Effects of Nanoformulated Plant Growth Regulator on Culturable Bacterial Population, Microbial Biomass and Enzyme Activities in Two Soil Types

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Abstract

Nanomaterials have many beneficial applications; however, their impacts on the environment necessitate an assessment. Understanding any possible negative effects of nano-based products on soil health needs to be performed before their extensive use in crop production and commercialization. Microorganisms are considered the most sensitive indicators of environmental stresses. Thus, this study assessed the effects of nanoformulated plant growth regulator (HormoGroe®) on the culturable soil bacterial population, dehydrogenase (DHA) and urease (UA) activities, and microbial biomass (MCB) in Lipa clay loam (LCL) and Sariaya sandy loam (SSL) over 75-day incubation period in the laboratory. Results showed the treatments had no significant effect on the culturable bacterial population. HormoGroe® had no adverse impact on DHA in both LCL and SSL. HormoGroe® significantly enhanced UA in SSL at 35 days after amendment (DAA) but significantly decreased UA in LCL at 75 DAA, while it decreased significantly the MCB at 35 and 1 DAA in LCL and SSL, respectively. Regardless of the amendments, LCL had higher DHA and UA but lower MCB than SSL. The effect of HormoGroe® and the non-nanomaterials on the parameters measured was influenced by soil type. In conclusion, HormoGroe® is safe for soil microbiome, mainly bacteria in crop production.

Keywords*:* agriculture, microbial activity, nanotechnology, nanomaterial, Philippines

1. Introduction

Nanotechnology is the most innovative field of the 21st century and has the potential to play a crucial role in food security, food safety and food production (Sabir *et al.*, 2014; Servin *et al*., 2015). Thus, extensive research has been conducted to commercialize nanomaterial (NM) products around the world (Sabir *et al.*, 2014). Nanomaterial may serve equally as additives mostly for controlled release and active constituents in plant protection or fertilizer products (Gogos *et al*., 2012). The use of nanotechnology aims to improve the farming systems and subsequently enhance crop yield while promoting sustainability in agriculture and the environment (Shang *et al.*, 2019).

As the world population increases, strategies for sustainable agriculture are needed to fulfill the global demand for food and other commercial products (Anderson *et al.*, 2017). One effort to promote plant growth is through the use of nanotechnology. The nanoformulated plant growth regulators like auxins, even at small levels, function by acting as biostimulants, thereby promoting plant growth (Juárez-Maldonado *et al*., 2019). In general, phytohormones are one of the signals in the regulation of the growth and development of plants (Sadiq *et al*., 2020). Phytohormones like auxins and indole-3-acetic acid (IAA) play significant functions in plant growth promotion. Moreover, auxin even stimulates plant development in response to various abiotic stresses or in the establishment and functioning of the arbuscular mycorrhiza symbiosis (Zhang *et al*., 2019; Quiroga *et al*., 2020). However, the extensive production and usage of nanomaterials with ultimate disposal in the environment lead to unintentional exposure of non-target environmentally beneficial bacteria, threatening the native soil inhabitants (Santimano and Kowshik, 2013).

Soil organisms are critically important since they directly influence soil ecosystem processes and soil quality. Thus, they serve as biological indicators; any factor, affecting soil microbial biomass, activity and populations, would greatly influence soil quality, sustainability and plant productivity (Hill *et al*., 2000; Dinesh *et al.*, 2012). Soil microbial biomass plays an important role in maintaining the soil structure, which facilitates the microbial metabolic processes and biogeochemical cycling of essential macro and micronutrients (Zhang *et al.*, 2016; Chaudhary *et al.*, 2021). Soil enzymes, which are strongly associated with microorganisms, are closely related to the biophysicochemical characteristics of the soil. They are vital for the regulation of the formation of soil fertility including nutrient cycling in nature (Shiyin *et al.*, 2004; Makoi *et al.*, 2008; Purev *et al.*, 2012). Soil dehydrogenase, an intracellular enzyme, is

highly correlated with the microbial biomass and its activity and can be considered as a good measure for soil microbial oxidative activity (Von Mersi and Schinner, 1991; Camiña *et al*., 1998). Urease catalyzes the hydrolysis of urea into CO_2 and NH_3, thereby dictating the fate of urea (Maddela and Venkateswarlu, 2018).

HormoGroe® is a controlled-release nanoformulation of the plant growth regulators (auxins, cytokinins and gibberellins) derived from naturally occurring plant growth-promoting bacteria. Unlike other plant stimulants, these naturally occurring plant growth regulators have been shown to enhance seed germination and increase the survival of tissue-cultured plants, seedlings and stem cuttings (Fernando *et al.*, 2017). However, the effects of HormoGroe® on the soil microorganisms have not been evaluated. Investigating the effect of nanomaterials, including the nanoformulated plant growth regulators like HormoGroe®, on the biological activity in the soil and on the environment is highly relevant (Handy *et al*., 2008; Fernando *et al.*, 2017). In this study, the impact of HormoGroe® on the soil bacterial population, soil microbial biomass and enzyme activities in two soil types was investigated. These indicators can serve as criteria for the possible commercialization of this plant growth regulator.

2. Methodology

2.1 Soil Collection and Characterization

Two soil types were used for the experiments: Lipa clay loam (LCL) and Sariaya sandy loam (SSL). The LCL soil samples were collected from the Central Experiment Station, University of the Philippines Los Baños (UPLB), Laguna, Philippines, while the SSL soil samples were obtained from Barangay Canda, Sariaya, Quezon. Soil sampling was done by collecting the top 20 cm of the surface soil. Before the analysis and set up of experiments, visible debris was removed, and the soil was air-dried and passed through a 2-mm sieve. A portion of the collected soil was characterized chemically by quantitatively determining the pH in water (1:2.5), organic matter (OM) by Walkley and Black method (Walkley and Black, 1934), total N using the Kjeldahl method (Blake, 1965), exchangeable K by flame photometer method (Pratt, 1965), cation exchange capacity (CEC) by ammonium acetate method (Chapman, 1965) and available P by Bray-2 method (Bray and Kurtz, 1945). The soil

particle size distribution was determined by the hydrometer method (Bouyoucos, 1962) and the soil textural class names were determined following the textural triangle of the United States Department of Agriculture (USDA) system (Rowell, 1994). The field capacity (FC) was also obtained using the gravimetric method (Blake, 1965). Selected physicochemical characteristics of the two soils are detailed in Table 1.

Table 1. Selected physicochemical properties of Sariaya and Lipa soils

	Lipa soil	Sariaya soil
pH (1:2.5 soil water)	5.40	5.80
OM (%)	3.11	1.40
CEC ($cmol_c\ kg^{-1}$)	40.06	14.09
Total N (%)	0.19	0.08
Available P (ppm)	10.50	348.50
Exchangeable K ($cmol_c\ kg^{-1}$)	2.05	1.99
Sand (%)	27.23	64.58
Silt (%)	39.61	28.94
Clay (%)	33.16	6.48
Texture	Clay loam	Sandy loam
Field capacity (%)	50.8	33.1

2.2 Experimental Design

The experiment was conducted at the Division of Soil Science (DSS)-Agricultural Systems Institute (ASI), College of Agriculture and Food Science (CAFS), UPLB (121° 14' 40.84" E, 14° 09' 34.55" N) from July 2018 to September 2018. The nanoplanthormone (HormoGroe®) and HormoGroe® carrier were obtained from the Nanobiotechnology Laboratory of the National Institute of Molecular Biology and Biotechnology, UPLB. An incubation experiment was performed in sterile 10" by 12" polypropylene containers each filled with 500-g sieved moist soil (100% moisture content at field capacity). The moist soil was pre-incubated for a week prior to the addition of amendments. A 2 x 4 factorial experiment was carried out in the Soil Microbiology Laboratory of the Division of Soil Science, UPLB at room temperature. The treatment factors were two types of soils (LCL and SSL) and three types of amendment HormoGroe® (50 ppm), HormoGroe® carrier (phosphatidylcholine) (50 ppm), standard IAA (stdIAA) (50 ppm), and

unamended soil as control. The treatments were replicated three times and laid out in a split-plot completely randomized design (CRD) (Gomez and Gomez, 1984). Samples were collected at one, seven and 14 days after amendments (DAA) for culture-dependent analysis of soil bacteria. Simultaneously, assessment of dehydrogenase and urease (modified buffer method) activities and microbial biomass were also performed at 1, 35 and 75 DAA.

2.3 Enumeration of Soil Culturable Bacteria

Indirect viable plate count was performed to determine the culturable soil bacterial population by pour plating in asparagine mannitol agar medium (Thorton, 1922) at 10^{-4}, 10^{-5} and 10^{-6} dilution. Nystatin was added before pouring the medium at a rate of 50 mg/L. Each dilution was plated in duplicate, allowed to solidify, inverted, wrapped with paper and incubated at room temperature (~29 °C) for five days. After five days, the bacterial colonies were counted. The valid count is between 25 to 250 colonies for bacteria (Breed and Dotterrer, 1916). Bacterial colonies were expressed as colony-forming units per gram of dry soil (CFU/g dry soil).

2.4 Dehydrogenase Activity (DHA) Assay

Soil dehydrogenase activity assay was performed according to the modified procedure of Tabatabai (1982). About 1-g soil was placed in a screw-capped tube to which 1-mL 3% (w/v) 2,3,5-triphenyl tetrazolium chloride (TTC) was added, stirred and incubated for 96 h at 27 °C. After incubation, 10-mL ethanol (EtOH) was added to the mixture and vortexed (Cat. No. 34524-200, CENCO Instruments, Netherlands) for 30 s. The tube was incubated for 1 h to allow the suspended soil to settle. About 5-mL of the supernatant was transferred to a clean test tube, and the absorbance was measured at 485 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland). The result was reported in µg triphenyl formazan (TF) g^{-1} dry soil 96 h^{-1}.

2.5 Urease Activity (UA) Assay

Urease activity assay was performed using the modified buffer method (Kandeler and Gerber, 1988). A 5-g soil sample was mixed with 2.5 mL of 720-mM urea, respectively, and incubated for 2 h at 37 °C (Dash *et al.*, 1981). After incubation, the control sample was treated with 2.5-mL of 720 mM urea and all samples, including the control, were added with 30-mL of acidified 2 M potassium chloride (KCl). The samples were shaken for 30 min on a rotary shaker (3520, Lab-Line Instruments, United States) and filtered using

Whatman filter paper number 41. A 1.5-mL aliquot was taken and placed in an Eppendorf tube and centrifuged at 10,000 x g for 5 min. A 1-mL supernatant solution was mixed with 9-mL sterile distilled water, 5-mL sodium salicylate/NaOH solution and 2-mL dichloroisocyanuric acid. The solution was incubated at room temperature for 30 min and the absorbance was determined using a spectrophotometer at 660 nm and the result was reported in μg NH_4-N g^{-1} 2 h^{-1}.

2.6 Determination of Microbial Biomass (MCB) by Substrate-Induced Respiration (SIR)

Standardization of the amount of glucose as the substrate and the maximum duration for the incubation of samples were determined based on the procedure by Swain *et al.* (1978). Each soil type was standardized prior to laboratory experiments involving nanomaterials. Standardization was performed by adding and mixing 10 to 70 mg/1 g glucose to glass jars containing 10 g of soil. The glass wire, which served as the stand, along with a plastic cup containing 10-mL of standard 0.04-N NaOH was immediately placed inside the jar, covered and sealed tightly. The samples were then incubated for 5 h. The amount of carbon dioxide (CO_2) released was determined by the titrimetric method using standard 0.04 N of hydrochloric acid (HCl) with barium chloride ($BaCl_2$) and phenolphthalein as indicators. The amount of CO_2 released, as computed using Equation 1, was plotted against glucose concentration. The maximum amount of glucose consumed was used for observing the microbial biomass throughout the experiments involving nanomaterials. Exactly, the D-glucose used were 500 and 100 mg for every 10 g of soil samples for SSL and LCL, respectively.

$$mg\ CO_2/10\ g\ soil = \frac{(b \times N \times 88)\ mg\ CO_2}{10\ g\ soil} \quad (1)$$

Using the maximum amount of glucose used, the time for initial biomass emission (z), median biomass emission at first-generation (A), and median biomass emission generation at second-generation (B) were determined within a 10-h incubation with 1 h of the sampling interval. Equations were generated depending on the curve plotted and the value of the proportional unit, z, signifying the volume (in mL) of standard 0.04 HCl used for titration. Using the value of z, the value of microbial biomass was computed. For LCL, z = 0.714B-1.714A while for SSL, z = 4.348B-5.348A. The standardized SIR procedure was utilized to measure microbial biomass throughout the

experiment. The calculation for microbial biomass was performed using Equation 2.

$$Microbial\ biomass\ (\mu g\ C/10\ g\ soil) = \frac{z \times N \times 12 \times 10^6}{10\ g\ soil} \quad (2)$$

where *b* is the difference between the delivered volume of HCl of the control and the delivered volume of HCl of the sample; *N* is the concentration of HCl in normality; *z* is the proportional unit.

2.7 Data Analysis

Two-way analysis of variance (ANOVA) according to Gomez and Gomez (1984) was performed using the Statistical Tool for Agricultural Research (STAR) version 2.0.1 to assess the effects of treatment factors on culturable soil bacteria, DHA and UA assays, and microbial biomass determination. The least significant difference (LSD) test at α = 5% was used to determine whether the means differed significantly.

3. Results and Discussion

3.1 Effect on Soil Culturable Bacteria

The nanoplanthormone (HormoGroe®) did not show a significant effect on the soil culturable bacterial population at α = 5% as shown in Table 2. This may suggest that HormoGroe® did not pose any harm to the culturable bacterial population in both LCL and SSL. This is despite the possibility that nanomaterials may affect the bacteria by interfering in their biological processes such as the behavior of cell membranes, biochemical pathways in cells and their genetic code (Klaine *et al*., 2012).

It was observed that at one DAA, although not significant, the HormoGroe® and HormoGroe® carrier had higher culturable bacteria compared with the control. This could be due to the phosphatidylcholine (a glycerophospholipid) present in both treatments, which is a surface-acting agent that allowed the utilization of hydrophobic substances in the soil by bacteria; hence, promoting bacterial growth. These hydrophobic substances are hydrophobic components of organic matter that act as soil aggregate-binding agents (Piccolo and Mbagwu, 1999). It was observed that bacteria prefered hydrophilic

compounds (i.e., compounds such as in root exudates or polysaccharides of plant tissues) over hydrophobic compounds since hydrophilic substances interact with the cell surface of bacteria prior to entering the cells (Piccolo and Mbagwu, 1999; Xia *et al.*, 2020). However, hydrophobic substances can enter the bacterial cells through surface-mediated transport or solubilization in the presence of surfactants (Fickers *et al.*, 2005).

Table 2. Effect of HormoGroe® nanoplanthormone on the culturable bacterial population in Lipa clay loam and Sariaya sandy loam

Soil type	Amendment	log (CFU/g dry soil)		
		1 DAA	7 DAA	14 DAA
Lipa clay loam	Control	6.05^{ns}	6.83^{ns}	5.32^{ns}
	stdIAA	6.33	6.10	5.39
	HormoGroe®	6.52	6.11	4.91
	HormoGroe® carrier	6.51	6.20	4.55
Sariaya sandy loam	Control	5.98^{ns}	6.25 ^{ns}	4.82^{ns}
	stdIAA	5.99	6.20	4.94
	HormoGroe®	5.98	6.08	5.22
	HormoGroe® carrier	6.59	6.10	4.82

^{ns} – not significant; DAA – days after amendment; sdtIAA – standard indole-3-acetic acid

At seven DAA, the phosphatidylcholine-formulated treatments, HormoGroe® and HormoGroe® carrier had lower culturable bacterial population relative to the control. Perhaps, the nutrients in the soil had depleted due to the exponential growth of bacteria as it approached seven DAA. Hartman and Richardson (2013) noted that biomass, including bacterial biomass, was limited by nitrogen needed to build proteins, but the high phosphorus demands of ribosomes limit the rates of protein synthesis. Their findings remain consistent with the model of cellular metabolism.

3.2 Effect on DHA

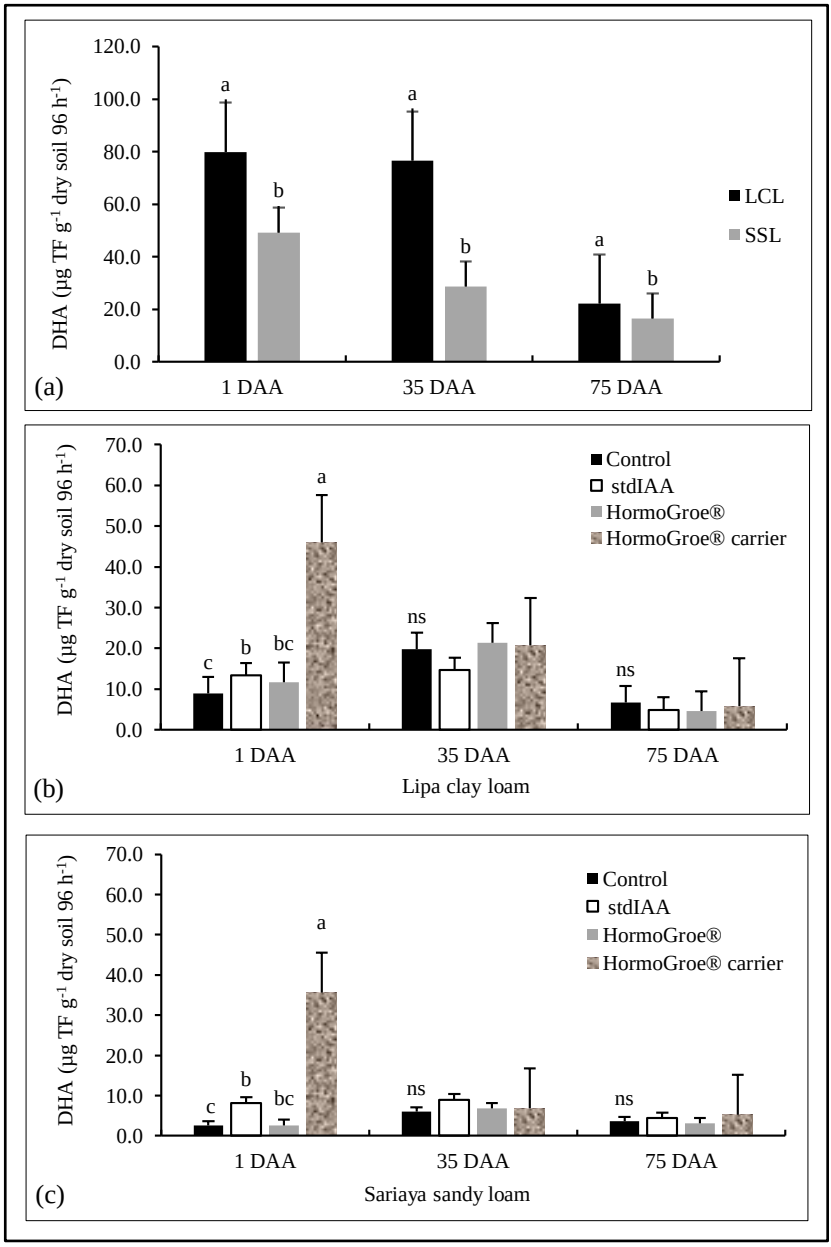
A significant main effect on DHA between soil types at one, 35 and 75 DAA was observed (Figure 1a). Also, a significant difference among amendments was shown at one DAA at α = 5% for both LCL (Figure 1b) and SSL (Figure 1c). A comparison in soil DHA among amendments in LCL and SSL showed a significant increase in DHA in HormoGroe® carrier-amended soil. This could be due to the property of the HormoGroe® carrier as being a surface-acting agent. Moreover, HormoGroe®-amended soils did not exhibit a

significant difference in DHA compared with the controls. Parr and Norman (1965) noted that surfactants are involved in complex growth phenomena such as differentiation, induction, or auxin-mediated events. Surfactants are used to reduce the surface tension of aqueous solutions to enhance the wetting of tissues for the penetration of various test compounds. The addition of surfactants in the soil system might have benefited the microorganism. According to Sunde *et al.* (2017), biosurfactants that can reduce the surface tension or bridge the hydrophobic-hydrophilic boundaries are required to support growth from microorganisms to mammals. Examples of these biosurfactants are the naturally occurring surface-active and interfacially active polypeptides used to achieve a reduction of surface tension, stabilization of emulsions, surface motility, or attachment to interfaces.

Moreover, the introduction of surfactants caused an increase in the percolation of water; however, this period of rapid water drainage was followed by decreased water percolation. Therefore, percolation resulted in faster nonreactive solute transport than in the absence of surfactants (Karagunduz *et al.*, 2015). The presence of surfactant increases the availability of nutrients in the soil solution and the rate of solute transport. These processes must have allowed the microorganisms in the soil access to the nutrient supply that is important for their growth. Hence, HormoGroe® carrier-amended soil had a significant level of DHA compared with other materials used.

Bacterial motility is a key mechanism for survival in a patchy environment. However, their motility in soils is hindered by inherent heterogeneity, pore space complexity and dynamics of microhydrological conditions. Bacterial colonies grew fast under matric potentials greater than -0.5 kPa (wet) while the rate of bacterial growth decreased significantly at -2 kPa. Thus, bacteria experience reduced cell motilities with decreasing matric potential on rough surfaces resulting in suppressed growth and colony expansion (Wang and Or, 2010). Perhaps, the HormoGroe® carrier used in this study increased the matric potential at a level that allowed the bacteria to grow faster and consequently increased DHA.

At one, 35 and 75 DAA, a significant main effect between soil types was observed (Figure 1a). The LCL exhibited higher DHA than SSL which may be attributed to the physicochemical properties affecting the microbial community. Particularly, LCL had a finer particle size than SSL.



DAA – days after amendment; sdtIAA – standard indole-3-acetic acid; error bars represent the standard error of the mean (n = 3); different letters indicate significant difference among means ($p < 0.05$) according to LSD test.

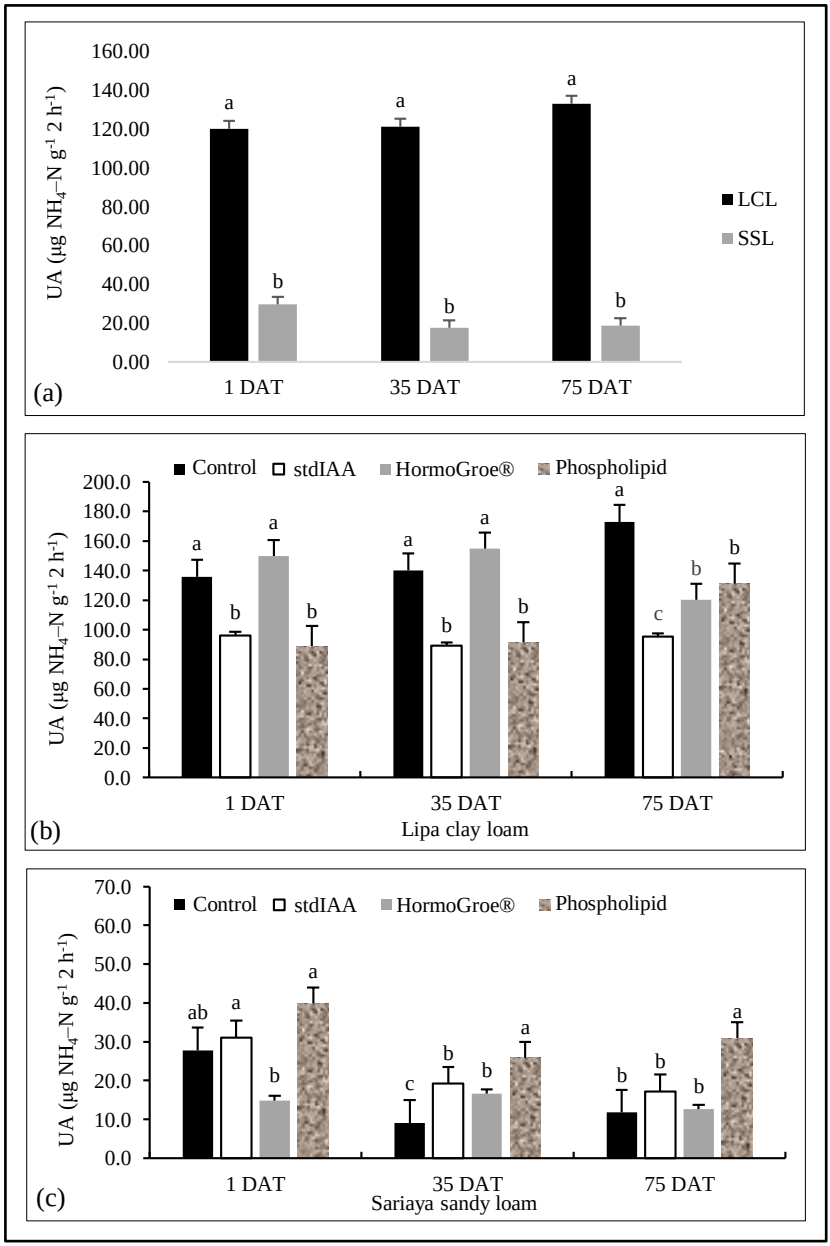
Figure 1. Differences in DHA between soil types (a) and the effect of amendments on soil DHA of LCL (b) and SSL (c)

In the study conducted by Sessitsch *et al*. (2001), particle size significantly affected the microbial community structure yielding higher diversity of microbes in small size fractions than in coarse ones. Thus, a decrease in microbial community structure, as affected by soil texture, might cause a decrease in soil DHA (Wolińska and Stepniewska, 2012). They concluded that DHA is related to the quantitative changes in the microorganism population and connected with living microbial cells. Another factor for the higher DHA of LCL than SSL is the level of organic matter. DHA reaches higher values in soils with higher total organic carbon content (Wolińska and Stepniewska, 2012).

3.3 Effect on UA

The results showed a significant interaction effect on UA between the soil type and amendments at one, 35 and 75 DAA. The simple effect of each treatment for each soil type is shown in Figure 2 at α = 5%. Comparison of the means showed significant differences in UA among treatments for LCL and SSL. A significant decrease in the UA of LCL amended with stdIAA and HormoGroe® carrier at one, 35 and 75 DAA was observed. Moreover, the UA of HormoGroe®-amended LCL was significantly higher than HormoGroe® carrier amendment. HormoGroe® (phosphatidylcholine as the carrier of the plant growth regulator) in LCL may have stimulated the growth of microbial population up to 35 DAA, which was possibly affected by the increase in the soil matric potential. However, an increase in the microbial population might lead to a decrease in the UA of the soil. Under steady-state conditions, 79-89% of the urease activity of the soil examined by Paulson and Kurtz (1969) was due to urease adsorbed on soil colloids; an increase in microbial population reduced this percentage temporarily until a new steady state was reached. In contrast to LCL, HormoGroe® carrier-amended SSL soil showed higher UA than SSL amended with HormoGroe®. HormoGroe®-amended LCL was able to reduce the UA in the soil at one DAA while it increased the UA at 35 DAA.

The significant main effect on soil urease activity between soil types was observed at one, 35 and 75 DAA (Figure 2a). The obvious difference in UA between the two soil types might probably be due to the differences in the level of soil colloids, which include the organic matter and clay fractions, with LCL having OM and percent clay at 122 and 512%, respectively, higher than that of SSL.



DAA – days after amendment; sdtIAA – standard indole-3-acetic acid; error bars represent the standard error of the mean ($n = 3$); different letters indicate significant difference among means ($p < 0.05$) according to LSD test.

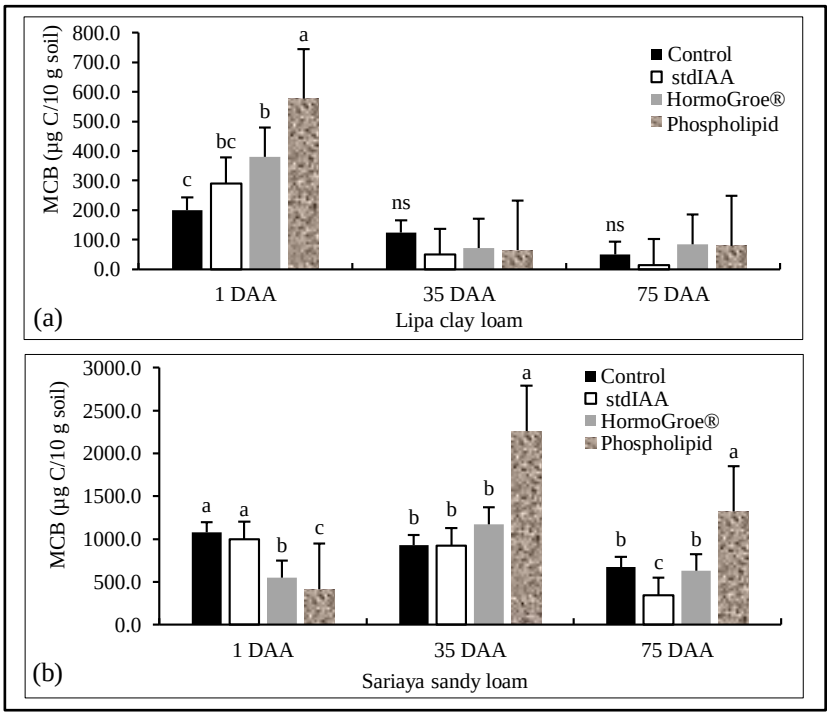
Figure 2. The observed differences in UA between soil types (a) and interaction effect on soil UA between soil types and amendments in LCL (b) and SSL (c)

Burns *et al*. (1972) suggested that urease exists in the soil as an enzyme-organic matter complex to protect the enzyme against the activities of proteolytic enzymes and yet permits the diffusion of substrate molecules to and product molecules from active enzyme sites. Using multiple regression analysis, Chakrabarti *et al*. (2004) revealed that the stabilization of UA in studied soils was caused by the complexation of organic matter and enzymes; the variation of soil properties in different soils influenced UA. It was also concluded by Wyszkowska *et al*. (2005) in their study on dehydrogenase and urease that enzymatic activity was dependent on the type of soil.

According to Dharmakeerthi and Thenabadu (1996), the remarkable long-term stability of urease is due to organo-mineral complexes in the soil. Humic and fulvic acid at pH below 10.4 are negatively charged, while urease is net positive below pH 5.2 or net negative at pH 5.2. Humic substances form strong complexes with oppositely charged proteins leading to changes in enzyme activities (Li *et al*., 2013). Considering that the pH of LCL (5.4) was lower than SSL (5.8) as shown in Table 1, perhaps the stability of free urease in SSL decreased and at the same time the urease-high molecular weight humic acid in LCL was increased (Marzadori *et al.*, 2000). Additionally, the higher UA of LCL was probably due to the urease-organic matter complex leading to a more stable urease. The degradation of free urease may be one of the reasons for the changes in UA in SSL since free urease is susceptible to the action of proteases (Marzadori *et al.*, 2000).

3.4 Effect of HormoGroe® Nanoplanthormone on Soil MCB

Results revealed a significant interaction effect on MCB between soil types and amendments at 1, 35 and 75 DAA at α = 5% (Figure 3). It was observed that, in both the LCL (Figure 3a) and SSL (Figure 3b), the HormoGroe® carrier amendment significantly increase soil MCB. This could be because of the HormoGroe® carrier as the surfactant, which had possibly increased soil solution percolation (Karagunduz *et al*., 2015). This process allowed the microorganisms in the soil to access the nutrient supply that is important for their growth. Also, greater percolation and, thus, distribution of solute mineral and soluble organic materials in SSL amended with nanocarrier than in LCL might have occurred that allowed the microorganisms access to these growth factors; hence, favoring microbial growth at 35 and 75 DAA in SSL.



DAA – days after amendment; sdtIAA – standard indole-3-acetic acid; error bars represent the standard error of the mean (n = 3); different letters indicate significant difference among means ($p < 0.05$) according to LSD test.

Figure 3. Effect of HormoGroe® nanoplanthormone on soil MCB in LCL (a) and SSL (b)

4. Conclusion and Recommendation

The use of HormoGroe® had no adverse effect on the culturable soil bacterial population, soil DHA and UA. However, a decrease in soil MCB in sandy loam soil was observed one day after the addition of HormoGroe®. Results may indicate that HormoGroe® may be safely used in crop production. Since the effect of nanoformulated plant growth regulator on soil microorganisms may differ in laboratory conditions and the natural environment, there is a need to investigate the effects of HormoGroe® in pot and field trials.

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6. References

Anderson, A.J., McLean, J.E., Jacobson, A.R., & Britt, D.W. (2017). CuO and ZnO nanoparticles modify interkingdom cell signaling processes relevant to crop production. Journal of Agricultural and Food Chemistry, 66(26), 6513-6524. https://doi.org/10.1021/acs.jafc.7b01302

Blake, C.A. (1965). Methods of soil analysis: Part 1: physical and mineralogical properties. Madison, Wisconsin, USA: American Society of Agronomy.

Bouyoucos, G.J. (1962). Hydrometer method improved for making particle size analysis of soils. Agronomy Journal, 54, 464-465. https://doi.org/10.2134/agronj1962.00021962005400050028x

Bray, R.H., & Kurtz, L.T. (1945). Determination of total, organic and available phosphorus in soils. Soil Science, 59, 39-45. http://dx.doi.org/10.1097/00010694-194501000-00006

Breed, R.S., & Dotterrer, W.D. (1916). The number of colonies allowable on satisfactory agar plates. Journal of Bacteriology, 1(3), 321-331.

Burns, R.G., El-Sayed, M.H., & Mclaren, A.D. (1972). Extraction of a urease-active organo-complex from soil. Soil Biology and Biochemistry, 4(1), 107-08. https://doi.org/10.1016/0038-0717(72)90048-X

Camiña, F., Trasar-Cepeda, C., Gil-Sotres, F., & Leirós, C. (1998). Measurement of dehydrogenase activity in acid soils rich in organic matter. Soil Biology and Biochemistry, 30(8-9), 1005-1011. https://doi.org/10.1016/S0038-0717(98)00010-8

Chakrabarti, K., Sinha, N., Chakraborty, A., & Bhattacharyya, P. (2004). Influence of soil properties on urease activity under different agro-ecosystems. Archives of Agronomy and Soil Science, 50(4-5), 477-483. https://doi.org/10.1080/03650340410001692998

Chapman, D.D. (1965). Determination of exchangeable Ca, Mg, K, Na, Mn and effective cation exchange capacity in soil. In C.A. Black (Ed.), Methods of soil analysis (pp. 902-904). Madison, USA: American Society of Agronomy.

Chaudhary, P., Sharma, A., Chaudhary, A., Khati, P., Gangola, S., & Maithani, D. (2021). Illumina based high throughput analysis of microbial diversity of maize rhizosphere treated with nanocompounds and *Bacillus* sp. Applied Soil Ecology, 159, 103836. https://doi.org/10.1016/j.apsoil.2020.103836

Dash, M.C., Mishra, P.C., Mohanty, P.K., & Bhatt, N. (1981). Effects of specific conductance and temperature on urease activity in some Indian soils. Soil Biology and Biochemistry, 13, 73-74.

Dharmakeerthi, R.S., & Thenabadu, M.W. (1996). Urease activity in soils: A review. Journal of the National Science Foundation of Sri Lanka, 24(3), 159-195.

Dinesh, R., Anandaraj, M., Srinivasan, V., & Hamza, S. (2012). Engineered nanoparticles in the soil and their potential implications to microbial activity. Geoderma, 173, 19-27. https://doi.org/10.1016/j.geoderma.2011.12.018

Fernando, L.M., Bautista, H.N.F., Parami, J.M.K., Mendoza, D.J.R., Lopez, I.A.K., Ocampo, A. M., Delfin, E.F., Duiloy, R.H., Lapoot, C.C., Paterno, E.S., Merca, F.E., & Lantican. M.Q. (2017). Pilot scale production of nanoencapsulated plant growth regulator (BIOTECH Nano-Plant Growth Regulator) for the production of high value crops (Unpublished Terminal Report). Philippines: DOST-PCIEERD.

Fickers, P., Benetti, P.H., Waché, Y., Marty, A., Mauersberger, S., Smit, M.S., & Nicaud, J.M. (2005). Hydrophobic substrate utilisation by the yeast *Yarrowia lipolytica*, and its potential applications. FEMS Yeast Research, 5(6-7), 527-543. https://doi.org/10.1016/j.femsyr.2004.09.004

Gogos, A., Knauer, K., & Bucheli, T.D. (2012). Nanomaterials in plant protection and fertilization: Current state, foreseen applications, and research priorities. Journal of Agricultural and Food Chemistry, 60(39), 9781-9792. https://doi.org/10.1021/jf302154y

Gomez, K.A., & Gomez, A.A. (1984). Statistical procedures for agricultural research (2nd ed.). New Jersey, United States: John Wiley and Sons.

Hartman, W.H., & Richardson, C.J. (2013). Differential nutrient limitation of soil microbial biomass and metabolic quotients (qCO_2): Is there a biological stoichiometry of soil microbes? PLoS One, 8(3), e57127. https://doi.org/10.1371/journal.pone.0057127

Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maldonado-Ramirez, S., Lynch, S.T., & Nelson, E.B. (2000). Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology, 15(1), 25-36. https://doi.org/10.1016/S0929-1393(00)00069-X

Juárez-Maldonado, A., Ortega-Ortíz, H., Morales-Díaz, A.B., González-Morales, S., Morelos-Moreno, Á., Sandoval-Rangel, A., Cadenas-Pliego, G., & Benavides-Mendoza, A. (2019). Nanoparticles and nanomaterials as plant biostimulants.

International Journal of Molecular Sciences, 20(1), 162. https://doi.org/10.3390/ijms20010162

Kandeler, E., & Gerber, H. (1988). Short-term assay of soil urease activity using colorometric determination of ammonium. Biology and Fertility of Soils, 6, 68-72. https://doi.org/10.1007/BF00257924

Karagunduz, A., Young, M.H., & Pennell, K.D. (2015). Influence of surfactants on unsaturated water flow and solute transport. Water Resources Research, 51(4), 1977-1988. https://doi.org/10.1002/2014WR015845

Klaine, S.J., Koelmans, A.A., Horne, N., Carley, S., Handy, R.D., Kapustka, L., Nowack, B., & von der Kammer, F. (2012). Paradigms to assess the environmental impact of manufactured nanomaterials. Environmental Toxicology and Chemistry, 31(1), 1-14. https://doi.org/10.1002/etc.733

Li, Y., Tan, W., Koopal, L.K., Wang, M., Liu, F., & Norde, W. (2013). Influence of soil humic and fulvic acid on the activity and stability of lysozyme and urease. Environmental Science and Technology, 47(10), 5050-5056. https://doi.org/10.1021/es3053027

Maddela, N.R., & Venkateswarlu, K. (2018). Insecticides-soil microbiota interactions. Switzerland: Springer, Cham.

Makoi, J.H., & Ndakidemi, P.A. (2008). Selected soil enzymes: Examples of their potential roles in the ecosystem. African Journal of Biotechnology, 7(3), 181-191.

Marzadori, C., Francioso, O., Ciavatta, C., & Gessa, C. (2000). The influence of the content of heavy metals and molecular weight of humic acids fractions on the activity and stability of urease. Soil Biology and Biochemistry, 32(13), 1893-1898.

Parr, J., & Norman, A.G. (1965). Considerations in the use of surfactants in plant systems: A review. Botanical Gazette, 126(2), 86-96.

Pratt, P.F. (1965). Potassium. Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties, 9, 1022-1030.

Paulson, K.N., & Kurtz, L.T. (1969). Locus of urease activity in soil. Soil Science Society of America Journal, 33(6), 897-901.

Piccolo, A., & Mbagwu, J.S. (1999). Role of hydrophobic components of soil organic matter in soil aggregate stability. Soil Science Society of America Journal, 63(6), 1801-1810.

Purev, D., Bayarmaa, J., Ganchimeg, B., Ankhtsetseg, B., & Anumandal, O. (2012). Catalase, protease and urease activity in some types of soil. Mongolian Journal of Chemistry, 13, 16-18.

Quiroga, G., Erice, G., Aroca, R., Zamarreño, Á.M., García-Mina, J.M., & Ruiz-Lozano, J.M. (2020). Radial water transport in arbuscular mycorrhizal maize plants under drought stress conditions is affected by indole-acetic acid (IAA) application. Journal of Plant Physiology, 246, 153115. https://doi.org/10.1016/j.jplph.2020.153115

Rowell, D.L. (1994). Soil science: Methods and applications. England, UK: Addison Wesley Longman Singapore Publishers P. Ltd.

Sabir, S., Arshad, M., & Chaudhari, S.K. (2014). Zinc oxide nanoparticles for revolutionizing agriculture: Synthesis and applications. The Scientific World Journal, 2014, 925494. https://doi.org/10.1155/2014/925494

Sadiq, Y., Zaid, A., & Khan, M.M.A. (2020). Adaptive physiological responses of plants under abiotic stresses: Role of phytohormones. In M. Hasanuzzaman (Ed.), Plant ecophysiology and adaptation under climate change: Mechanisms and perspectives I (pp. 797-824). Singapore: Springer.

Santimano, M.C., & Kowshik, M. (2013). Altered growth and enzyme expression profile of ZnO nanoparticles exposed non-target environmentally beneficial bacteria. Environmental Monitoring and Assessment, 185, 7205-7214. https://doi.org/10.1007/s10661-013-3094-6

Servin, A., Elmer, W., Mukherjee, A., De la Torre-Roche, R., Hamdi, H., White, J.C., Bindraban, P., & Dimkpa, C. (2015). A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. Journal of Nanoparticle Research, 17(2), 1-21. https://doi.org/10.1007/s11051-015-2907-7

Sessitsch, A., Weilharter, A., Gerzabek, M.H., Kirchmann, H., & Kandeler, E. (2001). Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. Applied and Environmental Microbiology, 67(9), 4215-4224. https://doi.org/10.1128/AEM.67.9.4215-4224.2001

Shang, Y., Hasan, M., Ahammed, G.J., Li, M., Yin, H., & Zhou, J. (2019). Applications of nanotechnology in plant growth and crop protection: A review. Molecules, 24(14), 2558. https://doi.org/10.3390/molecules24142558

Shiyin, L., Lixiao, N., Panying, P., Cheng, S., & Liansheng, W. (2004). Effects of pesticides and their hydrolysates on catalase activity in soil. Bulletin of Environmental Contamination and Toxicology, 72(3), 600-606. https://doi.org/10.1007/s00128-004-0286-3

Sunde, M., Pham, C.L., & Kwan, A.H. (2017). Molecular characteristics and biological functions of surface-active and surfactant proteins. Annual Review of Biochemistry, 86, 585-608. https://doi.org/10.1146/annurev-biochem-061516-044847

Swain, A., Bastiray, A., & Jitendra, K. (1978). Estimation of soil microbial biomass using substrate induced respiration: An experimental review study with loamy soil of North Bangalore. http://dx.doi.org/10.13140/2.1.2139.9049

Tabatabai, M.A. (1982). Soil enzymes. In A.L. Page, R.H. Miller & D.R. Keeney (Eds.), Methods of soil analysis, part 2, chemical and microbiological properties (pp. 903-947). Madison, Wisconsin, USA: ASA-SSSA.

Thorton, H.G. (1922). On the development of a standardised agar medium for counting soil bacteria, with special regard to the repression of spreading colonies. Annals of Applied Biology, 9(3-4), 241-274. https://doi.org/10.1111/j.1744-7348.1922.tb05958.x

Von Mersi, W., & Schinner, F. (1991). An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. Biology and Fertility of Soils, 11(3), 216-220. https://doi.org/10.1007/BF00335770

Walkley, A., & Black, I.A. (1934). An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science, 37, 29-38.

Wang, G., & Or, D. (2010). Aqueous films limit bacterial cell motility and colony expansion on partially saturated rough surfaces. Environmental Microbiology, 12(5), 1363-1373. https://doi.org/10.1111/j.1462-2920.2010.02180.x

Wolińska, A., & Stępniewska, Z. (2012). Dehydrogenase activity in the soil environment. Dehydrogenases, 10, 183-210. https://doi.org/10.5772/48294

Wyszkowska, J., Kucharski, J., & Lajszner, W. (2005). Enzymatic activities in different soils contaminated with copper. Polish Journal of Environmental Studies, 14(5), 659-664.

Xia, Y., Chen, X., Zheng, X., Deng, S., Hu, Y., Zheng, S., He, X., Wu, J., & Su, Y. (2020). Preferential uptake of hydrophilic and hydrophobic compounds by bacteria and fungi in upland and paddy soils. Soil Biology and Biochemistry, 148, 107879. https://doi.org/10.1016/j.soilbio.2020.107879

Zhang, C., Nie, S., Liang, J., Zeng, G., Wu, H., Hua, S., Liu, J., Yuan, Y., Xiao, H., Deng, L., & Xiang, H. (2016). Effects of heavy metals and soil physicochemical properties on wetland soil microbial biomass and bacterial community structure. Science of the Total Environment, 557, 785-790. https://doi.org/10.1016/j.scitotenv.2016.01.170

Zhang, P., Jin, T., Kumar Sahu, S., Xu, J., Shi, Q., Liu, H., & Wang, Y. (2019). The distribution of tryptophan-dependent indole-3-acetic acid synthesis pathways in bacteria unraveled by large-scale genomic analysis. Molecules, 24(7), 1411. https://doi.org/10.3390/molecules24071411