Effect of ph on growth of oil palm seedlings and basal stem rot disease incidence in ganoderma inoculated plants

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Abstract

A study was conducted to examine the effect of pH on growth of oil palm seedlings and basal stem rot disease incidence in *Ganoderma* inoculated plants. Total of 120 *Ganoderma* infected rubber wood blocks were allocated to four treatments (pH 5, 6, 7, and Control). Each treatment was replicated three times (10 plants per replicate). The results showed that disease incidence increased significantly from week 8 to 16 after inoculation with lower disease severity in the treated seedlings compared to control. Inoculated plants had lower growth, top, root dry weights and biomass yields per plant. The pH6G+ group appeared to have the lowest uptake for P, K, and Mg^{++}, whereas pH6G- has a greater uptake of N, P, Ca^{++}, and Mg^{++}. The pH5G+ displayed greater top dry weight while pH6G- showed lowest top dry weight per plant. In conclusion, pH levels reduced the growth and development of *Ganoderma* in oil palm seedlings.

Keywords: BSR, *Ganoderma boninense*, oil palm, pH, seedlings.

Introduction

Oil palm (*Elaeis guineensis Jacq.)* seedlings are predominantly prone to basal stem rot disease within few years after planting (Ariffin *et al.*, 2000). The expanse under oil palm husbandry has expanded rapidly in current years in Malaysia. With this fast increase in oil palm farming under monocultural settings, the incidence of basal stem rot (BSR) has continuous to be on the increase, especially along the coastal areas. A larger percentage of young oil palm seedlings have been reported to be attacked by *Ganoderma boninensis* in Peninsular Malaysia (Ho & Nawawi, 1985; Nawawi and Ho, 1990). The soil pH plays a crucial role in growth and development of *Ganoderma boninensis* in oil palm seedlings. The pH level in Malaysia soil as a result of mineral acid soils ranges from 3 to 5 (Shamshuddin *et al*., 2011). High salinity and low pH appear to suppress the incidence of BSR disease in oil palm (Gurmit, 1991). However, under laboratory environments, *Ganoderma boniness* is reported to grow at an optimal pH between 3.7 to 5.0 and temperature between 27-30°C (Nawawi and Ho, 1990). Under the field condition, this may suggest a vast range of growing condition for oil palm seedlings. For *Ganoderma*, pH and light are most essential factors for the growth of mycelium. Furthermore, the mycelia growth depends on factors such as pH, temperature, nutrient elements, and culture media. These factors significantly affect the growth of fungi in both laboratory and field conditions. The incidence of BSR in oil palm seedlings as caused by *Ganoderma boninense* has been an issue that continues to devastate the oil palm industry in the tropics. This disease appears to be influenced by soil pH level. Presently, no effective techniques have been documented by previous studies to obviate the incidence and severe damages done to young oil palm seedlings cause by *Ganoderma boninense*. Currently, there is no documented data on the effect of pH on the growth of oil palm seedlings and basal stem rot disease incidence. No data is obtainable on the influence of pH on *Ganoderma boninense* in the palm seedlings. Therefore, the aim of this study is to determine the effect of soil pH on shoot and root growth of oil palm seedlings and basal stem rot disease incidence in *Ganoderma* inoculated plant.

Materials and methods

Preparation of rubber wood block and inoculum

Total of 60 *Ganoderma* infected rubber wood blocks (6.0 × 6.0 × 12.0 cm) were prepared according to Khairuddin (1991). Each rubber wood was placed in a heat-resistant polypropylene bag (10.0 x 32.0 cm) and autoclaved (1.40 kg/cm^2 pressure) for 121 ºC for 25 min. Then, the blocks were soaked in Malt Extract Broth (MEB) in basins overnight. Following day, the blocks were again placed in similar bags, and 100 ml MEB was added to each bag. Next, the bags were tied with raffia string and autoclaved for the second time under previously mentioned conditions. Inoculum preparations were made by placing ten plugs (sized 5 mm) from eight days old mycelium cultures grown on potato dextrose agar (PDA) that was obtained using a core borer onto each surface of the autoclaved rubber wood blocks. Subsequently, these bags were carefully tied immediately to avoid contamination and incubated in the dark at 27 ± 1ºC for 12 weeks. Fully colonized (with *Ganoderma boninense*) uncontaminated blocks were used for inoculating the oil palm seedlings.

Soil pH determination

Different soil pH levels (pH 5, 6, and 7) were tested on the growth of *Ganoderma boninense* in oil palm seedlings and incidence of BSR disease. To determine the pH levels, air-dried soil was mixed with $CaCO_3$ (2.5, 5, and 7.5g $CaCO_3$ per 5kg soil respectively) two weeks before planting.

Seedling planting

Seedling planting was done in a greenhouse at University Putra Malaysia, Malaysia. Three months old seedlings were obtained from Sime Darby Plantation, placed in polythene bags containing 5 kg soils. The experiment was prepared using factorial arrangement in a Randomized Complete Block Design with three replicates per treatment (Control, pH 5, 6, and 7; 10 plants per replicate).

Disease incidence

Disease Incidence (DI) are based on quantitative assessment at four weeks intervals. DI denoted the number of seedlings visually evaluated as diseased type (leaves necrosis & chlorosis, with or without sporophore production) expressed as percentage, just as described by Campbell and Madden (1990):

DI = (Number of seedlings infected/ total number of seedlings evaluated) x 100.

The slope curves of DI data transformation was obtained by the monomolecular model (Monit) (Campbell & Madden, 1990).

Disease Severity Index (DSI)

The development of the disease in the seedling was evaluated by disease severity index (DSI). The symptoms were scored and calculated by procedure as described by Abdullah (2003):

0 = healthy seedlings; 1 = presence of three necrotic leaves; 2 = presence of more than three necrotic leaves; 3 = presence of fruiting body at the bowl; 4 = dying/dead seedling. The DSI was calculated at the end of study (16 weeks) using the following formula:

$$\text{Disease severity index (DSI)} = \frac{\Sigma\,(D \times W) \times 100}{\Sigma\, W \times 4}$$

Where: D: disease class (0, 1, 2, 3 or 4)

W: number of seedlings showing disease class per treatment.

N, P, and K determination

N, P, and K levels were measured by Auto-Analyzer (LACHAT Instrument, QuikChemFIA+ 8000 series), while Ca, and Mg were determined via Atomic Adsorption Spectrometer (AAS; PerkinElmer Analyst 400). Samples were taken for testing after four month. The plant tissues were

prepared by wet Digestion method (Campbell and Plank, 1998), and selected nutrients (Mg, and Ca) were determined with dry ashing method (Baker et al., 1964).

Seedling harvest

After four month of growing the seedlings, the plants were harvested. Fresh roots and shoots were collected and air dried and weights. All root samples were scanned using a scanner (EPSON PERFETION V700 PHOTO**)**.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) of SAS software (version 9.3) program, and the means are presented as Mean±SEM. The comparison between means was carried out using Tukey.

Results

The results of disease incidences and severity after *Ganoderma* inoculation are presented in Figure 1. After a Month of inoculation, all pH treated groups showed a significant ($P \leq 0.05$) increase in the disease incidence, however, by the second Month of inoculation pH5G+ showed lower while pH6G+ demonstrated higher increased compared to control plants (Con G+), although all groups are at increased. By the fourth Month, all group reached the peak of disease incidences, but the pH6G+ group still maintained higher infection. However, the disease severity in the treated seedlings (pH5, pH6, to pH7) at week 8 exhibited a significant ($P \leq 0.05$) decrease when compared to control plants (Figure 2). The control plants appeared to be more susceptible to *Ganoderma* infection than other seedlings. The severity was observed from week 8 until week 12 where the seedlings of pH treated plants had less infection compared to control plants; by week 16 the control group continue to display higher disease severity. Though, the pH6 group also showed some high disease severity near to the control group as compared with other groups. In response to *Ganoderma* infection, the seedlings mineral uptakes are significantly ($P \leq 0.05$) affected as presented in Table 2. The pH7G-, pH7G+, pH6G+, and ConG+ showed lower N uptake, while pH6G- indicated higher N uptake as compared to other groups. Interestingly, pH6G+ appeared to displayed lowest uptake for P, K, and Mg^{++}, whereas pH6G- has a greater ($P \leq 0.05$) uptake for N, P, Ca^{++}, and Mg^{++}. The effect of pH levels on root yields is presented in Table 1. Non-*Ganoderma* inoculated plants (Con G-, pH5G-, pH6G-, and pH7G-) showed a greater ($P \leq 0.05$; about triplicates) root lengths and tips yields when compared with inoculated seedlings (Con G+, pH5G+, pH6G+, and pH7G+). The surface areas of non-*Ganoderma* inoculated groups displayed a double increase when compared to inoculated seedlings, though, no significant difference exist among the groups. The root volumes of *Ganoderma* treated groups exhibited lower yield as compared with non-inoculated groups, though, the pH5G+ demonstrated more decline when compare with other treated groups. Non-*Ganoderma* inoculated plants (G-) demonstrated higher top dry weight per plant as compared with *Ganoderma* inoculated (G+). The *Ganoderma* treated seedlings of pH5G+ displayed greater top dry weight while pH6G- showed lowest top dry weight per plant. The root dry weight of the control plants remains steady not increased although that non-*Ganoderma* inoculated group has a higher yield per plant than *Ganoderma* inoculated ones. However, there was a sharp decrease in *Ganoderma* inoculated group from pH5G+ until pH6G+, which corresponded with an increase in non-*Ganoderma* inoculated of the same pH group. This trend was not observed in the other groups.

Discussion

The pH levels in the present study significantly decreased the growth and development of *Ganoderma boninense* in oil palm seedlings. It showed that pH had a negative effect on the growth of basal stem root disease caused by this fungus. Soil pH is an essential factor influences the growth and development of fungi (Kapoor and Sharm, 2014). The fungus can grow on a wide range of pH

between 3 to 11 (Kapoor and Sharm, 2014). The present study observed that pH5G+ showed lower *Ganoderma* disease incidence whereas pH6G+ demonstrated higher increased when compared with the control group (Con G+). Both higher and lower pH levels negatively affect the mycelia growth. However, Rai (2003) indicated that mycelia growth of *Ganoderma spp.* at acidic pH. Veena and Pandey (2006) showed that a range of 4.0- 6.5 pH levels are optimum for growth of *Ganoderma spp*. The current results agreed with previous findings. Though, the increased in disease incidence could be attributed to environment factor and soil type of the study area (soils collected from locations in Melaka). Recently, Parthiban *et al*. (2016) revealed that low pH of Selangor (Aeric Tropic Fluvaquent) and Peat (Typic Fibric Tropohemist) soil type has a higher incidence of BSR. This report clearly indicated that low pH increase the BSR disease in oil palm, which agreed with present result. Several reports indicated that environmental conditions (such as soil pH) may affect the development and spread of disease caused by a fungus (Gurmit, 1991; Alabouvette, 1999; Ploetz, 2000). The nature of the disease incidence as indicated by severity level may vary due to these environmental factors. High severity of disease in the present study was observed in control plants as compared with pH treated plants, though, pH6 plants also showed some high disease severity response near to the control plants as compared with other plants. This showed that pH6 plants were more susceptible to *Ganoderma* disease, in turn, increase the BSR infection. The results agreed with the previous studies on fungus (Gurmit, 1991; Alabouvette, 1999; Ploetz, 2000) that disease severity or development might be ascribed to environmental conditions especially pH levels. In the other word, the plants with pH5 and pH7 showed more resistance to the disease severity after the fungi disease infection. These pH groups had capabilities to suppress the *Ganoderma* infections. The soil condition and pH plays a substantial effect in suppressive strength on fungus disease such as Fusarium wilt (Alabouvette, 1999; Ploetz, 2000). Gurmit (1991) indicated that low soil pH and high salinity seemed to suppress the fungus disease in which BSR incidence was established to be lower on acid sulphate soils that in turn might had increase the plant biomass yield and the mineral uptake. The increase in root biomass yield per plant of non-infected seedlings (G-) as compared to infected seedlings could be attributed to optimal mineral uptake by the healthy plant as shown by present results. This might explained the effect of pH on the nutrient availability such as N, which was heavily affected by the pH levels and consequently the nutrients or mineral uptake by the oil palm seedlings, a similar observation was previously reported (Rosenani, *et al.*, 2016). The N was used by the plant for protein synthesis. Available P, K, Ca, and Mg concentrations reduced to more than 24% when oil palm was inoculated with *Ganoderma*, while non-inoculated seedlings had higher P, K, Ca, and Mg concentrations in the tissues. Oil palm can tolerate fairly low pH values. In the present study, the oil palm appeared to have tolerance for pH5 and pH6 which correspond with a reduction in BSR disease. Commonly reported pH values ranges between 4 and 5 which is considered favourable in Southeast Asia for commercial oil palm production (von Uexkull and Fairhurst, 1991; Goh 1995; Corley and Tinker, 2003; Paramananthan, 2003). In conclusion, pH levels reduced the growth and development of *Ganoderma* incidences and development in oil palm seedlings. It reduced the uptake for Available P, K, Ca, and Mg concentrations in inoculated plant. Inoculated plants had lower root biomass yields as well as lower top and root dry weights per seedling while non-inoculated had a higher yield per plant.

Reference

1. Abdullah, F., Ilias, G. N. M., Nelson, M., Izzati, N. A. M. Z., & Yusuf, U. K. (2003). Disease asessment and the efficacy of trichoderma as a biocontrol agent of basal stem rot of oil palms. *Research Bulatin Science Putra*, 1, 31-33.
2. Alabouvette C. 1999. Fusarium wilt suppressive soils: An example of disease-suppressive soils. *Australasian Plant Pathology* 28: 57-64.

3. Ariffin D, Singh G, and Lim T K. 2000. Ganoderma in Malaysia - current status and research strategy. In: Paper presented at Proceedings of the 1989 PORIM International Palm Oil Development Conference, Agriculture. Palm Oil Research Institute of Malaysia, Bangi, Malaysia.
4. Baker D E, Gorslinc G W, Smith C G, Thomas W I, Grube W E and Ragland J L. 1964. Techniques for rapid analysis of corn leaves for eleven elements. *Agronomy Journal* 56: 133–136.
5. Campbell C L and Madden L. 1990. Introduction to Plant Disease Epidemiology. John Wiley and Sons, USA.
6. Campbell CR,Plank CO(1998). Preparation of plant tissue for laboratory analysis. P 37-49. In Y.P. Kalra (ed) Handbook of Reference Method for Plant Analysis. CRC Press, Boca Raton, FL.
7. Corley R H V and Tinker P B. 2003. The oil palm. Fourth edition, World agricultural series. Blackwell Science Ltd, UK. p562.
8. Fang Q H and Zhong J J. 2002. Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of Ganoderma lucidum. *Process Biochemistry* 37(7):769-774.
9. Goh K J. 1995. In Basiron, Jalani and Chan (Eds.), Advances in oil palm research, Vol. I. Malaysian Palm Oil Board, Kuala Lumpur. p. 371-410.
10. Gurmit S. 1991. Ganoderma - the scourge of oil palm in the coastal area. *Planters* 67: 421-444.
11. Ho Y W and Nawawi A. 1985. Ganoderma boninense Pat. from basal stem rot of oil palm (Elaeis guineensis) in Peninsular Malaysia. *Pertanika Journal Tropical Agricultural Science* 8: 425–428.
12. Kapoor P and Sharm B M. 2014. Studies on different growth parameters of Ganoderma lucidum. *International Journal of Science, Environment and Technology* (3) 4: 1515 – 1524.
13. Khairudin, H. (1990). Basal stem rot of oil palm: incidence, etiology and control. *Master of Agriculture Science thesis, Universiti Pertanian Malaysia, Selangor, Malaysia, Selangor, Malaysia*.
14. Nawawi A. and Ho Y W. 1990. Effect of Temperature and pH on Growth Pattern of Ganoderma boninense from Oil Palm in Peninsular Malaysia. *Pertanika* 13(3): 303-307.
15. Paramananthan S. 2003. In Fairhurst, T.H., and R. Hardter (Eds.), Managing oil palm for large and sustainable yields. PPI/PPIC-IPI, Singapore. p. 27-57.
16. Parthiban K, Vanitah R, Jusoff K, Nordiana A A, Anuar A R, Wahid O and Hamdan A B. 2016. GIS Mapping of Basal Stem Rot Disease in Relation to Soil Series Among Oil Palm Smallholders. *American Journal of Agricultural and Biological Sciences* 11 (1): 2.12.
17. Ploetz R C. 2000. Panama disease: A classic and destructive disease of banana. *Plant Health Progress*.
18. Rai R D. 2003. Successful cultivation of the medicinal mushroom Reishi, Ganoderma lucidum in India. *Mushroom Research* 12: 87-91.
19. Shamshuddin J, Fauziah C I, Anda M, Kapok J and Shazana M A R S. 2011. Using Ground Basalt and/or Organic Fertilizer to Enhance Productivity of Acid Soils in Malaysia for Crop Production. *Malaysian Journal of Soil Science* 15: 127-146.
20. Veena SS and Pandey M. 2006. Evaluation of the locally available substrates for thecultivation of Indigenous Ganoderma lucidum isolate. Journal Mycology and Plant Pathology 36 (3): 434-438.
21. Von Uexkull H R and Fairhurst T H. 1991. IPI Bulletin No.12, International Potash Institute, Bern, Switzerland. 79p.

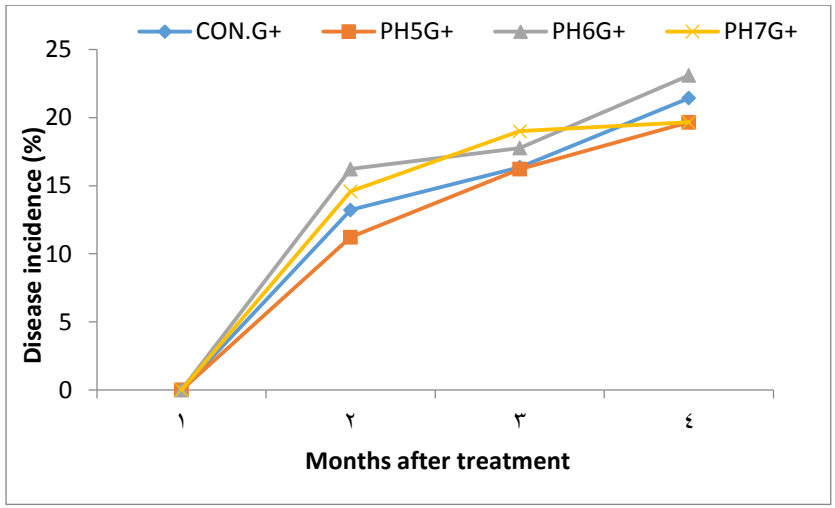

Figure 1. Effect of pH levels on disease incidence in *Ganoderma boninense* inoculated oil palm seedlings

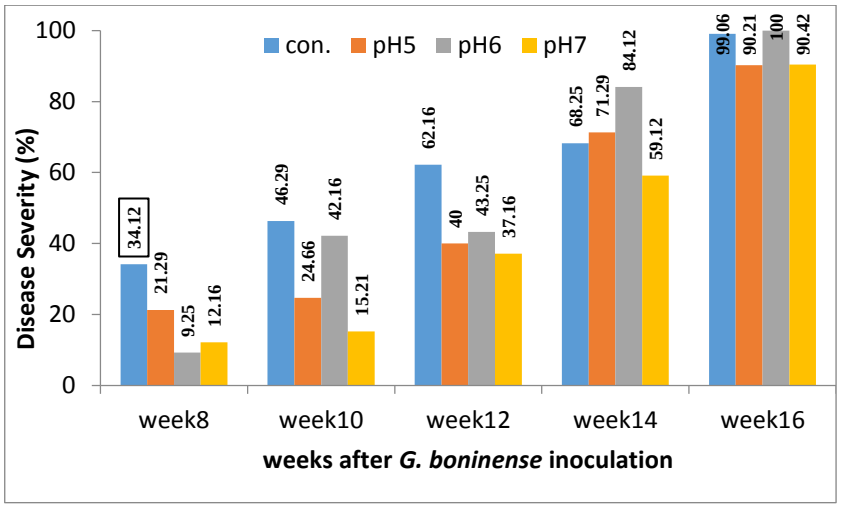

Figure 2. Effect of pH levels on disease severity in *Ganoderma boninense* inoculated oil palm seedlings

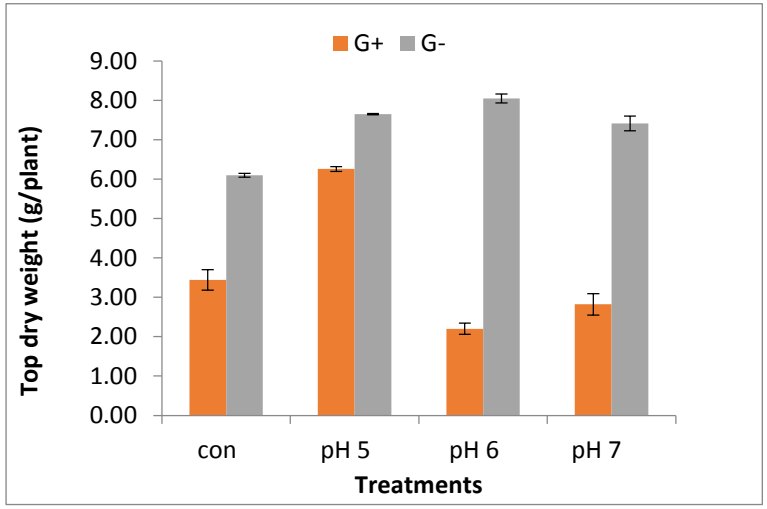

Figure 3: Effect of pH levels on top dry weight of oil palm seedlings after infected by *Ganoderma*

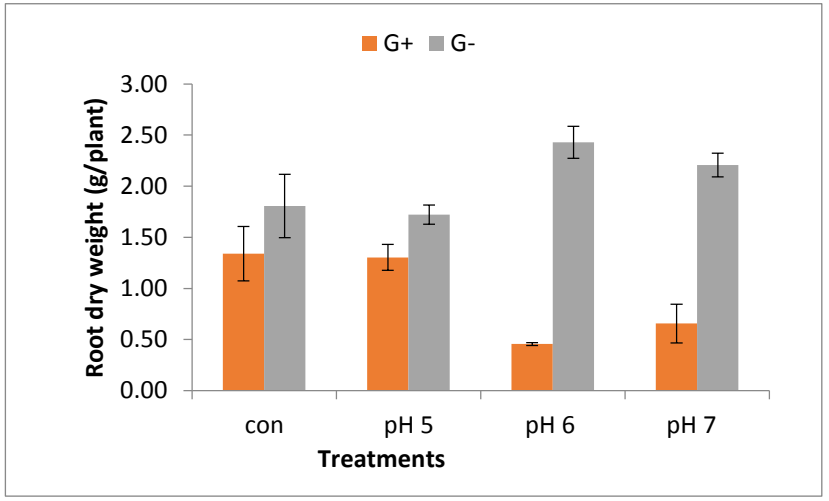

Figure 4: Effect of pH levels on root dry weight of oil palm seedlings after infected by *Ganoderma*

Table 1. Effect of pH levels on root biomass yields of oil palm seedlings after infected by *Ganoderma*

Treatments	Root Length (cm)	Surface area (cm^2)	Root volume. (cm^3)	Root tips
Con.G+	267.97±1.30^{bc}	319.86±1.22^{b}	35.45±4.12^{c}	1710.6±0.75^{cd}
Con.G-	754.85±2.50^{a}	582.85±0.81^{a}	38.99±0.38^{ab}	4492.1±2.29^{ab}
pH5G+	382.9493±1.35^{bc}	377.45±4.80^{b}	30.52±0.80^{bc}	2179.4±0.51^{bc}
pH5G-	948.20±3.20^{a}	676.85±3.63^{a}	39.47±0.45^{ab}	4374.8±2.10^{a}
pH6G+	203.75±1.40^{c}	283.14±0.61^{b}	36.99±0.35^{ab}	1181±1.60^{d}
pH6G-	966.86±0.68^{a}	697.93±4.14^{a}	43.09±1.17^{a}	4444±4.80^{a}
pH7G+	337.52±1.08^{b}	328.60±1.07^{b}	34.04±0.37^{bc}	1642.8±2.14^{cd}
pH7G-	918.69±0.51^{a}	654.04±5.16^{a}	42.30±0.82^{a}	4941±1.07^{a}

$^{a-d}$Mean±SEM without common subscripts in a column differ significantly at $P \leq 0.05$

Table 2. Effect of pH levels on mineral uptakes of oil palm seedlings after infected by *Ganoderma*

Treatments	N uptake (g/plant)	P uptake (g/plant)	K uptake (g/plant)	Ca^{++} uptake (g/plant)	Mg^{++} uptake(g/plant)
Con.G+	0.089± 0.007^{c}	0.048±0.003^{d}	0.168±0.018^{c}	0.0013±0.0001^{d}	0.0014±0.0001^{bc}
Con.G-	0.203±0.006^{b}	0.072±0.001^{c}	0.279 ±0.012^{b}	0.0027±0.0001^{c}	0.0027±0.0004^{a}
pH5G+	0.179 ±0.005^{b}	0.070±0.001^{c}	0.339±0.005^{ab}	0.0030±0.0001^{c}	0.0024±0.0001^{ab}
pH5G-	0.196 ±0.005^{b}	0.090±0.002^{ab}	0.405 ±0.010^{a}	0.0044±0.0001^{b}	0.0027±0.0002^{a}
pH6G+	0.076 ±0.002^{c}	0.026 ±0.001^{e}	0.134 ±0.004^{c}	0.0015±0.0002^{d}	0.0008±0.0001^{c}
pH6G-	0.293 ±0.019^{a}	0.094 ±0.001^{a}	0.380 ±0.010^{a}	0.0063±0.0003^{a}	0.0027±0.0002^{a}
pH7G+	0.073 ±0.010^{c}	0.033 ±0.003^{e}	0.188 ±0.019^{c}	0.0018±0.0002^{d}	0.0010±0.0001^{c}
pH7G-	0.0201±0.018^{b}	0.081±0.003^{bc}	0.353 ±0.019^{a}	0.0047±0.0001^{b}	0.0023±0.0001^{ab}

$^{a-d}$Mean±SEM without common subscripts in a column differ significantly at $P \leq 0.05$