Efficacy of Extraction Methods of *Moringa oleifera* Leaf Extract for Enhanced Growth and Yield of Wheat

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Abstract: A field study was conducted to assess effective method to get Moringa leaf extract, through its response on growth and yield traits of wheat. Extracts of whole or chopped and dry or fresh Moringa leaves were used exogenously i.e. pre-sowing seed soaking as well as water diluted solution based foliar spray application at tillering and booting stages. Significantly higher growth response in term of leaf area index, leaf area duration, and crop growth rate was observed when combination of 30 times diluted moringa leaf extract (MLE) was applied at both crop stages. Yield contributing traits of wheat such as fertile tillers, spikelet's spikelet's per spike, grains per spike, 1000 grain weight, biological and grain yields were recorded in significantly higher due to 30 times diluted fresh MLE followed by 20 times dilution of Moringa dried leaf powder (DLP). While, control treatment and hydro-priming showed at comparable results in the form of significantly lesser fertile tillers, grains per spike, 1000-grain weight and grain or biological yields. Conclusively, 30 times diluted MLE proved the best among the treatments combinations for improved wheat growth and yield however, the biochemical features responsible for such promotive response are yet to be investigated prior to dissemination of this technology to the farmer field.

Keywords: Moringa, Fresh leaf extracts, Dried leaf extract, wheat, growth, Yield.

INTRODUCTION

Moringa (*Moringa oleifera*) is native to tropical, semi-arid and subtropical areas of the world including sub-Himalayan plains of Pakistan, Bangladesh, Afghanistan and India [1]. High economic significance resulted in its wide cultivation in Central and Southern America, India, Malaysia, Sri Lanka, Indonesia and Philippines. *Moringa* is a drought resistant, fast growing, deciduous and perennial tree with white to creamy-white flowers, brown triangular pods and around 20 dark brown seeds per pod [2]. Moringa leaves contain Zeatin, a plant hormone belonging to group cytokinin (CKs) known for stress tolerance capabilities based on stay green characteristic adaptability [3]. CKs delay leaf senescence by increasing number of photosynthetic pigments hence, resist stressful environments [4]. In addition, all CKs in general and Zeatin in particular have dominant anti-aging role, increase the activity of antioxidant enzymes (such as catalases) and encourage growth, firmness of plant, resistant to pest and disease attacks, longer life span and heavier roots. The amount of Zeatin in various plants or even within the same plant may vary according to the growth phase season, temperature, endogenous site of biosynthesis in the plant body and in response to synthetic fertilizers application etc. Such composition justifies the increased growth and yield response of many field crops i.e. peanut, tomato, onion, corn and sugarcane from 20% to 35% with exogenous application of active ingredients of Moringa [2].

Moringa leaf extract (*MLE*) is an excellent promoter of germination and seedling growth of maize, rice, sorghum and wheat crops [5]. Enhanced germination of sorghum, reduced germination percentage of rice, increased hypocotyl length of wheat, reduced survival of sorghum seedlings and reduced hypocotyls in sorghum are few among responses of MLE treatment [6]. Moringa leaf water extract at different concentrations (20, 30 and 40 times water diluted) significantly increased leaf area, plant height and chlorophyll *a* or *b* contents under severe salinity and drought stresses [7]. Keeping in view the reported impacts of MLE, optimization of its extraction method to

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ISSN: 1814-8085 / E-ISSN: 1927-5129/18

obtain leaf extract of *Moringa* in relation to its method wise impact on growth and yield were evaluated at Agronomic Research Area, University of Agriculture Faisalabad, Pakistan (longitude 73·8°E, latitude 31·8°N, and altitude 184·4 masl).

MATERIALS AND METHODS

The study was carried out at Agronomic Research Area, University of Agriculture Faisalabad, Pakistan (longitude 73·8°E, latitude 31·8°N, and altitude 184·4 masl) during 2011- 12 by employing randomizes complete block design with four replications in 7.0m ×2.25m plots. Soil texture was clay loam. The crop was sown in 22.5 cm spaced rows with a single row hand drill at a separate 125 and kg on 1st of December 2011. Nitrogen and phosphorus fertilizers were applied @120 kg ha^{-1} and 100 kg ha^{-1}, respectively. Moringa dry leaf powder extract in 20, 30 and 40 times water diluted concentrations was obtained by drying leaves under shade and then ground to form powder using pestle and mortar which later was dissolved in water, overnight Moringa fresh chopped leaf extract was prepared by crushing fresh chopped Moringa leaves using pestle and mortar and soaked in water for 20, 30 and 40 times dilution for 24 h. Double control was used in which hydropriming + water spray was employed to nullify the effect of water used during seed priming and foliar spray whereas, a complete plot in every replication was kept without any seed treatment or foliar application. All the prepared Moringa extract concentrations were used simultaneously as seed priming agents in combinations with foliar sprays at both tillering and booting stages of the wheat crop. In any employed treatment Moringa extracts were first applied as pre sowing seed soaking agent and then the plants emerged from that seed were applied with same Moringa foliar applications at both tillering and botting stages of the wheat crop @ 160 L ha^{-1}. Hence, treatment combinations came into existence comprising of MLE of fresh leaves diluted in water for 30 times, Moringa dry leaf powder (DLP) diluted with water for 20, 30 and 40 times and fresh chopped 24 h water soaked MLE (CLE) diluted for 20, 30 and 40 times. Seed was soaked in each solution for 10 h keeping seed solution ratio 1:5. Both the treatments (no seed treatment and seed soaking with hydro-priming with tap water) were kept as double control for being a common pre-sowing practice. Seeds were dried under shade for 5 hours before sowing till approximately original weight of the seed for optimum moisture level in the seed was maintained. Similarly to exclude the effect of water used as diluting agent for Moringa concentrations, it was sprayed on plants grown with hydro-primed seed whereas check (no seed treatment) was also maintained with no foliar application.

Growth and Yield Related Traits

Leaf area index was calculated with plant samples taken from a randomly selected unit area of each plot. Leaf area duration (days), and crop growth rate crop growth rate (g m^{-2} day^{-1}) were calculated by employing standard procedures of Hunt [8]. The crop was harvested at maturity and data regarding agronomic and yield-related traits were recorded by adopting standard procedures.

Statistical Analyses

Statistical analysis (Statistics 8.1) was done using Fisher's analysis of variance technique and the least significant difference (LSD) test was applied at p= 0.05 to compare the treatment means [9].

RESULT AND DISCUSSION

The results indicated that the maximum leaf area index was recorded in 30 times diluted *moringa* leaf extract application followed by 20 times diluted *moringa* dry leaf powder at all growth intervals i.e. 45, 60, 75, 90 DAS whereas control and hydropriming + water spray gave the minimum leaf area index (Figure **1a**, **b**, **c** and **d**).

Diluted fresh Moringa leaf extract (30 times) significantly increased LAD and CGR, while minimum values was observed without any exogenous application (Figure **2**) at 45-60 DAS, 60-75 DAS, and 75-90 DAS. Since MLE is rich in zeatin, carotenoids, phenols, ascorbates, potassium and calcium that might have plant growth promoting capabilities, which increased the leaf area by decreasing chlorophyll degeneration [2].

A closer look of the Table **1** reveals that application of 30 times diluted MLE produced the highest number of productive tillers (382), number of grain spike^{-1} (37.17), 1000-grain weight (36.10g) and grain yield (4.15 t ha^{-1}) compared to all other tested treatments. In cereals, yield mainly depends on optimum plant density, number and size of grains. Many studies suggested that seed priming enhanced the crop yield [10, 11]. In this study, seed priming and foliar spray with different extraction methods of Moringa leaf extract increased the number of grains per spike and 1000-grain weight. Previous study elaborated that better

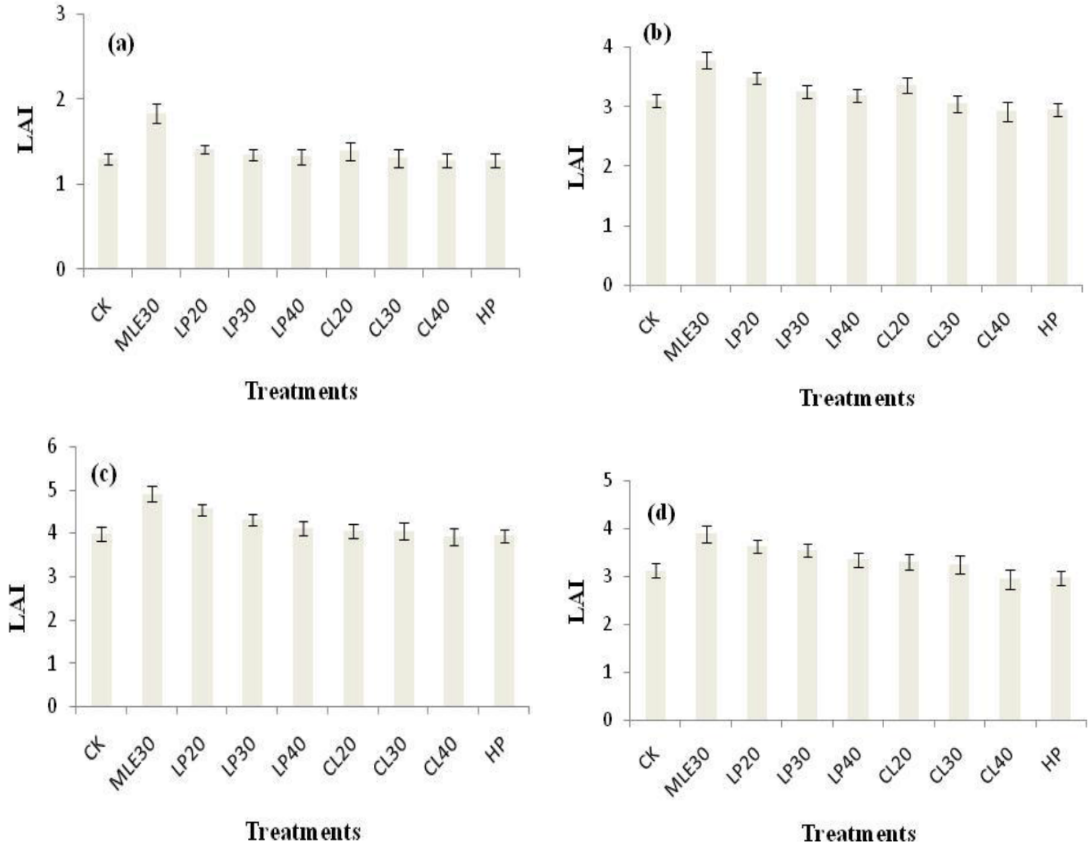
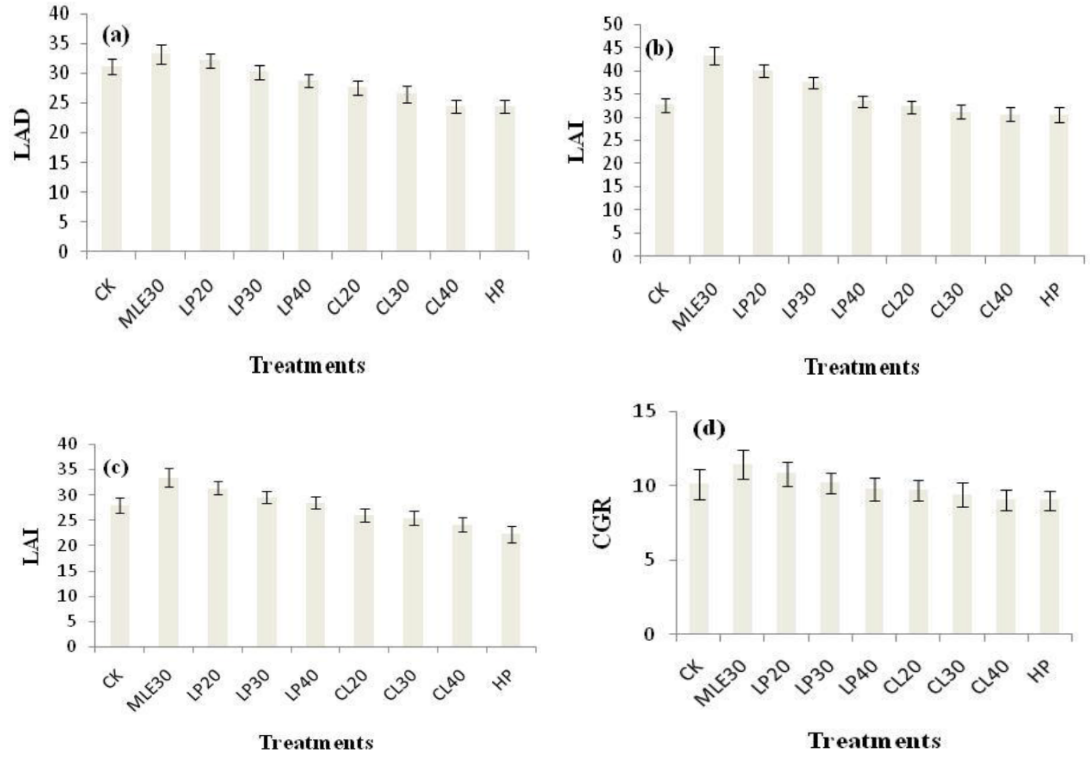


Figure 1: Effect of different extraction methods of *Moringa oleifera* leaf extract on leaf area index (LAI) [a:45 days after sowing, b: 60 days after sowing, c: 75 days after sowing, d: 90 days after sowing] of wheat. CK=Control (no spray), MLE = Fresh water diluted *Moringa oleifera* fresh leaf extract, DLP=water diluted Moringa oleifera dry leaf powder extract, CLE =water diluted *Moringa oleifera* dry leaf powder extract, CLE= *Moringa oleifera* fresh crushed leaves overnight soaked, HP=Hydropriming + water spray.



(Figure 2). Continued.

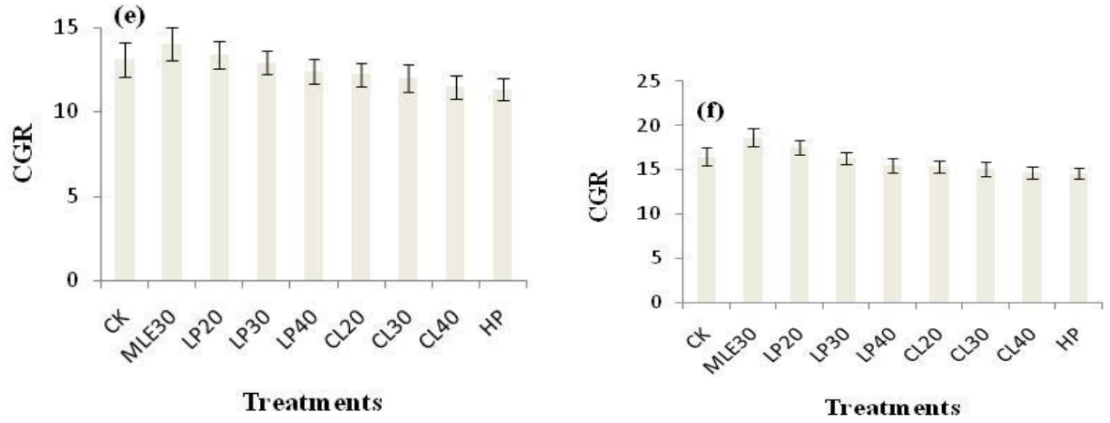


Figure 2: Effect of different extraction methods of *Moringa oleifera* leaf extract on leaf area duration (LAD) [a:45-60 days after sowing, b: 60-75 days after sowing, c: 75-90 days after sowing] and crop growth rate (CGR) [a:45-60 days after sowing, b: 60-75 days after sowing, c: 75-90 days after sowing] of wheat. CK=Control (no spray), MLE = Fresh water diluted *Moringa oleifera* fresh leaf extract, DLP=water diluted *Moringa oleifera* dry leaf powder extract, CLE =water diluted *Moringa oleifera* dry leaf powder extract, CLE=*Moringa oleifera* fresh crushed leaves overnight soaked, HP=Hydropriming + water spray.

Table 1: Effect of Different Extraction Methods of *Moringa oleifera* Leaf Extract on Yield Contributing Parameters of Wheat

Treatments	Fertile tillers m^{-2}	Grains per spike	1000 Grain weight (g)	Grain yield t ha^{-1}	Biological yield t ha^{-1}
CK	317.00 ±10.57e	34.52±0.10e	33.91±0.35d	3.67 ±0.07d	9.74 ±0.37e
MLE 30	382.00 ±8.18a	37.17 ±0.38a	36.61±0.18a	4.15 ±0.10a	12.35 ±0.26a
DLP 20	370.00 ±3.86ab	36.55±0.55ab	35.71±0.58b	4.03 ±0.06ab	12.16 ±0.22a
DLP 30	360.50 ±4.51bc	35.97±0.54bc	35.34±0.21b	3.95 ±0.04b	11.31 ±0.34b
DLP 40	340.00 ±4.14d	34.92±0.25e	35.29±0.29bc	3.76 ±0.06cd	10.78 ±0.20c
CLE 20	357.75 ±3.69bc	35.62±0.21cd	35.45±0.23b	3.91 ±0.06bc	11.39 ±0.13b
CLE 30	349.50 ±8.09cd	35.52±0.46cd	34.59±0.24cd	3.77 ±0.04cd	10.74 ±0.39c
CL 40	337.25 ±7.09d	35.10±0.60de	34.44±0.14d	3.71 ±0.03d	10.26 ±0.34d
HP	319.25 ±9.69d	34.52±0.30e	33.91±0.19d	3.67 ±0.09d	9.73 ±0.36e
LSD at P =0.05	16.708	0.819	0.722	0.164	0.478

CK=Control (no spray), MLE = Fresh water diluted *Moringa oleifera* fresh leaf extract, DLP=water diluted *Moringa oleifera* dry leaf powder extract, CLE =water diluted *Moringa oleifera* dry leaf powder extract, CLE=*Moringa oleifera* fresh crushed leaves overnight soaked, HP=Hydropriming + water spray.

partitioning of assimilates during grain filling stage may improve grain size [13]. Dietrich *et al*. [14] reported that cytokinin have positive correlation with final grain weight in maize. It has been found that Moringa leaves have ample concentration of zeatin, and cytokinin [2].

Therefore, it can be concluded that, Moringa leaf extract priming effectiveness might be due to its growth promoting quality. In present study foliar application of 30 diluted MLE significantly enhanced grain yield compared to other treatment. Lowest grain yield was recorded without any exogenous application (Table **1**). The crop yield of a crop is mainly influenced by photosynthetic rate. Foliar application of Moringa leaf extracts has ability to prevent premature leaf senescence that enhanced the leaf area with increased photosynthetic pigments [15]. The effectiveness of MLE with respect to better chlorophyll, antioxidants and higher plant growth was due to the presence of cytokinin which impart green color to crop plants [2, 16]. *Foidle et al.* [2] reported that MLE consists of significant contents of calcium (Ca), potassium (K) and cytokinin as well as ascorbates, and phenolics. Such compounds present in MLE not only increased seedling vigor but also trigger the plant to a good stand even under stressful conditions and improve grain yield. In present study seed treatment and foliar spray of 30 times diluted Moringa leaf extract at tillering and booting stage is statistically different but closely

followed by 20 times diluted Moringa dry leaf powder. This might be due to the ability of maintaining green color, throughout the grain filling period or due to remobilizing soluble carbohydrates (stem reserves) during grain filling period [17]. Fresh MLE can be used as a potential source to promote growth however; further research should be planed to explore the reasons of this enhancement. It conclusion seed treatment and foliar spray of 30 times diluted Moringa leaf extract increased crop yield by 13% and proved as a best extraction method. 20 times diluted Moringa dry leaf powder extract gave 10% more yield than control but statistically equal with 30 times diluted Moringa leaf extract. The present research study concludes that 30 times diluted Moringa leaf extract and 20 times diluted Moringa dry leaf powder can be used as growth enhancer.

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Received on 03-08-2017 Accepted on 09-03-2018 Published on 13-04-2018

https://doi.org/10.6000/1927-5129.2018.14.19