Effect of Humic Acid on the Growth, Yield, Nutrient Composition, Photosynthetic Pigment and Total Sugar Contents of Peas (*Pisum Sativum* L)

[1]AHMAD KHAN, [2]ALI RAZA GURMANI, [1]MUHAMMAD ZAMEER KHAN, [1]FAYYAZ HUSSAIN, [1]MUHAMMAD EHSAN AKHTAR AND [3]SADIA KHAN

[1]*Land Resources Research Institute, National Agricultural Research Centre, Islamabad, Pakistan.*

[2]*Department of Agriculture, Soil and Environmental Sciences Section, University of Haripur, Khyber Pakhtunkhwa, Pakistan.*

[3]*Department of Soil and Environment, Gomal University, D. I. Khan, Pakistan.*

gurmani_narc@yahoo.com*

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Summary: A pot experiment was conducted to evaluate the effects of humic acid (HA) applied as soil and foliar at 15, 30 and 45 ppm on the growth, biochemical content, nutrient concentrations and yield of peas. Soil as well as foliar application of HA increased the plant growth and grain yield of peas; however magnitude of increase was higher in soil application than foliar. Highest plant growth and grain yield was achieved with soil application of 15 ppm HA followed by 30 ppm and foliar application of 45 ppm HA respectively. Percentage increase in dry grain yield due to 15 ppm was 37%, with 30 ppm was 29% and foliar application of 45 ppm was 25%. Nutrient concentrations (P, K, Fe, Zn, Mn and Cu) were increased with soil and foliar application of HA. The concentrations of nutrients were relatively higher in shelf than grain. Maximum concentration of P, K and Fe was obtained with the soil application of HA at 15 ppm. Humic acid applied at 15, 30 as soil as well as foliar application at 45 ppm significantly increased chlorophyll, carotenoid and total sugar content. Our results indicate that soil application of HA at 15 and 30 ppm, while foliar application at 45 ppm can increase growth, nutrients concentration, chlorophyll content and yield of Peas in calcareous soil conditions.

Key words: *Pisum sativum* L, Humic acid, Growth, Yield, Nutrients, Chlorophyll, Carotenoid, Sugar.

Introduction

Pea (*Pisum sativum* L) is an important leguminous vegetable crop grown throughout the Pakistan. Pea is deemed to be one of the essential nutritional foods for the human beings. The pod of pea has grater amount of protein and carbohydrates. Soils of arid and semi arid region are generally alkaline and calcareous in nature having low organic matter, nutrient mining with intensive cultivation and imbalance fertilization causes nutrient deficiencies. High pH and low level of organic matter reduces solubility and mobility of macro and micro nutrients which causes decline in crop production and quality [1, 2]. Various measures were practiced to improve and sustain the fertility and productivity of soils in this region, which includes crop rotation, different ploughing techniques, green manuring, composting and use of farm yard manures. Besides these measures some organic fertilizers has increased to enhance the productivity of crops. Humic acid (HA) is one of the main organic fertilizers, which is an important component of humic substances. Humic acid is produced by the chemical and biological decomposition of organic material through the help of micronutrients. Humic acid is a vital component of soil organic matter which improves the growth of many plant species. It enhances soil fertility and improves physical and chemical characteristics of soil, like permeability, aeration, aggregation, water holding capacity, ion transport and availability through pH buffering [3, 4]. It was reported that application of HA increases organic matter and stimulate plant growth and crop production in different vegetable crops [3, 5]. Humic acid stimulates conversion of mineral nutrients into available forms, and increases seed germination viability and more prominent in root [6]. Humic acid is considered to increase the uptake of nutrient like nitrogen, phosphorus, potassium, calcium and magnesium making it more mobile and available to plant root system [7, 8]. Soil application of HA augments organic compounds like chlorophyll, sugar, protein content, total free amino acids and total soluble phenols in shoot and pods of snap beans [9]. It was reported that application of HA improves fertilizer use efficiency and increases the uptake of macro and micronutrients as well as potato tuber yield, starch and total soluble solids. Many reports showed that spraying plants with HA results better plant growth, number of pods per plant, pods weight, chlorophyll and protein content by increasing nutrient accumulation [10, 5].

Since Pakistani soils are highly deficient in organic matter having high pH and low availability of nutrients from the soils. The role of humic acid in various physiological and biochemical processes and nutrient uptake is well reported, however, its specific role in peas has yet to be explored. Therefore, a study was conducted to test the efficacy of HA applied as soil and foliar to increase growth, yield, nutrients availability and some biochemical contents in peas under soil pot conditions.

Results and Discussion

The Effects of HA on Dry Biomass

Plant height was improved by all the levels of soil and foliar application of humic acid (HA); however increase due to 15 and 30 ppm soil application as well as 45 ppm foliar application of HA was significant over control. Plant height was ranged from 49-62 cm. Maximum plant height was attained by the soil application of 15 ppm while lowest from the control (Table-1).

Table-1: Effect of different levels of humic acid as soil and foliar application on the plant height, shoot-root dry biomass and shelf dry weight of peas.

Treatments	Plant Height (cm)	Shoot dry weight (g)	Root dry weight (g)	Shelf dry weight (g)
T1	49±1.5 c	6.4±0.55 c	2.6±0.43 c	1.5±0.15 b
T2	62±2.0 a	9.5±0.89 a	4.0±0.18 a	2.1±0.21 a
T3	60±1.7 a	9.0±0.74 a	3.5±0.35 ab	2.0±0.30 a
T4	58±2.2 ab	8.5±0.80 bc	3.2±0.40 bc	1.8±0.12 ab
T5	52±3.3 bc	7.5±0.80 bc	3.0±0.42 bc	1.6±0.20 b
T6	54±4.0 bc	8.0±0.90 bc	3.3±0.60 bc	1.7±0.11 ab
T7	56±4.0 ab	8.5±0.60 ab	3.6±0.50 ab	1.9±0.14 a

Bars shows standard error (SE) of means value (n=3). Columns with different letters indicate significant difference at $P < 0.05$ (LSD test).

Shoot dry weight was also increased by the applied HA both as soil and foliar; however magnitude of increase was higher in soil application than foliar. Applied HA at 15 and 30 ppm as soil and 45 ppm as foliar was significantly increased shoot dry weight over control. Maximum shoot dry weight was achieved with soil application of 15 ppm HA, while lowest from the control (Table-1).

Root dry weight was also enhanced by the soil and foliar application of HA. Root dry weight was ranged from 6.4-9.5 g; relatively higher root dry weight was recorded with soil application of HA as compared to foliar. Highest root dry weight was recorded with 15 ppm soil application of HA; which was statistically at par with 30 ppm soil application of HA (Table-1).

Soil and foliar application of humic acid (HA) increased plant height, shoot and root dry weight (Table-1). The maximum increase was achieved by the soil applied HA at 15, 30 ppm and foliar applied HA at 45 ppm. Lulakis and Petsas [11] reported that uptake of water augmented nutrient absorbance by the roots was enhanced in the presence of humic acid, which enhances the improvement of roots. These results are agreed with our finding of the significant increase in root dry weight by the 15 and 30 ppm soil application of HA and 45 ppm foliar applied HA. El-Bassiony *et al.* [10] reported a significantly response of foliar application of HA on plant height, number of leaves and branches as well as fresh and dry weight of whole snap bean plants (*Phaseolus vulgaris* L.).

The Effects of HA on Yield and Yield Attributes

The effect of various applied HA levels on fresh pod yield, grain dry weight, number of pods per plant, number of seeds per plant and shelf dry weight was explained in Table-1 and 2. The mean fresh pod yield was ranged from 15-22 g, while number of pods per plant ranged from 5-7.1 and number of seeds per plant ranged from 26-41. Application of HA at 15, 30 and 45 ppm as soil, while 45 ppm as foliar was significantly increased fresh pod yield, grain dry weight, number of pods per plant and number of seeds per plant over control. Percent increase in fresh pod yield was 32, 26 and 21% respectively with the soil applied HA at 15, 30 and 45 ppm, while it was 8, 16 and 24% with foliar application of HA at 15, 30 and 45 ppm. Similarly soil applied HA increase grain dry weight over control was 37, 29 and 22 %; while due to foliar application was 9, 17 and 25% respectively. Maximum fresh grain yield, number of pods per plant and number of seeds per plant was recorded with the soil application of 15 ppm HA while lowest from the control treatment.

Table-2: Effect of different levels of humic acid as soil and foliar application on the fresh pod yield, grain dry weight, number of pods plant^{-1} and number of seeds plant^{-1} of peas.

Treatments	Fresh pod yield (g pod^{-1})	Grain dry weight (g)	No. of pods plant^{-1}	No. of seeds plant^{-1}
T1	15±3.5 c	5.0±0.46 c	5.0±0.58 c	26±2.3 c
T2	22±2.0 a	8.0±0.42 a	7.5±1.0 a	40±2.0 a
T3	20.3±2.2 ab	7.1±0.67 ab	7.1±0.33 ab	35±4.5 ab
T4	19.0±2.5 ab	6.4±54 abc	6.8±0.60 ab	34±2.0 b
T5	16.3±1.4 c	5.5±0.30 bc	6.0±0.60 bc	28±1.5 bc
T6	17.8±2.5 bc	6.0±0.80 bc	6.3±0.44 bc	30±1.5 bc
T7	19.8±1.3 ab	6.7±0.60 b	7.0±0.58 ab	33±1.1 b

Bars shows standard error (SE) of means value (n=3). Columns with different letters indicate significant difference at $P < 0.05$ (LSD test).

Shelf dry weight was increase with both the soil and foliar application of tested HA levels. Shelf dry weight was ranged from 1.5-2.1. Shelf dry weight was relatively higher by the soil applied HA as compared to foliar. Soil application of HA at 15 and 30 ppm and foliar application at 45 ppm was statistically at par with each other.

It was reported that humic substances increase the yield of several field crop in different studies [12]. Already increase in grain yield by the application HA in common vetch (*Vicia sativa* L) [13], cowpea (*Vigna unguiculata* L) [14], Snap bean [9] and common bean (*Phaseolus valgarous* L) [5]. Our results in the present study also showed that addition of humic acid increase plant height, number of pod plant^{-1}, number of seeds plant^{-1}, shelf dry weight and fresh pod yield. An increase in green pod yield, pod length and pod weight of snap bean plants (*Phaseolus vulgaris* L.) were recorded by the foliar application of humic acid [10].

The Effects of HA on Nutrient Element Concentrations in Grains and Shelf

The nutrient P, K, Fe, Zn, Cu and Mn concentrations in grain and shelf was illustrated in Table 3 and 4.

Phosphorus concentrations in grain ranged from 0.22-0.37 mg kg^{-1} while it ranged from 0.35-0.60 in shelf of peas. Phosphorus concentration was significantly raised with the soil applied HA at 15, 30 ppm and foliar applied 45 ppm over control in both grain and shelf of peas. Phosphorus concentrations were relatively higher in shelf than grains of peas. Maximum P concentration was achieved with 15 ppm soil application of HA while lowest from the control.

Potassium concentration in pea's grain ranged from 1.9-3.5 mg kg^{-1} while in shelf, it ranged from 3.5-6.2 mgkg^{-1}. Potassium concentration was improved by the various levels of applied HA in the form of soil as well as foliar; however increase due to 15 and 30 ppm soil application and 45 ppm foliar application was significant in both grain and shelf of peas. Soil application of HA at 15, 30 ppm and foliar application at 45ppm was statistically at par with each other in grain and shelf.

Iron concentration was relatively higher in shelf than grain of peas. Iron concentration in peas grain was ranged from 12.5-25.4, while it ranged from 50.3-85.0 in shelf. Concentration of Fe was significantly increased by all the three levels of soil and 45 ppm foliar applied HA in grains; while any increase in shelf was only significant with 15, 30 ppm soil applied HA and 45 ppm foliar applied HA. Highest Fe concentration (25.4 mgkg^{-1}) in grain and (85 mg kg^{-1}) in shelf was recorded with 15 ppm HA, while lowest with the control.

Zinc concentration in grain was ranged from 23-41 mg kg^{-1} while, it ranged from 37-58 mg kg^{-1} in peas shelf. Concentration of Zn was relatively higher by the foliar application as compared to soil application of HA in both grain and shelf. Zinc concentration was significantly increased by all the three levels of foliar applied HA in grain and shelf of peas. Maximum Zn concentration was recorded with foliar application of 45 ppm HA while lowest from the control in both plant parts.

Manganese concentration in grain was ranged from 7.3-15.3 while in shelf was 9.9-15. The response of Mn concentration in grain and straw was uneven. Highest Mn concentration in grains was recorded with soil applied HA at 30 ppm; while it was highest by the application of 15 ppm soil applied HA in pea's shelf.

Copper concentration in grain was ranged from 3.8-6.3, while in shelf was 5.5-8.3. Relatively higher concentration of Cu was recorded with foliar application of HA as compared to soil application. Maximum Cu concentration was recorded with 45ppm foliar application of HA, while lowest from control in both grain and shelf.

Table-3: Effect of different levels of humic acid as soil and foliar application on the nutrients (P, K, Fe, Zn, Mn and Cu) concentrations in grains of peas.

Treatments	P (%)	K (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
T1	0.22±0.03 c	1.9±0.23 c	12.5±2.3 c	23±2.1 d	7.3±0.62 c	3.8±0.47 c
T2	0.37±0.02 a	3.5±0.14 a	25.4±2.5 a	26±2.6 cd	10.3±1.7 bc	5.4±0.27 b
T3	0.35±0.02 a	3.2±0.25 ab	21.3±2.0 ab	27±1.4 cd	15.3±4.2 a	4.5±0.30 bc
T4	0.29±0.03 bc	2.9±0.3 ab	16±1.2 b	28±2.4 cd	13.5±2.3 ab	3.8±0.12 c
T5	0.26±0.04 bc	2.4±0.4 bc	14.5±4.2 bc	34±2.4 bc	8.0±1.7 c	5.9±0.53 ab
T6	0.29±0.03 bc	2.7±0.6 bc	15.0±2.0 bc	37±3.0 ab	12±3.0 b	5.8±0.21 ab
T7	0.35±0.03 a	3.1±0.56 ab	16±1.4 b	41±3.6 a	10.5±2.8 bc	6.3±0.40 a

Bars shows standard error (SE) of means value (n=3). Columns with different letters indicate significant difference at P < 0.05 (LSD test).

Table-4: Effect of different levels of humic acid as soil and foliar application on the nutrients (P, K, Fe, Zn, Mn & Cu) concentrations in shelf of peas..

Treatments	P (%)	K (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
T1	**0.35±0.04 c**	**3.5±0.21 c**	**50.3±6.8 c**	**37±3.5 c**	**9.0±1.9 b**	**5.5±0.80 c**
T2	**0.60±0.12 a**	**6.2±0.71 a**	**85.0±8.0 a**	**42±5.4 bc**	**15±2.4 a**	**6.3±0.90 bc**
T3	**0.55±0.13 ab**	**4.8±0.44 ab**	**70±9.5 ab**	**40±4.0 bc**	**14±2.0 a**	**6.0±0.82 bc**
T4	**0.48±0.03 b**	**4.5±0.33 bc**	**57±4.3 bc**	**42±6.0 bc**	**13±2.3 ab**	**6.7±1.0 bc**
T5	**0.41±0.06 bc**	**3.8±0.35 bc**	**58±8.7 bc**	**46±3.0 b**	**10±2.0 b**	**7.8±1.0 ab**
T6	**0.44±0.05 bc**	**4.7±0.40 ab**	**65±9.6 abc**	**54±3.0 a**	**11±2.4 ab**	**8.3±0.60 a**
T7	**0.50±0.05 ab**	**5.2±0.25 ab**	**70±9.5 ab**	**58±2.0 a**	**10±2.1 b**	**7.9±0.88 ab**

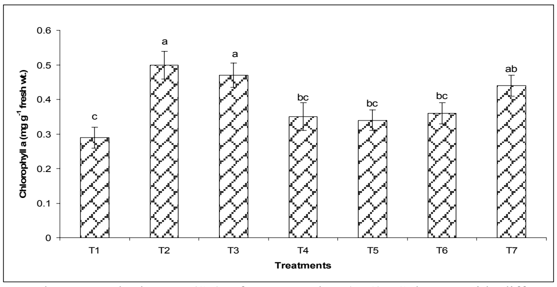
Bars shows standard error (SE) of means value (n=3). Columns with different letters indicate significant difference at $P < 0.05$ (LSD test).

In the present study higher growth and yield by the application of HA may be due to its better ability of nutrient uptake and accumulation in grain and shelf of peas. An increase in the uptake of nutrients like P, K, Ca, Mg, Zn and Fe concentrations was reported by the application of HA in shoot of snap bean under calcareous soil conditions [9]. Present results showed that concentrations of nutrients like P, K, Fe, Zn, Mn and Cu were improved by the soil and foliar application of HA (Table 3 and 4). The positive effect of HA on the uptake of nutrient might be due to their effect on the constancy of membrane permeability [15], and that is correlated by the surface activity of HA containing both hydrophilic and hydrophobic sites. Humic acid being an excellent organic substance; which enhance availability of nutrients from the soil [16, 17] by reducing soil pH, producing intermediate organic acid and ultimately enhancing the activity of micro organism in soil and releases the nutrients from the unavailable reserves. Improvement in nitrogen and phosphorus concentration in maize and wheat by the addition of HA was reported in alkaline soil condition [18]. Moreover, El-Ghamry *et al.* [19] reported a similar response of HA to increased N, P and K content in seed and straw of faba bean.

The Effects of HA on Chlorophyll, Carotenoid and Total Sugar Contents

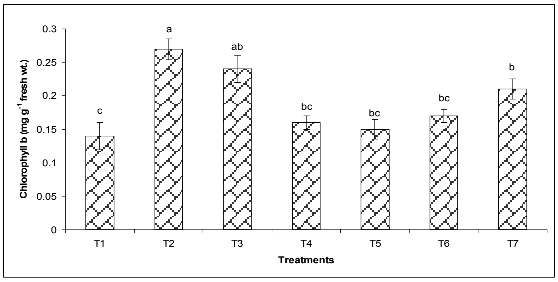
Humic acid applied at 15 and 30 ppm as soil and 45 ppm as foliar application significantly improved chlorophyll a and b contents. Maximum chlorophyll a and b contents were obtained with 15 ppm soil application of HA, while lowest from the control (Fig. 1 and 2). Any increase with the soil application of HA at 45 ppm and foliar application of HA at 15 and 30 ppm was non-significant.

Carotenoid contents were significantly increased by all the three levels of soil applied HA; however increase due to foliar application was only significant with 45 ppm. Maximum carotenoid contents were recorded with soil applied HA at 15 ppm while lowest with the control. Foliar application of 45 ppm HA was statistically at par with 30 and 45 ppm soil applied HA (Fig. 3).



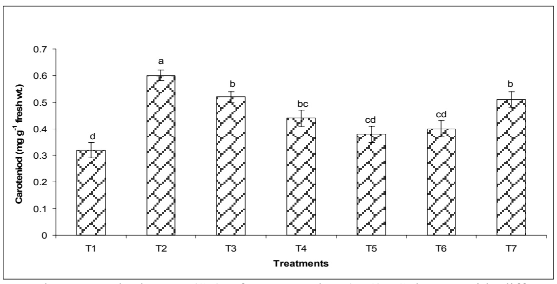
Bars shows standard error (SE) of means value (n=3). Columns with different letters indicate significant difference at $P < 0.05$ (LSD test).

Fig. 1: Effect of different levels of humic acid as soil and foliar application on chlorophyll a content of peas.



Bars shows standard error (SE) of means value (n=3). Columns with different letters indicate significant difference at $P < 0.05$ (LSD test).

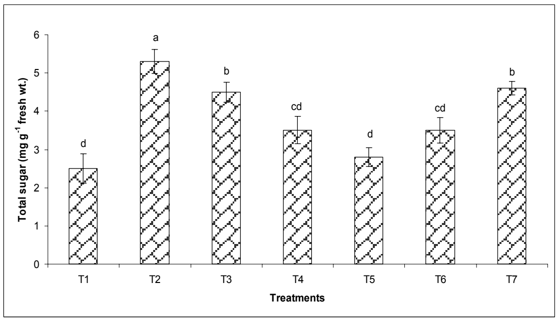
Fig. 2: Effect of different levels of humic acid as soil and foliar application on the chlorophyll b content of peas.



Bars shows standard error (SE) of means value (n=3). Columns with different letters indicate significant difference at $P < 0.05$ (LSD test).

Fig. 3: Effect of different levels of humic acid as soil and foliar application on the carotenoid content of peas.

Total sugar contents were significantly increased by all the tested treatments except foliar application of 45 ppm HA. Highest sugar contents were achieved with soil application of 15 ppm followed by 30 ppm and foliar application of 45 ppm HA respectively. Increase due to 45 ppm soil applied HA and 30 ppm foliar applied HA was statistically similar. Minimum sugar contents were recorded with control plants (Fig. 4).



Bars shows standard error (SE) of means value (n=3). Columns with different letters indicate significant difference at $P < 0.05$ (LSD test).

Fig. 4: Effect of different levels of humic acid as soil and foliar application on the total sugar content of peas.

All the biochemical contents of peas were affected by different levels of HA both as soil and foliar application. Soil application of 15 and 30 ppm HA significantly improved the chlorophyll a and b content in leaves of peas (Fig. 1 and 2). Similarly carotenoid and total sugar contents enhanced by the soil as well as foliar applications of HA (Fig. 3 and 4). The improvement in biochemical contents by the addition of HA could be due to related with the improvement of soil properties like aggregation, aeration, water holding capacity and increase capacity of ammune plant system [9]. Sahar *et al.* [20] reported an increase in sugar content of *Thuya orientalis*, L was obtained from plants supplied with 2.0 or 2.5% potassium humate. In addition, Chen and Aviad [21] added that humic acid increases plant chlorosis and thus enhanced photosynthetic pigments as well as increasing total sugar content in plants. Similarly, higher level of foliar application of HA increased the quality of snap bean by enhancing chlorophyll, fiber and total protein content [10].

Experimental

Material and Methods

The experiment was carried out in November 2010 in soil pot conditions in the glass house of National Agricultural Research Centre (NARC), Pakistan. Soils of the Ap horizon was collected from the field of NARC, Islamabad. Crushed and sieved soil was filled in 7 kg pot. A composite soil sample was taken to visualize the physico-chemical characteristics of the soil. Soil characteristics were pH, 8.0; calcium carbonate ($CaCO_3$) equivalent, 11.5%; organic matter, 0.75%; electrical conductivity (ECe), 0.56 dS m^{-1}; nitrogen, 0.042%; $NaHCO_3$ extractable P, 4.6 (mg kg^{-1}); ammonium acetate extractable K, 120 (mg kg^{-1}); AB-DTPA extractable Fe, 3.6; Zn, 0.80; Mn, 1.7; and Cu, 0.4 (mg kg^{-1}). All the soil tests were done by the method prescribed by Ryan *et al.* [22]. The experiment was laid out in randomized complete block design with seven treatments and three replications on a pea's cultivar (Climax). The detail of treatment is given in Table-5. Basal dose of NPK was applied at 87.5-100-62.5 mg kg^{-1} as N, P_2O_5 and K_2O in the form of Urea, di-ammonium phosphate and potassium sulphate. All the P and K were supplied at time of sowing, while N was supplied in three equal splits. Two plants per pot were raised in each pot. The biochemical attributes like chlorophyll a and b, carotenoid and total sugar contents were determined from the leaves at fruiting stage. The nutrient elements P, K, Fe, Zn, Mn and Cu were estimated from the pea's grains and shelf. Crop was harvested at maturity.

Chlorophyll Content, Total Sugar Contents and Nutrient Element Analysis

Chlorophyll a and b contents were estimated from the fresh leaves by dipping in 15 ml 80% ethanol in a test tube, covered with aluminum foil and extracted for 10 minutes in a water bath at 85°C. Sample was afterward cooled, and read at 663 and 645 nm optical densities by staying away from light using spectrophotometer (Unicam 8620). The chlorophyll a and b contents were calculated according to Arnon *et al.* [23]. Carotenoid contents from the fresh leaves were determined by measuring sample at 470 nm optical density using spectrophotometer (Unicam 8620) and the amount of these pigments was calculated by the method of Lichtenthaler and Wellburn [24].

Total sugar contents were determined from the fresh leaves by adopting the method of Dubois *et al.* [25]. Fully mature fresh leaves were immediately homogenized in a pestle and mortar by adding 10 mL of distilled water. Sample was centrifuged at 3000 × *g* for 5 min then subsequently inserted 1 mL of 80% (V/V) phenol. Then sample was incubated for 1 h at room temperature and added 5 mL concentrated

H_2SO_4. The absorbance of each sample was recorded at 490 nm.

Pea's grain and shelf from each treatment was analyzed for nutrients. Pea's grain and shelf were dried at 70°C for 48 h. Sample was ground using a pestle and mortar, stored in plastic bottles. Samples were digested in HNO_3:$HClO_4$ (2:1) mixture and analyzed for P, K, Fe, Zn, Mn & Cu on spectrophotometer and atomic absorption spectroscopy [22].

Statistical analysis was done by the help of Minitab software. Least significant difference was calculated by using analysis of variance by MSTAT-C software [26].

Table-5: Detail of treatment is given below.

Treatments	Detail
T1	Control (without application of HA)
T2	Soil application of HA at 15 ppm
T3	Soil application of HA at 30 ppm
T4	Soil application of HA at 45 ppm
T5	Foliar application of HA at 15ppm
T6	Foliar application of HA at 30 ppm
T7	Foliar application of HA at 45 ppm

HA, Humic acid.

Conclusions

Soil application of humic acid at 15 and 30 ppm as well as foliar application at 45 ppm significantly increased shoot-root biomass, grain yield, number of pods plant^{-1} and number of seeds plant^{-1}. Maximum P, K and Fe concentration was achieved with the soil application of HA at 15ppm in peas. Similarly chlorophyll a, b, carotenoid and total sugar contents respond better with the soil applied HA at 15ppm and eventually improved growth and yield of peas in calcareous soil conditions. Outcomes of the experiment suggested that soil application of HA at 15 ppm increased quantitative and qualitative yield of peas.

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