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Combined application of moringa leaf extract and chemical growth-promoters enhances the plant growth and productivity of wheat crop (*Triticum aestivum* L.)

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ABSTRACT

Wheat is regarded as a main staple crop in Pakistan because its contribution to the daily diet of a common man is 60%. Late sowing of wheat not only reduces yield but also affects efficiency of other production factors but it is not possible to absolutely avoid late sowing. Moringa leaf extract (MLE), exogenously applied either through seed or plant foliage, is known to boost field crops productivity under both normal and unfavorable conditions. A study was conducted to evaluate the crop growth enhancing potential of MLE alone and in combination with hydrogen peroxide, salicylic acid (SA) and ascorbic acid (ASA). The experimental design was a randomized complete block design with split plot arrangements having three replications. The treatments were control, hydropriming, MLE (3%) priming, MLE + H_2O_2 (2 μM) + SA (50 mg L^{-1}) + ASA (50 mg L^{-1}) priming, water spray, MLE spray and MLE + H_2O_2 + SA + ASA spray. Foliar treatments were applied twice during growth period at tillering and booting stages. Foliar application and priming approaches of MLE alone and with growth promoting substances were found to be better than control, and foliar effects were more prominent. Foliar application of MLE improved biological and grain yields and biochemical parameters as compared to control. It can be concluded that application of MLE as a priming agent improved the emergence parameters in normal and late sown wheat. Alone and blended MLE spray also enhanced the biochemical traits as well as growth attributes of wheat. Notably, application of moringa leaf extract alone portrays a beneficial role in terms of biological as well as economical yield of late sown wheat.

1. Introduction

After corn and rice, wheat is the third largest crop in the world as an essential source of carbohydrates for millions of people. Similarly, in Pakistan it is the main staple food having annual production of 25.75 million tonnes with a cultivation area of 9052,000 hectares. Due to the significant contribution of wheat in the daily diet (60% of caloric intake) of a common man, it holds a dominant position in agricultural policies of governmental and development sectors. Hence, there is an urgent need to explore different innovative and sustainable methods by agronomists and crop physiologists for improving growth and yield parameters of wheat, particularly because its productivity is negatively influenced by a number of factors, i.e. agronomic practices, plant protection measure and terminal heat stress due to climatic variability (Masood, 2015). Agronomics practices include non-availability of inputs (Kumbhare and Singh, 2016), inadequate seeds (Giri and Schillinger, 2003), weed infestation (Rasmussen, 2004; Weber and Hryñczuk, 2005), shortage of irrigation water (Zhang et al., 2017) and late sowing of wheat (Shan et al., 2015). Additionally, terminal heat stress and unexpected rain fall also reduce crop yield (Jena et al., 2017). Terminal heat stress decrease cytokinins content in the wheat plant that subsequently reduces grain quality and yield attributes (Shahid et al., 2017).

A set of physio-chemical, biological and integrated approaches is available for reducing yield losses (Bedada et al., 2016; Gaudin et al., 2015). Among them, the use of organic (biostimulants) and inorganic (nutrient and chemical agents) growth stimulators are considered viable approaches to compensate yield losses. Biostimulants are natural growth enhancers that stimulate crop yield via enhanced nutrient uptake and efficiency, improved tolerance to biotic and abiotic stresses and enhancement of the rhizospheric activities (Jardin, 2015). Natural

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sources like seaweed extracts, protein hydrolysates and amino acids, humic acid, fulvic acid, complex organic materials, chitin and chitosan derivatives, microbial inoculants, biochar and plant extracts are the most commonly used biostimulants in agriculture (EBIC, 2012; Jardin, 2015; Glodowska et al., 2016). *Moringa oleifera* leaf extract, sorghum water extract and mulberry water extracts are commonly used growth enhancers when applied as a seed priming agent and/or foliar spray. It has been scientifically proven that they positively modify plant growth and production with alterations in metabolic processes under different cultivation practices (Rady et al., 2013; Yasmeen et al., 2013; Khan et al., 2017a, 2017b). Moringa, among all the naturally occurring plant growth stimulants, has received enormous attention from the scientific community because of its rich source of growth hormones, antioxidants, vitamins and mineral nutrients in its leaves (Foidl et al., 2001; Yasmeen et al., 2013).

In literature, application of moringa leaf extract via a seed or foliage has been scientifically proven to boost emergence, seedling and plant growth as well as economical yield (Basra et al., 2011; Khan et al., 2017a). Seed priming, with MLE blended with salicylic acid, hydrogen peroxide, ascorbic acid and sorghum water extract, enhances germination parameters and seedling performance in maize (Imran et al., 2013). Foliar application of MLE along with benzyl amino purine and hydrogen peroxide at critical stages enhances wheat's growth, development and ultimate yield (Yasmeen et al., 2013). Similarly, Khan et al. (2017a) reported that leaf extract of white seeded moringa has a higher biostimulant potential regarding seed emergence and plant vigor.

Furthermore, a synergy was reported between moringa leaf extract and chemical growth promoters (i.e. salicylic acid, hydrogen peroxide and ascorbic acid) (Yasmeen et al., 2013). Salicylic acid (SA) is one of a variety of plant growth regulators (PGRs) that has been reported to effect various plant developmental processes (El-Tayeb, 2005). Foliar application of SA also increases the enzymatic activities of nitrate reductase and carbonic anhydrase which are very helpful in nutrient uptake. Ascorbic acid provides a pivotal role in the electron transport chain (ETC). It is an important factor for numerous key enzymes in the plants (Arrigoni and Detullio, 2000). Moreover, hydrogen peroxide (H_2O_2) can also be used as a plant signaling messenger, mediating the acquisition of tolerance to abiotic stress (Wahid et al., 2007). Many studies have been reported on the combined use of Moringa leaf extract and chemical growth stimulators in maize (Basra et al., 2011; Afzal et al., 2012). A study by our own research group reported on the role of the mentioned stimulants within greenhouses. To the best of our knowledge, no study has been conducted on the combined application of Moringa leaf extract, salicylic acid, ascorbic acid and hydrogen peroxide in wheat at field scale. The current study was designed with following objectives: (i) to investigate the biostimulant potential of Moringa leaf extract alone or in combination with chemical growth stimulants in normal and late sown wheat, (ii) to compare the efficiency of two application methods i.e. seed priming and foliar application.

2. Materials and methods

2.1. Extraction process of moringa leaves

Fresh, mature and healthy moringa leaves were collected from already established moringa trees at research area of Department of Agronomy, University of Agriculture, Faisalabad, Pakistan. Before extraction, leaves were rinsed and then stored at 0–4 °C in a deep freezer for 12 h. A locally assembled machine was used for extraction process (Yasmeen et al., 2013). The extracts were sieved and diluted 30 times with distilled water to prepare a 3% solution (Basra et al., 2011).

2.2. Experimental particulars

The experiment was carried out at the Agronomic Research Area, University of Agriculture, Faisalabad Pakistan. The textural class of the experimental site was recognized as loam. The other associated physio-chemical characteristics of soil used in the current experiment are presented in Table 1. The experimental design was a randomized complete block design (RCBD) with split plot arrangements having three replications and a net plot size of 5×2.25 m. The following treatment plan was followed for studying the mentioned objectives:

- Control; no exogenous application of MLE and chemical stimulants

Priming treatments;

- Hydropriming; seed soaked in distilled water for eight hours
- MLE priming; seeds soaked in MLE (3% solution)
- Blended priming; MLE (3%) + H_2O_2 (2 μM) + SA (50 mg L^{-1}) + ASA (50 mg L^{-1})

Foliar treatments;

- Water spray
- MLE (3%) spray
- Blended spray; (3%) + H_2O_2 (2 μM) + SA (50 mg L^{-1}) + ASA (50 mg L^{-1})

Priming treatments were applied before sowing of seeds in the field. In the priming treatments, seeds were soaked in an aerated water, solution of moringa leaf extract (MLE) and solution of MLE in combination with hydrogen peroxide (H_2O_2) @ 2 μM, salicylic acid (SA) @ 50 mg L^{-1} and ascorbic acid (ASA) @ 50 mg L^{-1} for eight hours (Imran et al., 2013). For seed priming, the ratio of seed weight to soaking solution was 1–5 (*w*/*v*) (Yasmeen et al., 2013). Before sowing of seeds, seedbed was prepared by using a cultivator (2 times) at field capacity followed by the same number of planking. Seeds were sown on in a well prepared fine seedbed with the help of single row hand drill. The interval between normal sown and late sown wheat was 20 days.

On the other hand, foliar treatments were applied twice during the growth period at critical stages, i.e. tillering stage and booting stage. A sprayer machine was used to apply the foliar treatments on the crop. Other agronomic practices were kept uniform. Necessary plant protection measures were adopted to keep crop free of weeds, insect, pests and diseases. The crop was harvested manually at 160 and 140 days after sowing (DAS) for normal and late sown wheat respectively. After one week of sun drying, spikes were threshed manually.

2.3. Seedling emergence evaluation

After seed sowing, emergence was counted on a daily basis until a constant count was achieved. Data regarding emergence and seedling vigor parameters were collected by following the ISTA protocols (ISTA, 2010). Mean emergence time (MET) was calculated according to Ellis and

Table 1
Physico-chemical properties of experimental soil

Determination	Values
Chemical analysis	
pH	7.83
EC (dS m^{-1})	1.73
Soil bulk density (Mg m^{-3})	1.43
Total Porosity (%)	45.40
Soil Infiltration Rate (mm hr^{-1})	25.40
Soil Hydraulic Conductivity (mm hr^{-1})	53.07
Total Nitrogen (mg kg^{-1})	0.52
Available phosphorus (mg kg^{-1})	9.46
Available Potassium (mg kg^{-1})	114.08
Soil Organic Carbon (g kg^{-1})	2.54
Physical analysis	
Sand (%)	46
Silt (%)	30
Clay (%)	25
Textural Class	Sandy clay loam

Roberts (1981) (MET = ΣDn/Σn). Emergence index (EI) was calculated by the following formula [EI = (number of emerged seedling(s)/days of first count) + ….. + (number of emerged seedlings/days of final count)] (AOSA, 1990). Time to 50% emergence (E_{50}) was calculated according to Farooq et al. (2005) [E50 = ti + {(N/2 – ni)/(nj – ni)} × (tj − ti)].

2.4. Crop growth and development

The growth parameters were recorded after 15 days of application of foliar treatment until maturity with an interval of 15 days. Leaf area index (LAI) was calculated on square meter basis by the ratio of leaf area to ground area with a formula given by Watson (1952).

Leaf area index = (Leaf area)/(Land area)

Leaf area duration (LAD) was measured according to Reddy et al. (2004) by following formula.

$$\mathrm{LAD} = (\mathrm{LAI}_1 + \mathrm{LAI}_2) \times (\mathrm{T}_2\text{–}\mathrm{T}_1)/2$$

Crop growth rate (CGR) was recorded four times during the course of study starting from 50 DAS and continuing to 95 DAS on a fortnightly basis. According to Reddy et al. (2004), CGR was calculated by the formula mentioned below;

$$\mathrm{CGR} = (\mathrm{W}_2\text{–}\mathrm{W}_1)/(\mathrm{T}_2\text{–}\mathrm{T}_1)$$

2.5. Biochemical analysis

Chlorophyll and total phenolic contents of flag leaf were determined at booting stage. Chlorophyll contents were calculated with the following formula described by Arnon (1949).

$$\text{Chlorophyll } a \text{ (mg g}^{-1}) = [(0.0127 \times \mathrm{A663}–0.00269 \times \mathrm{A645}) \times 100]/0.5$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}) = [(0.0229 \times \mathrm{A645}–0.00468 \times \mathrm{A663}) \times 100]/0.5$$

Total phenolic content in leaf samples was measured at booting stage by using a method as described by Ainsworth and Gillespie (2007). Flag leaf was harvested (approximately 20 mg). Leaf sample was homogenized by adding 2 ml of ice-cold 95% (vol/vol) methanol in each sample with ice-cold mortar. Then sample was incubated at room temperature for 48 h in the dark. Sample was centrifuged (13,000 for 5 min at room temperature) and supernatant was collected in a fresh microtube. 100 ml of sample supernatant and 200 ml 10% (vol/vol) Folin-Ciocalteau (FC) reagent were taken in a test tube and then vortexed thoroughly. 800 ml of 700 mM Na_2CO_3 was added into test tube and the assay tubes were inserted at room temperature for two hours. 200 ml of sample was taken from the test tube to run at the absorbance of 765 nm.

2.6. Yield parameters

Before harvesting, productive tillers were recorded per unit area. Plant height and spike length were measured using a meter rod. Numbers of spikelets and grains per spike were also recorded. 1000 grain weight was recorded by an electric balance. For the determination of biological yield, an area of $1m^2$ was harvested from each plot at the fully mature stage. Total wheat biomass was measured by using a weighing balance. The grains obtained from each plot after threshing were weighed by a weighing balance to record the grain yield. The harvest index was calculated as the ratio of grain yield to the biological yield using the following formula.

Harvest index = Grain yield/Biological yield × 100

2.7. Statistical analysis

All the recorded parameters regarding seedling emergence, plant growth, biochemical and yield parameters were analyzed and evaluated statistically using statistical package (Statistix 8.1). Microsoft Excel was used for calculations and graphical presentation. Comparison among different treatments were made with the help of an analysis of variance (ANOVA) at 95% of confidence interval. Different letters (a, b, c and d) were used to portray the significant difference among treatments via LSD as post-hoc test.

3. Results

3.1. Emergence parameters

All the priming treatments took significantly less time to start seed emergence as compared to control in normally sown wheat. Within treatments (priming), no statistical differences were recorded. While in the case of late sown wheat, application of MLE improved maximum seed emergence as compared to the control and other priming treatments (Fig. 1a). As seen in Fig. 1b, only application of MLE showed pronounced positive effect during 50% emergence time as compared to respective control and other priming treatments. Control (no priming) took more time to complete 50% emergence. Additionally, the same pattern was observed in normal and late sown wheat. A similar trend was observed for mean emergence time as in 50% emergence time (Fig. 1c). All the priming treatments improved emergence index relative to control but a significant effect was observed due to MLE application in

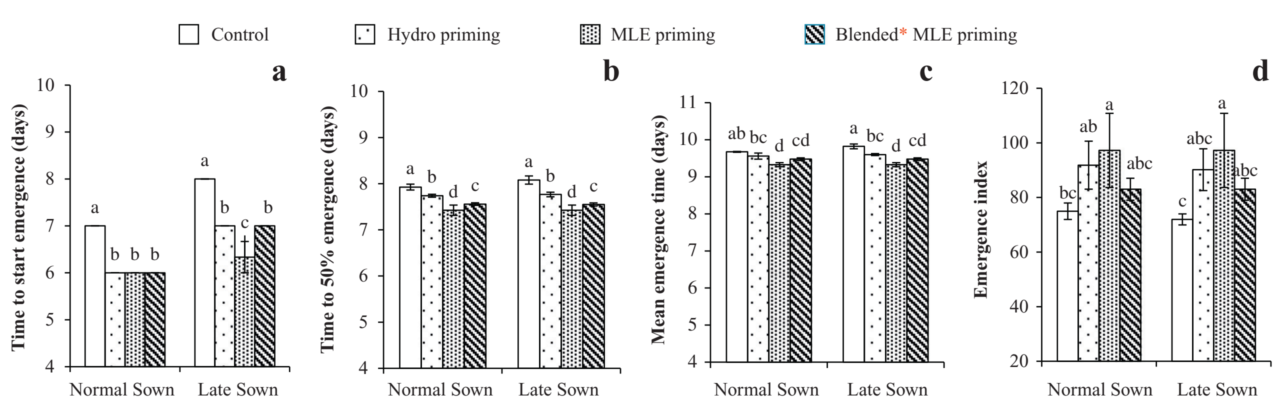

Fig. 1. Impact of alone and blended moringa leaf extract on emergence parameters (a–d) of wheat (three replicates) under field conditions. *3% solution of MLE in combination with hydrogen peroxide (H_2O_2) @ 2 μM, salicylic acid (SA) @ 50 mg L^{-1} and ascorbic acid (ASA) @ 50 mg L^{-1}.

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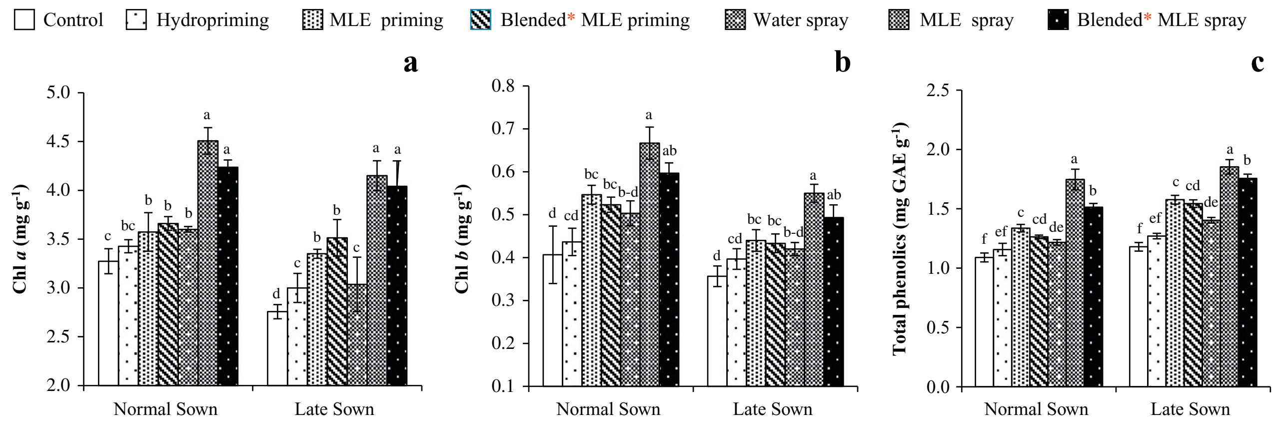

Fig. 2. Impact of alone and blended moringa leaf extract on biochemical parameters (a–c) of wheat (three replicates) under field conditions. *3% solution of MLE in combination with hydrogen peroxide (H_2O_2) @ 2 μM, salicylic acid (SA) @ 50 mg L^{-1} and ascorbic acid (ASA) @ 50 mg L^{-1}.

normal sown wheat. Moreover, the same trend was recorded in late sown wheat as well (Fig. 1d).

3.2. Biochemical parameters

Biochemical parameters were significantly affected by both priming and foliar application of MLE alone and blended MLE ($P < .05$). Maximum chlorophyll *a* contents were measured in MLE sprayed wheat (alone and blended form) in both sowing dates (Fig. 2a) while Fig. 2a depicts minimum chlorophyll *a* contents in control. Very interestingly, water spray had no effect on chlorophyll *a* contents in late sown wheat. Least chlorophyll *b* contents were observed in control and hydro priming while significantly highest in MLE alone and blended MLE spray treatments (Fig. 2b). At both sowing dates, priming and foliar treatments improved the total phenolic contents except hydro priming with respect to their control. Application of MLE alone and blended MLE showed pronounced effects on total phenolic contents. Over all higher total phenolics were observed in late sown wheat (Fig. 3c).

3.3. Growth parameters

Leaf area index (LAI) is the main physiological determinant for the crop yield. All the priming and foliar treatments increased LAI as compared to control. Within priming and foliar application, maximum LAI value was recorded from MLE primed seeds whereas minimum LAI value was recorded in control (Fig. 3a and b). At three sampling points (50, 65, 80 DAS), LAI values had a constantly increasing trend after that it gradually decreased up to maturity of the crop. All the priming and foliar treatments significantly improved leaf area duration (LAD) as compared to the control. At all sampling points maximum LAD was recorded in the MLE spray while minimum LAD in the control at both sowing dates. In reference to sowing time, maximum LAD was observed in normal sown wheat as compared to late sown wheat (Fig. 3c, d and e). Crop growth rate (CGR) is the product of total dry matter and LAI. As depicted from Fig. 4a–c, CGR was at its maximum at 65–80 DAS and 80–95 DAS, while the minimum CGR was at 50–65 DAS. Foliar spray of MLE enhanced the maximum CGR as compared to control. In

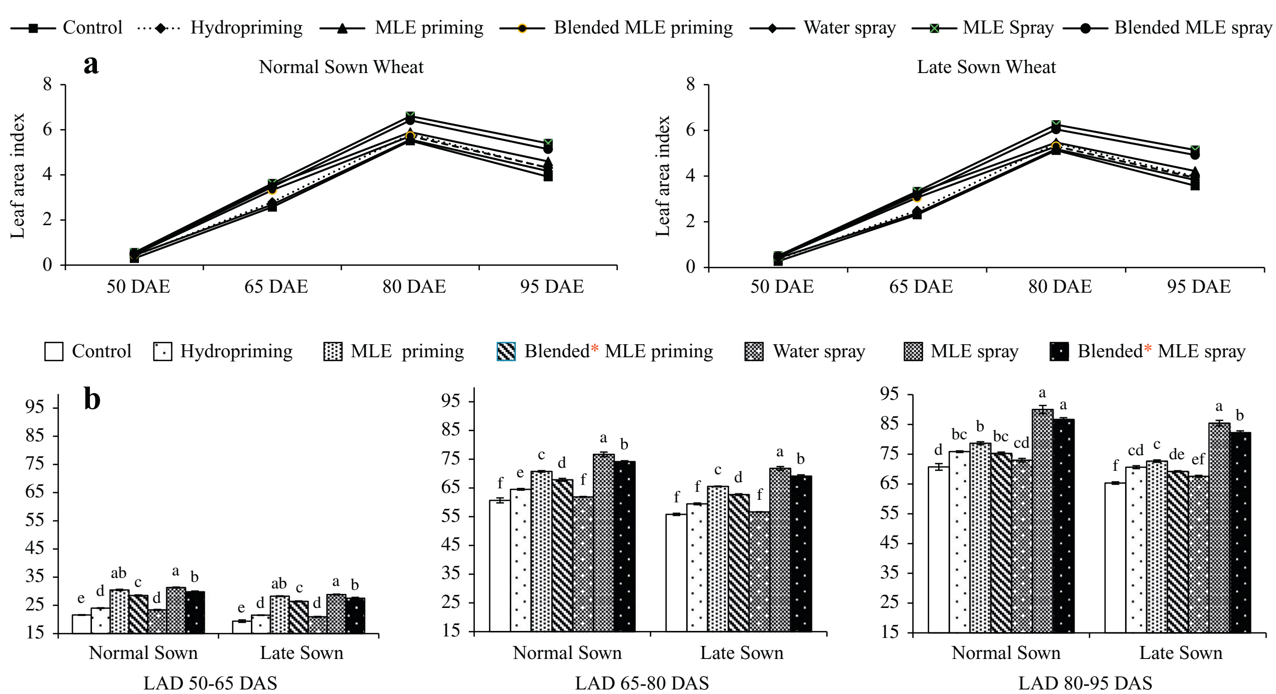

Fig. 3. Impact of alone and blended moringa leaf extract on leaf area index (a) and leaf area duration (b) of wheat (three replicates) under field conditions. *3% solution of MLE in combination with hydrogen peroxide (H_2O_2) @ 2 μM, salicylic acid (SA) @ 50 mg L^{-1} and ascorbic acid (ASA) @ 50 mg L^{-1}.

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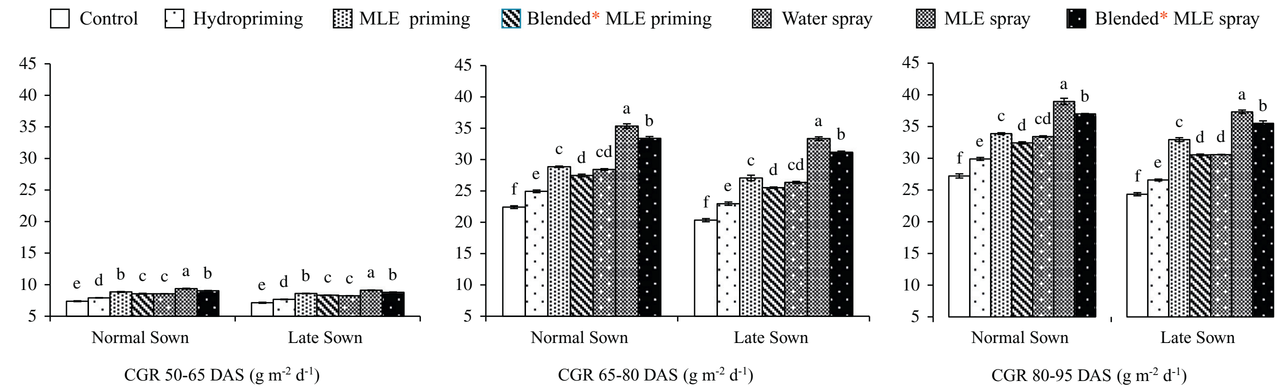

Fig. 4. Impact of alone and blended moringa leaf extract on crop growth rate of wheat (three replicates) under field conditions. *3% solution of MLE in combination with hydrogen peroxide (H_2O_2) @ 2 μM, salicylic acid (SA) @ 50 mg L^{-1} and ascorbic acid (ASA) @ 50 mg L^{-1}.

reference to sowing time, maximum CGR was observed in normal sown wheat while the minimum CGR was in late sown wheat. Foliar application of MLE increased the CGR even in late sown wheat.

3.4. Yield parameters

Statistical analysis of the data reveals that delayed sowing significantly affected tillering ability, tiller and spike length, number of spikelets per spike and grain numbers per spike ($P < .05$). An increase in the yield contributing parameters was observed due to priming as well foliar application of alone and blended MLE in crop plants raised by normal as well as late sowing (Table 2). Meanwhile the highest increase in yield parameters was found with MLE spray alone which was statistically at par with blended MLE spray. An important observation was the increase in grain numbers per spike in late sown crop plants due to foliar application of MLE alone (Table 3). An increase in 1000-grain weight was observed due to priming as well as foliar application of MLE alone and blended MLE in crop plants raised by normal as well as late sowing. Significantly higher 1000-grain weight was found in both MLE alone and blended MLE foliar spray (Table 3) in normal sown wheat. The minimum 1000-grain weight was observed in the late sown crop which received no exogenous treatments. A significant observation was the increase in 1000-grain weight in late sown crop due to foliar application of MLE alone.

Biological yield is the combination of grain and straw yield. It is the function of the genetic make-up of the crop, nutrient status of the soil, the exogenous application of minerals and growth enhancers, and the environmental conditions prevailing around the crop. Delayed sowing significantly affected biological yield. Exogenous application of MLE also affected this parameter. Maximum biological yield was found where MLE alone was sprayed (Table 4) on normally sown crop, while minimum biological yield was observed in the late sown crop which received no exogenous treatments. Delayed sowing also affected economic yield significantly. Maximum economic yield was recoded with MLE alone spray which was statistically at par with blended MLE spray (Table 4) in normal sown crop while minimum economic yield was observed in late sown crop which received no exogenous treatments. An important observation was the increase in economic yield of the late sown crop plants due to foliar application of MLE. Harvest index (HI) is an essential component showing the photosynthetic efficiency of a crop in the transformation of the photosynthesis into economic yield (Table 4). All treatments significantly improved HI as compared to control. Within priming and foliar application, maximum HI value was recorded from blended MLE spray in normal sown while minimum HI value was recorded by the same treatment in late sown wheat.

4. Discussion

Recently the use of biostimulants as a growth promoter and yield enhancer have received large degree of attention by plant physiologists. Biostimulants have been demonstrated to increase plant vigor and yield attributes by modifying plant physiology, increasing nutrient acquisition and enhancing the capacity of plants to cope with stress responses. One of the profound limitations for wheat productivity is late sowing. It is reported as a major contributor in yield reduction of wheat especially in the areas of Asian Sub-continent, including Pakistan. Advance occurrence of phenological stages and a decrease in the grain filling period, resulting in the decrease of final biological and economic yield of wheat crop due to late sowing have been reported by many scientists (Ugarte et al., 2007). High temperature is found to be involved in the reduction of dry accumulation and the number of grains in wheat varieties (Plaut et al., 2004). Under late sown conditions, all the yield contributing stages of wheat such as tillering, anthesis and grain filling are negatively affected by high temperature stress.

Table 2
Impact of alone and blended moringa leaf extract on yield parameters of wheat (three replicates) under field conditions.

Treatments	Tillers per unit area (m^{-2})		Plant height (cm)		Spike length (cm)	
	Normal sown	Late sown	Normal sown	Late sown	Normal sown	Late sown
Control	311.48 ± 13.09 c	251.33 ± 30.34 c	89.08 ± 0.59 d	85.61 ± 0.33 c	10.40 ± 0.26 b	9.24 ± 0.06 b
Hydropriming	326.33 ± 7.62 bc	268.67 ± 6.64 bc	91.89 ± 0.37 cd	86.19 ± 0.55 c	10.47 ± 0.07 b	9.41 ± 0.40 ab
MLE priming	350.00 ± 13.92 a	305.67 ± 26.44 a	93.77 ± 0.41 bc	89.83 ± 0.20 b	10.69 ± 0.29 b	9.56 ± 0.15 ab
Blended* MLE priming	345.67 ± 9.33 a	284.00 ± 9.71 ab	91.62 ± 0.59 cd	87.78 ± 0.35 bc	10.38 ± 0.28 b	9.54 ± 0.12 ab
Water spray	318.33 ± 4.05 c	255.67 ± 2.19 c	91.80 ± 0.50 cd	90.88 ± 0.30 b	10.42 ± 0.10 b	9.72 ± 0.32 ab
MLE spray	335.81 ± 9.36 ab	275.33 ± 6.94 bc	98.77 ± 0.80 a	96.39 ± 1.56 a	11.72 ± 0.53 a	10.55 ± 0.18 a
Blended* MLE spray	333.59 ± 15.20 a–c	259.67 ± 31.76 bc	96.76 ± 1.23 ab	95.21 ± 0.47 a	11.19 ± 0.36 ab	9.92 ± 0.12 ab
Mean	332.71 A	271.48 B	93.383 A	90.270 B	10.579 A	9.599 B
LSD	16.653	28.268	3.293	3.554	0.7892	0.9388

Means sharing the same letter did not differ significantly at $P < .05$.
* 3% solution of MLE in combination with hydrogen peroxide (H_2O_2) @ 2 μM, salicylic acid (SA) @ 50 mg L^{-1} and ascorbic acid (ASA) @ 50 mg L^{-1}.

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Table 3
Impact of alone and blended moringa leaf extract on yield parameters of wheat (three replicates) under field conditions.

Treatments	Spikelets per spike		Grain number per spike		1000-grain weight (g)	
	Normal sown	Late sown	Normal sown	Late sown	Normal sown	Late sown
Control	16.47 ± 0.24 c	15.40 ± 0.20 b	45.87 ± 2.17 d	37.00 ± 1.16 e	40.33 ± 0.33 b	39.67 ± 1.20 b
Hydropriming	16.80 ± 0.12 bc	15.40 ± 0.40 b	46.27 ± 1.75 d	38.67 ± 0.88 d	43.67 ± 0.88 ab	40.67 ± 1.20 ab
MLE priming	17.07 ± 0.07 b	15.67 ± 0.27 b	48.13 ± 1.95 cd	40.60 ± 1.17 bc	45.33 ± 1.20 a	42.33 ± 0.67 ab
Blended* MLE priming	17.00 ± 0.23 b	15.47 ± 0.07 b	50.40 ± 0.98 bc	41.33 ± 0.41 b	44.33 ± 0.88 ab	41.00 ± 1.16 ab
Water spray	16.92 ± 0.23 b	15.40 ± 0.31 b	49.47 ± 2.34 bc	39.73 ± 0.93 c	44.67 ± 1.20 ab	41.67 ± 0.88 ab
MLE spray	17.47 ± 0.18 a	16.13 ± 0.41 a	54.47 ± 1.25 a	44.20 ± 2.04 a	48.33 ± 0.88 a	44.67 ± 0.88 a
Blended* MLE spray	17.13 ± 0.41 ab	15.73 ± 0.18 b	51.80 ± 3.00 ab	41.47 ± 0.57 b	45.67 ± 0.67 a	44.00 ± 1.00 ab
Mean	16.979 A	15.600 B	49.505 A	40.143 B	44.619 A	42.000 B
LSD	0.3445	0.3963	2.9512	0.9235	4.763	4.9565

Means sharing the same letter did not differ significantly at $P < .05$.
* 3% solution of MLE in combination with hydrogen peroxide (H_2O_2) @ 2 μM, salicylic acid (SA) @ 50 mg L^{-1} and ascorbic acid (ASA) @ 50 mg L^{-1}.

Reduction in the biological and grain yield in wheat cultivars was recorded under late sown conditions in the current study.

In the present study, application of MLE, both alone and blended with growth promoting substances was linked with higher growth and yield attributes in normal and late sown wheat. All the priming treatments enhanced the emergence traits of wheat. Higher emergence traits (time to start emergence, time to 50% emergence, mean emergence time, emergence index) in wheat with respect to the control may be linked with an enhanced imbibition process, the breakage of seed coat and triggering of enzymatic activities due to seed priming (Anjum et al., 2011; Jena et al., 2017). Our study was in line with many other studies (Farooq et al., 2008; Zheng et al., 2016) that reported the effect of seed priming with improved emergence and seedling vigor. For example, Afzal et al. (2008) reported that priming of wheat seeds with MLE significantly improved the emergence and seedling vigor. Similarly, another study (Basra et al., 2011) reported the positive effects of seed priming with MLE on maize plants vigor and yield. Chemical stimulants may also be linked with higher growth initiation of plants as many (salicylic acid, ascorbic acid) are co-factors of enzymes. Ahmad et al. (2014) reported that exogenous application of salicylic acid, ascorbic acid and hydrogen peroxide improved the growth of maize seedling at early stages of growth. Foliar application of ASA, SA and H_2O_2 improved chlorophyll and leaf relative contents, seedling growth and enzymatic antioxidants activities in maize as compared to an untreated control (Ahmad et al., 2014). Improvement in plant growth attributes due to these chemical stimulants may also be linked with morphological and yield related traits of maize under field conditions. Seed priming with MLE and ascorbate enhances the chlorophyll and phenolic contents of maize seedling as compared to untreated seedlings (Basra et al., 2011). Seed priming enhances rate of metabolism which results in increase in speed of germination and emergence (Ashraf et al., 2008). The effectiveness of MLE with emergence parameters in the current study could also be linked with higher concentration of plant growth hormones, mineral nutrients and antioxidants such as zeatin, ascorbic acid, Ca and K (Fuglie, 1999; Foidl et al., 2001). Additionally, cytokinin improves metabolism of solutes to germinating plumule. It is also reported that SA application enhances cell division in the apical meristem of a wheat seedling resulting in higher plant growth and yield (Shakirova and Bezrukova, 1997). Hayat et al. (2005) also reported that wheat plants produced more leaves and biomass with exogenous SA application.

In biochemical parameters, application of MLE showed positive synergy with other chemical stimulants. The higher efficiency in biochemical parameters in the current study may be linked with the presence of various allelochemicals and secondary metabolites such as ascorbate, phenols (Foidl et al., 2001) and zeatin (Fuglie, 1999). Foidl et al. (2001) also stated that foliar application of MLE enhances chlorophyll "*a*" and "*b*" contents. Mona (2013) also concluded that foliar application of MLE improved photosynthetic rate, stomatal conductance along with plant growth hormones like cytokinins, gibberillins and auxins of rocket plant (*Erusa vesicaria*). Similar results were also reported by Abdalla (2013) that foliar application of MLE increased the plant height, fresh and dry weights, photosynthetic rates, the amount of each of chlorophyll *a* and *b*, stomatal conductance, total proteins, ascorbic acid and plant growth hormones like auxins, gibberillins and cytokinins of rocket plant (*Erusa vesicaria*).

Within growth parameters, leaf area is one of the main attributes which depicts economic yield of crops as LAI is typically associated with leaf surface. Maximum leaf area was observed where MLE alone was applied as a foliar spray. The increase in leaf area indices might be due to the cumulative effect of growth hormones as well micro and macro nutrients present in leaf water extract. Similarly crop growth rate is another important indicator of crop biological and economic yield. In the current study, crop growth rate was significantly increased by the foliar application of MLE. High crop growth might be due to higher leaf area as it is an indicator of higher photosynthesis rate and, higher carbohydrate production

Table 4
Impact of alone and blended moringa leaf extract on yield parameters of wheat (three replicates) under field conditions.

Treatments	Biological yield (kg ha^{-1})		Economic yield (kg ha^{-1})		Harvest index (%)	
	Normal sown	Late sown	Normal sown	Late sown	Normal sown	Late sown
Control	10,462 ± 157 e	8859 ± 209 d	3430 ± 87 e	2980 ± 82 d	32.80 ± 0.24 a	33.64 ± 0.17 a
Hydropriming	11,326 ± 252 de	9609 ± 237 d	3758 ± 71 de	3228 ± 92 cd	33.19 ± 0.17 a	33.59 ± 0.15 a
MLE priming	13,391 ± 225 bc	12,488 ± 177 a–c	4522 ± 64 a–c	4157 ± 67 ab	32.76 ± 0.80 a	33.28 ± 0.89 a
Blended* MLE priming	12,970 ± 190 bc	11,807 ± 131 bc	4290 ± 60 bc	3916 ± 165 ab	33.06 ± 0.54 a	33.17 ± 0.15 ab
Water spray	12,011 ± 228 cd	11,431 ± 295 c	3758 ± 120 cd	3728 ± 246 bc	33.69 ± 0.18 a	32.60 ± 0.38 ab
MLE spray	14,888 ± 343 a	13,144 ± 181 a	5010 ± 234 a	4391 ± 80 a	33.68 ± 0.57 a	33.40 ± 0.28 a
Blended* MLE spray	13,958 ± 252 ab	12,776 ± 71 ab	4736 ± 158 ab	4071 ± 80 ab	33.94 ± 0.09 a	31.87 ± 0.24 b
Mean	12,715 A	11,445 B	4215 A	3782 B	33.446 A	33.077 B
LSD	1451.7	1241.1	515.45	550.65	1.8965	4.957

Means sharing the same letter did not differ significantly at $P < .05$.
* 3% solution of MLE in combination with hydrogen peroxide (H_2O_2) @ 2 μM, salicylic acid (SA) @ 50 mg L^{-1} and ascorbic acid (ASA) @ 50 mg L^{-1}.

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that leads to enhanced crop growth. Another reason might be the presence of growth promoting hormones in MLE, which may have the potential to trigger a higher crop growth rate. Aregheore (2002) reported that MLE contains a growth hormone named zeatin which increases growth through an enhanced cell division rate.

Moreover, another significant finding of the present study was that application of all treatments improved grain yield and biological yield, however, application of MLE produced maximum grain and biological yield in normal and late sown wheat (Table 4). Higher grain yield of plants sprayed with MLE was due to increased spike length and the number of spikelets per spike. Similarly, higher grain yield with MLE alone spray was due to improvement in yield contributing factors i.e. number of grains per spike, number of spikelets per spike, grain weight, etc. MLE, an inexpensive, natural and rich source of important plant hormones and mineral nutrients plays a key role in the enhancement of wheat yield. Earlier, Yasmeen et al. (2012) also reported a 10% increase in wheat yield under late sown conditions due to a "stay green phenomenon,", enhanced LAD and increased grain-filling period with MLE application. Cytokinin is also present in MLE which might be involved in the stay green phenomenon.

Yield contributing factors are the most important indicators which have their direct role towards economic yield. Delayed sowing in wheat reduced the numbers of grains per spike and grain weight in this study. Similar findings report the impacts of post-anthesis stresses on assimilate translocation, grain-filling and grain size (Yang and Zhang, 2006). The reduction in yield contributing parameters may be linked with lower number of flowers per plant, pollen grains, pollen viability floral buds and higher flower abortion (Young et al., 2004). Foliar application of MLE has proved its importance in the enhancement of yield contributing parameters such as spike length, number grains per spike and 1000-grain weight. Exogenously applied MLE alone as foliar spray maximally improved the spike length, grains per spike and 1000-grain weight in comparison to the other treatments. Application of MLE as a foliar spray significantly increased 1000 gain weight, grain yield and biological yield due to "stay green phenomenon" throughout grain filling period. Application of MLE spray may be linked with grain filling photo assimilates translation that ultimately improved grain weight. Similar finding were also reported by Khan et al. (2017b).

On an overall basis, the application of moringa leaf extract alone or in combination with chemical growth stimulants showed positive results as indicated by emergence, biochemical, growth and yield parameters. Additionally, application of MLE is more indicated with late sown wheat. The findings of the current study also confirm the suitability of foliar application (as indicated by data presented here) compared to priming agent.

5. Conclusion

It is concluded that application of MLE as priming agent improved the emergence parameters in normal and late sown wheat. Alone and blended MLE spray also enhanced the biochemical traits as well as growth attributes of wheat. Finally, application of alone MLE as well as in combination with chemical stimulants showed elevated effects on wheat crop yield. Notably, the application of moringa leaf extract alone portrays a more beneficial role in both the biological and economical yield of late sown wheat.

Author contribution statement

Shahbaz Khan conducted this experiment and data were also collected and analyzed by Shahbaz Khan and Muhammad Nawaz. Shahzad MA Basra designed this experiment. First draft of the article was prepared by Shahbaz Khan and Imran Hussain. Final draft was also prepared by S. Khan after careful reading of Nikolaus Foidl.

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