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# Effects of Foliar Application of Brassinolide and Humic Acid on Protein and Nutrient Uptake of Black Gram (*Vigna mungo* L.)

**Kiran N More [a], Onkar R. Hirwe [a], Apeksha M Suryawanshi [b], Shital S. Patil [c], Shrikrishna B. Narale [d], Suraj N More [c], Harshraj Salunkhe [c] and Anita Jaswal [a*]**

*[a] Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara 144411, Punjab, India.*
*[b] Department of Animal Husbandry and Dairy science, Mahatma Phule Krishi Vidyapeeth, Rahuri 413722, Maharashtra, India.*
*[c] Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Phagwara 144411, Punjab, India.*
*[d] Department of Biochemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri, 413722, Maharashtra, India.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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**Corresponding author: E-mail: anita.27139@lpu.co.in;*

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## ABSTRACT

The field experiment was conducted during the *Kharif* season of 2022-2023 at the Lovely Professional University, Jalandhar, Punjab. To study the effect of brassinolide and humic acid on growth and yield of black gram. The experiment was laid out in a Randomized Block Design (RBD) having three replication and eight treatments by using MASH-114 variety. The treatments were: $T_0$-Control $T_1$-(100% RDF), $T_2$-RDF + Humic acid 20kg $ha^{-1}$ (Soil applied), $T_3$-RDF + Humic acid 0.1% (Foliar applied), $T_4$-RDF + Brassinolide 0.25ppm (Soil applied), $T_5$-RDF + Brassinolide 0.1ppm (Foliar applied), $T_6$-RDF + Humic acid 20kg $ha^{-1}$ + Brassinolide 0.25ppm (Soil applied), $T_7$-RDF + Humic acid 0.1%+ Brassinolide 0.1ppm (Foliar applied) were used for this study. According to the findings of a field investigation the protein and nutrient uptake black gram were considerably impact due to application of humic acid and brassinolide. The application of humic acid considerably enhanced the content and uptake of NPK as compared to the other treatments. Available NPK, uptake of NPK, content of NPK in plant after harvest of crop were increased significantly due to Humic acid 20kg $ha^{-1}$ +Brassinolide 0.25ppm Soil applied along with RDF and foliar application of Humic acid 0.1% + Brassinolide 0.1ppm respectively along with RDF in black gram. Humic acid and brassinolide significantly improved NPK content, uptake, and overall performance of black gram, with combined soil and foliar applications being most effective. Future studies should optimize doses, test across locations, and assess long-term soil and crop benefits to enhance nutrient efficiency and sustainable pulse production.


*Keywords: Protein; sustainability; brassinolide; humic acid; black gram.*

## 1. INTRODUCTION

One of the most significant pulse crops farmed throughout the nation in rainfed areas is the black gram [*Vigna mungo. L*]. This crop is grown across the country in many different kinds of cropping systems, including mixed crop, catch crop, and sequential crop. In India, the states of Andhra Pradesh, Bihar, Madhya Pradesh, Maharashtra, U.P., West Bengal, Punjab, Haryana, and Karnataka are among the most well-known for growing black gram. For milch cattle specifically, it is utilized as nutritional feed. Black gram, commonly known as urd, is grown in India as a green manuring crop (Asha and Lingakumar, 2015). One of the most significant pulse crops is the black gram. The Indian subcontinent is where the black gram first appeared. About 18,000 species comprise the family Leguminoseae, distinguished by its pods and alternate pinnate or trifoliate leaves. Grain legumes, also referred to as pulse, are members of the "papilionaceous" subfamily because their blossoms have a butterfly-like appearance.

In India, 4.4 million ha of land is used for the cultivation of black gram, which yields 2.8 million tonnes at a productivity of 632 $kg^{-1}$. It covers 3.37 lakh hectares with a 1.83 lakh tonnes total production and a 542 $kg^{-1}$ productivity. India is the greatest producer of pulse crops. Pulses are the most often consumed food item in Indian diets and provide the most practical solution to the issue of protein deficiency. Malnutrition related to protein and calories is a widespread issue in the nation. A severe protein deficiency is currently plaguing our nation's population, especially young children. Especially for vegans, pulses are the main source of protein, and they contribute (Rao et al., 2016). Black gram (*Vigna mungo*), a type of pulse, is one of the most significant crops farmed in India. It is eaten as "dal" (whole or split, husked and unhusked), or it can be parched. It is a key component in papad (Sangeetha & Mohan, 2020). It serves as both a crop for green manuring and nutritious feed, particularly for milch cattle. Each hectare of soil receives an addition of 42 kg of nitrogen. It has a dense root system that ties together soil particles to stop erosion. Black gram has roughly 25% protein, 60% carbohydrates, 10.9 percent moisture, 1.4 % fat, 0.9 % fiber, and 3.2% minerals and vitamins, contains 154 mg of calcium, 385 mg of phosphorus, 9.1 mg of iron, and a little quantity of vitamin B complex (Devaraju and Senthivel 2018).

“Because of their high nutritional value (protein content ranges from 17 to 27%) and flexibility to a wide range of agroecological and management variables, pulses are an important component of food grain crops” (Kumar et al., 2023). “Leguminous crops integrate well in crop rotation and cropping systems because they fix and use atmospheric nitrogen while also enhancing soil fertility. Pulse production is much lower than what is needed. As opposed to the 104 g per capita recommended by the FAO and WHO, India only provides 45 g of pulses per person each day" (Kumari et al., 2023). "Therefore, it presents a significant problem for agricultural experts to supply the nation's rapidly expanding population with pulses" (Jumrani, 2025).

"Brassinolide promotes plant cell elongation and division, which is its most notable physiological function. First, it increases the activity of DNA polymerase and RNA polymerase, promoting the synthesis of nucleic acids and proteins. Researchers have hypothesised that brassinolide induces cell division and elongation, prompts cell wall expansion, increases water absorption, and modifies the direction of microtubule distribution" (Liu et al., 2018). "Additionally, it can boost the movement of photosynthetic products, maintain a high level of soluble protein, and lift the cloud of growth inhibition. by increasing the photosynthetic rate and encouraging plant photosynthesis" (Wilhelm & Selmar, 2011).

"Humins, humic acid, and fulvic acid have received significant attention among the three humic compounds, and researchers have extensively investigated their effects on various crop plants" (Shahrajabian & Sun, 2024). "The portion of humic compounds known as humins is insoluble in both alkali and acid. Humin remains insoluble in water across all pH levels. Organic acids are unable to dissolve in water under acidic conditions; however, they can do so in alkaline environments. Humic acid is a complement fertilizer, not a fertilizer because it does not give plants nutrition directly. The plant's ability to absorb and receive more nutrients increases plant development. Humic acid is particularly useful for preserving soil nutrients so that they are always available to the plant" (Khaled & Fawy, 2011). "Humic acids are intermediates between humas in complexity and remain in the soil for a longer time, making them beneficial to crops" (Maffia et al., 2025). "Due to improved nutrient availability, increased metabolic activity, and quick and timely availability of micro and macronutrients, humic acid is applied topically actively expanding leaves resulting in a bigger expansion of the plant (Bakry et al., 2015). The present study was initiated to evaluate the effects of brassinolide and humic acid on the protein and nutrient uptakes of black gram.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site

The experiment was carried out at the fields of Lovely Professional University, Department of Agronomy, Lovely Professional University, Phagwara (Punjab) during the year 2022-23. This field is situated at $31^0$ 22'31.81 North Latitude and $75^0$ 23'03.02 East longitude, with an average elevation and with a mean sea level of 252 m.

#### 2.1.1 Climate and weather conditions

The test site enjoys a subtropical climate, with hot summer winds prevailing during the day and temperatures rising at night. The hottest months are May, June and July (mercury touches $38^0$C), and the corresponding temperatures in the last week of July. Winter comes in October.

#### 2.1.2 Soil properties

The soil at the experimental site is described as sandy loam mixed with the Hyperthermia family typical of Haplustept. The soil was excavated at a depth of 0-15 cm from different parts of the experimental site and its physical, chemical, and biological properties were examined. The test site contains a high sand content (77%) and is classified as sandy soil. Analysis reveals that the soil is low in organic carbon, nitrogen, and potassium, moderate in phosphorus, and somewhat acidic and alkaline. This shows the basic physicochemical properties of the soil.

### 2.2 Details of Treatments

A combination of Recommended doses of fertilizer (RDF) 20:40:20 (N:P: K kg $ha^{-1}$), Humic acid foliar application at 25 DAS and 15 Days after $1^{st}$ spray, Brassinolide foliar application at 30 DAS and 45 DAS tabulated in Chart 1.

**Chart 1. Treatments details**

| Treatments | Description |
|---|---|
| $T_0$ | Control |
| $T_1$ | (100%RDF) |
| $T_2$ | RDF + Humic acid 20kg $ha^{-1}$ (Soil applied) |
| $T_3$ | RDF + Humic acid 0.1% (Foliar applied) |

| Treatments | Description |
|---|---|
| $T_4$ | RDF + Brassinolide 0.25ppm (Soil applied) |
| $T_5$ | RDF + Brassinolide 0.1ppm (Foliar applied) |
| $T_6$ | RDF + Humic acid 20kg $ha^{-1}$ + Brassinolide 0.25ppm (Soil applied) |
| $T_7$ | RDF + Humic acid 0.1%+ Brassinolide 0.1ppm (Foliar applied) |

*Recommended doses of fertilizer (RDF) - 20:40:20 (N:P: K kg $ha^{-1}$), Humic acid foliar application – 25 DAS and 15 Days after 1st spray, Brassinolide foliar application – 30 DAS and 45 DAS.*

## 2.3 Variety Description

Certified seeds of a Black gram (MASH-114) were procured from Punjab Agriculture University, Ludhiana, located in Punjab. The seeds of the Black gram (MASH-114) are characterized by their small, upright, and densely-packed structure. They attain maturity within approximately 70 to 80 days. Each pod typically contains 6-7 bold, black seeds that possess excellent culinary qualities. Abundant podding can be observed in this variety. On average, a yield of 3.6 quintals of grain per acre can be expected. Notably, Black gram (MASH-114) exhibits resistance to diseases such as Cercospora leaf spot, bacterial leaf spot, and yellow mosaic virus.

## 2.4 Observations Recorded

### 2.4.1 Protein content

Protein analysis of grain was calculated by multiplying N content (%) by 6.25 (based on assumption that N content 16 % of protein).

Protein content (%) $= N\ content\ in\ \%\ X\ 6.25$

Protein yield (kg $ha^{-1}$) = $\frac{Protein\ content\ (\%)\ X\ grain\ yield\ (kg/ha)}{100}$

## 2.5 Chemical Analysis

### 2.5.1 N content in grain

N content in grain was determined by micro-Kjeldahl method as per procedure suggested by AOAC, 1995.

N uptake in grain (kg $ha^{-1}$) = $\frac{N\ content\ in\ grain\ (\%)\ X\ grain\ yield\ (kg/ha)}{100}$

### 2.5.2 P content in grain

P content in grain was determined by Wet digestion (Diacid) Vanadomolybdo phosphoric acid yellow colour method as per procedure outlined by Jackson, 1973.

P uptake in grain (kg $ha^{-1}$) = $\frac{P\ content\ in\ grain\ (\%)\ X\ grain\ yield\ (kg/ha)}{100}$

### 2.5.3 K content in grain

K content in grain was determined by flame photometry method as per the procedure outlined by Jackson, 1973.

K uptake in grain (kg $ha^{-1}$) = $\frac{K\ content\ in\ grain\ (\%)\ X\ grain\ yield\ (kg/ha)}{100}$

## 2.6 Statistical Analysis

Statistical analysis was conducted using SPSS VS-22 16 software to determine the significance of differences between various treatments. A significance level of 95% was used. The mean values with different letters indicate statistical significance at a p-value of less than 0.05.

# 3. RESULTS AND DISCUSSION

## 3.1 Effect of Humic Acid and Brassinolide on Protein content (%) and Protein Yield (kg $ha^{-1}$) of Black Gram

The protein content data presented in Table 1 and Fig. 1 clearly demonstrate that both soil and foliar applications of humic acid and brassinolide had a significant positive impact on the protein content of the crop. $T_7$ - (RDF + Humic acid 0.1%+ Brassinolide 0.1ppm through foliar application, exhibited the highest percentage of protein content at (28.75%). Similarly, the $T_6$ - (RDF + Humic acid 20kg $ha^{-1}$ + Brassinolide 0.25ppm to the soil, showed a protein content of (27.73%). These results indicate that both methods of application effectively increased the protein content in the crop. In comparison, the control - T0 had the lowest protein content percentage at (17.14%.) This treatment did not receive any additional applications of humic acid or brassinolide. The ($T_1$) which involved the application of 100% RDF, showed a protein content of (24.19%). It is worth noting that ($T_7$) and ($T_6$) treatments resulted in significantly higher protein content compared to both the control and ($T_1$) treatments.

The protein yield data presented in Table 1 and Fig. 2 revealed that both soil and foliar applications of humic acid and brassinolide resulted in a significant increase in protein yield. The treatment labeled as $T_7$- (RDF + Humic acid 0.1%+ Brassinolide 0.1ppm (Foliar applied), recorded the highest protein yield of (266.7 kg $ha^{-1}$). followed by $T_6$ - (RDF + Humic acid 20kg $ha^{-1}$ + Brassinolide 0.25ppm (Soil applied) with a protein yield of (244.4 kg $ha^{-1}$). The $T_0$ - (Control) exhibited the lowest protein yield of (111.6 kg $ha^{-1}$), followed by $T_1$ - (100% RDF) with a protein yield of (171.8 kg $ha^{-1}$).

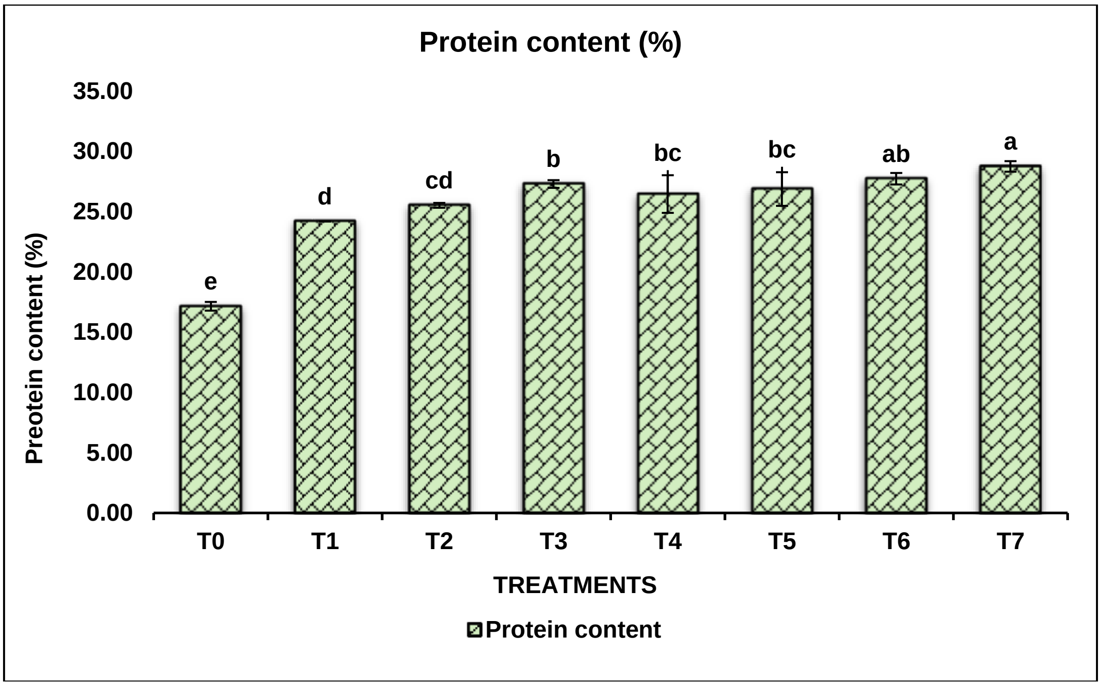


**Fig. 1. Effect of humic acid and brassinolide on the Protein content (%) of black gram**

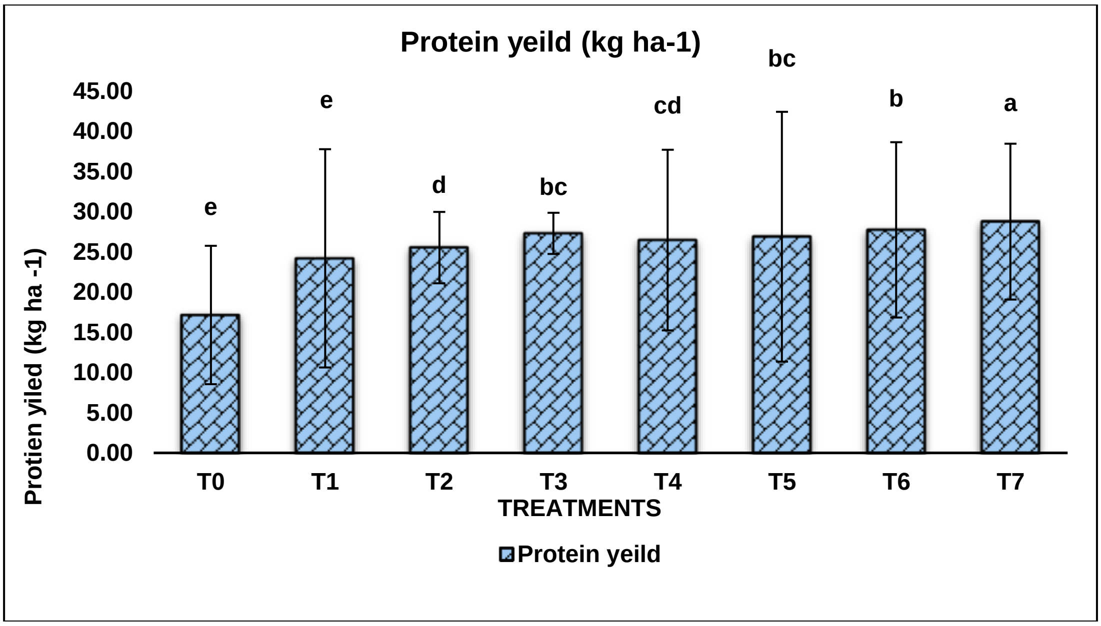


**Fig. 2. Effect of humic acid and brassinolide on the Protein yield (kg $ha^{-1}$) of black gram**

In summary, the application of humic acid and brassinolide, both as soil and foliar treatments, significantly increased the protein content and yield in the black gram plants the treatments ($T_7$) and ($T_6$) showed the highest protein yields, while the control treatment ($T_0$) had the lowest protein content and protein yield. This result showed that an increase in protein yield and protein content can be attributed to the direct and indirect effects of humic acid and brassinolide on plant growth. Humic acid and brassinolide have been found to modify membranes, enhance nutrient transport, improve photosynthesis, and increase the solubilization of micronutrients, which ultimately leads to improved protein synthesis. Saruhan et al., (2011) observed "a higher percentage of crude protein in millet with the application of humic acid treatments". Similarly, Sritharan et al., (2015) reported positive outcomes in black gram with soil application of a combination of 1% KCl and humic acid. Rajesh et al., (2014) demonstrated "improved yield in green gram through soil application of humic acid. Additionally, the foliar spray of vermicompost resulted in increased protein content in kernels. These findings indicate that the utilization of humic acid and brassinolide positively impacts protein yield and content in various crops by influencing nutrient transport, photosynthesis, and micronutrient availability".

Furthermore, Dudde et al., (1979) found that "applying a foliar spray of brassinolide (BR) to groundnut plants significantly increased nitrogen and phosphorus content in the leaves, particularly up to the peg formation stage. Han et al., (1988) also observed a notable increase in phosphorus uptake in tobacco plants with foliar application of BR, brassinolide treatment increased nitrogen absorption in rice". Similarly, Dogra and Thukral (1998) revealed "the positive effect of BR on nitrogen, phosphorus, and potassium absorption in maize. These findings support the idea that Brassinolide improves nutrient absorption, presumably by altering root permeability and cation exchange capacity. Thus, the current study's findings are consistent with prior research findings, demonstrating that BR has a good influence on nutritional content in plants".

## 3.2 Effect of Humic Acid and Brassinolide on N, P, K Content (%) in Grain of Black Gram

The findings in Table 2 and Fig. 3, Fig. 4 and Fig. 5 show that the different treatments used in the study had a considerable beneficial influence on the nitrogen (N), phosphorous (P), and potassium (K) content of the grain. These nutrients are critical for plant growth and development and for producing high-quality crop production.

### 3.2.1 Nitrogen content (%) in grain

The nitrogen content in grain was analyzed to assess the effects of different treatments on nutrient accumulation. Notably, $T_7$- (RDF + Humic acid 0.1%+ Brassinolide 0.1ppm through foliar application), exhibited the highest nitrogen content in the grain, recording an impressive value of (3.63%). This result was found to be statistically similar to the nitrogen content observed in $T_6$ - (RDF + Humic acid 20kg $ha^{-1}$ + Brassinolide 0.25ppm) was applied to the soil, resulting in a nitrogen content of (3.50%) in the grain. Conversely, the $T_0$ - (Control) demonstrated the lowest nitrogen content in the grain, with a value of (2.16%). followed by $T_1$, where 100% RDF was applied, resulting in a nitrogen content of (3.10%) in the grain. The observed trend indicates that the treatments involving the application of RDF, humic acid, and brassinolide (T7 and T6) positively influenced nitrogen accumulation in the grain (Table 2, Fig. 3).

These findings highlight the potential of foliar and soil applications of these substances in enhancing nitrogen content, and subsequently, the nutritional value of the grain. The application of humic acid and foliar spray of brassinolide (BR) and have been found to increase protein content in black gram seeds. Rajesh et al., (2014) and Chaitra (2018) observed that soil application of humic acid combined with foliar spray, resulted in higher protein content. The uptake of nutrients was enhanced by the stimulatory effect of humic substances on microbial activity in the soil. The increased protein content may be attributed to enhanced mineral uptake and enzyme activity, as indicated by Poole et al. (1983). These findings emphasize the importance of nutrient management and growth regulator application in maximizing protein yield in crops. The administration of humic acid in conjunction with RDF, which improved nutritional content and absorption was mostly responsible for the increase in NPK uptake. The rate of vegetative growth, physiological activity and metabolic activity was all higher, use of humic compounds enhanced the development of lateral root along with increase in the number of secondary roots and better availability of nutrients. Because humic acid is an excellent source, improved crop nutrient absorption may be due to the fact that humic compounds encourage microbial activity in the soil and boost nutrient uptake. It is a supplier of nitrogen, phosphorus, potassium and sulphur. Additionally, the supply of natural and supplemented nutrients was enhanced up to the harvest.

### 3.2.2 Phosphorus

The phosphorus content of grain was assessed in the study to determine the effect of different treatments on phosphorus absorption and buildup. The highest phosphorus content of (0.700%) was observed in $T_7$- (RDF + Humic acid 0.1%+ Brassinolide 0.1ppm through foliar application. This treatment resulted in the maximum phosphorus accumulation in the grain. Similarly, $T_6$ - (RDF + Humic acid 20kg $ha^{-1}$ + Brassinolide 0.25ppm to the soil, exhibited a phosphorus content of (0.700%) in the grain. This value was statistically similar to the phosphorus content recorded in $T_3$ - RDF + Humic acid 0.1% were applied through foliar application (Table 2, Fig. 4). These findings indicate that both foliar and soil applications of these treatments contributed to enhanced phosphorus uptake and subsequent accumulation in the grain. Conversely, the $T_0$ - control displayed the lowest phosphorus content in the grain, measuring (0.366%). This was followed by $T_1$, which received 100% RDF application, resulting in a phosphorus content of (0.500%) in the grain. Based on the observed results, the trend of phosphorus content in the grain followed the order: $T_7 > T_6 > T_3 > T_2 > T_5 > T_4 > T_1 > T_0$. This suggests that the application of RDF, combined with humic acid and brassinolide through foliar or soil application, positively influenced phosphorus uptake and accumulation in the grain.

### 3.2.3 Potassium

The maximum potassium content in grain (2.67%) was observed under $T_7$- (RDF + Humic acid 0.1%+ Brassinolide 0.1ppm (Foliar applied) which was statistically similar to $T_6$ - (RDF + Humic acid 20kg $ha^{-1}$ + Brassinolide 0.25ppm (Soil applied) having potassium content is (2.65%). The minimum potassium content (1.52%) was observed under $T_0$ - (Control) which was followed by $T_1$ - (100% RDF) (1.80%). The trend follows as $T_7>T_6>T_3>T_5>T_2>T_4>T_1>T_0$ (Table 2, Fig. 5). The application of humic acid and brassinolide, which enhance the formation of proteins and organic nitrogen molecules in plants, may be responsible for the rise in N, P, and K content. The combination of humic acid and Brassinolide with (RDF) enhances nutrient absorption, resulting in increased nitrogen, phosphorus, and potassium (NPK) uptake. This results in enhanced vegetative growth, physiological activity, and metabolic processes. Humic compounds also stimulate the

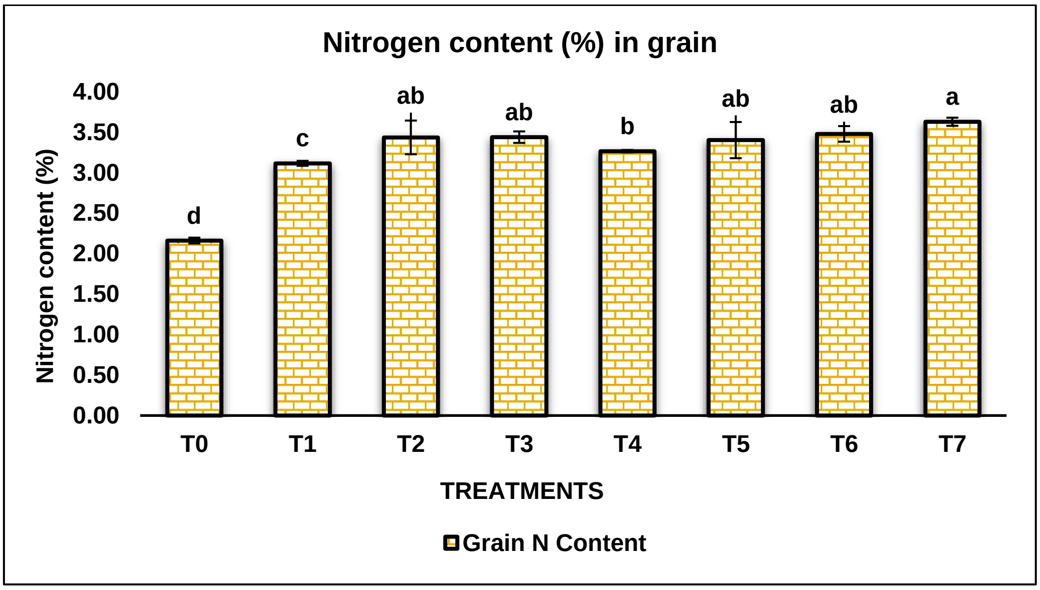


**Fig. 3. Effect of humic acid and brassinolide on the Nitrogen content (%) of black gram**

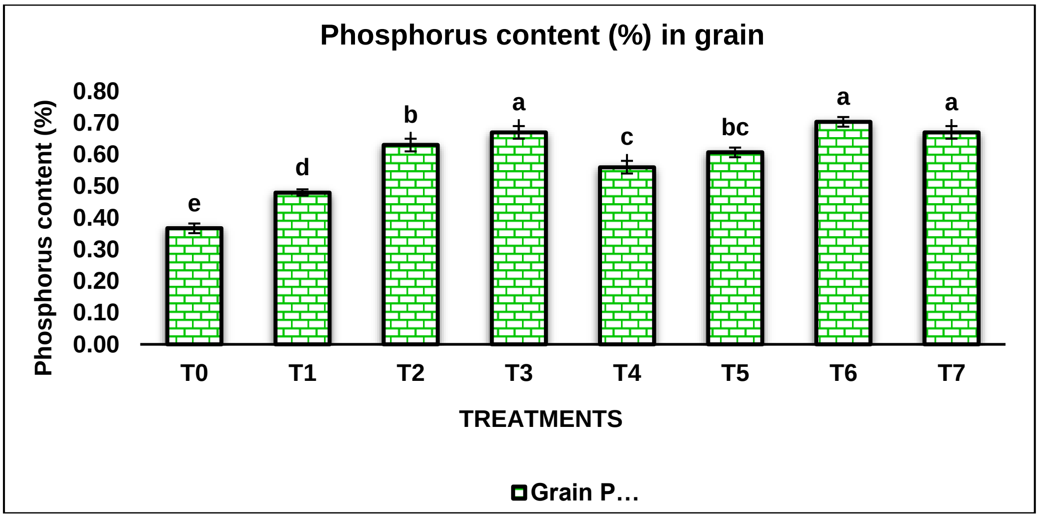


**Fig. 4. Effect of humic acid and brassinolide on the Phosphorus content (%) of black gram**

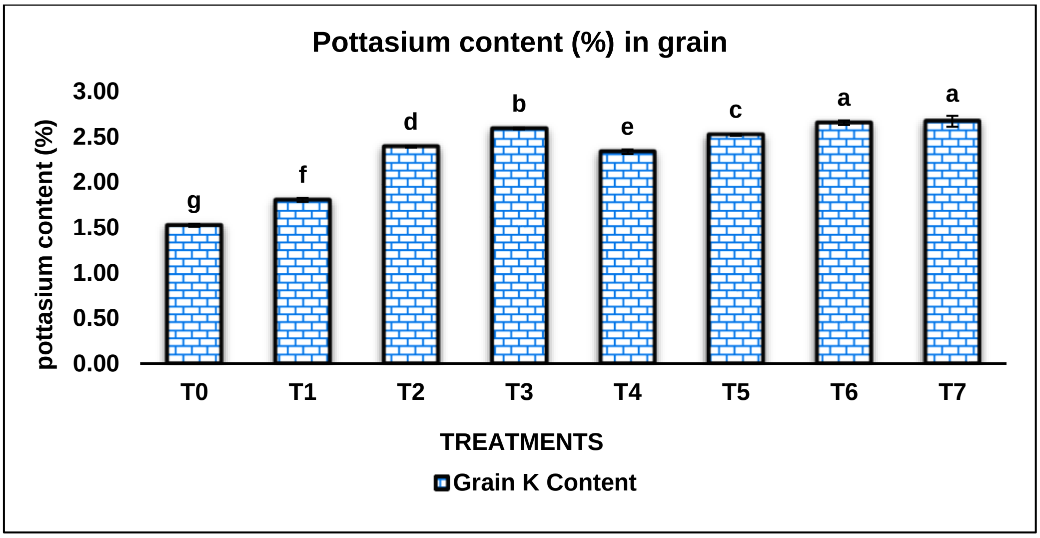


**Fig. 5. Effect of humic acid and brassinolide on the Potassium content (%) of black gram**

development of lateral roots and increase the number of secondary roots, improving nutrient availability for the plant. Moreover, humic acid enhances nutrient absorption by promoting soil microbiological activity. It serves as a supply of nitrogen, phosphorus, potassium, and sulphur, providing natural and supplemented nutrients to the crop. Overall, the use of humic acid improves nutrient supply and leads to increased nutrient uptake, ultimately enhancing crop growth and development.

The increase in protein content of black gram seeds attribute to the application of humic acid from farmyard manure (FYM), which contains nitrogen and sulfur. Nitrogen is necessary for protein synthesis because it is involved in the formation of amino acids and plant hormones, both of which are building blocks for proteins. Studies conducted by Rajesh et al*.,* (2014) in green gram and Chaitra (2018) in groundnut crops have shown that soil application of humic acid from vermicompost, combined with a foliar spray at a rate of 20 kg ha$^{-1}$ with a 0.2% concentration, resulted in increased protein content in kernels. The improved uptake of nutrients by the crop can be attributed to the stimulatory effect of humic substances on microbial activity in the soil. Humic acid acts as a good source of nitrogen, phosphorus, potassium, and sulphur, thereby increasing the availability of these nutrients to the crop. The increase in nitrogen content in response to the humic acid application may be due to its stimulatory effect on microorganisms capable of enhancing urease enzyme activity, leading to increased nitrogen availability in the soil and subsequent uptake by the crop. The foliar application of brassinolide (BR) and salicylic acid (SA) in mature green gram seeds resulted in higher amounts of sugar and starch. This can be attributed to enhanced carbon dioxide fixation, increased activity of carbohydrate-synthesizing enzymes, and efficient allocation of dry matter to reproductive sinks. These findings are consistent with previous studies by Sivakumar et al*.,* (2002) on pearl millet, which also showed positive effects of foliar application of plant growth regulatorsThe higher protein content in green gram seeds may be attributed to the increased uptake and mobilization of minerals for enzyme activity involved in the biosynthesis of major amino acids, which are crucial for protein synthesis. This supports the notion that enhancing protein content in seeds is strongly influenced by mineral uptake and enzyme activity, as noted by Poole et al*.,* (1983) and Nasiroleslami et al. (2021).

## 3.3 Effect of Humic Acid and Brassinolide on Grain N, P, K Uptake of Black Gram

The data presented in Table 3 and Fig. 6, Fig. 7 and Fig. 8 provides clear evidence that the various treatments employed in the study had a significant positive effect on the content of nitrogen (N), phosphorus (P), and potassium (K) uptake by grain.

### 3.3.1 Nitrogen uptake by grain

The nitrogen uptake by grain was evaluated across different treatments to assess their impact on crop growth and nutrient uptake. Among the treatments, $T_7$ - (RDF + Humic acid 0.1%+ Brassinolide 0.1ppm through the foliar application) exhibited the highest nitrogen uptake in the grain, recording an impressive value of (33.46%). This result was found to be comparable to the nitrogen uptake observed in $T_6$ - (RDF + Humic acid 20kg ha$^{-1}$ + Brassinolide 0.25 ppm through soil application), which demonstrated a nitrogen uptake of (30.26%) in the grain (Table 3, Fig. 6). Conversely, the control treatment (T0) showed the lowest nitrogen uptake in the grain, with a value of (14.63%). This was followed by $T_1$, where 100% RDF was applied, resulting in a nitrogen uptake of (22.73%) in the grain. These findings underscore the importance of incorporating specific treatments, such as the application of RDF, humic acid, and brassinolide, to enhance nitrogen uptake in grain crops.

### 3.3.2 Phosphorus uptake by grain

The phosphorus uptake by grain varied among different treatments. The highest phosphorus uptake (7.06%) was observed in $T_7$, where RDF, humic acid (0.1%), and brassinolide (0.1 ppm) were applied through foliar application. It was followed by $T_6$, which received RDF, humic acid (20kg ha$^{-1}$), and brassinolide (0.25 ppm) applied to the soil, resulting in a phosphorus uptake of (6.54%) in the grain. In contrast, the lowest phosphorus uptake (2.39%) was recorded in the $T_0$ - control, where no additional treatments were applied. $T_1$ - 100% RDF, exhibited a phosphorus uptake of (3.90%) in the grain. The trend of phosphorus uptake by grain follows the order: $T_7 > T_6 > T_3 > T_2 > T_5 > T_4 > T_1 > T_0$ (Table 3, Fig. 7). These results indicate that the application of RDF, humic acid, and brassinolide, especially through the foliar application ($T_7$) and soil application ($T_6$), positively influenced phosphorus accumulation in the grain.

### 3.3.3 Potassium uptake by grain

The potassium uptake by grain varied across different treatments. The highest potassium uptake (26.5%) was observed in $T_7$- RDF + Humic acid 0.1%+ Brassinolide 0.1ppm were applied through foliar application. It was closely followed by $T_6$ - (RDF + Humic acid 20kg ha$^{-1}$ + Brassinolide 0.25ppm applied to the soil, resulting in a potassium uptake of (25.1%) in the grain. In contrast, the lowest potassium uptake (9.0%) was recorded in the control treatment ($T_0$), where no additional treatments were applied. The $T_1$ - 100% RDF, exhibited a potassium uptake of (13.0%) in the grain. The trend of potassium uptake by grain follows the order: $T_7 > T_6 > T_3 > T_2 > T_5 > T_4 > T_1 > T_0$ (Table 3, Fig. 8). These findings indicate the beneficial effects of RDF, humic acid, and brassinolide treatment on increasing potassium absorption in grain. It appears that foliar applications of RDF, humic acid, and brassinolide ($T_7$), as well as soil applications of RDF, humic acid, and brassinolide ($T_6$), were very successful in boosting potassium accumulation in grain. Proper nutrient management practices, as demonstrated by these treatments, can contribute to improved crop quality and productivity.

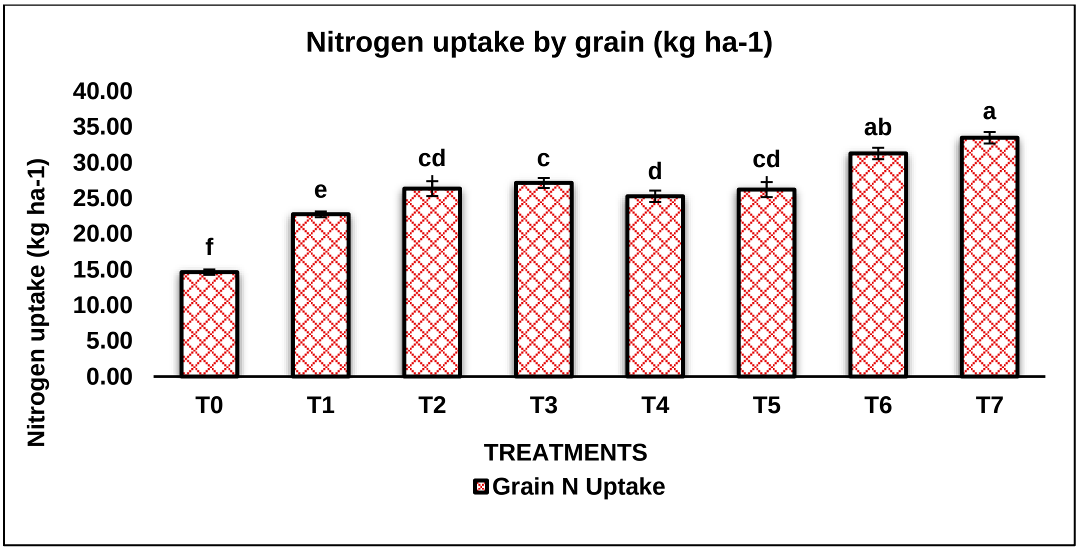


**Fig. 6. Effect of humic acid and brassinolide on the Nitrogen uptake (kg ha$^{-1}$) by black gram**

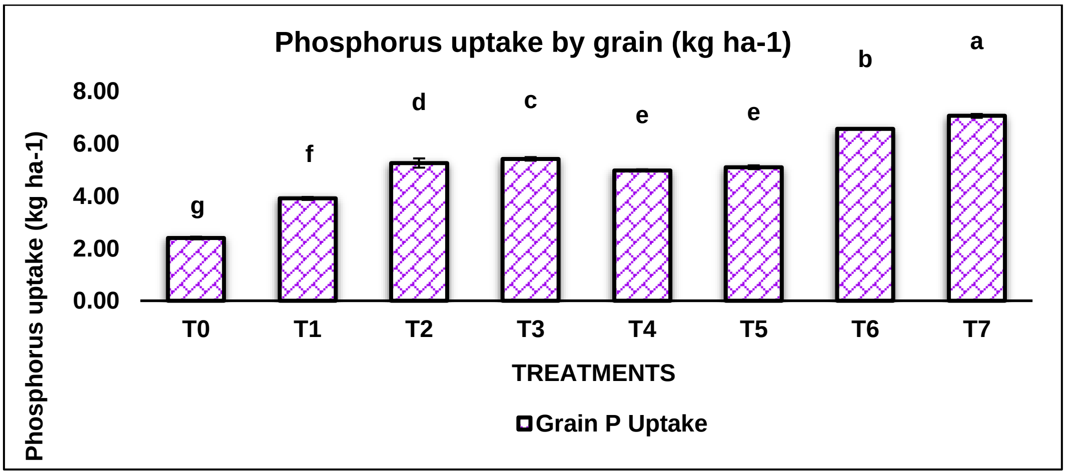


**Fig. 7. Effect of humic acid and brassinolide on the Phosphorus uptake (kg ha$^{-1}$) by black gram**

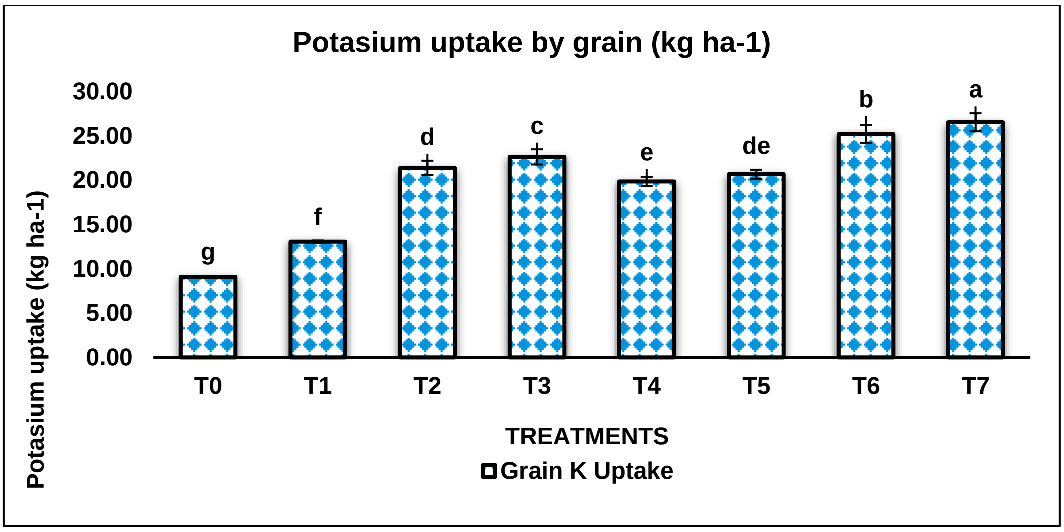


**Fig. 8. Effect of humic acid and brassinolide on the Potassium uptake (kg ha$^{-1}$) by black gram**

**Table 1. Effect of humic acid and brassinolide on the Protein content (%) and Protein yield (kg $ha^{-1}$) of black gram**

| Treatments | Protein content (%) | Protein yield (kg $ha^{-1}$) |
|---|---|---|
| $T_0$-Control | 17.14 $^{e}$ ± 0.35 | 111.6 $^{f}$ ± 8.60 |
| $T_1$-[100%RDF] | 24.19 $^{d}$ ± 0.00 | 171.8 $^{e}$ ± 13.56 |
| $T_2$-RDF + Humic acid 20kg $ha^{-1}$ (Soil applied) | 25.52 $^{cd}$ ± 0.19 | 207.4 $^{d}$ ± 4.43 |
| $T_3$-RDF + Humic acid 0.1% (Foliar applied) | 27.29 $^{b}$ ± 0.31 | 230.3 $^{bc}$ ± 2.56 |
| $T_4$-RDF + Brassinolide 0.25ppm (Soil applied) | 26.45 $^{bc}$ ± 1.56 | 213.6 $^{cd}$ ± 11.22 |
| $T_5$-RDF + Brassinolide 0.1ppm (Foliar applied) | 26.87 $^{bc}$ ± 1.39 | 231.2 $^{bc}$ ± 15.52 |
| $T_6$-RDF + Humic acid 20kg $ha^{-1}$ + Brassinolide 0.25ppm (Soil applied) | 27.73 $^{ab}$ ± 0.47 | 244.4 $^{b}$ ± 10.9 |
| $T_7$-RDF + Humic acid 0.1% + Brassinolide 0.1ppm (Foliar applied) | 28.75 $^{a}$ ± 0.44 | 266.7 $^{a}$ ± 9.6 |

**Table 2. Effect of humic acid and brassinolide on the Grain N, P, K (%) content of black gram**

| Treatments | N (%) | P (%) | K (%) |
|---|---|---|---|
| $T_0$-Control | 2.16 $^{d}$ ± 0.03 | 0.366 $^{e}$ ± 0.1 | 1.52 $^{g}$ ± 0.01 |
| $T_1$-[100%RDF] | 3.10 $^{c}$ ± 0.10 | 0.500 $^{d}$ ± 0.2 | 1.80 $^{f}$ ± 0.2 |
| $T_2$-RDF + Humic acid 20kg $ha^{-1}$ (Soil applied) | 3.43 $^{ab}$ ± 0.20 | 0.633 $^{b}$ ± 0.0 | 2.39 $^{d}$ ± 0.01 |
| $T_3$-RDF + Humic acid 0.1% (Foliar applied) | 3.46 $^{ab}$ ± 0.05 | 0.700 $^{a}$ ± 0.2 | 2.59 $^{b}$ ± 0.01 |
| $T_4$-RDF + Brassinolide 0.25ppm (Soil applied) | 3.30 $^{b}$ ± 0.10 | 0.566 $^{c}$ ± 0.1 | 2.33 $^{e}$ ± 0.02 |
| $T_5$-RDF + Brassinolide 0.1ppm (Foliar applied) | 3.43 $^{ab}$ ± 0.20 | 0.600 $^{bc}$ ± 0.1 | 2.52` $^{c}$ ± 0.01 |
| $T_6$-RDF + Humic acid 20kg $ha^{-1}$ + Brassinolide 0.25ppm (Soil applied) | 3.50 $^{ab}$ ± 0.10 | 0.700 $^{a}$ ± 0.2 | 2.56 $^{a}$ ± 0.02 |
| $T_7$-RDF + Humic acid 0.1% + Brassinolide 0.1ppm (Foliar applied) | 3.63 $^{a}$ ± 0.05 | 0.700 $^{a}$ ± 0.1 | 2.67 $^{a}$ ± 0.06 |

**Table 3. Effect of humic acid and brassinolide on Grain N, P, K uptake (kg $ha^{-1}$) of black gram**

| Treatments | N (kg $ha^{-1}$) | P (kg $ha^{-1}$) | K (kg $ha^{-1}$) |
|---|---|---|---|
| $T_0$-Control | 14.63 $^{f}$ ± 0.37 | 2.39 $^{g}$ ± 0.05 | 9.0 $^{g}$ ± 0.10 |
| $T_1$-[100%RDF] | 22.73 $^{e}$ ± 0.40 | 3.90 $^{f}$ ± 0.05 | 13.0 $^{f}$ ± 0.11 |
| $T_2$-RDF + Humic acid 20kg $ha^{-1}$ (Soil applied) | 26.33 $^{cd}$ ± 1.05 | 5.26 $^{d}$ ± 0.17 | 21.3 $^{d}$ ± 0.8 |
| $T_3$-RDF + Humic acid 0.1% (Foliar applied) | 27.13 $^{c}$ ± 0.70 | 5.42 $^{c}$ ± 0.06 | 22.6 $^{c}$ ± 0.8 |
| $T_4$-RDF + Brassinolide 0.25ppm (Soil applied) | 25.26 $^{d}$ ± 0.80 | 4.98 $^{e}$ ± 0.04 | 19.8 $^{e}$ ± 0.5 |
| $T_5$-RDF + Brassinolide 0.1ppm (Foliar applied) | 26.20 $^{cd}$ ± 1.05 | 5.10 $^{e}$ ± 0.07 | 20.6 $^{de}$ ± 0.5 |
| $T_6$-RDF + Humic acid 20kg $ha^{-1}$ + Brassinolide 0.25ppm (Soil applied) | 31.26 $^{ab}$ ± 0.80 | 6.54 $^{b}$ ± 0.02 | 25.1 $^{b}$ ± 1.0 |
| $T_7$-RDF + Humic acid 0.1% + Brassinolide 0.1ppm (Foliar applied) | 33.46 $^{a}$ ± 0.80 | 7.06 $^{a}$ ± 0.07 | 26.5 $^{a}$ ± 1.0 |

The findings show that applying humic acid and brassinolide as soil amendments and foliar sprays has a significant influence on nitrogen (N), phosphorus (P), and potassium (K) absorption in grain crops. This is attributed to the ability of these substances to enhance the absorption, translocation, and assimilation of various plant nutrients, resulting in a balanced and simultaneous utilization of these nutrients within the plants. Consequently, there is an increase in the production of dry matter, as well as improved availability of essential elements crucial for plant growth.

Humic acid plays a critical role in mobilizing phosphorus by forming complexes with organic matter in the soil, as indicated by Balasubramanian et al*.,* (1989). Furthermore, the balanced and simultaneous usage of different nutrient sources emerged as a key factor for the proper absorption, translocation, and assimilation of plant nutrients. According to Kadam et al*.,* (2010) and Wulandari et al. (2019) the combination treatment of organic nitrogen and fulvic acid substantially altered nitrogen, phosphorus, and potassium absorption in soybeans when compared to other treatments.

## 4. CONCLUSION

The application of humic acid and the use of plant growth regulators such as BR and SA have shown positive effects on protein content in various crops. Humic acid improves nutrient availability and uptake, while BR and SA enhance carbohydrate fixation, enzyme activity, and nutrient translocation, ultimately leading to increased protein synthesis in seeds. These findings align with previous research and highlight the importance of nutrient management and growth regulator application for optimizing protein yield in crops. The outcomes of this study highlight the relevance of using humic acid and brassinolide as part of nutrient management techniques to optimise nitrogen, phosphorous, and potassium absorption in grain crops. Humic acid acts as a facilitator for phosphorus mobilization and stimulates nutrient absorption, while the balanced utilization of multiple nutrient sources plays a vital role in promoting efficient absorption and utilization of plant nutrients. These results underscore the significance of incorporating humic acid and brassinolide in agricultural practices to maximize nutrient uptake and ultimately enhance crop productivity.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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