed the drought effects in maize (Ali et al. 2011), produced more and larger fruits and increased resistance against pests and diseases (Fuglie 2000) and enhanced the growth and yield of mung bean (Abohassan and Abusuwar 2018). MLE was also reported to mitigate the heavy metal stress like cadmium in the bean plant by increasing chlorophyll, plant growth, and antioxidant status (Howladar 2014).

Thiourea (TU) is a synthetic PGR and has been proven effective in breaking dormancy and stimulate germination (Mathur et al. 2006) by strengthening plants built-in mechanisms through physiological and molecular changes during biotic and abiotic stresses (Talukdar 2014). Several pieces of the research reported that exogenous TU application enhances heat stress tolerance in plants by reducing oxidative damage (Asthir et al. 2013), enhances salinity tolerance (Srivastava et al. 2011), reduces arsenic toxicity (Srivastava et al. 2014), and enhances plant resistance against parasitic weeds (Kannan and Zwanenburg 2014). Moreover, TU has effectively enhanced photosynthetic rate in cluster bean (Garg et al. 2006), water use efficiency in moth bean (Garg et al. 2003), and yield of mung bean (Singh and Rathore 2003, Mathur et al. 2006).

However, there is only limited literature available to show the effect of PGRs on the growth and productivity of mung bean. Moreover, no study has been conducted to determine the effect of natural and synthetic growth

regulators on the morphological, physiological, and yield-related character of mung bean irrigated with the sewage water. Therefore, the present study was conducted to evaluate the effect of irrigation water source and PGRs on the growth and yield of mung bean.

METHODOLOGY

Experimental Site and Layout

The experiments to determine the effect of sewage water and foliar applications of PGRs were performed in the spring season of 2016 and 2017 at Agronomic Research Farm, Bahauddin Zakariya University, Multan, Pakistan. Experiments were laid out in Randomized Complete Block Design (RCBD) with a factorial arrangement and replicated thrice. The net plot size for each replication was 3×2 m².

Experimental details

Factors under study included sources of irrigation water and foliar application of plant growth regulators (PGRs). Source of irrigation water including tube well water (TW), sewage water (SW) and mixed water (TW+SW) were placed in main plots while foliar application of distilled water (DW), Moringa leaf extract (MLE), and Thiourea (TU) were placed in sub-plots, while replication was placed in sub-sub plot.

During experiment conduction, four irrigations were applied including paleo watering during both years. Remaining irrigations were applied at 20, 35, and 50 days after sowing (DAS). For mixed water irrigation, an alternate irrigation method was used in which paleo watering was performed with SW followed by TW irrigation at 20 DAS, SW irrigation at 35 DAS, and TW irrigation at 50 DAS. Tube well water was taken from the farm tube well of the Agronomy Department, BZU, Multan while sewage water was taken from the sewerage system of BZU residential colony. Foliar application of 30 times diluted MLE (Yasmeen et al. 2013), 1000 ppm thiourea (Balai and Keshwa 2011), and distilled water was performed at 45 DAS using a hand sprayer.

Water analysis

Tube well and sewage water samples were collected to be analyzed by the procedure described by (Estefan et al. 2013). EC was 0.49 dSm⁻¹ and 0.12 dSm⁻¹, potassium concentration 1.5 and 30.78 mg/L, calcium concentration 30 and 51.1 mg/L, magnesium concentration 15 and 25.73 mg/L in tube well water and sewage water, respectively. Detailed sewage water analysis is presented in Annex. 1.

Preparation of Moringa Leaf Extract (MLE)

Fresh *Moringa oleifera* leaves were taken from the Agronomic research area of Bahauddin Zakariya University Multan and washed with distilled water before storing them at -5°C for 24 hours. Leaf extract was collected using a mechanical extractor (Yasmeen 2011), then extract was filtered using Whatman No. 1 filter paper followed by centrifugation at 8000 rpm for 15 min and 30 times dilution with distilled water prior foliar application.

Agro-Climatic Conditions and Meteorological Data

The meteorological data for both growing years is presented in Table 1.

Table 1 Meteorological data

Months	Mean Temperature (°C)		Relative Humidity (%)		Wind Velocity (km/hr)		Total Rainfall (mm)	
	2016	2017	2016	2017	2016	2017	2016	2017
March	21.95	21.7	77.5	68.35	4.8	4.3	20.1	0
April	28.5	30.0	86	53.5	5.0	5.5	13.1	5.7
May	34.35	33.98	74.3	63.05	6.5	6.68	2.0	0.1

Source: Pakistan Central Cotton Committee (PCCC), Multan

Soil Analysis

During both years, soil samples were collected from the experimental area before sowing and analyzed according to the procedure described by (Ryan et al. 2001). The soil texture of the experimental area was silt loam during both years having a pH 8.1 and 8.2, EC 2.13 and 2.15 $\mu\text{S cm}^{-1}$, organic matter 0.5% and 0.55%, available phosphorous 13.5 and 8.3 mg kg^{-1} , available potassium 260 and 255 mg kg^{-1} during the first and second year, respectively.

Crop Husbandry

The seedbed was prepared using respective irrigation water source and half the dose of recommended nitrogen and full phosphorous dose were applied at the time seedbed preparation. Seeds of *Vigna radiate* cv. NIAB Mung 2011 were sown on 1st March 2016 and 2017 with a hand drill while maintaining 30 cm row to row distance. Thinning was performed at 30 DAS to maintain a 10 cm plant to plant distance. Irrigation was performed as per treatment details. No disease was reported during the experiment in both years. Weeds were removed manually throughout the growing season of mung bean during both years. All other agronomic practices that are necessary to raise crops were kept the same in all experimental units. Harvesting was performed at 80 DAS.

Data Collection of Growth and Yield Parameters

At maturity, plants were harvested randomly from each experimental unit from three different points using a quadrat and weighed and averaged to measure biological yield (g m^{-2}) that was converted into biological yield (ton ha^{-1}). Grains of harvested plants were weighed to

determine economic yield (g m^{-2}) and converted into economic yield (ton ha^{-1}). 1000 grains were counted and weighed to obtain 1000 grain weight (g). 15 plants were randomly selected and tagged to record plant height (cm), number of pods per plant, and weight of pods per plant (g). Harvest index (%) was calculated by using the following formula (ur Rehman et al. 2005).

$$\text{HI} = \frac{\text{Economic Yield}}{\text{Biological Yield}} \times 100$$

Biochemical analysis

For biochemical analysis, leaves samples were taken before and after foliar application at 40 and 55 DAS and stored in liquid nitrogen immediately for transport from field to lab where they were preserved in a refrigerator at -20°C for further biochemical analysis. Chlorophyll a and b concentration was determined by following the methodology described by (Arnon 1949) using Agilent Cary 60 UV-Vis Spectrophotometer. Total soluble protein (mg g^{-1}) was determined using Bradford assay (Bradford 1976). Antioxidant enzymatic activity ($\text{IU min}^{-1} \text{mg}^{-1}$ protein) was also calculated by following the methodology described by (Giannopolitis and Ries 1977) for Superoxide dismutase (SOD) activity, and the procedure described by (Chance and Maehly 1955) was followed to determine Peroxide dismutase (POD) and Catalase (CAT) activity.

Statistical Analysis

MS Excel 2007 was used to record and arrange the data. Statistical variance analysis and treatments mean comparison was performed using Statistix 8.1 at 5% probability level.

Annex. 1 Sewage water analysis

Parameters	Unit	Results	Parameter	Unit	Results
pH	-	5.9	NO ₃ -N	mg/L	3.9
TSS	mg/L	76.5	Iron	mg/L	1.75
TDS	mg/L	610.9	Total Phosphorus	mg/L	16.93
EC	dS/m	0.12	Potassium	mg/L	30.78
DO		0.2	Magnesium	mg/L	25.73
BOD	mg/L	130.5	Calcium	mg/L	51.1
COD	mg/L	160.2	Total Coli forms	Count /100 ml	870
Alkalinity	mg/L	123.9	E-coli	Count /100 ml	3035
TN	mg/L	19.3	Turbidity	FAU	79
NH ₃ -N	mg/L	6.4	Color	Pt/co	595.7

RESULTS

Growth and yield analysis

Irrigation water source and foliar application of plant growth regulators significantly affected growth and yield of mung bean during both years of field trials (Tables 1 and 2). In 2016, irrigation with mixed water significantly affected all growth and yield-related parameters, however, in 2017, irrigation with sewage water significantly affected all physiological parameters under observation except the number of shoots and harvest index. No significant

differences were recorded among irrigation water sources for grain yield and 1000 grain weight during the second year of the experiment (2017). Among foliar applications, MLE spray significantly affected all growth and yield parameter in both years except the harvest index. In comparison to control, Thiourea spray also showed a significant effect on growth and yield of mung bean during both years except the number of leaves and the number of shoots in the second-year trial (2017) where the control treatment performed better than thiourea (Tables 1 and

2).

Table 1 Effect of Irrigation water source and foliar application of MLE and TU on growth and yield of mung bean during 2016

	Plant height (cm)	Shoot count	Leaves count	Pods count	Pods weight (g)	1000 Grain weight (g)	Biological yield (unit)	Grain yield (unit)	Harvest Index
TW	30.12 c	13.16 c	25.33 c	9.64 c	4.026 c	39.94 c	47.03 a	1.86 c	4.016 c
Mix	36.60 a	19.23 a	37.33 a	17.90 a	10.19 a	44.32 a	33.99 c	2.13 a	6.362 a
SW	34.61 b	15.11 b	28.01 b	11.77 b	4.31 b	40.78 b	42.24 b	1.90 b	4.557 b
DW	27.60 c	11.94 c	25.50 c	8.66 c	4.65 c	34.83 c	4.178 c	1.83 c	44.59 a
MLE	42.00 a	21.11 a	36.34 a	18.23 a	8.63 a	48.83 a	6.192 a	2.17 a	36.06 c
TU	31.73 b	14.44 b	28.83 b	12.42 b	5.25 b	41.38 b	4.564 b	1.88 b	42.61 b

Note: Different letters in the same column indicate a significant difference between treatments at 5% probability level. Where TW = Irrigation with tube well water; Mix = Irrigation with mixed (TW+SW) water; SW = Irrigation with sewage water; DW = Foliar application of distilled water; MLE = Foliar application of moringa leaf

extract; TU = Foliar application of Thiourea

Table 2 Effect of Irrigation water source and foliar application of MLE and TU on growth and yield of mung bean during 2017

	Plant height (cm)	Shoot count	Leaves count	Pods count	Pods weight (g)	1000 Grain weight (g)	Biological yield (unit)	Grain yield (unit)	Harvest Index
TW	35.86 c	10.49 c	15.46 b	9.82 c	7.00 b	53.56 a	4.911 b	2.02 a	40.98 b
Mix	37.63 b	12.32 a	15.56 b	10.93 b	7.00 b	53.11 a	4.666 c	2.01 a	43.83 a
SW	39.13 a	11.92 b	16.55 a	12.24 a	7.93 a	53.44 a	5.055 a	2.03 a	41.12 b
DW	37.66 b	10.66 b	14.72 b	9.089 c	6.36 c	52.33 b	4.322 c	1.94 b	45.50 a
MLE	39.71 a	13.42 a	18.84 a	12.97 a	8.36 a	55.89 a	5.577 a	2.13 a	38.44 c
TU	35.25 c	10.64 b	14.02 c	10.93 b	7.20 b	51.89 b	4.733 b	1.99 b	42.00 b

Note: Different letters in the same column indicate a significant difference between treatments at 5% probability level. Where TW = Irrigation with tube well water; Mix = Irrigation with mixed (TW+SW) water; SW = Irrigation with sewage water; DW = Foliar application of distilled water; MLE = Foliar application of moringa leaf extract; TU = Foliar application of Thiourea

MLE interaction with mixed water irrigation significantly affected all yield contributing factors during the first year while, in the second year, MLE spray on plants irrigated with sewage water produced the heaviest pods per plant bearing the heaviest 1000 grains followed by MLE and mixed water interaction (Table 4). The significantly higher biological and economical yield was also observed for MLE and mixed water irrigation interaction during 2016, while in the second year (2017), the interaction of MLE with sewage water produced significantly higher biological and economical yield, that was statistically equal and at par, respectively, for MLE and mixed water irrigation interaction (Table 5).

The interaction between the irrigation water source and PGR foliar application also showed a significant effect on all growth and yield contributing factors. Foliar application of MLE on mixed water irrigated plants provided with the best results in both years. All growth parameters under observation were significantly affected by the interaction of MLE and mixed water irrigation during both years of the experiment followed by MLE and sewage water irrigation interaction for plant height and number of shoots during both years (Table 3). Similarly,

Table 3 Mean comparison of interaction of water source and foliar application on growth traits of mung bean during 2016 & 2017

Irrigation source	Foliar spray	Plant height (cm)		No. of shoots		No. of leaves	
		2016	2017	2016	2017	2016	2017
Tube Well water	DW	24.03 h	39.23 c	11.13 e	11.77 d	23.16 g	17.57 c
	MLE	37.67 c	34.83 e	15.66 c	10.73 e	28.33 d	16.76 d
	TU	28.67 f	33.53 ef	12.67 d	8.96 g	24.5 f	12.07 fg
Mixed water	DW	27.60 g	37.13 d	11.70 e	11.67 d	28.33 d	14.73 e
	MLE	49.33 a	43.30 a	30.33 a	14.97 a	49 a	19.83 a

Sewage Water	TU	32.87 d	32.47 f	15.67 c	10.33 f	34.6 b	12.13 f
	DW	31.17 e	36.63 d	13.00 d	8.56 h	25 f	11.87 g
	MLE	39.00 b	41.00 b	17.33 b	14.57 b	31.7 c	19.93 a
	TU	33.67 d	39.77 bc	15.00 c	12.63 c	27.3 e	17.87 b

Note: Different letters in the same column indicate a significant difference between treatments at 5% probability level. Where, DW = Foliar application of distilled water; MLE = Foliar application of moringa leaf

extract; TU = Foliar application of Thiourea

Table 4 Mean comparison of interaction of water source and foliar application on yield contributing traits of mung bean during 2016 & 2017

Irrigation source	Foliar spray	Number of pods per plant		Pods weight per plant (g)		1000 Grain weight (g)	
		2016	2017	2016	2017	2016	2017
Tube Well water	DW	9.00 e	10.77 d	3.43 h	8.33 d	34.33 e	54.33 a-c
	MLE	10.33 d	9.13 f	5.00 d	6.03 g	45.67 b	54.00 bc
	TU	9.60 de	9.56 e	3.64 g	6.63 e	39.83 d	52.33 cd
Mixed water	DW	9.33 e	8.30 g	6.33 c	5.53 h	35.33 e	53.00 c
	MLE	30.03 a	15.03 a	16.20 a	9.23 b	55.00 a	56.66 ab
	TU	14.33 b	9.46 e	8.03 b	6.23 f	42.63 c	49.67 d
Sewage Water	DW	14.33 b	8.20 g	4.17 f	5.23 i	34.83 e	49.67 d
	MLE	13.33 c	14.76 b	4.70 e	9.83 a	45.83 b	57.00 a
	TU	9.00 e	13.77 c	4.06 f	8.73 c	41.67 c	53.67 c

Note: Different letters in the same column indicate a significant difference between treatments at 5% probability level. Where, DW = Foliar application of distilled water; MLE = Foliar application of moringa leaf extract; TU = Foliar application of Thiourea

Table 5 Mean comparison of interaction of water source and foliar application on yield of mung bean during 2016 & 2017

Irrigation source	Foliar Spray	Biological yield		Grain yield		Harvest Index	
		2016	2017	2016	2017	2016	2017
Tube Well water	DW	3.53 h	5.26 c	1.82 e	2.03 bc	51.46 a	38.73 d
	MLE	4.76 e	4.70 d	1.91 c	2.03 bc	40.18 d	42.93 c
	TU	3.74 g	4.76 d	1.85 d	2.00 c	49.45 b	41.30 c
Mixed water	DW	4.88 d	4.03 e	1.84 de	2.03 bc	37.62 e	49.33 a
	MLE	8.44 a	5.96 a	2.65 a	2.13 ab	31.38 h	35.53 e
	TU	5.76 b	4.00 e	1.90 c	1.86 d	32.97 g	46.63 b
Sewage Water	DW	4.11 f	3.66 f	1.84 d	1.76 d	44.69 c	48.43 ab
	MLE	5.37 c	6.07 a	1.96 b	2.23 a	36.62 f	36.87 de
	TU	4.18 f	5.43 b	1.90 c	2.10 bc	45.4 c	38.07 d

Note: Different letters in the same column indicate a significant difference between treatments at 5% probability level. Where, DW = Foliar application of distilled water; MLE = Foliar application of moringa leaf extract; TU = Foliar application of Thiourea

Biochemical analysis

Biochemical analysis presented in Table 6 and 7 indicate that irrigation water source significantly affected biochemical traits of Mung bean before and after foliar application of PGR during both years of study. Before foliar application; maximum chlorophyll content, antioxidant enzyme activity, and protein content were observed for mixed water irrigation during both years followed by sewage water irrigation that showed significant higher chlorophyll content and CAT activity than control during 2016, while during the second year, no significant difference for biochemical traits except SOD activity was observed between sewage water and tube well water irrigated plants. Similar results were observed after the foliar application of PGRs where mixed water irrigation significantly affected all biochemical traits during both years followed by sewage water irrigation (Table 6 & 7). Mean comparison of foliar application indicated that MLE

and TU foliar applications significantly affected all biochemical traits during both years when compared to the foliar application of distilled water. In 2016, MLE foliar application significantly affected all biochemical traits followed by foliar application of TU, however, no significant difference was observed between MLE and TU foliar application for POD and SOD activity during 2017. Moreover, significantly higher chlorophyll "a" content and CAT were observed for TU foliar application, while MLE foliar application showed significantly higher chlorophyll b and protein content in Mung bean during 2017. Mean comparison of interaction of irrigation water source and foliar application of PGR showed that during the first year of study, MLE interaction with mixed water irrigation had a significant effect on all biochemical traits followed by MLE and sewage water interaction. However, in 2017, MLE interaction with mixed water irrigation significantly enhanced chlorophyll and protein content while all antioxidant enzyme activity was highest for MLE and sewage water interaction (Tables 6 and 7).

Table 6 Effect of irrigation water source, foliar application of PGR and there interaction on chlorophyll "a & b" content, antioxidant enzyme activity, and protein content of Mung bean during 2016

Sampling time	SOV		CHL. A	CHL. b	SOD	POD(10 ⁻³)	CAT	PROTEIN	
Before foliar application	Irrigation water source	I1	0.745 b	0.523 b	0.056 c	0.422 b	0.850 a	17.152 b	
		I2	0.851 a	0.587 a	0.073 a	0.644 a	0.954 a	21.456 a	
		I3	0.732 b	0.528 b	0.063 b	0.444 b	0.902 a	18.462 b	
After foliar application	Foliar application	F1	0.843 c	0.640 c	0.058 b	0.511 b	0.874 c	17.951 c	
		F2	0.877 b	0.664 a	0.074 a	0.833 a	1.050 b	22.450 a	
		F3	0.892 a	0.654 b	0.074 a	0.800 a	1.095 a	20.661 b	
	Irrigation water source	I1	0.871 b	0.662 b	0.060 c	0.467 c	0.975 b	17.884 c	
		I2	0.983 a	0.713 a	0.078 a	0.900 a	1.060 a	23.152 a	
		I3	0.757 c	0.582 c	0.067 b	0.778 b	0.984 b	20.026 b	
	Interaction	Interaction	F1I1	0.861 d	0.661 cd	0.048 g	0.300 e	0.816 f	14.603 h
			F1I2	0.955 b	0.699 b	0.066 e	0.500 d	0.893 e	20.850 e
			F1I3	0.712 g	0.562 g	0.060 f	0.733 c	0.913 e	18.400 f
			F2I1	0.864 d	0.669 c	0.071 cd	0.567 d	1.140 b	17.730 g
			F2I2	1.007 a	0.736 a	0.081 b	1.000 b	1.043 c	25.657 a
			F2I3	0.759 f	0.586 f	0.069 de	0.933 b	0.966 d	23.963 b
		F3I1	0.887 c	0.657 d	0.062 f	0.533 d	0.970 d	21.320 d	
		F3I2	0.988 a	0.705 b	0.088 a	1.200 a	1.243 a	22.950 c	
		F3I3	0.799 e	0.599 e	0.073 c	0.667 c	1.073 c	17.713 g	

Note: CHL a = Chlorophyll a content in leaves; CHL b = Chlorophyll "b" content in leaves; SOD = Superoxide dismutase activity; POD = Peroxide dismutase activity; CAT = Catalase activity; I1 = Irrigation with tube well water; I2 = Irrigation with mixed water; I3 = Irrigation with sewage water; F1 = Foliar application of distilled water; F2 = Foliar application of MLE; F3 = Foliar application of thiourea

DISCUSSION

Water scarcity and mismanagement of wastewater have led to the usage of wastewater for irrigation purposes in developing countries. Wastewater is either used directly for irrigation or indirectly after it gets mixed with irrigation water due to mismanagement. The ability of sewage water to supply the soil with essential nutrients can have a positive effect on plant development, while, an excessive amount of nutrient and heavy metals can have a negative effect as well (Aboukhaled 1991, Abou-Seeda et al. 1997). Therefore, this research was conducted to evaluate the effect of sewage water usage on crop growth and yield.

Results revealed better vegetative growth of mung bean when irrigated with mixed water during both experimental years. (Dash 2012) also reported similar results while evaluating the effect of 50% diluted wastewater on the growth of wheat and rice. An increase in vegetative growth could be due to the presence of a moderate concentration of nutrients diluted by tube well water. The yield of mung bean plants irrigated with mixed water was also significantly higher than control. These results are consistent with the observation of (Capra and Scicolone 2007, Al-Hadithy et al. 2011) who observed an increase in growth and yield of growing crops in soils treated with different levels of sewage water. Sewage water when mixed with tube well water serves as fertilizer and helps in yield improvement of crops. Researches like

(Khan 2016) have reported a negative effect of sewage water because of excessive concentration of nutrients, however, no negative effects on growth or yield of mung bean were observed due to sewage water irrigation during 2016 or 2017. It can be due to the lesser toxicity of sewage water used during the experiment, however, sewage water analysis is always recommended before using it for irrigation.

Among plant growth regulators, moringa leaf extract spray significantly enhanced crop growth and yield. The presence of zeatin in moringa leaves can be a cause of better vegetative growth (Taiz and Zaiger 2006). Zeatin (a cytokinin) has been reported to enhance cell division, leaf area duration of crops, and postpone senescence (Foidl et al. 2001, Moyo et al. 2011, Yasmeen et al. 2014). Moreover, (Abbas et al. 2013) also reported an increase in photosynthetic pigments, antioxidant activity, and protein content due to the MLE application that leads to increased 1000 grain weight. These results were confirmed in the present study where MLE application produced significantly higher biological and grain yield by boosting photosynthetic pigments, protein content, and antioxidant activity in Mung bean during both years. Enhanced photosynthetic pigments, antioxidant activity, and protein content can be due to the presence of magnesium (Yaméogo et al. 2011), cytokinins (Zhang and Ervin 2008), and nitrogen (Elzaawely et al. 2017) in MLE.

MLE and mixed water irrigation interaction produced significantly better crop yield. Mung bean germinated and showed better growth characteristics when irrigated with mixed water. Similar plants when exposed to MLE application showed significantly higher chlorophyll content that leads to significantly higher biological yield during 2016 and 2017. Grain yield was significantly higher for MLE and mix water interaction in 2016 while at par with MLE and sewage water interaction during 2017. It can be due to antioxidant activity that was significantly

higher for mixed water and MLE interaction during 2016, while in 2017, sewage water irrigated plants in interaction with MLE produced significantly higher antioxidant activity. These results are in line with (Ayumi et al. 2004) and (Abbas et al. 2013) who reported that biological yield and grain yield are affected by chlorophyll content and antioxidant activity, respectively.

CONCLUSION

Direct or indirect usage of wastewater in agriculture is inevitable. Although, wastewater can be used as a source of irrigation water enrich with organic manure, yet, water analysis before its usage is highly recommended. Moreover, it was observed in the present research that the

toxicity of sewage water can be reduced by mixing it with fresh water to obtain better growth and yield. The use of plant growth regulators particularly natural PGRs are also recommended to help plants against any stress caused by sewage water toxicity. With ever-increasing water demand and water scarcity; it is very important to reuse the wastewater but with proper handling.

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REFERENCE

- Abbas R. N., Tanveer A., Khaliq A., Iqbal A., Ghaffari A., Matloob A., Maqsood Q. (2013) Maize (*Zea mays* L.) germination, growth and yield response to foliar application of *Moringa oleifera* Lam. leaf extracts. *Crop Environ* 4 (1):39-45
- Abohassan R., Abusuwar A. (2018) Effects of *Moringa oleifera* leaf extracts on growth and productivity of three leguminous crops. *Legume Res Int J* 41:114-119
- Abou-Seeda M., El-Aila H., Shehata A. (1997) Waste water treatment for irrigation purposes. 2.-sequential extraction of heavy metals in irrigated soil after one year. *Mansoura University Journal of Agricultural Sciences (Egypt)*
- Aboukhaled A. Towards safe and efficient management of waste water for crop production in the near east region. A paper presented at the 7th. In: World Congress on Water Resources, Morocco, 1991.
- Al-Hadithy A. H., Khmes H M., May E S., Luay Q H. (2011) Using of Rustumiya sewage water for irrigation:1-its effect on some soil properties and corn growth *Baghdad Science Journal* 8 (1):313-318
- Ali Z., Basra S. M. A., Munir H., Mahmood A., Yousaf S. (2011) Mitigation of drought stress in maize by natural and synthetic growth promoters. *J Agric Soc Sci* 7 (2):56-62
- Arnon D. I. (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant physiology* 24 (1):1
- Asthir B., Thapar R., Farooq M., Bains N. S. (2013) Exogenous Application of Thiourea Improves the Performance of Late Sown Wheat by Inducing Terminal Heat Resistance. *International Journal of Agriculture & Biology* 15 (6)
- Ayumi T., Masumi H., Ryoichi T. (2004) Chlorophyll metabolism and plant growth. *Kagaku Seibutsu* 42:93-98
- Balai L., Keshwa G. (2011) Effect of thiourea on yield and nutrient uptake of coriander (*Coriandrum sativum* L.) varieties under normal and late sown conditions. *Journal of Spices and Aromatic crops* 20 (1):34-37
- Barciszewski J., Siboska G., Rattan S. I., Clark B. F. (2000) Occurrence, biosynthesis and properties of kinetin (N⁶-furfuryladenine). *Plant growth regulation* 32 (2-3):257-265
- Basra S., Zahar M., Rehman H., Yasmin A., Munir H. Evaluating the response of sorghum and moringa leaf water extracts on seedling growth in hybrid maize. In: Proceedings of the international conference on sustainable food grain production: challenges and opportunities, 2009a. p 22
- Basra S., Zahoor R., Rehman H., Afzal I., Farooq M. Response of root applied brassica and moringa leaf water extracts on seedling growth in sunflower. In: Proceedings of the international conference on sustainable food grain production: challenges and opportunities, 2009b. p 23
- Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry* 72 (1-2):248-254
- Bråthe A., Andresen G., Gundersen L.-L., Malterud K. E., Rise F. (2002) Antioxidant activity of synthetic cytokinin analogues: 6-alkynyl- and 6-alkenylpurines as novel 15-lipoxygenase inhibitors. *Bioorganic & medicinal chemistry* 10 (5):1581-1586
- Capra A., Scicolone B. (2007) Recycling of poor quality urban wastewater by drip irrigation systems. *Journal of Cleaner Production* 15 (16):1529-1534
- Chance B., Maehly A. (1955) [136] Assay of catalases and peroxidases.
- Dash A. K. (2012) Impact of domestic waste water on seed germination and physiological parameters of rice and wheat. *IJRRAS* 12 (2):280-286
- Elzaawely A. A., Ahmed M. E., Maswada H. F., Xuan T. D. (2017) Enhancing growth, yield, biochemical, and hormonal contents of snap bean (*Phaseolus vulgaris* L.) sprayed with moringa leaf extract. *Archives of Agronomy and Soil Science* 63 (5):687-699
- Estefan G., Sommer R., Ryan J. (2013) Methods of soil, plant, and water analysis. A manual for the West Asia and North Africa region:170-176
- Faruqui N., Scott C., Raschid-Sally L. (2004) Confronting the realities of wastewater use in irrigated agriculture: Lessons learned and recommendations. Chapter 16:173-186

- Foidl N., Makkar H., Becker K. (2001) The potential of *Moringa oleifera* for agricultural and industrial uses. The miracle tree: The multiple attributes of *Moringa*:45-76
- Fuglie L. (2000) New uses of *Moringa* studied in Nicaragua. ECHO Development Notes 68:1-25
- Garg B., Burman U., Kathju S. Influence of thiourea on photosynthesis, nitrogen metabolism and yield of clusterbean under moisture deficit conditions. In: Abstracts of 2nd International Congress of Plant Physiology, 2003. p 158
- Garg B., Burman U., Kathju S. (2006) Influence of thiourea on photosynthesis, nitrogen metabolism and yield of clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) under rainfed conditions of Indian arid zone. *Plant growth regulation* 48 (3):237-245
- Ghosh A. K., Bhatt M., Agrawal H. (2012) Effect of long-term application of treated sewage water on heavy metal accumulation in vegetables grown in Northern India. *Environmental monitoring and assessment* 184 (2):1025-1036
- Giannopolitis C. N., Ries S. K. (1977) Superoxide dismutases: I. Occurrence in higher plants. *Plant physiology* 59 (2):309-314
- Govt.-Of-Pakistan (2016) Pakistan Economic Survey. Government of Pakistan Ministry of Finance http://www.finance.gov.pk/survey_1617.html
- Hicks R., Hird C. (2000) Soil and urban land use. *Soils: Their Properties and Management*:378-399
- Howladar S. M. (2014) A novel *Moringa oleifera* leaf extract can mitigate the stress effects of salinity and cadmium in bean (*Phaseolus vulgaris* L.) plants. *Ecotoxicology and Environmental Safety* 100:69-75
- Imran, Khan A. A., Inam I., Ahmad F. (2016) Yield and yield attributes of Mungbean (*Vigna radiata* L.) cultivars as affected by phosphorous levels under different tillage systems. *Cogent Food & Agriculture* 2 (1):1151982
- Iqbal S., Bhangar M. (2006) Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *Journal of food Composition and Analysis* 19 (6-7):544-551
- Kannan C., Zwanenburg B. (2014) A novel concept for the control of parasitic weeds by decomposing germination stimulants prior to action. *Crop Protection* 61:11-15
- Kannan V., Ramesh R., Sasikumar C. (2005) Study on ground water characteristics and the effects of discharged effluents from textile units at Karur District. *Journal of environmental biology* 26 (2):269-272
- Khan S. (2016): Impact of growth promoters on the storage life of moringa leaf extract as biostimulant in wheat. University of Agriculture, Faisalabad.,
- Kocak S., Tokusoglu O., Aycan S. (2005) Some heavy metal and trace essential element detection in canned vegetable foodstuffs by differential pulse polarography (DPP). *Electronic J Environ Agric Food Chem* 4:871-878
- Li W., Liu J., Ashraf U., Li G., Li Y., Lu W., Gao L., Han F., Hu J. (2016) Exogenous γ -aminobutyric acid (GABA) application improved early growth, net photosynthesis, and associated physio-biochemical events in maize. *Frontiers in plant science* 7:919
- Lubello C., Gori R., Nicese F. P., Ferrini F. (2004) Municipal-treated wastewater reuse for plant nurseries irrigation. *Water research* 38 (12):2939-2947
- Marcu M. G. (2005): *Miracle tree*. KOS Health Publications,
- Mathur N., Singh J., Bohra S., Bohra A., Vyas A. (2006) Improved productivity of mung bean by application of thiourea under arid conditions. *World J Agric Sci* 2 (2):185-187
- Moyo B., Masika P. J., Hugo A., Muchenje V. (2011) Nutritional characterization of *Moringa* (*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology* 10 (60):12925-12933
- Nagajyothi P., Dinakar N., Suresh S., Udaykiran Y., Suresh C., Damodharam T. (2009) Effect of industrial effluent on the morphological parameters and chlorophyll content of green gram (*Phaseolus aureus* Roxb.). *J Environ Biol* 30 (3):385-388
- Nath K., Singh D., Shyam S., Sharma Y. (2009) Phytotoxic effects of chromium and tannery effluent on growth and metabolism of *Phaseolus mungo* Roxb. *Journal of environmental biology* 30 (2):227-234
- Nouman W., Siddiqui M. T., Basra S. M. A. (2012a) *Moringa oleifera* leaf extract: An innovative priming tool for rangeland grasses. *Turkish Journal of Agriculture and Forestry* 36 (1):65-75
- Nouman W., Siddiqui M. T., Basra S. M. A., Afzal I., Rehman H. U. (2012b) Enhancement of emergence potential and stand establishment of *Moringa oleifera* Lam. by seed priming. *Turkish Journal of Agriculture and Forestry* 36 (2):227-235
- Ouzounidou G., Ilias I. (2005) Hormone-induced protection of sunflower photosynthetic apparatus against copper toxicity. *Biologia plantarum* 49 (2):223
- Pakistan-development-Statistics (2016). Bureau of statistics government of punjab lahore www.bos.gov.pk
- Palese A., Pasquale V., Celano G., Figliuolo G., Masi S., Xiloyannis C. (2009) Irrigation of olive groves in Southern Italy with treated municipal wastewater: effects on microbiological quality of soil and fruits. *Agriculture, ecosystems & environment* 129 (1-3):43-51
- Perveen S., Samad A., Nazif W., Shah S. (2012) Impact of sewage water on vegetables quality with respect to heavy metals in Peshawar, Pakistan. *Pakistan Journal of Botany* 44 (6):1923-1931
- Ryan J., Estefan G., Rashid A. (2001) Soil and plant analysis laboratory manual, International Centre for Agricultural Research in the Dry Areas (ICARDA). Aleppo and National Agricultural Research Centre (NARC), Islamabad, Pakistan
- Singh A., Sharma R. K., Agrawal M., Marshall F. M. (2010) Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food and Chemical Toxicology* 48 (2):611-619

- Singh S., Rathore P. (2003) Influence of phosphorus and thiourea on yield and economics of greengram [*Vigna radiata* var. aureus (L.) Wilczek].
- Srivastava A., Srivastava S., Mishra S., D'Souza S., Suprasanna P. (2014) Identification of redox-regulated components of arsenate (As V) tolerance through thiourea supplementation in rice. *Metallomics* 6 (9):1718-1730
- Srivastava A. K., Srivastava S., Penna S., D'Souza S. F. (2011) Thiourea orchestrates regulation of redox state and antioxidant responses to reduce the NaCl-induced oxidative damage in Indian mustard (*Brassica juncea* (L.) Czern.). *Plant physiology and biochemistry* 49 (6):676-686
- Taiz L., Zaiger E. (2006) *Plant physiology*. 4th edition. Sinauer Associates, Sunderland, MA, USA:764
- Talukdar D. (2014) Arsenic-induced oxidative stress and its reversal by thiourea in mung bean (*Vigna radiata* (L.) Wilczek.) Genotype. *Cent Euro J Exp Biol* 3:13-18
- Ullah H., Khan I., Ullah I. (2012) Impact of sewage contaminated water on soil, vegetables, and underground water of peri-urban Peshawar, Pakistan. *Environ Monit Assess* 184 (10):6411-6421. doi:10.1007/s10661-011-2429-4
- ur Rehman A., Saleem M., Naveed A. (2005) Genetic analysis of harvest index in mungbean. *Pak J Agric Sci* 42:66-70
- Yaméogo C. W., Bengaly M. D., Savadogo A., Nikiema P. A., Traore S. A. (2011) Determination of chemical composition and nutritional values of *Moringa oleifera* leaves. *Pakistan Journal of Nutrition* 10 (3):264-268
- Yasmeen A. (2011): Exploring the potential of moringa (*Moringa oleifera*) leaf extract as natural plant growth enhancer. UNIVERSITY OF AGRICULTURE FAISALABAD, PAKISTAN,
- Yasmeen A., Basra S., Farooq M., ur Rehman H., Hussain N. (2013) Exogenous application of moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. *Plant growth regulation* 69 (3):225-233
- Yasmeen A., Basra S. M. A., Ahmad R., Wahid A. (2012) Performance of late sown wheat in response to foliar application of *Moringa oleifera* Lam. leaf extract. *Chilean Journal of Agricultural Research* 72 (1):92
- Yasmeen A., Nouman W., Basra S. M. A., Wahid A., Hussain N., Afzal I. (2014) Morphological and physiological response of tomato (*Solanum lycopersicum* L.) to natural and synthetic cytokinin sources: a comparative study. *Acta Physiologiae Plantarum* 36 (12):3147-3155
- Zhang X., Ervin E. (2008) Impact of seaweed extract-based cytokinins and zeatin riboside on creeping bentgrass heat tolerance. *Crop Science* 48 (1):364-370