INTERNATIONAL JOURNAL OF APPLIED AND EXPERIMENTAL BIOLOGY
(ISSN PRINT: 2790-6523; ISSN ONLINE: 2790-6531)
2024, Vol. 3, NO. 1, 75-82.
doi: 10.56612/ijaaeb.v3i1.61
https://eminentscientists.com/

Effect of foliar application of naphthalene acetic acid on physio-morphological attributes and green biomass production of sorghum [*Sorghum bicolor* (L.) Moench.]

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Abstract
The present study was aimed to evaluate the impact of foliar application of naphthalene acetic acid (NAA) on the physio-morphological attributes and green biomass production of sorghum under field conditions at the Agronomic Research Field, Gomal University, Dera Ismail Khan, Pakistan. Varying concentrations of NAA (0, 45, 90, 135, and 180 mL ha^{-1}) were applied to sorghum seedlings at different intervals [30 and 90 days after emergence (AE)]. All plots equally received recommended agronomic practices and crop protection measures. The NAA foliar application in different levels caused a significant influence on the growth and biomass production of sorghum. The maximum plant tallness (434.3 cm), plant leaf area (419 cm^{2}), number of nodes (17.01 plant^{-1}), number of tillers (19.03 m^{-2}), and chlorophyll contents (56.97 µg cm^{-2}) were observed due to NAA supplementation at 90 days AE, while, the stem thickness (8.82 cm), photosynthetic efficiency (2.56%) and green biomass (34.72 t ha^{-1}) were observed in the plots which received the foliar spray of 180 mL ha^{-1} NAA followed by 135 mL ha^{-1}, that showed improvement in plant tallness (423.1 cm), plant leaf area (402.07 cm^{2}), number of nodes/plant (16.71), tiller numbers (189.81 m^{-2}), chlorophyll contents (56.59 µg cm^{-2}), stem thickness (8.69 cm), photosynthetic efficiency (2.56%) and green biomass (36.10 t ha^{-1}) compared to those in the control treatment. The findings of the study clearly depict that foliar application of NAA as 120 mL ha^{-1} at 90 days AE showed promising results in terms of enhanced production of green biomass of sorghum under arid agro-ecological conditions of Pakistan.

SECTION
Plant Biology (PB)

HANDLING EDITOR
Athar, H.R. (PB)

ARTICLE HISTORY
Received: 18 Feb, 2023
Accepted: 29 May, 2023
Published: 02 Jan, 2024.

KEYWORDS
Bioregulators;
Fodder;
Growth promoters;
Photosynthetic efficiency;
Plant productivity

Introduction

Sorghum (*Sorghum bicolor* L. Moench.) is one of the 5th most essential crops in the family of cereal crops after wheat, rice, maize and barley. It is cultivated for fodder, biofuel and grains (Khan et al., 2013; Ananda Galaihalage et al., 2020). The plant requires less water and can withstand in harsh environments where other crops show poor growth and yield. The increasing demand for food and fodder has led to considerable interest in sustainable agriculture in the changing climate scenarios, and sorghum could be the viable solution to farmers in dry regions under rainfed conditions. Sorghum is cultivated for multipurposes and is the chief source of calories (protein, vitamins and minerals) for millions of poor

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TO CITE THIS ARTICLE: Hussain, I., Ali, M., Ullah, M. (2024). Effect of foliar application of naphthalene acetic acid on physio-morphological attributes and green biomass production of sorghum [*Sorghum bicolor* (L.) Moench.]. *International Journal of Applied and Experimental Biology* 3(1): 75-82.

people (Kumari et al., 2016). To an estimate, in Pakistan, sorghum was cultivated on 242 thousand ha with a production of 149 thousand tons in 2019 (Anonymous, 2019). In the Khyber Pakhtunkhwa province of Pakistan, this crop was grown on an area of 4 thousand ha in 2015 and produced 2.33 thousand tons of grain yield with an average yield of 581 kg ha^{-1} (Anonymous, 2015).

Plant growth regulators (PGRs) are produced intrinsically by the plants, but crop plants may also respond to these foliar-fed chemicals (Dianaguiraman et al., 2013). This is because of their limited endogenous biosynthesis in plants for optimal crop growth and development (Ali et al., 2012). Besides, plant growth enhancers may substitute plant growth hormones when they are in low levels intrinsically (Abdelgadir et al., 2009; Fahad et al., 2015; Hirayama and Mochida, 2022). PGRs influence plant growth, development, metabolic and biological activities in plants, and ultimately improve fruiting bodies and yields (Hayat et al., 2010; Singh et al., 2017). Previous research has shown that PGRs may cause better plant water relations and photosynthetic rates (Jones et al., 2012; Benedict et al., 2013). Of different types of plant growth regulators, naphthalene acetic acid (NAA) being an analogue of auxins, is considered as an effective bioregulator which can promote growth both under normal and stress conditions by stimulating cell division and elongation (Gill and Bal, 2009; Chen et al., 2022; Ullah et al., 2022). Moreover, foliar application of NAA can increase the fruit size and quality of blueberries, plums, guavas, ber, and many other such crops, thereby improving final crop yield (Singh et al., 2017; Milic et al., 2018). Based on these findings, it was hypothesized that NAA, being a potential plant growth regulator, can promote growth of sorghum by regulating key physiological attributes in sorghum. Thus, the key objective of the current research was to examine how and up to what extent NAA application could regulate key physio-morphological traits, thereby promoting green biomass yield of sorghum under high-temperature regime.

Materials and Methods

An investigation was carried out at the experimental area of Gomal University, D.I. Khan, to investigate the influence of NAA applied at different time intervals to sorghum seedlings. The experiment was arranged in a randomized complete block design and replicated thrice in split-plot arrangements. The experiment included two factors, i.e., five concentrations of NAA (0, 45, 90, 135 and 180 mL ha^{-1}) in the main plots and three plant growth stages of sorghum [30, 60 and 90 days after emergence (AE)] in subplots. Hybrid sorghum "Jumbo+" seed @ 30 kg ha^{-1} was planted in the field during the 3rd week of July 2018 with a hand drill on ridges keeping 45 cm row to row distance in each plot of 4 × 3 m dimensions. The optimum level of fertilizers NPK (125-90-60 kg ha^{-1}) was managed by applying a complete dose of P, K, and half quantity of nitrogen during tillage operations, whereas the remaining 1/2 dose of nitrogen was applied at tillering. Irrigation was scheduled as per crop requirements. NAA (Planofix) was foliar sprayed by a knapsack sprayer @ 4.5% concentration. The effect of NAA on agronomic attributes like plant tallness, number of nodes, and internodes per plant were measured by randomly selecting five plants from each plot and then averaged. The leaf area per plant was measured by taking both sides (width + length) of each of 5 leaves $plant^{-1}$ randomly from each sub-unit with the help of a measuring scale by using the following formula:

$$\text{Leaf area} = \text{Length of leaf} \times \text{Width of leaf} \times \text{correction factor } (0.75) \times \text{number of leaves per plant}$$

The CF (correction factor) for the area of leaves per plant is referred to as the K coefficient and the fixed unit for sorghum is 0.75. The stem thickness was measured by random selection of 5 plants from each sub-unit by a Vernier Caliper (Series 530, accuracy 0.02 mm). Leaf chlorophyll concentration in 5 randomly tagged plants in every subplot was measured before heading with a portable photometer (SPAD-502Plus). The plant growth rate was calculated using the following formula:

$$\text{Crop growth rate} = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{1}{GA}$$

Where W_1 is the previous plant's dry mass, W_2 the last dry mass, T_1 the collection time of the previous sample and T_2 the collection time with 60 day interval from the previous weight recording time and GA is the ground area.

Photosynthetic efficiency was calculated by the formula presented by Stoskopf (1981). The green biomass (t ha^{-1}) was calculated by weighing the total harvested (wt) material in each unit and then converted to t ha^{-1}.

Statistical analysis of data

The data were recorded and subjected to analysis of variance (Steel et al., 1997) with a subsequent assessment of discrete treatment means worked out using the Tukey HSD test. ANOVA was calculated using “Statistix 8.1” computer software.

Results and Discussion

The present research was conducted to evaluate the influence of exogenously applied NAA (naphthalene acetic acid) on the agronomic and physio-morphological attributes of sorghum. NAA is a commercial plant growth regulator having an auxin nature, and it has been widely used as a foliar spray to encourage the fundamental processes of commercial crops. For example, application of NAA improves the photosynthetic activity of plants by enhancing the concentration of chlorophyll (Meena et al., 2017). Plants generally produce growth regulators in insufficient quantity under stress conditions, therefore, exogenous applications of PGR promotes plant growth and associated characteristics (Kato et al., 2014). However, the exogenous applications of NAA showed positive effects on different growth parameters in comparison with the control treatment (**Figure 1**; **Table 1**). The results showed that the application of 135 mL ha^{-1} NAA at 90 DAE or 60 DAE induced maximum height at maturity, while the minimum plant tallness was observed at 30 days AE in the untreated plots (**Table 1**). Enhancement in plant tallness and growth due to NAA application has also been reported in previous studies (Sivakumar et al., 2006; Almodares and Eraghizadeh, 2011; Basuchaudhuri, 2017; Baksh et al., 2017).

Leaf area per plant (cm^2) was affected significantly by the exogenous application of NAA (**Figure 1b**). Plants treated with 180 mL NAA ha^{-1} carried the leaves with a maximum area (419 cm^2). Similarly, the plants expanded their leaves up to the maximum extent at 90 DAE (**Table 1**). The application of different concentrations of NAA at different dates after emergence revealed that the maximum node number was counted in the plots treated with 180 mL NAA ha^{-1} at 90 days AE followed by the same level of NAA applied 60 days AE as well as 90 days AE (**Table 1**). Mean values indicated that the application of NAA as 135 mL ha^{-1} at 90 days AE produced maximum internode number (**Figure 1d**; **Table 1**). The minimum internode number was recorded in the untreated plants at 30 days AE (Table-1). The stem thickness of the sorghum plants showed a maximum response to NAA applied at different plant growth stages (**Table 1**). Maximum values of stem thickness were recorded in the plots which received 180 mL NAA ha^{-1} at 60 days AE or 30 days AE, while the untreated plants expressed the minimum value of this attribute at 30 days AE (Table-1). Mona et al. (2013) also reported that plants sprayed with NAA or a combination of IAA and NAA induced plants to have expanded leaves. The positive effect of the sole application of NAA or in combination with other growth hormones like GA_3 on the morphological characteristics of plants was also reported by Khan and Chaudhry (2006). Their findings revealed that exogenous application of varying levels of gibberellic acid blended with NAA influenced the morpho-physiological characters, thereby increasing the meristematic centers. The increase in number of nodes, internodes and stem thickness due to NAA was also reported by other researchers (Knoche et al., 2000; Djanaguiraman and Ramesh, 2013; Rajput et al., 2015; Viana et al., 2016; Ben et al., 2017; Macedo et al., 2017).

Sorghum plants treated with 180 mL NAA ha^{-1} at 90 days AE, also produced the highest chlorophyll contents, followed by the same level of NAA applied at the seedling stage (**Figure 1g**; **Table 1**), while the minimum chlorophyll was recorded during 30 days AE of control/untreated plants (**Table 1**). The results also showed a positive effect of NAA on photosynthetic efficiency, i.e., the ability of a plant to convert light energy into chemical energy by the synthesis of organic compounds (**Figure 1h**. The data revealed that the sorghum plants expressed maximum photosynthetic efficiency at 90 days AE, while the minimum photosynthetic efficiency was recorded at 30 days AE (**Table 1**). Independent of growth stage, the sorghum plants expressed maximum photosynthetic efficiency when NAA was sprayed at the rate of 135 mL ha^{-1} (Table-1). Stoskopf (1981) also reported a positive relationship between NAA levels and photosynthetic efficiency.

The results depicted that the use of NAA at different time intervals after emergence has significant effects on plant growth rate (**Figure 1i**). Mean values indicated that the NAA level of 180 mL ha^{-1} produced the highest plant growth rate when the application was done during 30 days AE or 60 days AE (**Table 1**). Moreover, the impact of varying concentrations of NAA at different dates after emergence had significant effects on the green biomass of the sorghum crop (t ha^{-1}) (**Figure 1j**). Averaged values showed that the NAA @ 135 mL per hectare had high green biomass yield when applied at 90 days AE, while the minimum green biomass yield was recorded when NAA was applied at 30 days AE (**Table 1**). Many factors like leaf area, tillering, plant tallness, plant growth rate, and photosynthesis are involved in the production of green biomass (Ben et al., 2017; Macedo et al., 2017). It has been reported elsewhere that the increase in the level of naphthalene acetic acid can improve photosynthetic rate (Viana et al., 2016; Begum et al., 2018), plant growth rate (Marais and Averbeke, 2009), and other physio-morphological attributes including green biomass production (Bonner et al., 1962; Stoskpof et al., 1981; Shuvan et al., 1995; Liu et al., 2012; Gemici, 2013; Ullah et al., 2017).

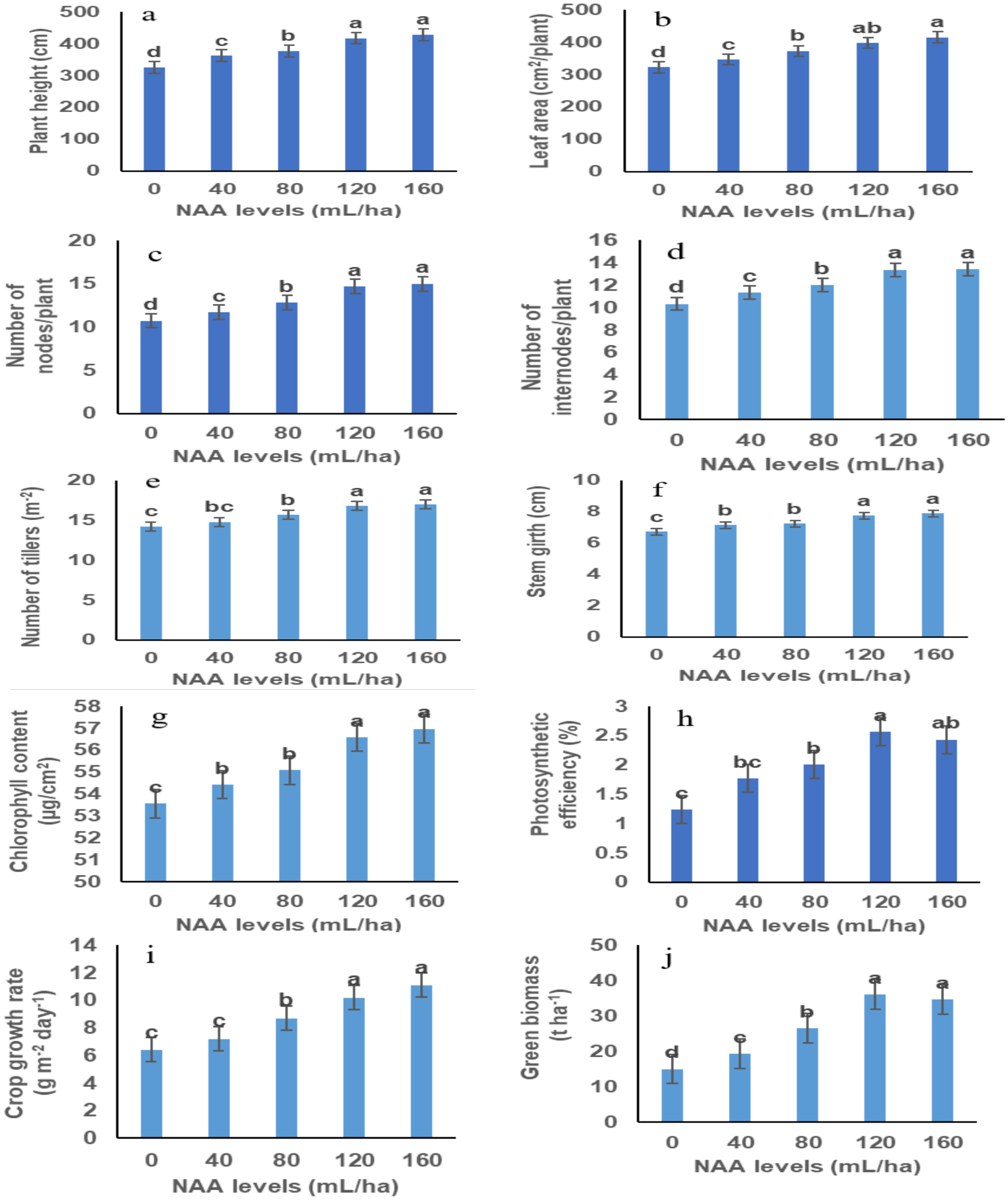

Figure 1: Effect of naphthalene acetic acid application on different growth parameters of sorghum (mean of data for three growth stages)

a) Plant tallness (cm); b) Leaf area (cm^2/plant); c) Number of nodes/plant; d) Number of internodes/plant; e) Number of tillers m^{-2}; f) stem thickness (cm); g) Chlorophyll contents ($\mu g\ cm^{-2}$); h) Photosynthetic efficiency (%); i) Plant growth rate ($g\ m^{-2}\ day^{-1}$); j) Green biomass ($t\ ha^{-1}$);

L1: 0 mL NA/ha; L2: 45 mL NAA/ha; L3: 90 mL NNA/ha; L4: 135 mL NAA/ha; L5: 180 mL NAA/ha; Means having same letter (s) in each attribute did not differ significantly at $P \leq 0.05$.

Table 1: Effect of different levels of NAA applied at different growth stages of sorghum plants on different growth parameters (S1=30 days AE; S2=60 days AE; S3=90 days AE)

Parameters	Growth stages	NAA (levels) $L_1 = 0$	$L_2 = 45$	$L_3 = 90$	$L_4 = 135$	$L_5 = 180$	Mean
PT	S1	299.9 h	323.2 g	352.7 f	384.2 e	412.6 d	354.4 c
	S2	323.1 g	366.8 e	374.9 e	405.4 d	430.8 c	380.2 b
	S3	360.7 ef	400.3 d	408.4 d	467.4 a	447.1 b	416.7 a
	Mean	331.3 d	366.9 c	382.1 b	423.1 a	434.3 a	
PLA	S1	320.8a	336.60b	367.24e	397.56i	410.26j	366.7 c
	S2	323.1a	344.02c	371.58f	398.68i	416.35k	370.7 b
	S3	325.3a	359.82d	381.62g	399.88i	421.42l	377.6 a
	Mean	325.8 c	349.4 c	376.2 b	402.1 ab	419.4a	
NNP	S1	11.73 h	12.53 g	13.66 f	14.56 e	16.0 d	13.70 c
	S2	10.63 g	11.83 f	12.97 e	13.87 d	14.83 c	12.83 b
	S3	11.80 f	12.80 e	13.87 d	17.63 a	16.13 b	14.45 a
	Mean	12.74d	13.74 c	14.85 b	16.71 a	17.1 a	
NIP	S1	10.29 i	11.49 h	12.33 g	14.29 ef	13.83 d	13.34 c
	S2	10.40 h	11.43 g	12.13 f	12.93 d	13.50 c	12.08 b
	S3	11.37 g	12.13 f	12.67 de	15.23 a	13.90 b	13.06 a
	Mean	12.45 d	12.39 c	13.08 b	15.40 a	14.45 a	
TN	S1	13.93 i	14.93 hi	15.93 gh	16.97 fg	17.97 ef	16.42 c
	S2	14.33 hi	15.00 eg	15.67 df	15.67 df	16.67 cd	15.47 b
	S3	16.00 ce	16.00 ce	17.00 c	20.00 a	17.67 b	17.53 a
	Mean	15.28 c	15.84 bc	16.73 b	18.81 a	19.03 a	
ST	S1	7.51 h	7.81 fh	8.04 eg	8.28 de	8.58 bd	8.27 b
	S2	6.77 gh	7.03 eh	7.27 df	7.40 ce	7.73 bc	7.24 b
	S3	6.80 gh	7.20 eg	7.30 de	8.50 a	8.26 b	7.61 a
	Mean	7.63 c	8.04 b	8.14 b	8.69 a	8.82 a	
PGR	S1	4.35 h	4.44 gh	6.40 fh	7.51 df	9.48 be	6.53 c
	S2	6.4 fh	7.2 eg	8.9 cf	9.7 bd	11.4 ab	8.7 b
	S3	8.6 cf	9.7 be	10.8 ac	13.2 a	12.5 a	11.0 a
	Mean	6.39 c	7.37 c	8.42 b	10.47 a	11.46 a	
Chl	S1	53.27	54.04	54.90	55.64	56.54	54.8 c
	S2	53.50	54.40	54.97	56.10	56.84	55.1 b
	S3	53.90	54.84	55.37	58.04	57.54	55.9 a
	Mean	53.56 c	54.43 b	55.08 b	56.59 a	56.97 a	
PE	S1	1.00	1.49	1.58	2.02	2.04	1.63 c
	S2	1.28	1.86	2.05	2.45	2.24	1.98 b
	S3	1.43	1.95	2.38	3.20	3.02	2.40 a
	Mean	1.24 c	1.77 bc	2.00 b	2.56 a	2.43 ab	
GB	S1	14.94 g	15.17 g	20.55 f	30.58 c	27.52 d	21.7 c
	S2	15.09 g	18.58 f	25.78 de	36.81 b	36.37 b	26.5 b
	S3	15.01 g	24.31 e	33.41 c	40.90 a	40.28 a	30.7 a
	Mean	15.01 d	19.35 c	26.58 b	36.10 a	34.72 a	

Plant tallness (cm); PLA = Plant leaf area (cm^2); NNP = Number of nodes/plant; NIP = Number of internodes/plant; TN = Tiller number (m^{-2}); ST = Stem thickness (cm); PGR = Plant growth rate ($g\ m^{-2}\ day^{-1}$); Chl = Chlorophyll content ($\mu g\ cm^{-2}$); PE = Photosynthetic efficiency (%); GB = Green biomass ($t\ ha^{-1}$)
L1: 0 mL NAA/ha NAA; L2: 45 mL NAA/ha; L3: 90 mL NAA/ha; L4: 135 mL NAA/ha; L5: 180 mL NAA/ha; Means having a similar letter (s) in each attribute did not differ significantly at $P \leq 0.05$.

Conclusion

Foliar application of 135 mL or 180 mL naphthalene acetic acid ha^{-1} at 90 days AE induced the sorghum plants to exhibit maximum height, increased number of leaves and nodes per plant, added stem thickness and tillers, improved photosynthetic efficiency and produced high amount of green biomass in sorghum. Therefore, foliar application of NAA at 135 mL ha^{-1} or 180 mL ha^{-1} at 90 days AE is suitable for the sorghum crop to exhibit maximum values of green biomass and enhanced regulation of physio-morphological attributes.

Declaration of Author(s), Editor(s) and Publisher

Acknowledgement

The authors gratefully acknowledge the lab and field staff of Department of Agronomy, Gomal University, D.I. Khan for their technical support throughout the whole experimentation.

Conflict of interest
The authors declare no conflict of interest.

Source of funding
None declared.

Contribution of authors
Conceptualized and designed the study, IH, MA. Conducted field experiments and wrote up the first draft of the manuscript, IH, MA. Reviewed and edited the manuscript, MU. All authors have read, reviewed and agreed to the publish the current version of the manuscript.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/journal management. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All authors contributed in designing and writing the entire article. All contributors have critically read and reviewed this manuscript and agreed for publishing in IJAaEB.

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