POTASSIUM HUMATE AMENDMENT REGULATES SOIL NPK SUPPLY AND GROWTH PARAMETERS OF POTATO (SOLANUM TUBEROSUM L.) IN A CALCAREOUS SOIL

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Abstract

Constrained nutrient supplies to plants grown in calcareous mineral soils are expected to diminish faster under a climate change scenario; therefore, we examined the potato vegetative and physiological responses to potassium (K) humate soil amendment applied in addition to the recommended doses of nitrogen-phosphorus-potassium (NPK) fertilizers. Hypothetically, K-humate regulates or improves NPK soil supplies and plant uptakes and hence, growth parameters of potato. Field experiments were conducted on potato (cv. Cardinal) grown at the Vegetable Research Farm, Bahauddin Zakariya University, Multan, Pakistan for two autumns (2010-2011). We used randomized complete block design with three repeats each of the NPK (0, 50, 75 and 100% of recommended dose: 120-80-80 kg ha⁻¹) and K-humate (0, 8, 12 and 16 kg ha⁻¹; 30% K) treatments. *Individual* soil NPK or K-humate application significantly improved ($p \le 0.05$) leaf chlorophyll content, final plant height, photosynthetic rate, stomatal conductance (S_c) and water-use-efficiency (WUE). We observed a significant interaction between NPK and K-humate for explaining additional significant improvements in these parameter values (except Sc; leaf chlorophyll 23%, plant height 41%, photosynthetic rate 34%, WUE 37%) as well as those of the number of leaves (55%) and leaf area (38%) per hill, and transpiration rate (27%). However, leaf chlorophyll content values in responses to 75 and 100% recommended doses of NPK did not differ significantly. Therefore, we conclude that K-humate can be used in calcareous soils in addition to the NPK fertilizer doses to mitigate the adverse effects of calcareousness (responsible for constraining the NPK uptake) by regulating the NPK soil supplies and plant uptakes, which improve potato vegetative and physiological growth parameters.

Key words: Humate, Nutrients, Physiological, Cardinal, Vegetative.

Introduction

Potato (Solanum tuberosum L.) is the third most important food crop worldwide regarding human consumption and is critical to global sustainable food security (Strange & Scott, 2005). In South Asia, current arid or semi-arid climate and calcareous nature of soils constrain the availability of nitrogen (N), phosphorus (P) and potassium (K) minerals to potato plant (Leytem & Mikkelsen, 2005). Calcareous soils accumulate significant free excess lime (calcium and/or magnesium carbonate) concretions and therefore limit fertilizer use efficiency (FUE) in soils and mineral nutrient availability to plants (Leytem & Mikkelsen, 2005). Moreover, soils under agricultural or forest ecosystems are expected to be drier and warmer under future climate scenarios (Strack et al., Munir et al., 2017a) which could pose an incremental risk to potato production in South Asian regions. While the effects of soil amendments on mineral nutrients availabilities are extensively studied, controlled field experimentation for testing K humate influence on calcareous soil N, P, and K availabilities and potato vegetative and physiological parameters remains limited.

Plant roots uptake mineral nutrients from soil solution in ionic forms: N as nitrate (NO₃⁻) or ammonium (NH₄⁺), P as phosphate (PO₄²⁻), and K as potash (K₂O⁺). The NPK are essential, and mobile nutrients (except P), and are usually the most limiting in calcareous soils of arid regions (Singh *et al.*, 2007). They play integral roles directly or indirectly in most plant growth processes, such

as chlorophyll synthesis, enzyme production, stomatal conductance (S_c), and WUE (Kanbi & Bhatnagar, 2005; Yan *et al.*, 2020).

The extreme temperature during summer diminishes soil organic matter (OM) (Munir *et al.*, 2015); that is why most calcareous soils in arid regions (South Asia) are deficient in OM (< 1%), and therefore, poor in health and structure. Humic substances could be alternative sources of OM and are frequently reported to improving soil chemical and biological properties, and plant growth and physiology (Brar *et al.*, 2000; Diacono & Montemurro, 2011). They are also found to improve soil physical condition, act like hormones, stimulate root and shoot development, and enhance plant nutrient uptake (Younis *et al.*, 2015; Abid *et al.*, 2017; Danish & Zafar-ul-Hye, 2019).

Potassium humate, a potassium salt of humic acid, has a very high humic acid content (~ 70%), and is used as a soil and plant amendment in combination with basal NPK fertilizer dose to regulate the bioavailability of nutrients (Khadka *et al.*, 2016; Mohammed & Saeid, 2020), and enhance plant biogeochemical mechanism rates such as photosynthesis (Mostafa, 2011; Sarwar & Shahbaz, 2020) to increase plant productivity. The combined application is a management principle to sustain soil fertility as well as crop production (Geerts & Raes, 2009). Hence a study aimed at investigating the combined NPK and K humate impact on potato vegetative and physiological responses was conducted on autumn potato crops in 2010-2011. We hypothesized that improvement in potato vegetative and physiological parameters in response to combined NPK and K humate application would be higher compared to individual applications. Our specific objectives were to analyze the combined soil NPK and K humate application effects on:

- 1. Vegetative responses (aerial stems, leaves and leaf area per hill, leaf chlorophyll content, and final plant height), and
- 2. Physiological responses [photosynthesis rate (P_r), transpiration rate (T_r), stomatal conductance (S_c) and water-use-efficiency (WUE)] of potato plant (cv. Cardinal) in calcareous soil.

Materials and Methods

Study area: During autumn cropping seasons of 2010-2011, experiments were conducted on potato (*Solanum tuberosum* L.) crop (cv. Cardinal) at the Vegetable Research Farm ($30^{\circ}15'49''$ N, $71^{\circ}30'35''$ E; elevation 411 m above sea level), Department of Horticulture, Bahauddin Zakariya University, Multan, Pakistan. According to climate data obtained from a meteorological station 9 km South-West at Central Cotton Research Institute Multan, the 30-yr (1981-2010) mean annual minimum and maximum air temperatures in this region are 10.1°C and 35.6°C, respectively, with mean annual precipitation of 80 mm. The textural and chemical analysis of soil is presented in Table 1. The soil was strongly calcareous with 6-10% (mean; n = 16) calcium carbonate equivalent, as determined by a field test (Luttmerding *et al.*, 2010).

Experimental design: A cultivated land area of 16335 ft² was chosen, equally divided into 48 plots (16 treatments \times 3 replications; each plot = 340 ft^2) laid out in a triplicate randomized complete block design (RCBD), and prepared for potato seeding following Beukema & Zaag (1990). The plots were applied with three repeats of 16 treatment combinations (4×4) of NPK (0, 50, 75 and 100% of the recommended nutrient doses) and K humate (0, 8, 12, and 16 kg ha⁻¹; 30% K). The recommended (best) mineral N, P₂O₅, and K₂O doses for potato crop in this region are 120 (N), 80 (P), and 80 (K) kg ha⁻¹. Potato crop yield rarely responds to higher than recommended nutrient doses (e.g., Trehan et al., 2001; Güler, 2009). The N, P, and K nutrient sources used for soil application were urea (46% N), single superphosphate (18% P₂O₅) and sulphate of potash (50% K_2O), respectively. Full doses of P_2O_5 and K_2O , and $1/3^{rd}$ doses of N and K humate were incorporated to the soil at the seedbed preparation stage, and the remaining amounts each of N and K humate were applied in two split doses after 30 and 60 days of seeding. Standard crop management practices such as irrigation, weeding and plant protection were adapted following Brar et al., (2000) to grow the crops during the study years.

Autumn potato tubers were planted during the third week of September and harvested during the third week of January. Responses of vegetative (aerial stems, leaves and leaf area per hill, leaf chlorophyll content, final plant height), and physiological parameters (P_r , T_r , S_c , and WUE) to individual and combination treatments were measured for two study seasons.

Methodologies

Vegetative parameters: Seventy-five days old, ten plants were randomly selected in each plot for assessments of vegetative parameters per plot and treatment. Counting determined the numbers of aerial stems and leaves per hill. Leaf chlorophyll content in term of SPAD value (soil plant analysis development) was measured on the third leaf from the top and ~0.5 cm from leaf tip by using a chlorophyll meter (SPAD-502, Minolta, Japan) following Chang and Robison (2003). Leaf-area per hill was measured with a portable leaf area meter (LI-3000C, Lincoln, Nebraska USA). Final plant height from the soil surface to the last extended mature leaf was measured using a metering rod, and the respective averages per plot and treatment were computed.

Physiological parameters: Plants measured for vegetative responses were also assessed for physiological parameters: gas exchange characteristics such as P_r , T_r , and S_c were evaluated using a CI-340 Handheld Portable Photosynthesis System (CID Bio-Science Inc, WA, USA). The WUE was calculated as, WUE = P_r/T_r . All physiological measurements were made on a leaf surface area of ~6.3 cm² during 0930 to 1150 h, and under the defined ranges of the atmospheric conditions: pressure = 99.04-99.24 kPa, photosynthetically active radiation (PAR) = 1880-1892 µmol m⁻² s⁻¹, and leaf chamber temperature = 33.1-41.3°C.

Statistical analysis: All data were statistically analyzed using Statistix® 8.1 software (Tallahassee, FL 32317, USA). A repeated measure analysis of variance (ANOVA) with an RCBD was used to evaluate the individual and interactive effects of four NPK fertilizer and four K humate treatments on five vegetative and four physiological variables of the potato crop. Since the same plots were measured in each of the study seasons (2010-2011) for the response variables, the year was taken as repeated measures within the same model. Between-years variables values were virtually the same; therefore, the year was dropped as a fixed effect and considered only as a repeated measure. Difference between treatment means was compared by Tukey's honest significance difference test at $p \le 0.050$.

Table 1. Physicochemical characteristics and mineral nutrient status of the study soil.

Soil depth (cm)	Texture (class)	Organic matter (%)	рН	ECe (dSm ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Calcareousness (CaCO ₃ equivalent) (%)
0-15	Clay Loam	0.63	8.30	2.01	6.9	140	8.4
15-30		0.41	8.24	2.00	5.7	121	9.1

Values are mean (n = 28). Soil samples were collected prior to seedbed preparation

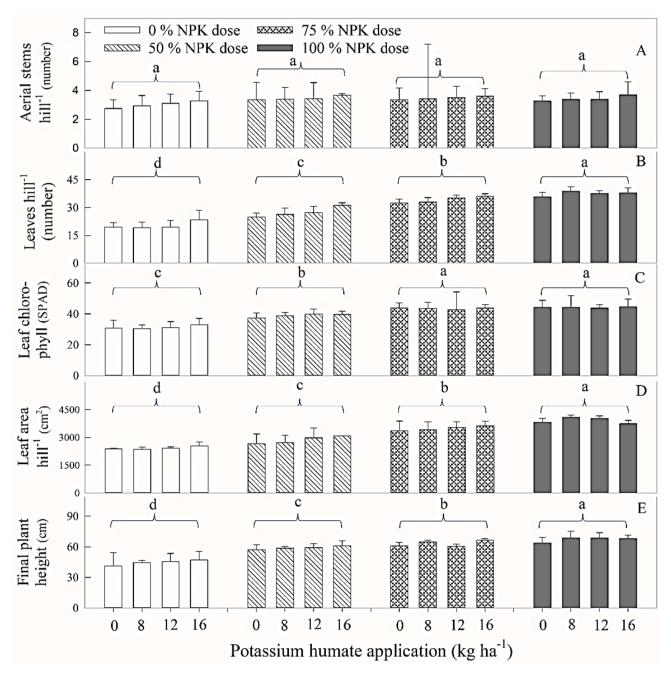


Fig. 1. Mean values of vegetative parameters: A) aerial stems per hill, B) leaves per hill, C) leaf chlorophyll content, D) leaf area per hill, and E) final plant height, in response to NPK (0, 50, 75, 100% of recommended dose – 120-80-80 kg ha⁻¹) and potassium humate (0, 8, 12, 16 kg ha⁻¹) treatments, individual and combined. Each bar shows \pm SD of the mean, n = 20 (10 plants \times 2 years).

Results

Overall, there was an NPK-K humate interaction resulting in significant crop vegetative and physiological responses (p = 0.041, 0.037, respectively) over two growing seasons (2010-2011). Individual and interactive effects of NPK and K humate treatments on plant vegetative and physiological parameters are provided:

Vegetative parameters

Aerial stems per hill: The number of aerial stems per hill did not respond to applied NPK doses (0, 50, 75, 100% of 120-80-80 kg ha⁻¹; Fig. 1A; Table 2), compared to a significant positive response to K humate (a soil

conditioner) when applied in addition to recommended NPK doses (Fig. 1A; Table 2). The maximum numbers of aerial stems per hill were recorded for the plots applied with the highest and second highest doses of K humate, i.e., 16 kg ha⁻¹ and 12 kg ha⁻¹, respectively.

Leaves and leaf area per hill, leaf chlorophyll content, final plant height: Generally, the soil applications of NPK levels significantly increased the leaf chlorophyll content and final plant height measured after 75 days of potato crop planting (Fig. 1C, 1E; Table 2); however, no significant responses of numbers of leaves and leaf area per hill measured at the end of the two growing seasons were noticed in response to the NPK doses (Fig. 1A, 1C; Table 2). Application of K humate in addition to recommended NPK doses significantly increased all the studied potato vegetative parameters except aerial stems per hill (Fig. 1A-AE; Table 2). Although general linear increases in all parameters' values in responses to the gradient of NPK applications from 0-100% were observed; however, no significant differences in leaf chlorophyll content in responses to 75% and 100% recommended NPK doses were found (Fig. 1C).

Physiological parameters: The NPK levels significantly enhanced P_r , Sc, and WUE (Fig. 2A, 2C, 2D; Table 2) with largest values in plants grown under highest dose of NPK (100% of the recommended dose) and minimum in control treatment. However, there were exceptions: no differences in T_r and WUE responses to 50% and 75% of recommended NPK doses, and S_c responses to no NPK and 50% recommended NPK doses could be found.

Application of K humate in addition to NPK, significantly improved P_r and WUE among measured

physiological parameters (Fig. 2; Table 2). There was an interactive effect of NPK and K humate to significantly increase P_r , T_r and WUE of the crop leaves. The minimum or no increases in P_r , T_r , Sc and WUE were recorded from plants grown under control conditions (without NPK and K humate), while the maximum values were recorded from those grown with the highest or 2^{nd} highest levels of both NPK (100%, 75% of recommended dose) and K humate (12, 16 kg ha⁻¹).

Relationships of NPK and K humate soil applications with growth parameters: The individual and interactive relationships of NPK and K humate soil applications with the vegetative and physiological parameters are shown in Fig. 3A and 3B and Table 2. The NPK levels (without K humate) were found significantly correlated with leaf chlorophyll content, final plant height, P_r , S_c and WUE. Addition of K humate significantly improved all the growth parameters except aerial stems per hill and S_c .

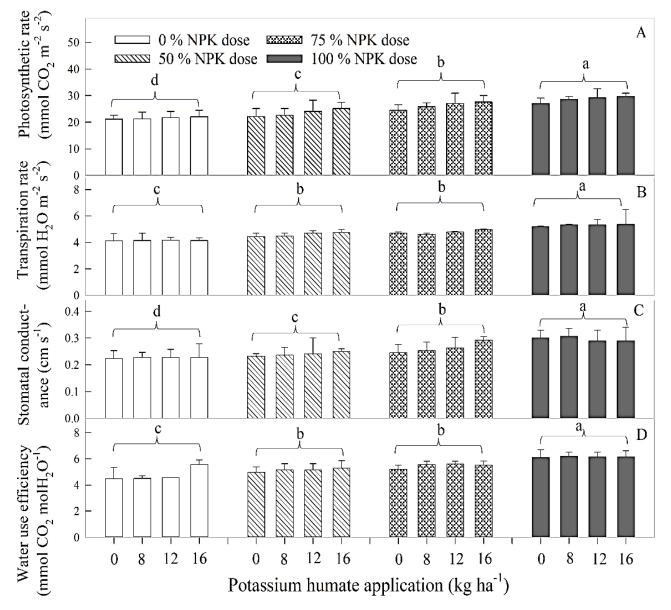


Fig. 2. Mean values of potato physiological parameters: A) photosynthesis rate, B) transpiration rate, C) stomatal conductance, and (D) water-use-efficiency in response to NPK (0, 50, 75, 100% of recommended dose – 120-80-80 kg ha⁻¹) and potassium humate (0, 8, 12, 16 kg ha⁻¹) treatments, individual and combined. Each bar shows \pm SD of the mean, n = 20 (10 plants × 2 years).

parameters of potato (cv. Cardinal) crop grown in calcareous soil during autumn 2010-2011

 $= -0.8 + 0.02 x_1 + 0.03 x_2; p = 0.073$ $y = 0.4 + 0.02 x_1 + 0.01 x_2; p = 0.194$ $y = 1.2 + 0.04 x_1 + 0.05 x_2; p = 0.020$ $y = 1.5 + 0.02 x_1 + 0.03 x_2; p = 0.024$ $y = 3.5 + 4.72 x_1 + 0.04 x_2; p = 0.049$ $y = 1.6 + 0.02 x_1 + 0.03 x_2; p = 0.026$ $= 0.6 + 0.02 x_1 + 0.03 x_2; p = 0.009$ $y = 0.6 + 0.02 x_1 + 0.03 x_2; p = 0.001$ $y = 1.2 + 0.02 x_1 + 0.03 x_2; p = 0.001$ (% recommended dose, kg ha⁻¹) NPK + Potassium Humate Values in bold are significant at $p \le 0.050$ $\mathbf{x_1} + \mathbf{x_2}$ У The recommended dose of NPK in the region is 120-80-80 kg ha⁻¹ $-0.8 + 0.06 x_2$; p = 0.225 $y = 1.4 + 0.06 x_2; p = 0.046$ $y = 3.3 + 0.07 x_2$; p = 0.217 $y = 1.6 + 0.06 x_2; p = 0.037$ $0.5 + 0.06 x_2; p = 0.035$ $y = 0.4 + 0.02 x_2; p = 0.225$ $y = 1.2 + 0.06 x_2; p = 0.039$ $= 0.5 + 0.06 x_2; p = 0.081$ $y = 1.2 + 0.09 x_2; p = 0.061$ **Potassium Humate** (kg ha⁻¹) X2 - || $y = 1.5 + 0.04 x_1; p = 0.043$ $= 1.6 + 0.04 \ x_1; \ p = 0.042$ = -0.8 + 0.04 x₁; p = 0.030 $y = 0.4 + 0.04 x_1$; p = 0.248 $y = 3.4 + 9.44 x_1$; p = 0.245 $y = 1.2 + 0.03 x_1$; p = 0.044 $= 0.6 + 0.03 x_1; p = 0.097$ $0.6 + 0.03 x_1; p = 0.026$ $= 1.2 + 0.07 x_1; p = 0.064$ (% recommended dose) were used. NPK × No significant difference between years (p = 0.871); therefore, mean values ₹ || λ λ У Water-use-efficiency (mmolCO2 molH2O⁻¹) Leaf chlorophyll content (SPAD value) Photosynthesis rate (μ mol CO₂ m⁻² s⁻¹) Transpiration rate (mmol $\rm H_2O~m^{-2}~s^{-1}$) Stomatal conductance (mmol $m^{-2} s^{-1}$) Aerial stems hill⁻¹ (number) Þ Final plant height (cm) Leaves hill⁻¹ (number) leaf area hill⁻¹ (cm²) Parameter

Discussion

Potato aerial stems per hill did not respond to NPK application; however, a significant interactive response to a subsequent application of K humate was observed. The parameter value depends primarily on the number of buds on the planted seed tuber, and we preferred the tubers with approximately an equal number of buds. Moreover, meagre amounts of NPK is taken up by the tubers at this early growth stage (Henricksen & Molgaard, 2005) due to low nutrient requirement and little root coverage. Only the soil conditioning agent (e.g., K humate) may result in an increased number of stems per hill at early potato growth stage due to favourable root penetration conditions (Noor et al., 2011). The phenomenon was also explained by Lambers et al., (2008) that the increase in aerial stems number, in response to K humate, was due to augmented physiological processes such as cell division and allocation of fresh carbon to new vascular tissue formation, which consequently enhanced the vegetative growth.

While the numbers of leaves and leaf area per hill did not respond to the soil application of NPK, significant improvements in these two parameters and leaf chlorophyll content and final plant height were recorded in response to a subsequent soil addition of K humate. Similar positive responses of the vegetative parameters to the increasing NPK gradient were attributed to the enhancement in soil nutrient supplies and plant uptake due to subsequent additions of humic substances (Henricksen & Molgaard, 2005; Khadka et al., 2015) that support vegetative growth and photosynthetic/ meristematic activity (Ewing, 1995; Singh et al., 2007), and accumulation of plant carbohydrates (Diacono & Montemurro, 2011) by improving soil structure and redox potentials across soilnutrient-root continuum. The observed stagnant chlorophyll content response to 100% of recommended NPK dose could be due to higher energy use for carbohydrate formation and transport and accumulation in tubers as elaborated by Goffart et al., (2008).

The crop plants showed the highest leaf chlorophyll content at the highest K humate application rate (16 kg ha⁻¹) similar to the findings of Kanbi & Bhatnagar (2005). The overall better performance of the vegetative characteristics under K humate applications could be due to its reported ability to enhance soil biological activity (Brar *et al.*, 2000), increase soil cation exchange capacity, improve NK fertilizer use efficiency, and promote root growth and plant height (Trehan *et al.*, 2001).

The increases in physiological responses along the increasing NPK gradient were due likely to the firm N control on photosynthetic and meristematic activities that result in enhancing the carbon sequestration and related P_r , S_c and, WUE activities (Van Loon, 1981). Rubisco formation also stimulates physiological parameters by using P (Cen & Sage, 2005) whose requirements in soil and plant would likely increase due to the introduction of high yielding cultivars (Güler, 2009) and the atmospheric enrichment of CO_2 in the face of climate change (Munir *et al.*, 2017b).

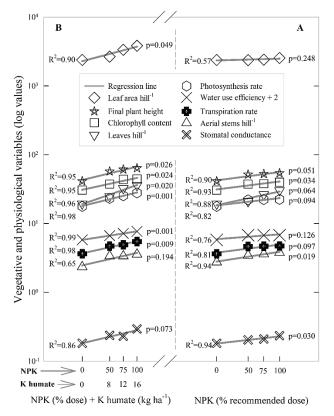


Fig. 3. 1:1 relationships of potato growth parameters with NPK treatments alone (A; 0, 50, 75, 100% of recommended dose – 120-80-80 kg ha⁻¹), and in combination with K humate (B; 0, 8, 12, 16 kg ha⁻¹). All relationships are significant at $p \le 0.05$. Each point is a mean, n = 20 (10 plants $\times 2$ years).

Our findings of incremental responses of potato growth parameters to K humate application in addition to recommended NPK doses support the reported positive responses of tomato yield to supplemental humic substances (Mostafa, 2011). In contrast, a few of the parameters values (aerial stems per hill and S_c) were not affected in response to the highest K humate level applied in addition to the maximum NPK dose. It is well established that leaf K nutrient primarily increases the stomatal conductance of CO2 by increasing the turgor pressure of stomata (Wang et al., 2013); however, further K supply (after maximum CO₂ conductance is reached) enhances the vapour conductance which results in decreased sensitivity of maximum CO2 uptake to increasing vapour conductance (Brodribb & Holbrook, 2006; Brodribb & McAdam, 2011). These findings of exponential CO2 conductance rates support the earlier established role of K in enhancing the physiological attributes of the leaf (gas exchange characteristics) in most plant types (Brodribb & McAdam, 2011).

The crop growth improvement in response to the added soil potassium amendment is due to its high mobility in both soil solution and plant root/stem/leaf (Kanbi & Bhatnagar, 2005). The K⁺ is the proton pump controlling ion at the root hair membrane and the most abundant univalent cation in plant cells. It plays a vital role in the regulation of P_r and T_r (Véry & Sentenac, 2003) which in turn is an essential factor in controlling WUE and S_c (Tuzet *et al.*, 2003); however, we did not observe improvement in S_c in response to additive

application of K humate which might be offset by the significant increases in leaf area and number of leaves per hill we observed in response to this amendment. Potassium humate is well known for conditioning the soil by improving the aggregate structure and water holding capacity (Mostafa, 2011) and hence supporting the shallow and inefficient potato root system known for a reduced ability to uptake nutrients and water from unconditioned calcareous soils.

Conclusions

Soil NPK application improves the leaf chlorophyll content, final plant height, photosynthesis rate, stomatal conductance and water-use-efficiency. Remarkably, the addition of K humate interacts with NPK minerals and results in incremental improvements in all studied vegetative and physiological parameters except aerial stems per hill and stomatal conductance. Therefore, to improve the potato vegetative and physiological parameters, K humate can be used in calcareous soils, in addition to NPK, to mitigate the adverse effects of calcareousness that constrain the uptake of NPK minerals and stress the potato plant growth parameters.

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