ISSN 1807-1929
Revista Brasileira de Engenharia Agrícola e Ambiental
Brazilian Journal of Agricultural and Environmental Engineering
v.25, n.10, p.657-663, 2021
Campina Grande, PB – http://www.agriambi.com.br – http://www.scielo.br/rbeaa

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v25n10p657-663

Humic acid and nitrogen dose application in corn crop under alkaline soil conditions[1]

Aplicação de doses de ácido húmico e nitrogênio na cultura do milho em solo alcalino

Kamran Azeem[2], Farah Naz[3], Arshad Jalal[4]*, Fernando S. Galindo[4], Marcelo C. M. Teixeira Filho[4] & Farhan Khalil[2]

[1] Research developed at Peshawar, Khyber Pakhtunkhwa, Pakistan
[2] University of Agriculture Peshawar/Faculty of Crop Production Sciences, Peshawar, Pakistan
[3] Department of Soil and Water Conservation, Haripur Khyber Pakhtunkhwa, Pakistan
[4] Universidade Estadual Paulista, Campus Ilha Solteira, Ilha Solteira, SP, Brazil

HIGHLIGHTS:

Humic acid and nitrogen doses have a positive influence on nitrogen and organic matter concentration in alkaline soil.
Humic acid and nitrogen doses improve nitrogen concentration in corn plants.
Humic acid and nitrogen doses increase corn growth and yield.

ABSTRACT: Humic acid (HA), as a bio-stimulant and a major component of organic matter (OM), can improve plant physiology, soil fertility, and nutrient availability, mainly in low OM soils. Nitrogen (N) is one of the most important nutrients that affect several metabolic and biochemical activities, leading to improved plant development. This study was conducted to investigate the combined effect of HA and N doses on soil organic matter (SOM) and total N concentration, N uptake, corn growth, and grain yield under conventional tillage at Peshawar, Pakistan. Treatments were tested in a randomized block design with four replicates arranged in a factorial scheme 3 × 4 + 1. The respective doses of HA (1.5, 3,0 and 4.5 kg ha^{-1}) were applied at the corn sowing, whereas N doses (80, 120, 160, and 200 kg ha^{-1}) were applied in three splits (1/3 at sowing, 1/3 at the V5 stage, and remaining 1/3 at the tasselling stage) with one control (no HA and N). The application of HA, regardless of the applied doses, had positive effects on SOM, N concentration, N uptake, corn development, and grain yield. However, the application of 4.5 kg ha^{-1} of HA was the most effective in promoting SOM (0.83%) and total N (0.31%), shoot biomass (10610 kg ha^{-1}), N uptake (1.13%), and grain yield (3780 kg ha^{-1}), even when combined with the N doses of 80, 120 and 160 kg N ha^{-1}. Increasing N doses positively influenced SOM, N concentration, N uptake, and corn growth. The greatest grain yield was obtained at 150 kg ha^{-1} of N regardless of HA applied doses.

Key words: *Zea mays* L., grain protein, organic fertilizer, nitrogen fertilization

RESUMO: Ácidos húmicos (AH), como bio-estimulante e componente principal da matéria orgânica (MO), podem beneficiar a fisiologia das plantas, fertilidade do solo e disponibilidade de nutrientes, principalmente em solos com baixo teor de MO. O nitrogênio (N) é um dos nutrientes mais importantes que exerce diversas atividades metabólicas e bioquímicas, levando a um melhor desenvolvimento da planta. Esta pesquisa foi desenvolvida visando investigar o efeito combinado de doses de HA e doses de N na matéria orgânica do solo (MOS), teor de N total, absorção de N, crescimento do milho e produtividade de grãos sob cultivo convencional em Peshawar, Paquistão. O delineamento utilizado foi em blocos casualizados com quatro repetições, dispostos em esquema fatorial 3 × 4 + 1. As doses de AH (1,5; 3,0 e 4,5 kg ha^{-1}) foram aplicadas em ocasião da semeadura do milho, enquanto que as doses de N (80, 120, 160 e 200 kg ha^{-1}) foram aplicadas parceladamente (1/3 na semeadura, 1/3 no estágio V5 e 1/3 no florescimento) com um controle (sem HA e N). A aplicação de AH aumentou a MOS e N no solo, a absorção de N beneficiou o crescimento e produtividade de grãos de milho, independentemente das doses de N. Entretanto, a aplicação de 4,5 kg ha^{-1} of HA foi mais eficiente em promover maior SOM (0,83%) e N total (0,31%), biomassa da parte aérea (10610 kg ha^{-1}), absorção de N (1,13%) e produtividade de grãos (3780 kg ha^{-1}), mesmo quando associado às doses de N de 80, 120 e 160 kg ha^{-1} de N. O aumento das doses de N influenciou positivamente a MOS, o teor de N, a absorção de N, o desenvolvimento do milho e a maior produtividade de grãos foi obtida com 150 kg ha^{-1} de N, independentemente das doses de AH.

Palavras-chave: *Zea mays* L., proteína nos grãos, fertilizante orgânico, adubação nitrogenada

• Ref. 236730 – Received 16 Apr, 2020
* Corresponding author - E-mail: arshad.jalal@unesp.br
• Accepted 06 Apr, 2021 • Published 09 May, 2021
Edited by: Hans Raj Gheyi

Introduction

Corn (*Zea mays* L.) is one of the most important grain crops in the world. Millions of people worldwide consume it as diet food directly, whereas feeding to animals indirectly (Bijanzadeh et al., 2019). Corn is a multipurpose crop (Khaliq et al., 2004) with great nutritional values and contains about 72% starch, 10.4% proteins, and 4.5% fats, minerals, and non-cholesterol oil (Aslam et al., 2011). However, the yield potential of corn in developing countries is low due to poor-quality seed and imbalanced nutrients application, especially nitrogen (N) (Bijanzadeh et al., 2019). Nitrogen has a critical function in the photosynthetic activities, quality, and yield of cereal crops, such as corn (Gojon, 2017).

Nitrogen has an intensified role in agricultural and non-agricultural ecosystems. Therefore, N use efficiency is required to enhance grain crops with urgency to cope with climatic and environmental risks (Xia et al., 2017). The fertilization and availability of N from different sources (Jalal et al., 2020) in soil and plants can be synergistically increased with HA application (Niaz et al., 2016). According to Azeem et al. (2015), combined N and HA applications prominently improved physiological and yield indexes in corn.

Humic acid (HA) is water-soluble and naturally occurring organic acid in soil organic matter (SOM) which enhance microbial activities and uptake of nutrients (Liu et al., 2019), also stimulate cell permeability and plant growth and result in greater crop yield (Morozesk et al., 2017). Humic acid stimulates microbial growth and activities in the rhizosphere due to the biochemical decomposition of animal and plant residues (El-Howeity et al., 2019), improving photosynthetic efficiency soil-water physical properties (Shah et al., 2018b). HA regulates several metabolic, hormonal, biochemical, molecular, and physiological activities and triggers different biotic and abiotic stresses in the rhizosphere (Shah et al., 2018b). Humic acid can improve soil physical conditions and nutrients assimilation to the plants (Olaetxea et al., 2018). Around 1 kg ha^{-1} of HA may improve the yield of several crops and soil physio-chemical attributes up to 20% (Sharif et al., 2003); however, these improvements are more dependent on the source, concentration, and molecular weight of humic substance application (Nardi et al., 2000).

Pakistani soils are naturally alkaline with low macro-nutrients and organic matter availability (Arif et al., 2017). Besides, socio-economic issues, environmental losses, and unawareness of the farmer community of Pakistan constrained the supply and utilization of N fertilization, therefore decreasing N use efficiency (Shah & Ahmad, 2006; Shah et al., 2018a). Therefore, a study was performed using HA in combination with N doses in alkaline soil to improve organic matter and nutrient availability in corn crops, leading to greater crop growth and grain yield. This study hypothesized that the application of HA combined with N doses would benefit corn growth and grain yield by increasing organic matter and total N availability in soil, leading to a higher N uptake. This study aimed to investigate the combined effect of HA levels and N doses on soil organic matter and total N content, N uptake, corn growth, and grain yield in clayey alkaline soil in Pakistan.

Material and Methods

The study was performed during the corn-growing season of 2016 (June-September). The experiment was set up in silty-loam soil under the conventional tillage system, located at Peshawar, Khyber Pakhtunkhwa, Pakistan, at 34° 1’ N, 71° 33’ E, and altitude of 450 m above sea level. The soil of the experimental area was classified as Alfisol, according to the Soil Survey Staff (2014).

The climatic condition of the experimental area was classified as semi-arid subtropical according to the Köppen classification. The region has cool winters and hot summer with annual rainfall from 350 to 500 mm. A small quantity (about 30-40%) of the total annual rainfall occurs in winter due to the cold air currents from Mediterranean and Gulf disturbances. With this, most of the rainfall (around 60 to 70%) occurs in summer due to hot air currents from the Bay of Bengal with a high temperature of 30 to 40 °C (digital weather station from the agronomy research farm, University of Agriculture Peshawar). Maximum and minimum temperature and rainfall during the growing season recorded are shown in Figure 1.

The soil was collected from the 0-0.20 cm soil layer before and after the experiment implantation for physio-chemical analysis. The composite soil samples were analyzed for organic matter, texture, pH, ECe, and available P and K content (Table 1). Soil texture was determined by the pipette method as described by Tagar & Bhatti (2001). Soil organic matter was determined by the wet digestion method as described by Nelson & Sommers (1996). Soil pH was estimated according to the methodology of McLean (1983), and available P and K were determined according to the methodology proposed by Soltanpour & Schwab (1977). Post-harvest soil was analyzed for total and mineral N by the Kjeldahl method according to the proposed methodology of Bremner & Mulvaney (1982). The results of soil physio-chemical analysis are summarized in Table 1.

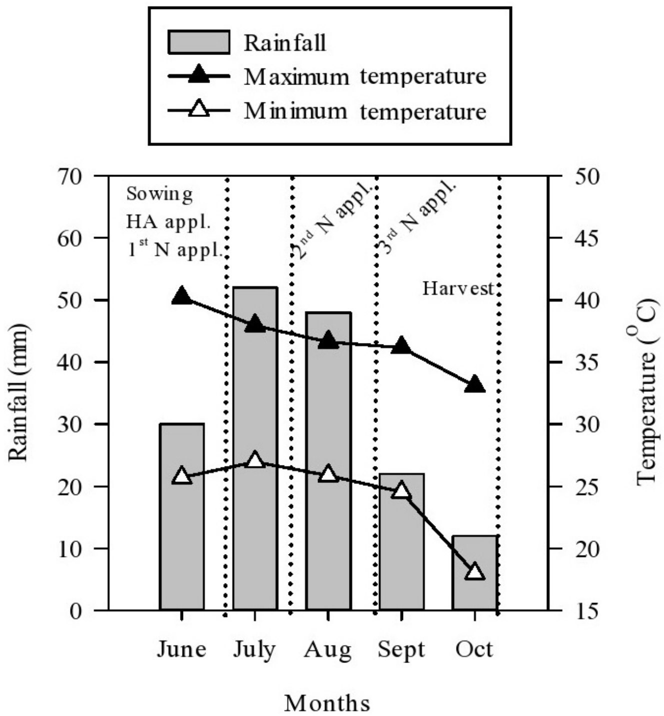


Figure 1. Rainfall, maximum and minimum temperature at the experimental area from June to October 2016

Table 1. Pre-experiment soil physio-chemical analysis and pre- and post-experiment total and mineral soil nitrogen status

Soil property	Unit	Values
Clay	%	2.8
Silt	%	50.0
Sand	%	47.2
Texture	-----	Silty loam
pH (1:5)	-----	7.9
Electrical conductivity (1:5)	dS m^{-1}	0.19
Organic matter	%	0.41
Phosphorus	Ppm	2.86
Potassium	mg L^{-1}	120.8
Nitrogen concentration	**Total nitrogen (%)**	**Mineral nitrogen (mg kg^{-1})**
Soil before fertilization	0.166	36.75
Soil after fertilization		
80 kg N ha^{-1}	0.218	41.13
120 kg N ha^{-1}	0.281	42.87
160 kg N ha^{-1}	0.332	44.62
200 kg N ha^{-1}	0.341	45.52
Control	0.091	28.36

The experimental design was a randomized block design with four repetitions arranged in a factorial scheme 3 × 4 + 1. The treatment combination was comprised of three HA levels (1.5, 3.0, and 4.5 kg HA ha^{-1}) applied at sowing, and four N doses (80, 120, 160, and 200 kg N ha^{-1}) split applied 1/3rd at sowing, 1/3rd at V5 stage, and the remaining 1/3rd at tasseling stage. One additional treatment without HA and N application (control) was added. Nitrogen was applied using urea (46% N) as the source, obtained from Fauji fertilizer company limited®. HA (Energizer® - 50% HA, 51-57% C, 4-6% N, and 0.2-1.0% P) was obtained from Warble chemicals private limited.

The experimental area was irrigated for weeds germination and maintaining field capacity. The area was plowed with a cultivator and harrowed with a discing harrow. Azam corn variety was sown with a seed drill. Later on, thinning was done at the second unfolding of second trifoliate leaves to maintain recommended plant population of 60000 plants ha^{-1}. A plot size of 15 m^2 was composed of four rows spaced at 75 cm apart. The length of the rows was 5 m, with plants spaced at 25 cm. Nitrogen doses were according to the treatments, and an additional 90 kg of phosphorus (P_2O_5) ha^{-1} and 60 kg of potassium (K_2O) ha^{-1} were applied at sowing. Urea (46% N), diammonium phosphate (46% P_2O_5), and potassium sulfate (50% K_2O) were used as sources of N, P_2O_5, and K_2O, respectively. The crop was irrigated according to its water needs with a surface irrigation system using the Warsak Canal system of the Kabul river. Simultaneously, the N treatments were applied in the area on the same day to incorporate N in each plot. The other agronomic and crop practices were uniformly maintained for all treatments.

The plant height was measured from the base of the plant to the tip of the last opened leaf. Shoot biomass was recorded by harvesting four central rows in each plot, sun-dried, and weighed on an electronic scale. Harvest index was the result of the proportion of kernel dry weight to the total plant dry weight. Grain yield was determined by harvesting the plants contained in the useful area of each plot. After harvest, the grains were quantified in kg ha^{-1} and corrected to 13.0% moisture (wet basis). Organic matter in soil was determined by the modified method of Nelson & Sommers (1996). The nitrogen content in soil, straw, and grains was determined by following the Kjeldahl method according to the proposed methodology of Bremner & Mulvaney (1982). Grain protein was determined from the percent N in grain determined by the Kjeldahl method as; Grain protein content = N% × 6.25 (AOAC, 1992).

All data were initially tested for normality using the Shapiro and Wilk test and Levene's homoscedasticity test ($p \le 0.05$), which showed the normally distributed data ($W \ge 0.90$). The data was analyzed by ANOVA in a two-way factorial design with additional treatment, HA doses, and N doses, and their interactions considered fixed effects in the model using the ExpDes package. The means were compared when significant factors or interactions were observed using the Tukey test ($p \le 0.05$). Regression analysis was used to discern whether there was a linear or non-linear response to N doses. The heatmap was developed by calculating the Pearson's correlation ($p \le 0.05$) using the corrplot package to evaluate the relationship among the SOM, N concentration in plant and soil, corn yield components, and grain yield, using R software (R Core Team, 2015).

Results and Discussion

The application of 4.5 kg HA ha^{-1} positively influenced plant height compared to the application of 1.5 and 3.0 kg HA ha^{-1} promoting an increase of 11.6 and 6.5%, respectively (Table 2). Besides, the application of 4.5 kg HA ha^{-1} positively influenced straw N concentration (an increase of 8.7%) compared to 1.5 kg HA ha^{-1} (Table 2). Also, shoot biomass and grain yield were greater with the application of 4.5 kg (an increase of 5.6 and 14.6%) and 3.0 kg HA ha^{-1} (an increase of 4.4 and 10.4%), respectively, compared to the application of 1.5 kg HA ha^{-1} (Table 2). Similarly, Baldotto et al. (2019) reported an increase of 15% in corn grain yield according to the HA application. The corn yield and yield components were improved with the application of HA (Öktem et al., 2018).

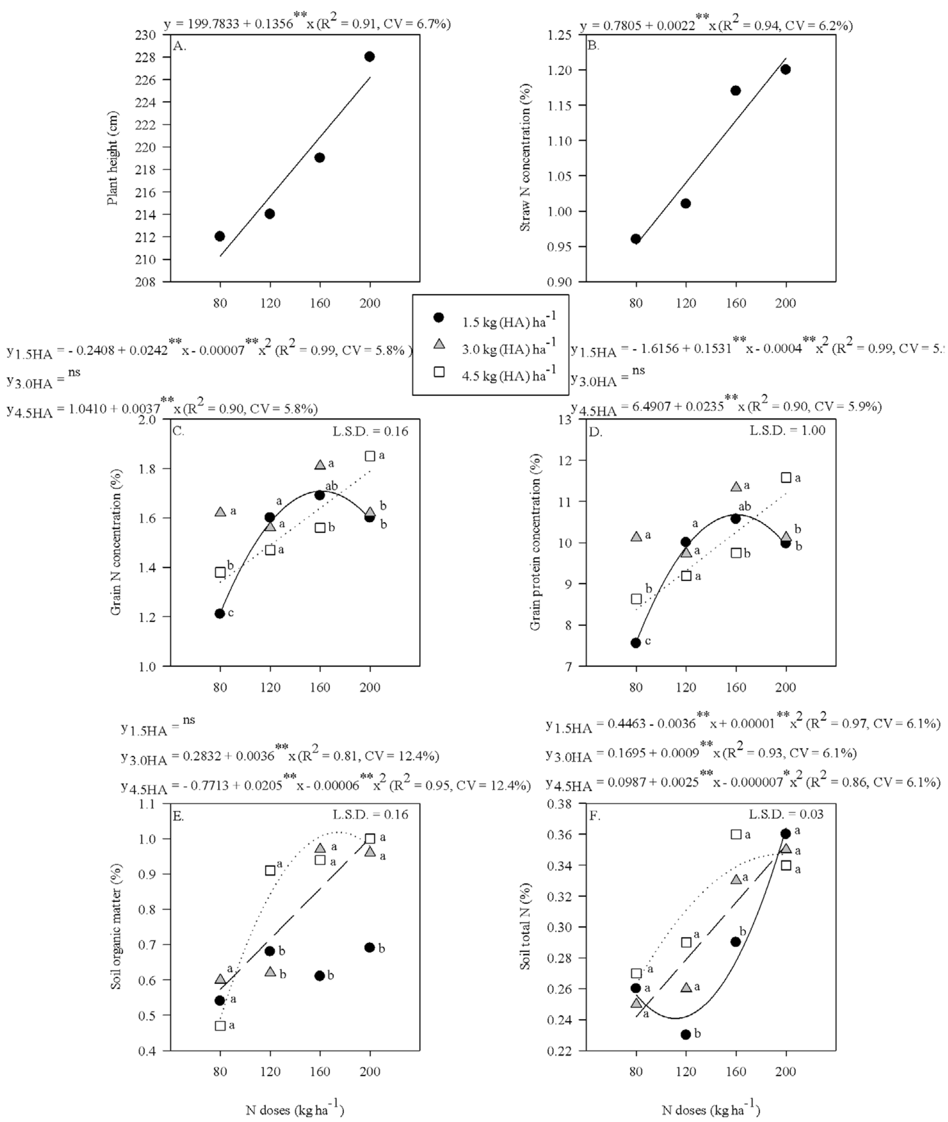
Regarding the effect of N doses, plant height and straw N concentration responded linearly to N increasing doses (Figures 2A and B). Shoot biomass and grain yield responded non-linearly to N doses up to 158 and 150 kg N ha^{-1}, respectively (Figures 3A and C). Also, factorial treatments were observed with greater plant height (an increase of 29%), straw N concentration (an increase of 49.3%), grain N concentration (an increase of 49.1%), grain protein concentration (an increase of 49%), SOM (an increase of 63.0%), soil total N (an increase of 20.0%), shoot biomass (an increase of 18.2%), harvest index (an increase of 8.5%) and corn grain yield (an increase of 29.7%) compared to control treatments (Table 2). These results are supported by Oktem et al. (2010), who described that corn yield and quality were improved with N fertilization up to an extent while further increase in fertilization cause decrease in corn attributes.

Grain N concentration and grain protein concentration were greater with the application of 3 kg HA ha^{-1} when combined with 80 and 160 kg N ha^{-1} (Figures 2C and D). However, grain N and grain protein concentrations were greater with the application of 4.5 kg HA ha^{-1} when combined with 200 kg N

Table 2. Plant height, straw N, grain N and grain protein concentrations, soil organic matter, soil total N, shoot biomass, harvest index, and corn grain yield according to humic acid and N doses

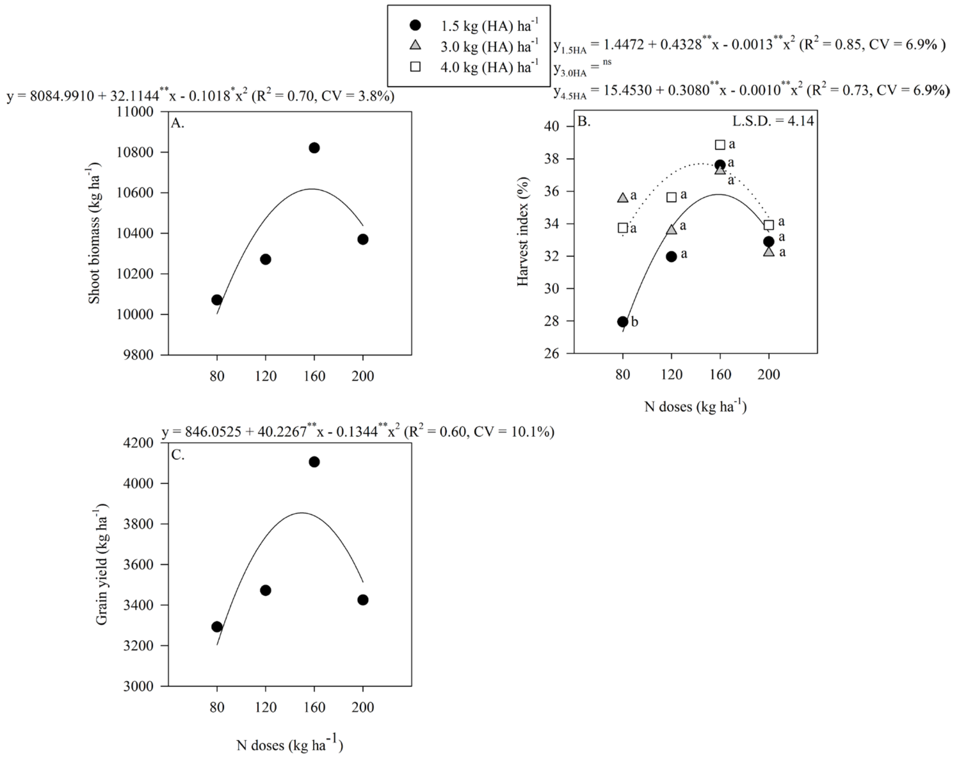
	Plant height (cm)	Straw N concentration	Grain N concentration	Grain protein concentration	Soil organic matter	Soil total N	Shoot biomass	Harvest index	Grain yield
		(%)				(%)	($kg\ ha^{-1}$)	(%)	($kg\ ha^{-1}$)
Humic acid doses ($kg\ ha^{-1}$)									
1.5	207 b†	1.04 b	1.52	9.52	0.63	0.28	10051 b	32.6	3299 b
3.0	217 b	1.09 ab	1.65	10.30	0.79	0.30	10489 a	34.6	3642 a
4.5	231 a	1.13 a	1.57	9.79	0.83	0.31	10610 a	35.5	3780 a
LSD	12.86	0.06	0.08	0.50	0.08	0.02	346	2.07	316
Nitrogen doses ($kg\ ha^{-1}$)									
80	212	0.96	1.40	8.76	0.54	0.26	10071	30.9	3292
120	214	1.01	1.54	9.64	0.73	0.26	10271	32.5	3472
160	219	1.17	1.69	10.60	0.84	0.32	10821	36.2	4106
200	228	1.20	1.67	10.70	0.88	0.35	10370	32.2	3425
Control vs Factorial									
Control	169 b	0.73 b	1.06 b	6.63 b	0.46 b	0.25 b	8786 b	31.6 b	2756 b
Factorial	218 a	1.09 a	1.58 a	9.88 a	0.75 a	0.30 a	10383 a	34.3 a	3574 a
F-test									
Humic acids (HA)	11.305**	7.602**	8.127**	8.090**	4.111*	10.131**	5.282**	6.367**	7.417**
N doses (N)	3.137*	9.025**	6.063**	6.039**	6.632**	9.377**	4.626**	13.07**	11.908**
HA × N	1.498ns	0.348ns	10.092**	10.323**	7.974**	5.482**	1.030ns	2.693*	2.226ns
Control vs Factorial	44.558**	30.557**	21.404**	21.828**	21.722**	20.482**	31.787**	16.321**	10.127**

Means in the column followed by different letters are significantly different at p ≤ 0.05 by Tukey test; **, *, ns - Significant at p ≤ 0.01 and p ≤ 0.05, and not significant, respectively, by F-test; LSD - Least significant difference



Different letters correspond to a significant difference at p ≤ 0.05 by Tukey test; *, ** and ns - Significant at p ≤ 0.01 and p ≤ 0.05, and not significant by F test, respectively

Figure 2. Plant height (A) and straw N concentration (B) according to N doses, the interaction between humic acid doses and N doses in grain N (C), grain protein (D), soil organic matter (E), and soil total N (F) concentration



Different letters correspond to a significant difference at $p \leq 0.05$ by Tukey test; *, ** and ns - Significant at $p \leq 0.01$ and $p \leq 0.05$, and not significant by F test, respectively

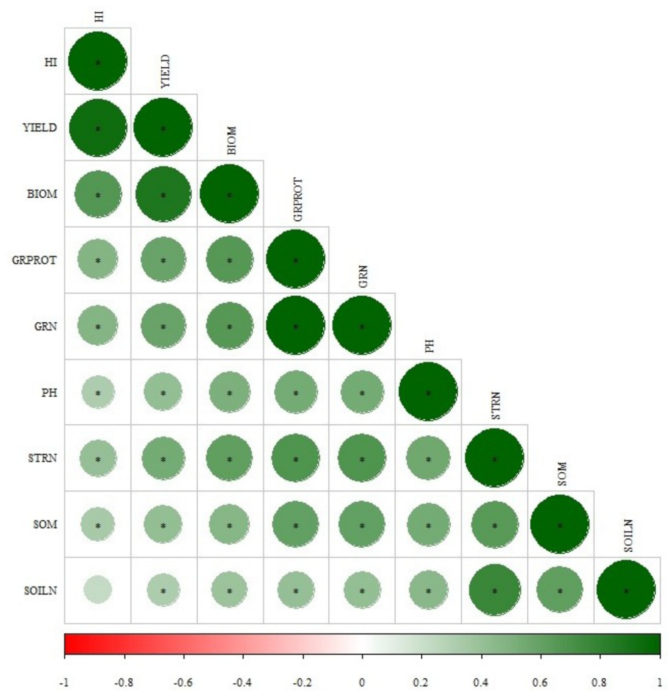
Figure 3. Shoot biomass (A) and corn grain yield (C) according to N doses, and interaction between humic acid doses and N doses in harvest index (B)

ha^{-1} (Figures 2C and D). Both grain N concentration and grain protein concentration responded non-linearly to increasing N doses up to 173 and 191 kg N ha^{-1} when 1.5 kg HA ha^{-1} was applied (Figures 2C and D). In contrast, grain N concentration and grain protein concentration responded linearly to N doses when 4.0 kg HA ha^{-1} was applied (Figures 2C and D). Niaz et al. (2016) indicated that integrated application of HA and N prominently improved N utilization in plant and soil with greater production of corn in calcareous soils.

Soil organic matter was greater with the application of 4.5 kg HA ha^{-1} when combined with 120 kg N ha^{-1} (Figure 2E). Additionally, organic matter was lower with the application of 1.5 kg HA ha^{-1} when combined with 160 and 200 kg N ha^{-1} (Figure 2E). Soil organic matter responded linearly to N application doses when 3.0 kg HA ha^{-1} was applied; however, it responded non-linearly when 4.5 kg HA^{-1} was applied (up to 170 kg N ha^{-1}) (Figure 2E). Similarly, soil total N was lower with the application of 1.5 kg HA ha^{-1} when combined with 120 and 160 kg N ha^{-1} (Figure 2F). Soil N responded non-linearly to increasing N doses when 1.5 and 4.5 kg HA ha^{-1} were applied (up to 180 and 179 kg N ha^{-1}, respectively) (Figure 2F). Also, soil total N responded linearly to N doses when 3.0 kg HA ha^{-1} was applied (Figure 2F). Also, the harvest index was lower with the application of 1.5 kg HA ha^{-1} when combined with 80 kg N ha^{-1} (Figure 3B). Harvest index responded linearly to increasing N doses when 1.5 kg HA ha^{-1} was applied and non-linearly when 4.5 kg HA ha^{-1} was applied (up to 154 kg N ha^{-1}), respectively (Figure 3B).

Humic acid is a bio-stimulant substance that can improve biochemical and physical properties of soil with increased uptake of macro and micronutrients (Shah et al., 2018b; El-Howeity et al., 2019), in especial N, which result in improved several physio-biochemical functions of plants (Olaetxea et al., 2018). The physiological effect of HA is partially explained by the structural resemble of them to plant hormones such as auxins, which participate in cell expansion and root initiation, among other physiological effects (Baldotto et al., 2019). Plants obtain N from organic and inorganic sources and are functional units of amino acid, protein and nucleic acid, and structural unit of chlorophyll and cell wall, influencing plant metabolism (Krapp, 2015; Gojon, 2017; Ghimire et al., 2017). Several studies exhibited that HA can reduce the harsh impacts of stress and enhanced plant development (Olaetxea et al., 2018; Shah et al., 2018b; El-Howeity et al., 2019). Also, HA can stimulate seed germination, nutrient uptake and accelerates root and shoot growth (Baldotto et al., 2019). According to Khan (2019), integrated application of HA and N can increase the growth and development of corn plants. Therefore, HA application combined with N doses could increase SOM, N concentration, and N uptake in the corn plant, reflecting in increased plant growth and grain yield as verified in this study. The positive Pearson's correlation between SOM, N concentration, N uptake, grain yield, and yield components support the hypothesis of the present study that the application of HA combined with N doses would benefit corn development and grain yield by increasing organic matter and total N availability in soil, leading to a higher N uptake (Figure 4).

Nitrogen is the most demanded nutrient by corn crops and directly affects crop development and yield (Lollato et al., 2019). This nutrient plays an important role in plant nutrition and development (Lollato et al., 2019), such as synthesis and production of phytohormones, coenzymes, nucleic acids, secondary metabolites, chlorophyll, and proteins (Krapp, 2015; Gojon, 2017; Ghimire et al., 2017). There is a lot of studies



STRN - Straw N concentration; GRN - Grain N concentration; GRPROT - Grain protein concentration; PH - Plant height; SOM - Soil organic matte; SOILN - Soil total N; BIOM - Shoot biomass; HI - Harvest index and YIELD - Grain yield

Figure 4. Heatmap showing the Pearson's correlation among plant height, straw N, grain N and grain protein concentration, soil organic matter, soil N concentration, shoot biomass, harvest index, and corn grain yield

reporting the benefits of N fertilization in different cereal crops such as corn (Ichami et al., 2019), wheat (Omara et al., 2020), and rice (Tatsumi et al., 2019). The optimum amount of N required for best cereal production depends on many factors, including soil type, indigenous nutrient concentration in the soil, weather conditions, and others (Sanchez et al., 2019). Therefore, studies aiming at determining the best N management practices related to N doses combined with HA application should be performed under different soil and weather conditions.

Conclusions

1. Regardless of the doses, the application of humic acids (HA) provided positive effects on soil organic matter (SOM), N concentration, N uptake, corn development, and grain yield.

2. The application of 4.5 kg HA ha^{-1} was the most effective in promoting SOM and total N, corn growth, N uptake, and grain yield alone and even in combination with N doses.

3. Increasing N doses positively influenced SOM, N concentration, N uptake, corn growth, and grain yield. The greater corn grain yield was obtained with 150 kg N ha^{-1} regardless of HA applied doses.

4. Is was verified positive improvements in corn production variables and N uptake due to HA application in combination with split N doses.

5. Application of 4.5 kg HA ha^{-1} and 150 kg N ha^{-1} is recommended for improved corn growth, grain yield, and overall plant and soil N concentration in the Peshawar region of Pakistan.

Literature Cited

AOAC. Association of Official Analytical Chemists. Official methods of analysis. Washington, DC: AOAC, 1992. 1115p.

Arif, M.; Ilyas, M.; Riaz, M.; Ali, K.; Shah, K.; Haq, I. U.; Fahad, S. Biochar improves phosphorus use efficiency of organic-inorganic fertilizers, maize-wheat productivity and soil quality in a low fertility alkaline soil. Field Crops Research, v.214, p.25-37, 2017. https://doi.org/10.1016/j.fcr.2017.08.018

Aslam, M.; Iqbal, A.; Zamir, M.S.; Mubeen, M.; Amin, M. Effect of different nitrogen levels and seed rates on yield and quality of maize fodder. Crop Environment, v.2, p.47-51, 2011.

Azeem, K.; Shah, S.; Ahmad, N.; Shah, S.T.; Khan, F.; Arafat, Y.; Naz, F.; Azeem, I.; Ilyas, M. Physiological indices, biomass and economic yield of maize influenced by humic acid and nitrogen levels. Russian Agricultural Sciences, v.41, p.115-119, 2015. https://doi.org/10.3103/S1068367415020020

Baldotto, M. A.; Melo, R. O.; Baldotto, L. B. Field corn yield in response to humic acids application in the absence or presence of liming and mineral fertilization. Semina: Ciências Agrárias, v.40, p.3299-3304, 2019. https://doi.org/10.5433/1679-0359.2019v40n6Supl2p3299

Bijanzadeh, E.; Naderi, R.; Egan, T. P. Exogenous application of humic acid and salicylic acid to alleviate seedling drought stress in two corn (*Zea mays* L.) hybrids. Journal of Plant Nutrition, v.42, p.1483-1945, 2019. https://doi.org/10.1080/01904167.2019.1617312

Bremner, J. M.; Mulvaney, C. S. Kjeldhal method. In: Method of soil analysis. Part-2: Chemical & microbiological properties. Madison: American Society of Agronomy, 1982. p.903-948.

El-Howeity, M. A.; Abdel-Gwad, S. A.; Elbaalawy, A. M. Effect of bio-organic amendments on growth, yield, nodulation status, and microbial activity in the rhizosphere soil of peanut plants under sandy soil conditions. Journal of Soil Sciences and Agricultural Engineering, v.10, p.299-305, 2019. https://doi.org/10.21608/jssae.2019.43220

Ghimire, B.; Riley, W. J.; Koven, C. D.; Kattge, J.; Rogers, A.; Reich, P. B.; Wright, I. J. A global trait-based approach to estimate leaf nitrogen functional allocation from observations. Ecological Applications, v.27, p.1421-1434, 2017. https://doi.org/10.1002/eap.1542

Gojon, A. Nitrogen nutrition in plants: Rapid progress and new challenges. Journal of Experimental Botany, v.68, p.2457-2462, 2017. https://doi.org/10.1093/jxb/erx171

Ichami, S. M.; Shepherd, K. D.; Sila, A. M.; Stoorvogel, J. J.; Hoffland, E. Fertilizer response and nitrogen use efficiency in African smallholder maize farms. Nutrient Cycling in Agroecosystems, v.113, p.1-19, 2019. https://doi.org/10.1007/s10705-018-9958-y

Jalal, A.; Azeem, K.; Teixeira Filho, M. C. M.; Khan, A. Enhancing soil properties and maize yield through organic and inorganic nitrogen and diazotrophic bacteria. In: Sustainable crop production. London: IntechOpen, 2020. p.165-178. https://doi.org/10.5772/intechopen.92032

Khaliq, T.; Mahmood, T.; Masood, A. Effectiveness of farm yard manure, poultry manure and nitrogen for corn productivity. International Journal of Agriculture and Biology, v.2, p.260-263, 2004.

Khan, S. A.; Khan, S. U.; Qayyum, A.; Gurmani, A. R.; Khan, A.; Khan, S. M.; Ahmed, W.; Mehmood, A.; Amin, B.A.Z. Integration of humic acid with nitrogen wields an auxiliary impact on physiological traits, growth and yield of maize (*Zea mays* L.) varieties. Applied Ecology and Environmental Research, v.17, p.6783-6799, 2019. https://doi.org/10.15666/aeer/1703_67836799

Krapp, A. Plant nitrogen assimilation and its regulation: a complex puzzle with missing pieces. Current Opinion in Plant Biology, v.25, p.115-122, 2015. https://doi.org/10.1016/j.pbi.2015.05.010

Liu, M.; Wang, C.; Wang, F.; Xie, Y. Maize (*Zea mays* L.) growth and nutrient uptake following integrated improvement of vermicompost and humic acid fertilizer on coastal saline soil. Applied Soil Ecology, v.142, p.147-154, 2019. https://doi.org/10.1016/j.apsoil.2019.04.024

Lollato, R. P.; Figueiredo, B. M.; Dhillon, J. S.; Arnall, D. B.; Raun, W. R. Wheat grain yield and grain-nitrogen relationships as affected by N, P, and K fertilization: A synthesis of long-term experiments. Field Crops Research, v.236, p.42-57, 2019. https://doi.org/10.1016/j.fcr.2019.03.005

McLean, E. O. Soil pH and lime requirement. In: Methods of soil analysis: Part 2 - Chemical and microbiological properties. Madison: American Society of Agronomy, 1983. p.199-224. https://doi.org/10.2134/agronmonogr9.2.2ed.c12

Morozesk, M.; Bonomo, M. M.; Souza, I. da C.; Rocha, L. D.; Duarte, I. D.; Martins, I. O.; Dobbss, L. B.; Carneiro, M. T. W. D.; Fernandes, M. N.; Matsumoto, S. T. Effects of humic acids from landfill leachate on plants: An integrated approach using chemical, biochemical and cytogenetic analysis. Chemosphere, v.184, p.309-317, 2017. https://doi.org/10.1016/j.chemosphere.2017.06.007

Nardi, S.; Pizzeghello, D.; Gessa, C.; Ferrarese, L.; Trainotti, L. A low molecular weight humic fraction on nitrate uptake and protein synthesis in maize seedlings. Soil Biology & Biochemistry, v.32, p.415-419, 2000. https://doi.org/10.1016/S0038-0717(99)00168-6

Nelson, D. W.; Sommer, L. E. Total C, organic C and organic matter. In: Spark, D. L. (ed.). Method of soil analysis. Part 3: Chemical methods. Madison: American Society of Agronomy, 1996. p.961-1010. https://doi.org/10.2136/sssabookser5.3.c34

Niaz, A.; Yaseen, M.; Shakar, M.; Sultana, S.; Ehsan, M.; Nazarat, A. Maize production and nitrogen use efficiency in response to nitrogen application with and without humic acid. Journal of Animal and Plant Sciences, v.26, p.1641-1651, 2016.

Öktem, A.; Çelik, A.; Öktem, A.G. Effect of humic acid seed treatment on yield and some yield characteristic of corn plant (*Zea mays* L. indentata). Journal of Agricultural, Food and Environmental Sciences, v.72, p.142-147, 2018.

Oktem, A.; Oktem, A. G.; Emeklier, H. Y. Effect of nitrogen on yield and some quality parameters of sweet corn. Communications in Soil Science and Plant Analysis, v.41, p.832-847, 2010. https://doi.org/10.1080/00103621003592358

Olaetxea, M.; Hita, D. de; Garcia, C. A.; Fuentes, M.; Baigorri, R.; Mora, V.; Garnica, M.; Urrutia, O.; Erro, J.; Zamarreño, A. M.; Berbara, R. L. Hypothetical framework integrating the main mechanisms involved in the promoting action of rhizospheric humic substances on plant root-and shoot-growth. Applied Soil Ecology, v.123, p.521-537, 2018.

Omara, P.; Aula, L.; Dhillon, J. S.; Oyebiyi, F.; Eickhoff, E. M.; Nambi, E.; Fornah, A.; Carpenter, J.; Raun, W. Variability in winter wheat (*Triticum aestivum* L.) grain yield response to nitrogen fertilization in long-term experiments. Communications in Soil Science and Plant Analysis, v.51, p.403-412, 2020. https://doi.org/10.1080/00103624.2019.1709489

R Core Team. A: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2015. Available on: https://www.r-project.org/ . Accessed on: Dec. 2019.

Sanchez, I. I.; Fultz, L. M.; Lofton, J.; Haggard, B. Soil biological response to integration of cover crops and nitrogen rates in a conservation tillage corn production system. Soil Science Society of America Journal, v.83, p.1356-1367, 2019. https://doi.org/10.2136/sssaj2019.02.0051

Shah, S.; Hussain, M.; Jalal, A.; Khan, M. S.; Shah, T.; Ilyas, M.; Uzair, M. Nitrogen and sulfur rates and timing effects on phenology, biomass yield and economics of wheat. Sarhad Journal of Agriculture, v.34, p.671-679, 2018a. https://doi.org/10.17582/journal.sja/2018/34.3.671.679

Shah, Z.; Ahmad, M. I. Effect of integrated use of farm yard manure and urea on yield and nitrogen uptake of wheat. Journal of Agriculture and Biological Science, v.1, p.60-65, 2006.

Shah, Z. H.; Rehman, H. M.; Akhtar, T.; Alsamadany, H.; Hamooh, B. T.; Mujtaba, T.; Daur, I.; Al Zahrani, Y.; Alzahrani, H. A.; Ali, S.; Yang, S. H. Humic substances: Determining potential molecular regulatory processes in plants. Frontiers in Plant Science, v.9, p.263, 2018b. https://doi.org/10.3389/fpls.2018.00263

Sharif, M.; Khattak, R. A.; Sarir, M.S. Residual effect of humic acid and chemical fertilizers on maize yield and nutrient accumulation. Sarhad Journal of Agriculture, 19, 543-550, 2003.

Soil Survey Staff. Keys to soil taxonomy, 12.ed. USDA. Natural Resources Conservation Service, Washington, DC, 2014.

Soltanpour, P. N.; Schwab, A. P. A new soil test for simultaneous extraction of macro-and micro-nutrients in alkaline soils. Communications in Soil Science and Plant Analysis, v.8, p.195-207, 1977. https://doi.org/10.1080/00103627709366714

Tagar, S.; Bhatti, A. Physical properties of soil. Soil Science, p.130-140, 2001.

Tatsumi, K.; Abiko, T.; Kinose, Y.; Inagaki, S.; Izuta, T. Effects of ozone on the growth and yield of rice (*Oryza sativa* L.) under different nitrogen fertilization regimes. Environmental Science and Pollution Research, v.26, p.32103-32113, 2019. https://doi.org/10.1007/s11356-019-06358-6

Xia, L.; Lam, S.K.; Chen, D.; Wang, J.; Tang, Q.; Yan, X. Can knowledge-based N management produce more staple grain with lower greenhouse gas emission and reactive nitrogen pollution? A meta-analysis. Global Change Biology, v.23, p.1917-1925, 2017. https://doi.org/10.1111/gcb.13455