



# Influence of Humic Acid on Yield and Physiological Attributes of Greengram (*Vigna radiata* L.)

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## Authors' contributions

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

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## ABSTRACT

A field experiment was conducted to study the influence of humic acid on physiological attributes of greengram (*Vigna radiata* L.) in loamy sand at Agronomy Instructional Farm, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Gujarat during *kharif* of 2023. Nine treatment combinations comprising three levels each of soil application of humic acid (0, 3 and 6 kg/ha) and foliar application of humic acid (0, 250 and 500 ppm) were evaluated in randomized block design with factorial concept and replicated four times. Soil application of 6 kg humic acid ha<sup>-1</sup> at the time of sowing and foliar application of 250 ppm humic acid at 30 and 45 days

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after sowing (DAS) besides recommended dose of fertilizer viz., 20:40:00 N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O kg ha<sup>-1</sup> led to notably higher protein content in seeds, increased protein yield, and enhanced nitrogen and phosphorus content, and uptake in both seeds and stover. This treatment also improved grain and straw yields. The lowest protein content, protein yield, nutrient content and uptake in seed and stover at harvest, as well as grain and straw yields, were observed in plots that did not receive soil or foliar application of humic acid.

**Keywords:** Humic acid; greengram; foliar application; nutrient content; uptake; yield.

## 1. INTRODUCTION

Greengram (*Vigna radiata* L.), often referred to as mung bean, is a versatile legume known for its rich protein content and ability to thrive in various agroclimatic conditions. Greengram contains about triple fold protein as compared to cereals. This versatile legume boasts a unique ability to fix atmospheric nitrogen (30-40 kg N ha<sup>-1</sup>), enriching the soil and reducing reliance on synthetic fertilizers (Patel *et al.*, 2021). Greengram's short growth duration makes it ideal for intensive crop rotations, optimizing land use. Being a close growing legume cover crop; it also helps in reducing soil erosion and thereby indirectly improving the soil fertility.

After green revolution, exponential increase in the usage of chemical fertilizers happened. Even though it was necessary to feed the growing population of India, the consistent use of these over a long duration without giving much attention to organic manures and fertilizers have rendered the soil less productive. Organic matter has beneficial effects on soil structure, quality, microbial biome and has the potential to sequester carbon. Also, through adsorption and complex forming nature, it reduces the leaching of nutrients and keep the soil fertile and productive. It is crucial at this moment to re-evaluate fertilization methods, emphasizing the use of organic fertilizers and amendments. The low analysis nature of organic manures, bulkiness and handling difficulties are some of the reasons they are less preferred by many. Humic substances, however, offer a promising alternative, addressing these challenges while delivering similar benefits.

Humic acid, often referred to as the 'dark gold of agriculture' is a key component of soil organic matter. It is alkali soluble and has a molecular weight ranging from 10,000 to 100,000 Daltons (Da). Humic acid has a carbon content of 51-57%, nitrogen content of 4-6%, phosphorus content of 0.2-1% and minute quantities of other micronutrients (Haworth, 1971). Other than

improving the soil structure and quality, humic acid also found to exert its influence on plant growth, yield and quality through various mechanisms. Therefore, an experiment was carried out to analyse the influence soil and foliar applications of humic acid have on physiological parameters of greengram.

## 2. MATERIALS AND METHODS

The field experiment was conducted at Agronomy Instructional Farm, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Banaskantha, Gujarat during *khariif* season of the year 2023. The farm is situated at a latitude of 24° 19' North and a longitude of 72° 19' East, at an elevation of 154.52 metres above mean sea level. The soil at the experimental site was loamy sand, with a pH of 7.58 and an electrical conductivity of 0.120 dS m<sup>-1</sup>. Initial status of organic carbon, available N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O were 0.27%, 158.2 kg ha<sup>-1</sup>, 45.8 kg ha<sup>-1</sup>, and 250.4 kg ha<sup>-1</sup>, respectively.

The experiment was demonstrated in Factorial RBD using nine treatment combinations including three levels each of soil application (0, 3 and 6 kg ha<sup>-1</sup>) and foliar application of humic acid (0, 250 and 500 ppm), replicated four times. Greengram variety GM 4 was sown at a recommended seed rate of 17.5 kg ha<sup>-1</sup> with a spacing of 45 x 10 cm. The recommended fertilizer dose of 20:40:00 kg ha<sup>-1</sup> was applied uniformly to all treatments using urea and DAP. The fertilizers were placed in furrows just before sowing and lightly covered with soil. Humic acid granules (98% humic acid) were applied to the respective plots at the time of sowing, whereas the liquid humic acid, containing 18% humic acid, was diluted with required amount of water to achieve 250 and 500 ppm solutions. Humic acid solutions were freshly prepared and sprayed at 30 and 45 days after sowing (DAS). Harvesting was done at physiological maturity. The produce from net plots was sun dried, and seed and stover yields were recorded for each plot. Seed and stover samples collected from the respective

net plots were then oven-dried at 60 °C for 24 hours and subsequently ground using a mechanical grinder for further analysis. For nitrogen and phosphorus estimation, the powdered plant samples (seed and stover) were digested and their nitrogen and phosphorus content were determined by using the Micro-Kjeldahl's digestion method and Vanadomolybdo phosphoric acid yellow colour method, respectively (Jackson, 1973). The protein content in seeds was determined by taking the nitrogen percentage and multiplying it by 6.25, in accordance with the method established by Gupta *et al.* (1972). To calculate the protein yield, the following formula was used:

$$\text{Protein yield } \left( \frac{\text{kg}}{\text{ha}} \right) = \frac{\text{Protein content in seed (\%)} \times \text{Seed yield } \left( \frac{\text{kg}}{\text{ha}} \right)}{100} \quad (1)$$

Seed, stover and total plant uptake of nitrogen and phosphorus were calculated using the below given formula:

$$\text{Nutrient uptake } \left( \frac{\text{kg}}{\text{ha}} \right) = \frac{\text{Nutrient content (\%)} \times \text{Yield } \left( \frac{\text{kg}}{\text{ha}} \right)}{100} \quad (2)$$

Statistical analysis of data was done as per the methodology suggested by Cochran and Cox (1957).

### 3. RESULTS AND DISCUSSION

#### 3.1 Yield

A marked improvement in both seed yield (800 kg ha<sup>-1</sup>) and stover yield (1457 kg ha<sup>-1</sup>) was observed with the soil application of 6 kg ha<sup>-1</sup> humic acid, which was significantly superior to the other treatments (Table 1). These results are in close conformity with the findings of Tripura *et al.* (2017) and Nalia and Sengupta (2019).

Furthermore, the foliar application humic acid at 250 ppm at 30 and 45 DAS, significantly enhanced seed yield (751 kg ha<sup>-1</sup>) and stover yield (1314 kg ha<sup>-1</sup>) compared to the rest of the treatments. The results were comparable to those obtained with 500 ppm dosage. These findings are consistent with the observations of earlier studies by Deotale *et al.* (2014) and Lingaraju *et al.* (2016).

Izquierdo *et al.* (2023) reported a significant improvement in soybean yield with the use of humic biostimulants in on-farm trials conducted at 53 localities in Uruguay over a period of eight

years. A similar study on rice conducted by Izquierdo *et al.* (2024) documented a significant effect on rice yield over the same period with foliar application of humic biostimulants.

The increased grain yield may be attributed to humic acid's positive influence on soil properties and improved photosynthate translocation. Additionally, enhanced growth parameters and nutrient uptake likely contributed to greater biomass production and stover yield.

#### 3.2 Quality Parameters

Data presented in Table 2 shows that the protein content and protein yield in seeds of greengram were significantly influenced due to soil and foliar application of humic acid. Soil application of humic acid @ 6 kg ha<sup>-1</sup> resulted in significantly higher protein content (22.65%) and protein yield (180.56 kg ha<sup>-1</sup>) compared to the other treatments. The lowest protein content and protein yield were observed in control plots. These results are in close agreement with those of Thenmozhi *et al.* (2004) and Sritharan *et al.* (2015).

Foliar application of 250 ppm humic acid at 30 and 45 DAS produced significantly higher protein content (21.62%) and protein yield (162.66 kg ha<sup>-1</sup>) compared to control. The treatment remained statistically at par with foliar spray of humic acid @ 500 ppm at 30 and 45 DAS. The lowest protein content and protein yield were recorded with water spray. Similar results were observed by Deotale *et al.* (2014), with the application of 250 ppm humic acid through vermicompost wash in greengram.

The protein levels in seeds primarily reflect their nitrogen content. The hormonal effects of humic acid may enhance respiratory enzyme activity, improve cell permeability, and boost nutrient absorption, which could lead to greater nitrogen uptake and subsequently higher protein levels and yields (Chen & Aviad, 1990; Nardi *et al.*, 2002; Trevisan *et al.*, 2010).

The interaction effect due to soil and foliar application of humic acid on quality parameters of greengram was found non-significant, as per the data provided in Table 2.

#### 3.3 Nutrient Content

Data pertaining to the effect of soil and foliar application of humic acid on nitrogen and

**Table 1. Seed yield and stover yield of greengram as influenced by soil and foliar application of humic acid**

Treatments	Yield (kg ha <sup>-1</sup> )	
	Seed	Stover
<b>[A] Soil application of humic acid (S)</b>		
S <sub>1</sub> : 0 kg ha <sup>-1</sup>	680	1161
S <sub>2</sub> : 3 kg ha <sup>-1</sup>	744	1306
S <sub>3</sub> : 6 kg ha <sup>-1</sup>	800	1457
S.Em. (±)	19	34
C.D. at 5%	55	100
<b>[B] Foliar application of humic acid (F)</b>		
F <sub>1</sub> : Water spray	703	1240
F <sub>2</sub> : 250 ppm	751	1314
F <sub>3</sub> : 500 ppm	770	1371
S.Em. (±)	19	34
C.D. at 5%	55	100
<b>Interaction (S×F)</b>	NS	NS
C.V.%	8.74	9.05

**Table 2. Effect of soil and foliar application of humic acid on protein content and protein yield of greengram**

Treatments	Protein content (%)	Protein yield (kg ha <sup>-1</sup> )
<b>[A] Soil application of humic acid (S)</b>		
S <sub>1</sub> : 0 kg ha <sup>-1</sup>	20.24	137.50
S <sub>2</sub> : 3 kg ha <sup>-1</sup>	21.47	160.25
S <sub>3</sub> : 6 kg ha <sup>-1</sup>	22.65	180.56
S.Em. (±)	0.29	4.49
C.D. at 5%	0.84	13.10
<b>[B] Foliar application of humic acid (F)</b>		
F <sub>1</sub> : Water spray	20.86	147.17
F <sub>2</sub> : 250 ppm	21.62	162.66
F <sub>3</sub> : 500 ppm	21.88	168.49
S.Em. (±)	0.29	4.49
C.D. at 5%	0.84	13.10
<b>Interaction (S×F)</b>	NS	NS
C.V.%	4.66	9.75

phosphorus content (%) of greengram are presented in Table 3. Interaction effect of soil and foliar applications of humic acid with respect to nitrogen content in seed and stover of greengram were found non-significant. Significantly higher nitrogen content in seed and stover (3.62% and 0.806%, respectively) was recorded with the soil application of 6.0 kg humic acid ha<sup>-1</sup> over rest of the treatments. This treatment also resulted in significantly higher phosphorus content in seed and stover (0.471% and 0.169%, respectively). Conversely, the lowest nitrogen and phosphorus content in both seed and stover at harvest was observed in the control plots. A substantial improvement in nutrient content following the soil application of

humic acid was reported by Hyder *et al.* (2014) and Khan *et al.* (2018).

Foliar application of humic acid exerted a significant influence on the nutrient content of greengram seed and stover. Spraying 250 ppm humic acid at 30 and 45 DAS showed significantly higher nitrogen and phosphorus content in the seed (3.46% and 0.457%, respectively) and stover of greengram (0.769% and 0.163%, respectively), compared to the control. However, the results were statistically at par with 500 ppm humic acid. These findings are in line with those reported by Ananthi and Vanangamudi (2014), Deotale *et al.* (2014) and Meena *et al.* (2022).

Humic acid stimulates the root growth, enhances soil enzyme activity, and promotes the secretion of root exudates, leading to a surge in microbial population in the rhizosphere (Chaitra & Math, 2018). This increased microbial activity, improves the mineralization and solubilization of organic matter, thereby enhancing nutrient availability for plant uptake. Furthermore, the chelating nature of humic acid significantly boosts the uptake of essential nutrients (Mayhew, 2004). This improved nutrient uptake results in increased dry matter accumulation and nutrient content in the plant.

### 3.4 Nutrient Uptake

An analysis of data provided in Table 4 showed significant variations in nutrient uptake across different levels of humic acid. However, interaction between soil and foliar applications of humic acid with respect to nutrient uptake was found to be non-significant. Notably, the application of 6.0 kg of humic acid per hectare resulted in a marked increase in nitrogen and phosphorus uptake by greengram seeds (28.98 and 3.77 kg ha<sup>-1</sup>, respectively) and stover (11.72 and 2.46 kg ha<sup>-1</sup>, respectively). This treatment also led to the highest total uptake of both nutrients. The lowest nutrient uptake in both seeds and stover was observed in the control. Similar findings regarding enhanced phosphorus uptake in wheat following the soil application of humic acid were reported by Tahir *et al.* (2011). Likewise, Sivakumar *et al.* (2007) and Tripura *et al.* (2017) observed an increase in nitrogen uptake with the soil application of humic acid.

Significantly higher nitrogen and phosphorus uptake by the seed (26.02 and 3.43 kg ha<sup>-1</sup>, respectively) and stover (10.05 and 2.14 kg ha<sup>-1</sup>, respectively) was recorded with foliar application of 250 ppm humic acid at 30 and 45 DAS. Subsequently, total nitrogen and phosphorus uptake (36.07 and 5.57 kg ha<sup>-1</sup>, respectively) was also higher under this treatment. These observations were significantly greater than the control, but comparable to the foliar application of 500 ppm (Table 4).

Khaled and Fawy (2011) found that foliar applications of 0.1% and 0.2% humic acid significantly improved phosphorus uptake in maize. Similarly, Lingaraju *et al.* (2016) noted that soybean showed greater nitrogen and phosphorus uptake when 0.1% humic acid was applied during flower initiation, compared to other treatments and the control group.

Nitrogen and phosphorus uptake by greengram varied markedly with the application of humic acid. Enhanced root growth, along with an increased number of lateral roots and root hairs, may contribute to improved nutrient absorption (Canellas *et al.*, 2002). Humic acid stimulates respiration, raising the demand for inorganic phosphorus needed for ATP synthesis, thereby leading to higher phosphorus uptake (Smidova, 1960). Additionally, it forms humo-phospho complexes, which enhance the availability and uptake of phosphorus (Balasubramanian *et al.*, 1989). Improved nitrogen uptake may be attributed to increased activity of ammonifiers and nitrifiers.

**Table 3. Nutrient content in seed and stover of greengram as influenced by soil and foliar application of humic acid**

Treatments	Nitrogen content (%)		Phosphorus content (%)	
	Seed	Stover	Seed	Stover
<b>[A] Soil application of humic acid (S)</b>				
S <sub>1</sub> : 0 kg ha <sup>-1</sup>	3.24	0.717	0.438	0.157
S <sub>2</sub> : 3 kg ha <sup>-1</sup>	3.44	0.768	0.455	0.162
S <sub>3</sub> : 6 kg ha <sup>-1</sup>	3.62	0.806	0.471	0.169
S.Em. (±)	0.05	0.01	0.005	0.001
C.D. at 5%	0.13	0.04	0.014	0.004
<b>[B] Foliar application of humic acid (F)</b>				
F <sub>1</sub> : Water spray	3.34	0.739	0.445	0.160
F <sub>2</sub> : 250 ppm	3.46	0.769	0.457	0.163
F <sub>3</sub> : 500 ppm	3.50	0.783	0.462	0.165
S.Em. (±)	0.05	0.01	0.005	0.001
C.D. at 5%	0.13	0.04	0.014	0.004
<b>Interaction (S×F)</b>				
C.V.%	4.66	5.46	3.53	3.16

**Table 4. Nutrient uptake by greengram as influenced by soil and foliar application of humic acid**

Treatments	Nitrogen uptake (kg ha <sup>-1</sup> )			Phosphorus uptake (kg ha <sup>-1</sup> )		
	Seed	Stover	Total	Seed	Stover	Total
<b>[A] Soil application of humic acid (S)</b>						
S <sub>1</sub> : 0 kg ha <sup>-1</sup>	22.11	8.33	30.44	2.98	1.82	4.80
S <sub>2</sub> : 3 kg ha <sup>-1</sup>	25.58	9.97	35.56	3.39	2.12	5.50
S <sub>3</sub> : 6 kg ha <sup>-1</sup>	28.98	11.72	40.70	3.77	2.46	6.22
S.Em. (±)	0.72	0.29	0.75	0.08	0.06	0.11
C.D. at 5%	2.09	0.84	2.17	0.24	0.16	0.31
<b>[B] Foliar application of humic acid (F)</b>						
F <sub>1</sub> : Water spray	23.60	9.22	32.82	3.14	1.98	5.12
F <sub>2</sub> : 250 ppm	26.02	10.05	36.07	3.43	2.14	5.57
F <sub>3</sub> : 500 ppm	27.05	10.75	37.80	3.56	2.27	5.83
S.Em. (±)	0.72	0.29	0.75	0.08	0.06	0.11
C.D. at 5%	2.09	0.84	2.17	0.24	0.16	0.31
<b>Interaction (S×F)</b>	NS	NS	NS	NS	NS	NS
C.V.%	9.71	10.00	7.26	8.55	8.95	6.71

#### 4. CONCLUSION

Soil and foliar applications of humic acid have notably improved yield, quality parameters, nutrient content and uptake in greengram. Soil application of 6 kg humic acid per hectare and foliar spray of 250 ppm humic acid at 30 and 45 DAS besides recommended dose of fertilizer viz., 20:40:00 N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O kg ha<sup>-1</sup> have resulted in a significant improvement in the yield and physiological attributes of greengram.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors 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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