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Soil Science Original article

# Nutritional improvement of wheat by foliar application of moringa leaf extract

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Abstract		
A field experiment was conducted at the Soil Science Field Laboratory of Bangladesh Agricultural University (BAU), Mymensingh, during rabi season to evaluate the effect of foliar application of moringa leaf extract on produc- tivity and nutrient uptake efficiency of wheat plants. The experiment was laid out in a randomized complete block design with six treatments and three replications. The treatments were T1 (Control), T2 [moringa leaf extract		
(MLE) sprayed only at tillering stage], T3 (MLE sprayed at tillering and jointing stages), T4 (MLE sprayed at tillering, jointing and booting stages), T5 (MLE sprayed at tillering, jointing, booting and heading stages) and T6 (MLE sprayed only at heading stage). The application of moringa leaf extract significantly increased nutrient content as well as nutrient uptake by grain and straw. The content of N. P. K and S in both grain and straw of wheat was		
the highest in T4 treatment which produced the maximum biological yield of 9.05 t ha <sup>-1</sup> . Again, treatment T4 resulted in the highest total uptakes of N, P, K and S in wheat, which were 131.91, 15.55, 122.27, and 24.16 kg ha <sup>-1</sup> , respectively. The results of this study indicate that foliar application of MLE can potentially be a viable option to increase biological yield and nutrient uptake efficiency of wheat plants, particularly N, P, K and S. In this study, the foliar application of moringa leaf extract on tillering, jointing and booting stages of the crop showed the best performance and therefore, it might be used due to its eco-friendly nature for yield enhancement as well as nutrient enrichment in wheat.		
<b>Keywords:</b> Moringa leaf extract, foliar application, wheat, biological yield, nutrient uptake		

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#### 1 Introduction

Wheat (*Triticum aestivum* L.) is the staple food for the people of many countries in the world. It is the second most important cereal crop cultivated in Bangladesh next to rice. Wheat as a grain crop has a great importance in human nutrition and industrial uses. The total area and the annual production of wheat in this

country cover around 1.78 lakh ha and 10 lakh m tons, respectively (BBS, 2017). Although the production of wheat in Bangladesh is increasing day by day the average yield of wheat in this country is still low as compared to that of other Asian countries like China, India, and Pakistan. Bangladesh is a densely populated country and the food demand has been increasing day by day due to high population growth. In the coming

decades, the agriculture of this country will face a great challenge to meet the food demand of the growing population. Hence, it is imperative to increase wheat yield in Bangladesh as a sufficient increase in wheat production has the potential to meet the demand of ever rising population (Mainuddin and Kirby, 2015). In recent years, rapid increase in global temperature due to climatic change and population growth and their consequences on crop productivity, has been a major concern throughout the world. Climate change leading to global warming has already produced a radical change in temperature regimes in developing countries including Bangladesh and that will impact strongly on production of wheat which is temperature-sensitive (Hossain and da Silva, 2012). Therefore, systematic integration of environmental and economic development measures as well as judicial fertilization and proper management practices are necessary for sustainable wheat production in Bangladesh. It is a crying need to use safe alternate natural source of plant nutrients because continuous use of inorganic fertilizers as a source of plant nutrients causes not only soil degradation but also environmental pollution (Abdalla, 2013).

Phytohormones are chemical substances produced within the plants that regulate cellular processes including growth and differentiation of tissues (Neumann KH, 2009). Phytohormones can be used to increase yield per unit area as they influence every phase of plant growth and development (Maishanu et al., 2017). Among different growth regulators, cytokinins are involved in cell division in plant roots and shoots promoting food production. Zeatin is the most common form of naturally occurring cytokinin in plants. Importantly, the leaves of moringa oleifera plant contain significant amount of zeatin whose concentration could be as high as 200  $\mu$ g /g of leaves depending on the plant species (Basra et al., 2011; Abdalla, 2013; Davies, 2015). Moringa oleifera is one of the 13 species of genus Moringa and family Moringaceae. It is a well-known vegetable crop in Bangladesh and is considered as an important food source in many parts of the world. From the last couple of decades many scientists highlighted the industrial and medicinal value of this plant and considered it as one of the world's most useful trees as different parts like leaves, roots, seeds contain significant amount of minerals, proteins, vitamins,  $\beta$ -carotene, amino acids, phenolics, cytokinin, flavonoids and antioxidants (Khalafalla et al., 2010; Adebayo et al., 2011; Nambiar et al., 2005; Anwar et al., 2006; Makkar et al., 2007; Busani et al., 2011).

The cumulative effects of hormones, proteins, minerals, vitamins, essential amino acids, glucosinolates, isothiocyanates and phenolics in extract of *Moringa oleifera* leaves make it a novel, natural biostimulant which after application can enrich the crop with higher nutrient content, improve plant antioxidant system, boost the growth and improve the yield performance. Recently many scientists reported the growth and yield enhancement by applying moringa leaf extract (MLE) in various horticultural crops and some field crops under normal as well as stress conditions (Shehu and Okafor, 2017; Aluko et al., 2017; Matthew, 2016; Ahmad et al., 2016; Afzal et al., 2015; Emongor, 2015; Ozobia, 2014; Abbas et al., 2013; Mohammed et al., 2014; Abdalla, 2013; Mvumi et al., 2013; Yasmeen et al., 2012; Basra et al., 2011; Phiri, 2010; Nouman et al., 2012).

Application of MLE is a low-cost and environment friendly technology (Nouman et al., 2012) and small scale and commercial farmers would be benefitted if they use it as a supplement or substitute to inorganic fertilizers. Since fresh moringa leaves contain lots of vitamins, zeatin and many nutrient elements, it can be used as an effective organic fertilizer that is capable of increasing yield from 10 to 45% according to crop species (Maishanu et al., 2017). The use of MLE as a possible plant growth enhancer can provide a relatively environmentally safe, easily accessible and affordable means of improving crop production to meet the growing demand of food.

Very few scientific reports are found which show the effect of MLE on field crops and therefore it becomes necessary to investigate the role of MLE as a bio-organic fertilizer for field crops in Bangladesh. The present study was conducted to determine the role of MLE at different growth stages of wheat plants on biological yield and nutrient uptake.

#### 2 Materials and Methods

#### 2.1 Experimental site and soil

The experiment was conducted at the Soil Science Field Laboratory of Bangladesh Agricultural University (BAU), Mymensingh during rabi season of the year 2015. The soil of the experimental site belongs to Sonatala series of Non-calcareous Dark Grey Floodplain soils of Old Brahmaputra Floodplain (AEZ-9). The soil was silt loam in texture having pH 6.31, organic matter content 1.15%, total N 0.11%, available P 10.98 ppm, exchangeable K 0.25 me% and available S 9.78 ppm.

#### 2.2 Treatments and design of the experiment

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications and six treatments which were T1 (Control), T2 (MLE sprayed only at tillering stage), T3 (MLE sprayed at tillering and jointing stages), T4 (MLE sprayed at tillering, jointing and booting stages), T5 (MLE sprayed at tillering, jointing, booting and heading stages), and T6 (MLE sprayed only at heading stage). The experimental area was divided into three blocks representing the replications and each block was subdivided into six unit plots where the treatments were randomly distributed. The total number of plots was 18 and the size of the unit plot was  $4m \times 2.5m$ . The spacing between blocks was 1 m and the plots were separated from each other by 0.5 m bund.

#### 2.3 Seed sowing

BARI Gom-26, one of the high yielding varieties of wheat in Bangladesh was used as a test crop for this field experiment. The line to line spacing was 25 cm and the seeds were sown on the 6th of December, 2015 in the prepared plot @ 125 kg ha<sup>-1</sup> in lines. After sowing the seeds were covered with soil by hand. A strip of the same crop was grown as border crop around the experimental field.

#### 2.4 Fertilizer application

During final land preparation, the full doses of triple super phosphate (TSP), muriate of potash (MoP), gypsum, zinc oxide, and boric acid fertilizers were applied as basal to all the plots to supply P, K, S, Zn and B, respectively. The rates of P, K, S, Zn and B were 20, 60, 10, 3, and 2 kg ha<sup>-1</sup>, respectively according to the Fertilizer Recommendation Guide (BARC, 2012). Nitrogen was applied from urea in three equal splits and the dose of N was 100 kg ha<sup>-1</sup>. The first split of urea was also applied as basal during final land preparation where the second split and the third split were applied as top dressing at 30 DAS (days after sowing) during crown root stage and at 56 DAS during booting stage of wheat, respectively.

# 2.5 Preparation of MLE and application of MLE in the field

Fresh and young leaves of moringa were collected from mature plants located at different homestead and roadside areas of BAU Campus, Mymensingh. For extract preparation, 100 g clean leaves were ground using a mortar and pestle with a small amount of water (10 mL/100 g fresh leaves). The leaf juice was filtered through a cheese cloth and extracted by hand pressure followed by re-filtering through Whatman filter paper No.2. The extract was diluted 33 times with distilled water @ 1:32 (v/v) according to the method developed by Fuglie (2000). The prepared MLE was then sprayed directly onto the wheat plants @ 25 mL plant<sup>-1</sup> using hand sprayers as per treatments with special attention for complete coverage of plants with MLE. The remaining extract was stored at 0 °C temperature in a refrigerator for further application. The spraying of MLE was performed several times at different growth stages of the crop namely tillering, booting, jointing and heading. Water was

sprayed in the control plots and adequate attention was paid to avoid drifting of spray materials from one plot to another.

#### 2.6 Intercultural operations

Irrigation was provided two times at 25 and 55 DAS of the crop. Thinning was done at 25 DAS to maintain 20 plants per line. The other intercultural operations such as weeding and pesticide application were done as and when necessary.

#### 2.7 Crop harvesting and data collection

The crop was harvested on the 7 April, 2016 at full matured stage and data on the yield were recorded. The represented plant samples (both grain and straw) were collected plot-wise for chemical analysis.

# 2.8 Preparation and analysis of plant samples

The leaf samples of moringa as well as grain and straw samples of wheat were dried in an oven at 65 °C temperature for about 48 hours and then ground by a grinding machine to pass through a 20-mesh sieve. The ground plant materials were stored in paper bags separately and placed in a desiccator. These plant samples were analyzed for determination of macronutrient contents namely N, P, K and S. The total N, available P, exchangeable K and available S of plant samples were determined following semi-micro Kjeldahl method, modified Olsen method, NH4OAc extraction method and CaCl<sub>2</sub> extraction method, respectively. The macronutrient content in dry moringa leaves is shown in Table 1.

Table 1. Nutrient contents in dry moringa leaves

Nutrient elements	Concentration (%)
Nitrogen	$5.04\pm0.06$
Phosphorus	$0.34\pm0.02$
Potassium	$0.97\pm0.05$
Sulphur	$0.10\pm0.01$

After chemical analysis of grain and straw samples of wheat, the nutrient uptake was calculated from the nutrient content and yield of the crop using the following formula:

$$TU = \frac{NC \times Y}{100} \tag{1}$$

where, TU = total nutrient uptake (kg ha<sup>-1</sup>), NC = nutrient concentraiton (%), and Y = yield (kg ha<sup>-1</sup>).

#### 2.9 Statistical analyses

The collected data were compiled and tabulated for statistical analysis. Analysis of variance (ANOVA)

was done as per randomized complete block design with the help of computer package MSTAT-C. The mean differences were compared by Duncan's New Multiple Range Test at  $p \le 0.05$  (Gomez and Gomez, 1984).

#### 3 Results

#### 3.1 Biological yield

The application of MLE exerted a significant influence (p = 0.008) on biological yield of BARI Gom-26 (Fig. 1). The biological yield ranged from 6.54 to 9.05 t ha<sup>-1</sup>. The maximum biological yield (9.05 t ha<sup>-1</sup>) was noted in treatment T4 (MLE sprayed at tillering, jointing and booting stages) and the minimum yield (6.54 t ha<sup>-1</sup>) was found in control treatment. The biological yieldof BARI Gom-26 may be ranked in the order of T4>T5>T6>T3>T2>T1. Again, the percent increase in biological yield of wheat over control due to different treatments ranged from 18.35 to 38.38%.



Figure 1. Biological yield of BARI Gom-26 as influenced by application of moringa leaf extract

## 3.2 Nutrient content and uptake by wheat

#### 3.2.1 Nitrogen content and uptake

Results presented in Table 2 showed that the nitrogen (N) content of wheat grain and straw was significantly influenced (p = 0.009) by application of MLE. The content of grain-N varied from 1.22 to 2.06% while that of straw-N ranged from 0.91 to 1.06%. For both grain and straw samples, the highest values were found in treatment T4 (MLE sprayed at tillering, jointing and booting stages) and the lowest values in treatment T1 (control). Moringa leaf extract caused a significant variation in N uptake by grain, straw and in total by BARI Gom-26 as shown in Table 2. The values for grain-N uptake, straw N-uptake and total N uptake varied from 32.04 to 74.57, 35.67 to 57.34 and 67.71 to 131.91 kg ha<sup>-1</sup>, respectively. The maximum values

for grain-N uptake, straw-N uptake and total N uptake were observed in T4 treatment and the minimum values were noted in control treatment.

#### 3.2.2 Phosphorus content and uptake

Application of MLE significantly affected (p = 0.023) the phosphorus (P) content of grain but not of straw of BARI Gom-26 (Table 3). The content of grain-P varied from 0.17 to 0.23% whereas the content of straw-P from 0.10 to 0.13%. In both grain and straw samples, the highest P content was noted in treatment T4 (MLE sprayed at tillering, jointing and booting stages) and the lowest in treatment T1 where no MLE was sprayed. Again, a significant variation in P uptake by grain, straw and in total uptake by wheat was found in this study (Table 3). The values for grain-P uptake, straw P-uptake and total P-uptake ranged from 4.52 to 8.24, 3.92 to 7.31 and 8.44 to 15.55 kg ha<sup>-1</sup>, respectively. In the same manner, the maximum values for grain-P uptake, straw-P uptake and total P uptake were observed in T4 treatment and the minimum values in control treatment.

#### 3.2.3 Potassium content and uptake

The potassium (K) content of wheat grain was significantly influenced (p = 0.008) by MLE application although that of straw remained unaffected (Table 4). The content of grain-K varied from 0.61 to 0.98% while that of straw-K from 1.18 to 1.59%. In case of grain-K content as well as straw-K content, the highest values and the lowest values were found in treatment T4 (MLE sprayed at tillering, jointing and booting stages) and treatment T1 (control), respectively. In addition, results expressed in Table 4 showed that foliar spray of MLE significantly affected K uptake by grain, straw and in total by BARI Gom-26. The K uptake by grain, straw and in total varied from 15.97 to 35.60, 46.29 to 86.67 and 62.26 to 122.27 kg ha $^{-1}$ , respectively. Similarly in all three cases of K uptakes (grain, straw and total), the highest values were observed in T4 treatment and the lowest in control treatment.

#### 3.2.4 Sulphur content and uptake

Addition of MLE significantly affected (p = 0.01) the sulphur (S) content of both grain and straw of BARI Gom-26 as shown in Table 5. The content of grain-S and straw-S varied from 0.21 to 0.26% and 0.23 to 0.28%, respectively. Similar with other nutrient elements, the maximum S content of grain and straw was noted in treatment T4 with MLE at tillering, jointing and booting stages and the minimum S content in treatment T1 without MLE. Further, the S uptake by grain, straw and in total by wheat was significantly influenced by the treatments under study (Table 5). The S uptake by grain, straw and in total ranged from

Treatment	N content (%)		N uptake (kg ha $^{-1}$ )		Total N uptake
	Grain	Straw	Grain	Straw	$(\text{kg ha}^{-1})$
T1	1.22 b	0.91 c	32.04 c	35.67 c	67.71 d
T2	1.85 a	0.98 abc	57.72 b	45.3 b	103.00 b
Т3	1.95 a	1.02 ab	61.23 ab	48.04 b	109.27 b
T4	2.06 a	1.06 a	74.57 a	57.34 a	131.91 a
T5	1.92 a	0.96 bc	66.24 ab	46.37 b	112.61 b
T6	1.25 b	0.99 abc	39.63 c	46.93 b	86.56 c
$\overline{\text{SE}(\pm)}$	0.37	0.01	3.9	1.53	5.02
CV (%)	5.97	6.52	7.65	8.69	8

Table 2. Nitrogen content and uptake by BARI Gom-26 as influenced by different treatments

Table 3. Phosphorus content and uptake by BARI Gom-26 as influenced by different treatments

Treatment	P content (%)		P uptake (kg ha $^{-1}$ )		Total P uptake	
	Grain	Straw	Grain	Straw	(kg ha <sup>-1</sup> )	
T1	0.17 b	0.1	4.52 c	3.92 b	8.44 c	
T2	0.21 ab	0.11	6.67 b	5.05 b	11.72 abc	
T3	0.21 ab	0.11	6.64 b	4.93 b	11.56 c	
T4	0.23 a	0.13	8.24 a	7.31 a	15.55 a	
T5	0.21 ab	0.12	7.29 b	5.94 ab	13.23 ab	
Т6	0.18 ab	0.11	5.72 bc	5.31 b	11.03 abc	
$\overline{\text{SE}(\pm)}$	0.03	0	0.3	0.26	0.52	
CV (%)	7.36	14.64	9.64	11.89	11.89	

Table 4. Potassium content and uptake by BARI Gom-26 as influenced by different treatments

Treatment	K content (%)		K uptake (kg ha $^{-1}$ )		Total K uptake
	Grain	Straw	Grain	Straw	$(kg ha^{-1})$
T1	0.61 d	1.18	15.97 c	46.29 c	62.26 d
T2	0.79 ab	1.29	24.70 b	59.40 bc	84.12 c
Т3	0.81 ab	1.51	25.46 b	71.21 b	96.67 bc
T4	0.98 a	1.59	35.60 a	86.67 a	122.27 a
T5	0.86 ab	1.41	29.73 b	68.21 ab	97.93 bc
T6	0.73 bc	1.4	23.24 b	66.83 ab	90.07 bc
$\overline{\text{SE}(\pm)}$	0.03	0.06	1.56	3.41	4.68
CV (%)	5.51	8.5	4.97	10.14	8.61

Table 5. Sulphur content and uptake by BARI Gom-26 as influenced by different treatments

Treatment	S content (%)		S uptake (kg $ha^{-1}$ )		Total S uptake	
	Grain	Straw	Grain	Straw	$(kg ha^{-1})$	
T1	0.21 b	0.23 b	5.50 c	9.02 c	14.52 c	
T2	0.23 ab	0.26 ab	7.02 b	11.78 b	18.80 b	
T3	0.24 ab	0.26 ab	7.54 b	12.25 b	19.78 b	
T4	0.26 a	0.28 a	9.23 a	14.93 a	24.16 a	
T5	0.24 ab	0.26 ab	8.28 ab	12.56 b	20.84 b	
T6	0.23 ab	0.25 ab	7.29b	11.64 b	18.93 b	
$\overline{\text{SE}(\pm)}$	0.01	0	0.33	0.46	0.75	
CV (%)	3.96	3.39	4.65	3.73	3.99	

In a column figures with same letters do not differ significantly as per DMRT at 5% level of probability. SE = Standard error and CV = Coefficient of variation. Here T1= Control, T2= MLE sprayed only at tillering stage, T3= MLE sprayed at tillering and jointing stages, T4= MLE sprayed at tillering, jointing and booting stages, T5= MLE sprayed at tillering, jointing, booting and heading stages, T6= MLE sprayed only at heading stage.

5.50 to 9.23, 9.02 to 14.93 and 14.52 to 24.16 kg ha<sup>-1</sup>, respectively. In all cases, the highest uptakes were observed in T4 and the lowest uptakes in control.

#### 4 Discussion

In recent years the discovery of MLE as a biostimulant for improving the performance of many agricultural crops has gained much significance (Rady and Mohamed, 2015). Yield enhancement of crops by spraying MLE was reported by many researchers (Mvumi et al., 2013; Abbas et al., 2013; Emongor, 2015; Matthew, 2016; Shehu and Okafor, 2017). The present study clearly showed that the foliar application of moringa leaf extract at critical growth stages (tillering, jointing and booting stages) of wheat can increase the biological yield of the crop (Fig. 1). This finding is partially similar to that of Yasmeen et al. (2012) who reported higher yield performance of wheat by spraying MLE at four growth stages (tillering, jointing, booting and heading).

Recently, Afzal et al. (2015) suggested that 3% moringa leaf juice applied as a foliar spray at critical growth stages (tillering and booting stages) has the potential to increase the yield of late-sown wheat. Again, Brockman and Brennan (2017) reccommended that application of MLE at tillering and booting stages can be a viable option to increase yield of wheat. It was observed from our experiment that the higher the frequency of moringa leaf extract application during the vegetative growth stages of the crop, the higher the yield of the crop.

Some researchers (Mvumi et al., 2013; Abdalla, 2013) suggested that the presence of phytohormone namely cytokinin might be involved in the enhancement of growth and yield of a crop after spraying MLE. However, a thorough research is necessary to clarify the role of phytohormones in MLE. In this study, use of MLE increased biological yield upto 38% and these results are in confirmation with those of Maishanu et al. (2017) who stated that MLE can improve crop yield in the range of 10-45%.

The present study also showed that the uptake and accumulation of various nutrients namely N, P, K and S in grain and straw of wheat were significantly increased due to application of moringa leaf extract (Table 2 to Table 5). For both grain and straw, the highest contents as well as uptakes of N, P, K and S were found in T4 treatment with MLE sprayed at tillering, jointing and booting stages while the lowest contents and uptakes of N, P, K and S were noted in T1 treatment without MLE. Previously Merwad and Abdel-Fattah (2017) reported the highest percent increase of N, P, and K uptake by wheat (157%, 320%, and 116%, respectively for straw while 226%, 329%, and 163%, respectively for grain) from 4% MLE application. According to Merwad (2017), the P and K use efficiency of wheat could be increased by 30% due

to application of MLE at booting stage. The results of our study were accorded to those of several comparable studies of some researchers (Sivakumar and Ponnusami, 2011; Abdalla, 2012) suggesting that the increased uptake and accumulation of some macro nutrient elements such as N, P, K and S in several plants under investigation as a consequence of application of moringa leaf extract. Recently, Merwad (2017) found the highest percent increase of N, P, and K accumulation in pea (145%, 374%, and 172%, respectively for shoot and 212%, 296%, and 252%, respectively for seed) with foliar application of 4% MLE. Nasir et al. (2016) also showed that application of 3% MLE could increase N, P and K contents (1.35, 1.09 and 1.89 folds, respectively) of Kinnow mandarin leaves and when MLE was applied with other nutrients it could increase the nutrient uptake by leaves. In the same manner, significant increase in the leaves nutrient uptake (N, P and K) of rocket plant (Abdalla, 2013) and pear tree (El-Hamied and El-Amary, 2015) was reported with foliar application of MLE.

Different parts of M. oleifera plants have been reported to be a rich source of important nutrient elements (Yameogo et al., 2011; Busani et al., 2011) and thus moringa leaf extract can boost plants to uptake beneficial elements, to increase the nutrient status and eventually to attain optimum growth and productivity.

#### 5 Conclusions

Now-a-days, natural plant growth stimulants are intensively studied for improving plant performance under normal and stress situations because they are ecofriendly, cheap and easily available. *Moringa oleifera* is one of the best natural biostimulants, enriched with phytohormones, phenolics, vitamins and minerals that can play a vital role in increasing crop growth and improving crop with higher nutritional value.

The present study suggests that foliar application of moringa leaf extract has the potential to increase the nutrient status of wheat plants and the highest frequency of MLE application during the critical growth stages of the crop would be most suitable for better crop performance. Based on the findings of the present study it may be concluded that the use of MLE as a biostimulant should be encouraged and introduced to our farmers as an effective means of increasing yield and improving nutritional quality of crop in order to develop sustainable agricultural practice in our country. However, further research needs to be performed to elucidate the field efficacy of MLE on various cereal crops as well as horticultural crops and to ascertain its application frequency according to the crop species.

#### **Conflict of Interest**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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