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Original article

# Integrated use of phosphorus and growth stimulant (actibion) improves yield and quality of forage sorghum (*Sorghum bicolor* L.)

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ABSTRACT

*Background and objectives:* Sorghum (*Sorghum bicolor* L.) is an important summer fodder all over the world. Phosphorus (P) is an important macronutrient which plays significant role in the growth and development of crop plants. The P is deficit in most of the Pakistani soils. Bio-stimulants are organic compounds that could enhance vegetative growth, development, nutrients' uptake and tolerance of the plants to abiotic stresses.

*Methods:* This two-years field experiment determined the effect of different P levels and growth stimulant (actibion) on yield and quality of forage sorghum. The P levels were 0, 30, 60 and 90 kg ha$^{-1}$ and bio-stimulant (1250 ml ha$^{-1}$) was applied at 20, 30 and 40 days after sowing (DAS), while distilled water was sprayed as control.

*Results:* The results revealed that the highest values for plant height (241.70 and 237.03 cm), stem diameter (1.59 and 1.41 cm), number of leaves per plant (15.4 and 14.80), leaf area per plant (3016.8 and 2575.3 cm$^2$), chlorophyll contents (46.17 and 44.34), fresh forage yield (47.83 and 45.33 ton ha$^{-1}$) and dry matter yield (13.84 and 13.02 ton ha$^{-1}$) were recorded with 60 kg ha$^{-1}$ P and bio-stimulant application at 30 DAS. The similar trend was observed for crude protein (9.53 and 13.01 %). Total ash contents, plant P contents (0.17 % and 0.16 %) were improved when 90 kg ha$^{-1}$ P was applied along with bio-stimulant application at 30 DAS. However, no application of P and bio-stimulant resulted in the highest values of NDF and ADF.

*Conclusion:* It is concluded that 60 kg ha$^{-1}$ P application along with foliar application of bio-stimulant at 30 DAS is optimum for improving yield and quality components of forage sorghum.



## 1. Introduction

Sorghum (*Sorghum bicolor* L.), a member of the family Poaceae is a tall annual plant with adventitious roots (Barkworth, 2003). Sorghum plays an important role in food security of some of the poorest parts of the world (Mundia et al., 2019). Globally, sorghum is 5th most important cereal crops in the world (Iqbal et al., 2010) and cultivated on an area of 0.41 mha with 6.3 million tones annual production (Anonymous, 2008). It is known for high production potential, even on marginal lands. Forage sorghum is a heat resistance crop and produces high biomass (Reddy and Reddy, 2003).

In Pakistan sorghum is known as 'Jowar' which is vital kharif crop and grown for fodder and grain purposes (Iqbal et al., 2010). Sorghum is an essential feed in dairy and livestock sector in dry and short supply seasons. Hence, dual purpose is favored which produces stover and grains (Hall et al., 2014). After pearl millet (*Pennisetum glaucum* L.) sorghum is the cheapest source of micronutrients and energy. Sorghum is a virtuous source of >20 minerals and contains high amounts of potassium (K), phosphorus (P), zinc (Zn) and iron (Fe) (Dicko et al., 2006). It requires low inputs and known as quick growing grass and recommended more than other fodders. However, there are many reasons behind poor quality and low

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production of forage sorghum in Pakistan. These include inappropriate sowing method, selection of inappropriate varieties, malnutrition, improper harvesting stage, presence of hydrocyanic acid contents and low protein contents (Chattha et al., 2017). Among essential nutrients, P is an essential nutrient (Dixon et al., 2020) and 2nd most important deficient macronutrient (Munir and Ranjha, 2004). It plays significant role in the respiration, photosynthesis, cell enlargement, and cell division (Mengel et al., 2001). It strengthens cereal straw, hasten flowering and maturity of crops, increase seed formation and root development (Gupta et al., 2016). Pakistani soils are P-deficit (Ali et al., 2020) due to calcareousness of soil ($CaCO_3$ > 3.0 %) or soil alkaline reaction (pH > 7.0) or low level of soil initial P. In Pakistan, P use as fertilizer on fodder crops is limited, which reduces production and quality of fodder (Khan et al., 2003). The availability of high yielding and fertilizer-responsive sorghum cultivars has shown great interest in fertilizer research (Bughdady, 2016). Among fertilizers, P directly improves the quantity and quality of fodder production. The P application gradually increases leaf area, stem diameter, plant height, number of leaves and forage yield (Mahmud et al.. 2013).

Abiotic stresses are major factors that reduce ~ 50 % yield of important crops (La-Pena and Hughes, 2017). Plant bio-stimulants have gained great interest from the agrochemical industry and farmers because of their ability to enhance nutrient use efficiency and increase abiotic stress tolerance of crops (Rajabi Hamedani et al., 2020). Different products categorized as plant growth stimulants (PGS) are used with herbicides, fungicides, and insecticides (Calvo et al., 2014). Growth stimulants are active biological compounds having ability to promote growth and development and enhance metabolisms when applied in minute quantity. They contain hormones, amino acids, enzymes, vitamins, microelements, and many other compounds (Edmeades, 2002). Basically, bio-stimulants are not nutritious products, but they have capability to improve uptake of soil nutrients (Brown & Saa, 2015). Bio-stimulants have direct hormonal effects (Subler et al., 1998). Bio-stimulants have positive impact on the plants, but their effects depend on cultivar and plant species (Sultana et al., 2005). They can be applied by using foliar spray or soil application. Foliar application needs low amounts of bio-stimulants and absorbed quickly and directly by leaves through mesophyll cells, cuticular and epidermal cells (Fernandez et al., 2016). Present field study was performed to understand the effect of P and growth stimulant (actibion) application timings on yield and quality of forage sorghum.

## 2. Materials and methods

### 2.1. Experimental site description

The field experiment was conducted during summer seasons of 2018 and 2019 at Agronomic research site, College of Agriculture, University of Sargodha, Pakistan. The soil of experimental area was loamy having pH of 7.4 and 7.6, E.C of 3.67 and 3.78 $\mu Scm^{-1}$, organic matter contents 0.91 % and 0.88 %, total N 0.049 % and 0.042 %, available K 268 and 250 ppm, available P 7.90 and 7.40 ppm and saturation percentage was 39 % and 37 % during 2018 and 2019, respectively.

### 2.2. Experimental details

Experiment was laid out in randomized complete block design (RCBD) with split plot arrangement and each treatment had three replications during both years. The experiment comprised of two factors, i.e., P levels and bio-stimulant application timing. The P fertilizer levels were 0, 30, 60, 90 kg $ha^{-1}$ and actibion (1250 ml $ha^{-1}$) was applied at 20, 30 and 40 days after sowing (DAS), while distilled water was applied as control. Crop was sown manually by single row drill. The N was applied @ 90 kg $ha^{-1}$ and 1/3rd of N was applied before sowing and remaining was applied in two splits (with 1st and 2nd irrigation). The sorghum seeds were planted on well prepared seedbed during last week of April 2018 and 2019 to obtain maximum germination percentage. The seeds rate was kept 75 kg per hectare. Thinning was done at early stages of crop development to provide maximum space for plant growth. Furadan (8 kg $ha^{-1}$) was applied to protect the crop against stem borer. Foliar application of growth stimulant: 'actibion' which contains (alanine 0.52, arginine 1.73, aspartic acid 1.21, cystine 0.78, glycine 0.78, histidine 0.22, leucine 0.78, glutamic acid 3.14, lysine 0.72, phenylalanine 0.54, methionine 0.08, proline 1.15, threonine 1.14, serine 1.51, tyrosine 0.26, isoleucine 0.59 and valine 0.85 %) @ 1250 ml $ha^{-1}$ was done at 20, 30 and 40 DAS. For weed control, hoeing was done at different intervals. First irrigation was applied immediately after completion of germination, while subsequent two irrigations were applied to crop according to the requirements. The sorghum crop was manually harvested at 50 % panicle emergence (70 DAS).

### 2.3. Crop harvesting and data recording

During both years sorghum was harvested at 70 DAS. Ten plants were randomly selected at harvest from every subplot and plant height (cm), number of leaves per plant, stem diameter (cm), leaf area per plant ($cm^2$) and chlorophyll contents were recorded. For fresh forage yield (t $ha^{-1}$) entire treatment plots were manually harvested by sickle and weighed on digital field balance and converted in tons per hectare. For dry matter yield, ten plants were taken randomly from every subplot, chopped by using fodder cutter machine and thoroughly mixed. The sample fresh weight was recorded. Afterwards, the chopped material was taken and completely dried in an oven for 3 days at 72 °C. Dry matter content (%) was calculated. The dry matter contents (%) were intended from dry weight of sample.

$$\text{Dry matter content } (\%) = \frac{\text{dry weight}}{\text{fresh weight}} \times 100$$

To calculate ash content (%), 5 g of powdered sample was taken in a China dish ($W_1$) and samples were placed at 550–650 °C for 6–7 h in a muffle furnace until grey ash obtained. The sample was cooled in the desiccator, and remaining contents were weighed ($W_2$) and percentage was calculated by method proposed by AOAC (2002).

$$\text{Ash content } \% = \frac{W2}{\text{sample weight}} \times 100$$

For determination of neutral detergent fiber content (%), 1 g of powdered sample was taken in a conical flask. After that 0.50 g of sodium sulphate was added and then 100 ml neutral detergent mixture was added. For temperature settlement, flask was fitted with air condenser. The flask was heated gradually for 1-hour, cooled and purified by using suction pump four times with hot distilled water and then once with acetone solution and dried normally. After drying crucible residues were shifted and placed in oven at 105 °C for 1 h. Crucible was placed for 10 min in desiccator after drying and NDF was calculated as follows.

$$\text{Neutral detergent fiber content } \% \text{ (NDF)} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

To find out acid detergent fiber contents (%), NDF residues were shifted to 500 ml of flask and acid detergent fiber solution was added (100 ml) into flask and fixed with an air condenser. The

sample was heated till boiling point for 2 to 3 min and then cooled and again heated for 60 min. The contents were filtered after removing from condenser by suction pump. Then the residues were washed thrice with water and once with acetone. Residues were shifted to a pre-weighted crucible and put this crucible at 105 °C in oven for 24 h. Then the crucible was cooled in desiccator. The ADF% was calculated by using the following equation.

$$\text{Acid detergent fiber content } (\%)(\text{ADF}) = \frac{\text{Weight of ADF residue}}{\text{Weight of sample}} \times 100$$

Crude protein content (%) was calculated by determination of N. A 1 g of powdered sample was taken in kjeldahl digestion flask to which 30 ml of $H_2SO_4$ and 10 g of digestion mixture were added and heated until green liquid was formed. Green liquid was shifted to 250 ml flask after proper cooling and flask was filled up to the given mark. The 10 ml aliquot material was taken in micro distillation apparatus and 15 ml NaOH (40 %) was taken. Nitrogen was evolved as ammonia and collected in flask containing 4 % boric acid. Then methyl red was added and bromocresol green as an indicator. Distilled material was titrated against N/10 $H_2SO_4$ until red color was obtained. Amount of N was calculated from the acid used in the process of titration. Crude protein (CP) percentage was calculated by multiplying reading with 6.25, procedure proposed by the AOAC (2002).

$$\text{Nitrogen } (\%) = \frac{A - B}{\text{Volume of Digested Sample}} \times 100 \times 0.0014$$

The total plant P content (%) was obtained by the method proposed by AOAC (2002). One-gram dry grinded plant sample was taken and $HNO_3$ and $HClO_4$ at the rate of 20 ml and 10 ml respectively were added and placed on the hot plate. When volume of sample reduced to 3 ml after heating, the obtained mixture was cooled and shifted in a 100 ml flask and final volume was prepared by distilled water addition. After that 50 ml flask was taken and 5 ml of aliquot was added and then 5 ml of ammonium vanadate and ammonium molybdate (color developing reagents) was added and made the volume up to 50 ml by adding water and placed for half an hour. The intensity of color was noted by the help of spectrophotometer (Beckman) at the wavelength of 470 nm. Reading was noted from standard curve.

### 2.4. Statistical analysis

Data recorded was analyzed statistically by Fisher's analysis of variance (ANOVA) method and their means were compared by Tukey's HSD test at 95 % probability (Steel et al., 1997). The normality and homogeneity of variance were tested prior to ANOVA. The data were normally distributed; therefore, met the normality assumption for executing ANOVA. Two-way ANOVA was used to infer the significance and Tukey's HSD post-hoc test was used to compare the means where ANOVA denoted significant differences.

## 3. Results

### 3.1. Growth, yield, and yield contributing traits

The interactive impact of different P levels and growth stimulant application timing was significant for growth and yield attributes (plant height at maturity, stem diameter, number of leaves per plant, leaf area per plant, chlorophyll content, fresh forage yield, dry matter content and dry matter yield) of forage sorghum during both study years (Tables 1 and 2). The highest plant height at maturity (241.70 and 237.03 cm), stem diameter (1.59 and 1.41 cm), leaf area (3016.8 and 2575.3 $cm^2$), number of leaves per plant (15.4 and 14.80), chlorophyll content (46.17 and 44.34), fresh forage yield (47.83 and 45.33 ton $ha^{-1}$) and dry matter yield (13.84 and 13.2 ton $ha^{-1}$) were recorded when crop was fertilized with 60 kg $ha^{-1}$ P and growth stimulant application at 30DAS during both years. Interactive effect of 60 kg $ha^{-1}$ P and growth stimulant application at 20 DAS produced statistically similar values of these traits. However, no P and growth stimulant application (control treatment, only distilled water) resulted in the lowest values of plant height (161.53 and 154.87 cm), number of leaves per plant (12.13 and 11.63), stem diameter (1.062 and 1.05 cm), leaf area (2235.9 and 1951.7 $cm^2$), chlorophyll content (27.50 and 21.83), fresh forage yield (35.16 and 30.33 tons $ha^{-1}$) and dry matter yield (8.23 and 6.24 ton $ha^{-1}$).

### 3.2. Quality traits

The interactive impact of different P levels and growth stimulant application timing was significant for quality traits (crude protein, NDF, ADF, plant P content and ash content) (Tables 2 and 3). Slightly different trend was observed with respect to quality traits and most of the quality parameter were better at combination of 90 kg $ha^{-1}$ P and growth stimulant application at 30 DAS followed by 60 kg $ha^{-1}$ P and growth stimulant application 30 DAS. The highest values of crude protein (9.67 and 13.01 %) was observed with 60 kg $ha^{-1}$ P and growth stimulant application at 30 DAS during both years. The highest values for ash content (13.31 % and 12.85 %), and plant P content (0.17 and 0.16 %) were recorded with 90 kg P $ha^{-1}$ and growth stimulant application at 40 DAS. The lowest values of most of the quality traits were recorded in control treatment. The least value of crude protein (8.14 and 6.24 %) and P content (0.08 and 0.06 %) were recorded in control treatment. The lowest values of NDF (62.23 % in 2018 and 54.86 % in 2019) and ADF (37.23 % in 2018 and 33.20 in 2019) were observed when P was applied at the rate of 60 kg $ha^{-1}$ with growth stimulant application at 30 DAS. The highest value for both NDF and ADF during both years was observed in control treatment.

## 4. Discussion

The P levels and timing of growth stimulant application have significant effect on yield and quality attributes of forage sorghum in the current study. The increase in sorghum yield parameters (plant height, stem diameter, leaf area per plant, number of leaves per plant, chlorophyll contents) and fresh and dry forage yield was observed by the interactive effect of 60 kg $ha^{-1}$ P and growth stimulant application at 30 DAS. This effect is attributed to role of P and growth stimulant in plant growth and development. Phosphorus is involved in strengthening and extension of roots, stem strengthening and elongation, synthesis of energy rich nucleotide (ATP) and increase in respiration rate. Better functioning of P resulted in better growth of roots and stem and better utilization of other macro and micronutrients from soil in the current study, which correlate the better performance of all yield contributing parameters. The highest plant productivity can be achieved by application of the best management of all agricultural practices.

The management and sustainable cultural practices are highly needed and must be preferable. For instance, foliar application of amino acids may enhance plant productivity and improve product quality. Our results showed better plant performance when foliar application of growth stimulant was done after 30 DAS which is related to delayed senescence process in older leaves in plants. Growth stimulants are single or multi-ingredient plant extracts containing protein, vitamins, hormones, and some biological active compounds. Growth stimulant can be treated as systematic agent which may easily penetrate through cuticle and reached easily to

**Table 1**
Interactive effect of phosphorus and growth stimulant (actibion) application on plant height, stem diameter, leaf area and leaf number of forage sorghum.

| Treatments | Plant height (cm) | | Stem diameter (cm) | | Leaf area ($cm^2$) | | Number of leaves $plant^{-1}$ | |
|---|---|---|---|---|---|---|---|---|
| | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| **Interaction Phosphorus Level × Bio-stimulant** | | | | | | | | |
| **P1 × B1** | 161.53 g | 154.87 g | 1.06 h | 1.06e | 2235.9i | 1951.7e | 12.1f | 11.63e |
| **P1 × B2** | 183.7efg | 178.03ef | 1.20 fg | 1.21b-e | 2411.0fgh | 2072.3cde | 13.3def | 12.96cde |
| **P1 × B3** | 191.5def | 187.2cde | 1.28efg | 1.27a-d | 2487.0e-h | 2125.7cde | 14.3a-d | 13.63a-d |
| **P1 × B4** | 176.97 fg | 175.63 fg | 1.17gh | 1.15de | 2372.1ghi | 2023.0e | 13.6b-e | 12.60de |
| **P2 × B1** | 199.43 c-f | 183.77def | 1.20 fg | 1.19cde | 2349.7hi | 2029.1de | 12.4ef | 11.86e |
| **P2 × B2** | 209.53 bcd | 205.53bcd | 1.35de | 1.32a-d | 2510.2efg | 2214.8b-e | 13.6cde | 13.00c |
| **P2 × B3** | 219.87 abc | 220.87ab | 1.43bcd | 1.33a-d | 2580.0de | 2175.4bcde | 14.8abc | 14.23abc |
| **P2 × B4** | 205.93 b-e | 201.27b-e | 1.31def | 1.34a-d | 2479.5e-h | 2100.1cde | 13.3def | 13.03cde |
| **P3 × B1** | 210.53 bcd | 197.87b-f | 1.43bcd | 1.39ab | 2567.8def | 2376.0abc | 13.4def | 13.06cde |
| **P3 × B2** | 225.93 ab | 219.93ab | 1.52ab | 1.40ab | 2847.3b | 2497.3ab | 14.9ab | 14.63ab |
| **P3 × B3** | 241.70 a | 237.03a | 1.59a | 1.41a | 3016.8a | 2575.3 a | 15.4a | 14.80a |
| **P3 × B4** | 216.73 bcd | 211.07bc | 1.48abc | 1.39ab | 2774.8bc | 2358.1a-d | 14.5a-d | 14.20abc |
| **P4 × B1** | 220.97 abc | 213.97ab | 1.49abc | 1.38abc | 2747.8bc | 2357.7a-d | 14.3a-d | 13.96a-d |
| **P4 × B2** | 208.33 bcde | 204.67bcd | 1.38cde | 1.38abc | 2615.5cde | 2228.6b-e | 13.9bcd | 13.60a-d |
| **P4 × B3** | 201.13 b-f | 197.47b-f | 1.35de | 1.36a-d | 2499.8e-h | 2186.7b-e | 13.5cde | 13.16b-e |
| **P4 × B4** | 213.33 bcd | 209.33bc | 1.42bcd | 1.35abc | 2709.3bcd | 2399.3abc | 14.2a-d | 13.80a-d |
| **LSD** | 22.90 | 22.89 | 0.128 | 0.156 | 147.34 | 365.0 | 1.33 | 1.38 |

Means having similar letters do not differ significantly at 5% probability level.
**$P_1$**: No phosphorus, **$P_2$**: Phosphorus 30 $kgha^{-1}$, **$P_3$**: Phosphorus 60 $kgha^{-1}$, **$P_4$;** Phosphorus 90 $kgha^{-1}$.
**$B_1$**: Distilled water application (Control), **$B_2$**: Bio stimulant application 20 DAS, **$B_3$**: Bio stimulant application 30 DAS, **$B_4$**: Bio stimulant application 40 DAS.

**Table 2**
Interactive effect of phosphorus and growth stimulant (actibion) application on chlorophyll contents, fresh forage yield, DM yield and crude protein contents of forage sorghum.

| Treatments | Chlorophyll Content | | Fresh forage yield (t/ha) | | Dry Matter Yield | | Crude Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| **Interaction Phosphorus Level × Bio-stimulant** | | | | | | | | |
| **P1 × B1** | 27.50f | 21.83f | 35.16e | 30.333f | 8.23i | 6.24h | 8.14i | 6.24g |
| **P1 × B2** | 30.46def | 25.16ef | 37.33e | 35.16def | 9.5gh | 8.10fgh | 8.25i | 8.01d-g |
| **P1 × B3** | 36.16bcd | 30.50cd | 42.00cd | 37.83b-e | 10.05gh | 8.70def | 8.38h | 8.70c-f |
| **P1 × B4** | 28.46ef | 22.46ef | 37.33e | 34.50ef | 9.40ghi | 7.29gh | 8.22i | 7.29fg |
| **P2 × B1** | 31.06def | 25.80cde | 37.83e | 36.20cde | 8.99hi | 7.94fgh | 8.57g | 7.94efg |
| **P2 × B2** | 36.53bcd | 31.23abc | 42.66bcd | 41.00abc | 11.41cde | 10.24bcd | 8.64g | 10.24bcd |
| **P2 × B3** | 40.33abc | 35.33abc | 44.83b | 41.16abc | 11.48cde | 9.97b-e | 8.84f | 9.97b-e |
| **P2 × B4** | 34.43c-f | 29.10b-e | 41.83cd | 37.33b-e | 10.60efg | 8.23efg | 8.68g | 8.23d-g |
| **P3 × B1** | 35.86cde | 39.00b-e | 41.00d | 39.00b-e | 11.18def | 9.69c-f | 9.35e | 9.69b-e |
| **P3 × B2** | 42.16ab | 42.10ab | 44.16bc | 42.10ab | 13.31ab | 11.72ab | 9.55bcd | 11.72ab |
| **P3 × B3** | 46.16a | 44.34a | 47.83a | 45.33a | 13.84a | 13.02a | 9.71a | 13.01a |
| **P3 × B4** | 41.26abc | 41.50a-d | 44.16bc | 40.50a-d | 12.39bcd | 10.14b-e | 9.53cd | 10.14b-e |
| **P4 × B1** | 41.83abc | 41.45abc | 44.83b | 41.83abc | 12.54bc | 10.66 bc | 9.42de | 10.66bc |
| **P4 × B2** | 39.43abc | 39.33a-d | 43.16bcd | 40.33a-d | 11.23def | 10.14bcd | 9.57bc | 10.14b-e |
| **P4 × B3** | 37.73bcd | 40.23a-d | 43.00bcd | 40.83a-d | 11.48cde | 10.03bcd | 9.67ab | 10.03b-e |
| **P4 × B4** | 41.03abc | 39.98ab | 45.16ab | 42.76ab | 12.39bcd | 10.78bc | 9.57bc | 10.78abc |
| **LSD** | 5.98 | 6.01 | 2.61 | 6.032 | 1.22 | 1.92 | 0.136 | 2.05 |

Means having similar letters do not differ significantly at 5% probability level.
**$P_1$**: No phosphorus, **$P_2$**: Phosphorus 30 $kgha^{-1}$, **$P_3$**: Phosphorus 60 $kgha^{-1}$, **$P_4$;** Phosphorus 90 $kgha^{-1}$.
**$B_1$**: Distilled water application (Control), **$B_2$**: Bio stimulant application 20 DAS, **$B_3$**: Bio stimulant application 30 DAS, **$B_4$**: Bio stimulant application 40 DAS.

active sites. Therefore, growth stimulants when applied in small quantities may enhance synthesis of natural growth promoters, stimulate plant metabolism and assimilation (Basak, 2008). Growth stimulants may enhance root growth and activity ultimately enhance nutrient uptake.

Improvement in fresh fodder yield and yield contributing traits of sorghum seemed to the result of increased nutrients uptake, cell division, and natural growth promoting substances in cell. Our findings are supported by Roy and Khandaker (2013). The increase in plant height by P application may be due to its promising effect on cell enlargement and cell division. Kocira (2019) studied the impact of growth stimulant (amino acid) on the soybean yield and concluded that foliar application of growth stimulant significantly enhanced the plant height (38 %) than control. The possible reason is existence of micro and macro elements and growth enhancing substances in growth stimulants such as cytokinin and auxin. Safdar et al. (2012) observed the highest number of leaves per plant (13.53) at 90 kg P $ha^{-1}$, whereas the lowest (12.40) number of leaves per plant were recorded in control. Low number of leaves might be due to more movement of photosynthates towards root under suboptimal conditions of P (Alkhader et al., 2013). Effect of P × growth stimulant was significant and is quite alike with the findings of Biya (2018) who recorded maximum stem girth (1.00 cm) when 34.5 kg P was applied over control which gave minimum stem girth (0.85 cm). Islam et al. (2017) reported that 90 kg $P_2O_5$ $ha^{-1}$ gave the highest leaf area (128.3 $cm^2$) of wheat, while no P application produced the lowest leaf area (122.5 $cm^2$). Reason for the highest leaf area is that P can enhance numerous physiological processes, cell division, cell enlargement and respiration. Plant bio-stimulant contains amino acids (aspartate, alanine and glutamate) which might be helpful

**Table 3**
Interactive effect of phosphorus and growth stimulant (actibion) application on ash contents, NDF, ADF and plant phosphorus contents of forage sorghum.

| Treatments | Ash Content (%) | | NDF content (%) | | ADF content (%)) | | Phosphorus content (%) | |
|---|---|---|---|---|---|---|---|---|
| | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| **Interaction Phosphorus Level × Bio-stimulant** | | | | | | | | |
| **P1 × B1** | 7.42i | 6.85h | 79.02a | 73.38a | 54.36a | 51.02a | 0.08h | .060f |
| **P1 × B2** | 9.85h | 9.32g | 69.40a-d | 62.12ab | 44.40a-e | 41.06a-e | 0.083h | .0733def |
| **P1 × B3** | 10.10fgh | 9.57fg | 66.44cd | 59.71ab | 41.44cde | 38.10b-e | 0.093gh | .0867b-f |
| **P1 × B4** | 9.93gh | 9.45fg | 67.17bcd | 62.81ab | 42.17b-e | 37.50cde | 0.083h | 0.700ef |
| **P2 × B1** | 10.30fgh | 9.65fg | 78.75a | 69.41ab | 53.75ab | 49.08ab | 0.096fgh | 0.083c-f |
| **P2 × B2** | 10.27fgh | 9.88efg | 68.86a-d | 61.22ab | 43.86a-d | 39.20b-e | 0.113d-g | 0.093b-f |
| **P2 × B3** | 10.56efg | 9.99d-g | 65.19cd | 58.83ab | 40.19de | 35.52e | 0.120cde | 0.103b-f |
| **P2 × B4** | 10.46e-h | 9.99d-g | 66.22cd | 61.55ab | 41.22cde | 36.55de | 0.106efg | 0.083c-f |
| **P3 × B1** | 10.65ef | 10.37def | 78.29ab | 66.05ab | 52.62abc | 47.95a-d | 0.116c-f | 0.093b-e |
| **P3 × B2** | 11.13de | 10.78cde | 71.88a-d | 68.40ab | 46.88a-d | 42.22a-e | 0.126cde | 0.103b-e |
| **P3 × B3** | 12.08bc | 11.43bc | 62.23d | 54.86b | 37.23e | 33.20e | 0.133bcd | 0.110bcd |
| **P3 × B4** | 11.61cd | 10.89cd | 64.37cd | 56.37b | 51.20a-d | 34.70e | 0.130bcd | 0.110bcd |
| **P4 × B1** | 12.10bc | 11.60bc | 75.53abc | 63.18ab | 39.85de | 48.20abc | 0.136bc | 0.113bc |
| **P4 × B2** | 12.62b | 12.12ab | 64.85cd | 56.61b | 39.85de | 36.85cde | 0.150ab | 0.123b |
| **P4 × B3** | 13.31a | 12.85a | 62.87d | 58.58b | 39.37de | 34.70e | 0.17a | 0.16a |
| **P4 × B4** | 13.29a | 12.79a | 62.70d | 56.00b | 37.70e | 34.23e | 0.136bc | 0.113bc |
| **LSD** | 0.614 | 1.030 | 11.07 | 15.83 | 11.44 | 11.48 | 0.0217 | 0.029 |

Means having similar letters do not differ significantly at 5% probability level.
**$P_1$**: No phosphorus, **$P_2$**: Phosphorus 30 kgha$^{-1}$, **$P_3$**: Phosphorus 60 kgha$^{-1}$, **$P_4$;** Phosphorus 90 kgha$^{-1}$.
**$B_1$**: Distilled water application (Control), **$B_2$**: Bio stimulant application 20 DAS, **$B_3$**: Bio stimulant application 30 DAS, **$B_4$**: Bio stimulant application 40 DAS.

to increase the leaf area, chlorophyll contents and ultimately increases the photosynthetic activity (Carillo et al., 2019).

Chlorophyll content at harvest support plants to absorb light energy so it is the most important component for photosynthesis. If plant has more chlorophyll, it absorbs more quantity of light and ultimately produces more yields. Chlorophyll contents trend in this experiment is quite parallel with the results of Guo et al. (2016) who concluded that P deficiency decreases the chlorophyll contents of rice and affect photosynthesis. The photosynthetic phase imbalance due to P deficiency which may cause increase in reactive oxygen species, which alters functions of plants by damaging enzymes, lipids, protein, and photosynthetic pigments. Sujatha and Vijayalakshmi (2017) concluded that foliar application of growth stimulant at flowering and vegetative stages of black gram (*Vigna mungo* L.) increased the total chlorophyll (46.62 %). Plant growth stimulant contains amino acids (aspartate, alanine and glutamate) which might be helpful to increase the chlorophyll contents and ultimately increases the photosynthetic activity (Carillo et al., 2019).

Fresh forage yield is cumulative result plant density, stem diameter, plant height and number of leaves and leaf area. The increase in these parameters in the present study is due to the possible fact that P increased the growth of root which explored more nutrients and moisture from soil. In addition to this, P improves the photosynthesis and several other plant physiological functions which ultimately enhance all yield contributing parameters. Sadiq et al. (2017) described that 120 kg P ha$^{-1}$ gave maximum biological yield (13,006 kg ha$^{-1}$) of maize, whereas control treatment resulted in the lowest biological yield (9241 kg ha$^{-1}$). Increased nutrient availably increased the leaf number, chlorophyll contents, LAI and photosynthetic activity which increased the fresh yield. Dry matter yield (DMY) is a key component and presents the total contents in forage without water. Our findings are alike with the findings of Safdar et al. (2012) who noted rise in DMY with increased dose of phosphorus. Maximum dry matter yield (6.07 tons ha$^{-1}$) of rainfed maize was found at 90 kg P ha$^{-1}$ while the lowest DMY was noted where no P was applied. The rise in DMY may be due to improvement in leaf area (Mouri et al., 2019). Magalhaes et al. (2016) found that growth stimulant significantly increased total dry matter of cassava.

Crude protein (CP) is a rough estimate of protein and important factor of forage quality. Trend in crude protein contents is similar with Roy and Tudu (2003). The probable fact is that P participates in protein synthesis (Mengel et al., 2001). Roy et al. (1997) who found higher crude protein (19.33 %) at 80 kg P ha$^{-1}$. De-Lima et al. (2019) described that growth stimulant application significantly improved crude protein contents in *Urochloa* hybrid (HD-364).

Ash content percentage describes the total amount of minerals present in crop. Increase in ash contents in plants are confirmed by Rashid and Iqbal (2018). Popko et al. (2018) described that growth stimulant significantly improved the grain ash contents of wheat. The NDF and ADF contents are basically the structural components of plant, mainly the cell wall. Forage of good quality generally contains lower NDF and ADF contents. Decrease in NDF and ADF in this study is related to decrease in non-digestible components of cell like lignin and cellulose. Results are confirmed by Pholsen and Suksri (2004), Godlewska and Ciepiela (2016), they reported that Kelpak (growth stimulant application) reduced 6 % NDF and ADF contents as compared to control (no growth stimulant). The reason is that growth stimulant contains various plant growth regulators which may be helpful in improving the quality of crop. The reason to increase in plant phosphorus contents is that phosphorus fertilizer performs a significant part in development and proliferation of roots hence more uptake of nutrient occurred, and bio-stimulant contains amino acid, ascorbic acid, riboflavin and some macro and micronutrients. Nadeem et al. (2014) concluded that in cowpea, application of phosphorus (40 kg ha$^{-1}$) increased the plant phosphors contents (0.14 %). Abdelgawad et al. (2018) who reported that foliar applied bio-stimulant improved the phosphorus contents in leaves of lettuce. The phosphorus percentage was significantly increased due to foliar application of ascopin and super biomin respectively than where no bio stimulant was applied.

## 5. Conclusion

Application of P and growth stimulant is important for achieving high yield and good quality forage sorghum. It is concluded that 60 kg P application per hectare along with foliar application of growth stimulant at 30 DAS increased yield and quality components of sorghum. Therefore, this combination is recommended for improving forage yield and quality of sorghum.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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