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Original Research Article

Improving the growth of *Centella asiatica* using surfactant modified natural zeolite loaded with NPK nutrients

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Abstract
There is great interest in using sustainable fertilizer to enhance medicinal plant quality such as *Centella asiatica*, which is known for its various medicinal properties. Here, we examined the performance of NPK-Organo-Zeolite (NPKOZ) as a controlled release fertilizer for the growth of *C. asiatica*. Natural zeolite that has been modified with surfactant hexadecyltrimethyl ammonium (HDTMA) and addition with ammonium (N), phosphate (P) and potassium (K) was characterized with X-ray diffraction and Fourier transform infrared spectroscopy. In a leaching study, it was found the NPKOZ showed fewer losses of nutrients. For the plant growth study, a comparison was made with eight different treatments including chemical fertilizers monoammonium phosphate and monopotassium phosphate for 70 days. Morphological (number of flowers, branches and leaves, specific leaf area and plant biomass) and biochemical growth parameters (N, P, K and chlorophyll contents) of the plant were analysed. Second cultivation was also conducted to compare potted, hydroponic and verticulture systems with or without NPKOZ. The NPKOZs treatments resulted in the highest number of leaves, branches and flowers among all treatments. In addition, the chlorophyll contents were also the highest with NPKOZs application. The NPK contents in the *C. asiatica* plants were comparable with that of chemical fertilizers tested. This study showed that NPKOZ fertilizer with verticulture system is a suitable sustainable controlled release fertilizer for the improvement of the growth of *C. asiatica*.

Keywords: *Centella asiatica*, Surfactant modified zeolite, Controlled release fertilizer

Introduction

Vegetables contribute an important part in malnutrition problems and resulted in a higher rate of mortality such as accident happened in Mali and Nigeria (Keatinge et al., 2011). Plant herbs especially the leafy or parts of soft flowering offered valuable fibre and medicinal properties. One of many potential vegetable that needs to be improved is *Centella asiatica (Pennywort)* that belongs to the family of Apiaceae. *C. asiatica* which flourishes in damp places could be eaten as a raw vegetable in Malaysia and Indonesia and mostly found in tropical and subtropical countries such as India, Southeast Asia and South Africa (Huda-Faujan et al., 2009; Gohil et al., 2010). Different types of phytochemical compounds such as triterpene acid, flavonoids and alkaloids are useful in medicinal problems. Pennywort represents one of the herbs that can treat numerous ethnomedical and pharmacological problems, for example as a wound dressing, antioxidant properties as well as reducing Parkinson's disease symptoms (Jin et al., 2015; Nataraj et al., 2017). Thus, studying the improvement of *C. asiatica* yield is very important because of its high valuable properties and application.

The usage of excess fertilizers in agriculture could harm the environment such as leaching of nutrients from the fertilizers (Lubkowski, 2016). This nutrients leaching contributes significantly to the eutrophication and groundwater contamination (Penuelas et al., 2009). For example in China and Taiwan, the problem with nitrogen leaching from agricultural activities that contaminates groundwater becoming a national major concern (Xu et al., 2010, Adhikari and Chen, 2013). Besides, Penuelas *et al.* (2009) documented the effect of algal bloom caused by the excess of phosphorus in fertilizer in Catalonia, Spain. Furthermore, Ah Tung *et al.* (2009) also reported that N and K fertilizers implementation in oil palm showed a concern for groundwater quality in Malaysia. There are various methods in combating nutrients leaching and one of them is the application of a slow release fertilizer (SRF) that can reduce nutrients losses, lowering fertilization expenditure and increased crop yields (Sempeho, 2014).

In this part, a 3-dimensional aluminosilicates mineral material called zeolite is a candidate of choice as a controlled release fertilizer for cultivation because it has high adsorption efficiency (Polat et al., 2004). Raw zeolite is inadequate to help plant growth since it lacks in major plant nutrients. Macronutrient like N is needed to improve the stimulate the green growth production of the plant, P as an important phosphate source for nucleic acid and K provides a vital role in photosynthesis activity (Prajapati and Modi, 2012). The zeolite can only adsorb and retain cationic elements such as ammonium and potassium because of the negative charge of the zeolite framework. Fortunately, zeolite efficiency can be improved by modifying its external surface using cationic surfactant molecules (Reháková et al., 2004; Bansiwal et al., 2006; Bhardwaj et al., 2012). The surfactant modified zeolite can hold P element and the combination of zeolite itself could hold N and K nutrients. One of the major characteristics of this fertilizer is the ability of slowly released the nutrients. Nevertheless, the related particular reports only mentioned single N-loaded or P-loaded surfactant modified zeolite (SMZ) as a fertilizer (Li, 2003; Bansiwal et al., 2006; Bhardwaj et al., 2012). The introduction of N, P and K onto zeolite is a better way of increasing nutrients retention capacity in medicinal plant production especially for the *C. asiatica* growth. Furthermore, there are diverse methods on how to grow a plant, including the outdoor and indoor techniques. Basically, improvements from year to year are made to culture the plant in a way of lessening the fertilizer, water and spaces used. Common ways of planting a plant include potted technique, which have different container types that can be made from ceramic, plastic or fibre waste. In addition, a liquid based culture known as a hydroponic method excels in regulating humidity, nutrients percentage and quality of water (Rajpurkar, 2016). Vertical farming or verticulture is another form of cultivating the plant but in a vertical condition (Sitawati et al., 2016). These three cultivation methods also showed a possibility of decreasing nutrient leaching when incorporated with suitable fertilizer. The basic concept of this study was to compare the system used to grow *C. asiatica* with NPK-Organo-Zeolite (NPKOZ) and conventional fertilizers with the development of new technique in growing the herb by using NPKOZ. Thus, it is hoped to bring a valuable solution to increase the desired yield of *C. asiatica* and reducing environmental concern for leaching problems by applying slow release fertilizer (SRF) (Sempeho, 2014).

Materials and Methods

Preparation and Characterization of NPK-Organo-Zeolite

Zeolite from Indonesia was obtained from Provet Group of Companies Sdn Bhd, Serdang, Selangor. Monoammonium phosphate (MAP) and monopotassium phosphate (MKP) fertilizers were supplied by Greentrade Sdn Bhd while Ferti 45 (F45) fertilizer was obtained from Fertiland Trading Co.. The surfactant hexadecyltrimethyl ammonium bromide (HDTMA-Br) and potassium chloride (KCl) were supplied by Qrec (Asia) Sdn Bhd. The preparation process of NPK-Organo-Zeolite was referred to Malek et al. (2014) which was begin with the addition of 40 g zeolite (zeo) with KCl and stirred overnight by using a magnetic stirrer. Then, the mixture was filtered with 185 mm Macherey-Nagel filter paper and dried in an oven at 80°C overnight. Next, the solid mesh was ground with mortar and pestle into powder form and sieved. The steps were repeated three times to ensure most K ions are located inside the zeolite framework forming K-zeo. Next, 10 g of K-cli were mixed with 4 mM of HDTMA and Organo-K-zeo (OKZ) was obtained after the grinding process. Lastly, 40 g of OKZ was added into MAP to form the final product which is NPKOZ. Each product formed was characterized by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) with ATR (Attenuated Total Reflectance) sampling technique (Malek, 2011; Malek et al., 2014).

Elemental Analysis of Fertilizers

All fertilizers were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) Perkin Elmer Optima at Ayer Hitam Agriculture Institute (IPAH), Johor for K and the content of trace elements (%) is shown in Table - 1. The determination of N content (%) in the materials was conducted by using Kjeldahl method and P content (%) with atomic absorption spectroscopy (AAS), AAnalyst 300, Perkin Elmer at Institute of Veterinary Malaysia, Johor.

Column Leaching Study

The release patterns of nutrients from 8 different treatments in 15 days were determined by column leaching study (Li, 2003; Malek et al., 2015). The treatments were applied by top dressing on top of the sand and it was done in triplicates and the experimental setup includes retort stand, transparent plastic cup, 185 mm filter paper and 200 ml collection plastic cup. Each cup (top) was filled in with approximately 200 g of sand and fixed amount of fertilizers (Table - 2). Then, 100 ml of distilled water was poured into each cup for every 24 hours and leachate was then collected and stored in 50 ml plastic bottle. NANOCOLOR® Test 1-05 (Ref 918 05) and NANOCOLOR® Test 1-77 (Ref 918 77), Macherey-Nagel were used to measure ammonium (NH_4^+) and phosphate in the leachate solution, respectively. Whereas, K nutrient (K^+) in the leachates was analysed using ICP-OES.

Table -1: Elemental analysis of the materials used in the study

No.	Materials	N (%)	P (%)	K (%)	Zn (%)	Mn (%)	Fe (%)
1	Zeo	0.00 ± 0.00	0.02 ± 0.00	0.20 ± 0.00	27.33 ± 0.88	128.67 ± 3.71	0.33 ± 0.03
2	MAP	14.38 ± 0.01	1.11 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3	MKP	0.00 ± 0.00	1.11 ± 0.03	31.37 ± 0.85	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4	F45	18.29 ± 0.38	0.37 ± 0.01	14.57 ± 0.20	15.00 ± 0.58	102.67 ± 0.88	0.10 ± 0.00
5	NPKOZ	0.69 ± 0.00	0.14 ± 0.00	2.83 ± 0.03	15.67 ± 1.76	110.00 ± 4.73	0.53 ± 0.07

Table - 2: List of treatments for column leaching study

No.	Treatments	Description
1	Control	Sand (200 g) (No treatment)
2	Zeo	Sand (200 g) + Zeolite (5 g)
3	MAP	Sand (200 g) + Monoammonium phosphate (5 g)
4	MKP	Sand (200 g) + Monopotassium phosphate (5 g)
5	F45	Sand (200 g) + F45 (5 g)
6	NPKOZ5	Sand (200 g) + NPK-Organo-Zeolite (5 g)
7	NPKOZ10	Sand (200 g) + NPK-Organo-Zeolite (10 g)
8	NPKOZ15	Sand (200 g) + NPK-Organo-Zeolite (15 g)

Cultivation Process of *Centella asiatica* for Fertilizers Comparison

Firstly, the soil pH was maintained at pH 7 and sandy loamy soil was used throughout the experiment (Devkota and Jha, 2009). *C. asiatica* was obtained from Institute of Veterinary Malaysia, Johor. The *C. asiatica* was cultivated and grouped for eight different treatments as in Table - 2 in a semi-greenhouse for 35 days. At least 3 leaves per plantlet were planted into a mixture of sand and soil (40%:60%) that was evenly mixed (Devkota and Jha, 2009). The harvest period was done after 70 days of cultivation (Zainal, 2005). Morphological growth parameters like number of leaves, branches, flowers, leaf area (LA) (cm^2) and biggest specific leaf area (BSLA) (cm^2/g) were measured. Meanwhile, the total fresh biomass (TFB) and total dried biomass (TDB) of *C. asiatica* were weighed and then dried in an oven at 60°C for 48 hours (Devkota and Jha, 2009). Determination of the biochemical growth parameters was conducted after harvesting the plants including the estimation of total chlorophyll content in leaf using acetone which were determined their absorbance values at 662, 646, and 470 nm (Pompelli et al., 2013). Lastly, the leaves samples were analysed for N, P and K contents in *C. asiatica*.

Cultivation Process of *Centella asiatica* for Systems Comparison

For systems comparison, three different cultivation techniques with or without NPKOZ was compared with similar propagation, soil pH and location study. The morphological and biochemical growth parameters used in the second comparison were also similar with previous procedure. However, the system used in this study is the potted technique with fixed rectangular shape made from plastic material (48.5 cm × 19.5 cm × 14.5 cm). The second system was based on hydroponic method by the Deep Water Culture (DWC) system with little modification (57.0 cm × 43.0 cm × 13.5 cm) (Okemwa, 2015). The last method which is verticulture system required the usage of vertical gardening concept by using polyvinyl chloride (PVC) with the height of 72.5 cm and 8.6 cm in diameter. A total of eight holes were made and the PVC was then submerged in small pot filled with 2 kg of soil for stability.

Statistical Analysis

The data from each parameter were analysed by computing software, Statistical Package for the Social Sciences (SPSS) software version 16. A one-way analysis of variance (ANOVA) test involving one species (*C. asiatica*), morphological growth parameters and eight treatments was interpreted. The post hoc Duncan Multiple Range Test (DMRT) was conducted to compare the means and find the existence of variation. Lastly, Pearson Correlation test was carried out to identify the correlation between the parameters with the significance level of ($p \leq 0.05$).

Results and Discussion

Characterization of NPK-Organo-Zeolite

XRD patterns of modified zeolite (K-zeo, OKZ and NPKOZ) were compared with raw zeolite shown in Figure 1(i). Most of the detected materials consist of natural zeolite clinoptilolite (C) and some quartz and mordenite as its impurities (Treacy and Higgins, 2007). Characterization of the prepared materials by FTIR spectroscopy was performed to detect the presence of HDTMA molecules in the sample (Figure 1(ii)). There are important bands were observed (in circle) in the FTIR spectra of the surfactant modified zeolites (Organo-K-zeo (2921 and 2850 cm^{-1}) and NPKOZ (2923 and 2851 cm^{-1}) samples). Both bands were in line with the finding from previous study (Malek, 2011) where these bands proved the presence of CH_3 asymmetric and CH_2 stretching of the HDTMA molecules, respectively. The FTIR peaks below 1000 cm^{-1} represent Si-O-Al and Si-O-Si bonding properties of the zeolite framework with a weak peak at 793 cm^{-1} for the symmetric bonding. In addition, the strong intensity of the wide peak at 1008 cm^{-1} is referred to asymmetric bonding which similarly observed at bands around 1000 cm^{-1} and 500 cm^{-1} (Sharuddin *et al*. 2014). The presence of clinoptilolite can also be seen in many specific bands ranging from 3400 and 3700 cm^{-1} bands (Mansouri et al., 2013).

The negative charge of the zeolite surfaces made it possible to attract other cations especially ammonium and potassium cations and also cationic surfactant HDTMA-Br. The attachment of HDTMA-Br which has polar head surrounded with a charge on the zeolite surfaces creates surfactant modified zeolite (SMZ) and eventually, the SMZ has the ability in adsorbing anionic compounds such as phosphate or nitrate (Haggerty and Bowman, 1994). From the XRD, the crystalline structure of the modified zeolite unchanged and stable after undergoes several reaction processes which are heating and nutrients loaded processes (Malek *et al*. 2015). Thus, the stable ionic nature of the zeolite framework helped attracting the cationic charge for instance, ammonium and potassium cations as well as amphiphilic compounds of HDTMA-Br surfactant in attaching phosphate ions towards the formation of NPKOZ. It is possible that the zeolite will remain intact as a medium in the soil (Reháková et al., 2004; De Smedt et al., 2015).

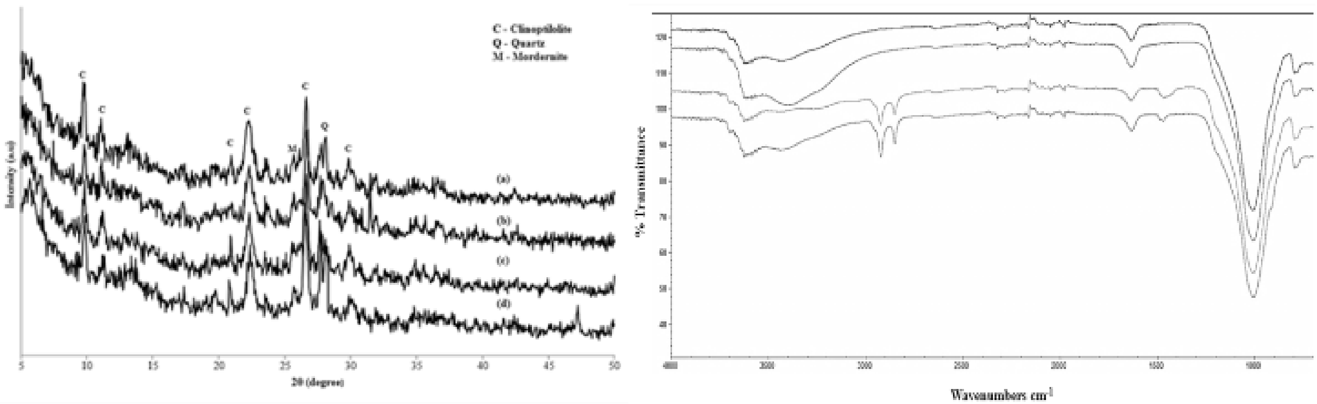

Figure 1

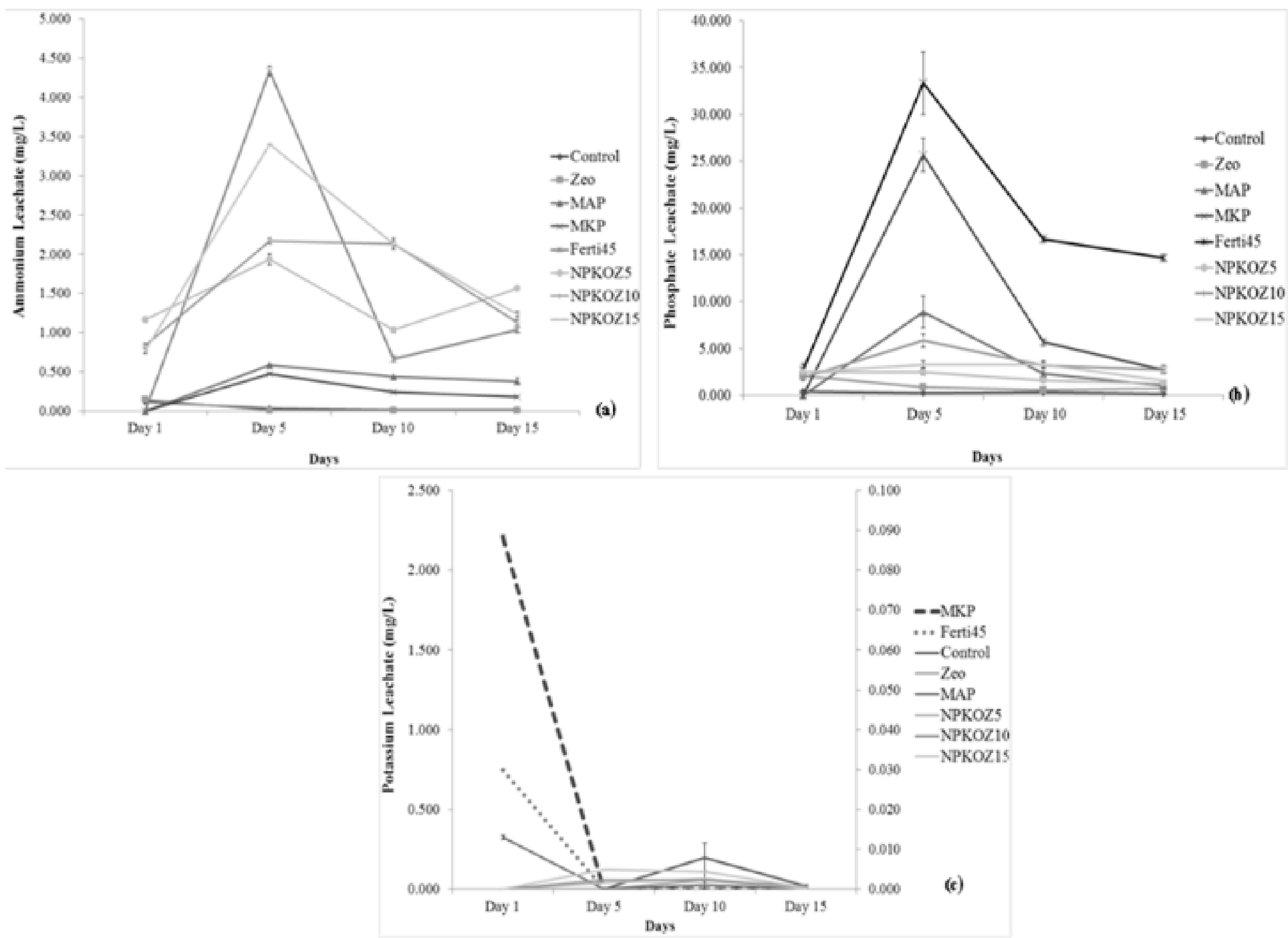

Figure 2

Column Leaching Study

Based on Figure 2, all fertilizer samples released nutrients at maximum level at day 5 and gradually decreased until day 15. A high phosphate ion was found to release from Ferti 45 fertilizer on day 5 as compared to other samples (Figure 2b). The phosphate released percentage by Ferti 45 as compared to NPKOZ exceeded more than 500%. Furthermore, the result of ammonium leaching was similarly recorded by Ferti 45 fertilizer where it had a dramatic loss from day 5 to 10. For the potassium released (K^+) in the column leaching study, monopotassium phosphate fertilizer released the highest K^+ on day 1, which is linked to its highest K content (Figure 2c). Ferti 45 fertilizer also marked second in rapid losses of K^+ ion while the NPKOZ only released K^+ on day 5 afterward in a small quantity. The absence of physical nutrients carrier such as zeolite in the chemical fertilizer resulted in a higher loss of respective nutrients from chemical fertilizers.

In column leaching study, the initial detection of nutrients in the first week was considered normal and leached out until the value is detected over the course (Lou et al., 2015). The chemical fertilizer such as F45 was observed to release more nutrients than their counterpart, NPKOZ fertilizer. At higher concentration of P in fertilizer, it can be indicated that the increasing P will also increase its leaching content. The slow release of phosphate ion from NPKOZ happens because the SMZ has capabilities in adsorbing phosphate ions from aqueous solution (Bansiwal, 2006; Hamzah et al., 2014). Meanwhile, the strong attraction of K^+ towards zeolite anionic framework is achieved by cation exchange property of the zeolite (Li et al., 2013). The incorporation of N and K inside the zeolite is not only contributes to nutrients loss but also a better performance for plant growth (Li et al., 2013).

Comparison between NPKOZ and Conventional Fertilizers towards the *C. asiatica* Growth

The morphological growth parameters in Table - 3 showed that NPKOZ15 has the highest number of leaves after 70 days of cultivation. In contrast, MAP with a lot of ammonium element for leaves development did not resulted in a high number of leaves. Moreover, the branches numbers and number of flowers were higher in NPKOZ10 and NPKOZ15 than other treatments. Interestingly, even though F45 had the highest TFB, the biggest reduction of its weight after heating treatment into the TDB was notifiable. On the other hand, the NPKOZs treatments increased steadily for its TFB and TDB than other systems except for the Ferti 45 system. Based on the result of leaves number, six out of eight treatments mean score were considered rejecting the null hypothesis except for control ($M = 2.33$, $SE = 0.33$) and NPKOZ15 ($M = 12.67$, $SE = 1.45$) that did not differ significantly from other treatments. In term of the biggest leaf area (cm^2) (BLA) and the biggest specific leaf area (cm^2/g) (BSLA) selected during harvesting time, F45 application contributed to the highest value compared to other systems. However, NPKOZ5 application resulted as good as MAP and MKP fertilizers performance. In overall, the increased in the amount of NPKOZ used (5, 10 and 15 g) resulted in the increased morphological growth parameters. There are two major biochemical growth parameters presented in this study; chlorophyll contents and N, P, and K contents for the growth of *C. asiatica* (Figure 3). In this study, the application of NPKOZ5, NPKOZ10 and NPKOZ15 dominated the total chlorophyll content (TCC) while F45 resulted in the lowest TCC value. In term of N and K contents, F45 showed the highest percentage while NPKOZs treatments were comparable with that of MAP and MKP. On the other hand, the number of branches, leaves and flowers along with the changes of TFB to TDB showed that the NPKOZs application was better than other treatments which are known for its slow release properties. There was a strong, positive correlation between number of branches ($r = 0.948$, $n = 24$, $P \leq 0.01$), number of flowers ($r = 0.817$, $n = 24$, $P \leq 0.01$), TFB ($r = 0.908$, $n = 24$, $P \leq 0.01$), TDB ($r = 0.866$, $n = 24$, $P \leq 0.01$), and BLA ($r = 0.522$, $n = 24$, $P \leq 0.01$) towards the number of leaves in Table - 3. A similar results pattern can be seen in parameter number of branches and chlorophylls. Reháková *et al.* (2004) suggested that the zeo loaded with certain nutrients might thrive longer during vegetation, as the nutrients were released slowly. This finding might be contributed in the making of NPKOZ that was added with the MAP at the last stage of the preparation. By the supplement of MAP onto the surfactant layer, NPKOZ was able to discharge the ammonium element that helped in leaf formation and phosphate element for the cell nucleic development for the growth of the plant stem. Besides that, the last nutrient to be released is K element inside NPKOZ that regularly known to induce the growth of flowers (Hitchmough, 2008). The presence of flower as an essential organ for the plant reproduction could have been related to the previous numbers of branches formed, where the flower arose from beneath. In term of biochemical parameters, chlorophyll is one of the main component in the photosynthesis process. Chlorophyll also served as parts of compounds in antioxidant property, namely the carotenoid. This outcome showed a valuable property of NPKOZ as a single solution to increase the chlorophylls content in the leafy plant. Although the uptakes of NPK are fewer by the whole plant for NPKOZs application compared to F45, the initial deduction was that the NPKOZs are slightly better than its counterpart. The ability of NPKOZ to release the nutrients as discussed in the characterization section might provide a sufficient source of nutrients even with fewer amounts.

Comparison between Cultivation System towards the *C. asiatica* Growth

In morphological growth parameters based on Table - 5, plant treated by both NPKOZ Pot (M = 20.00, SE = 2.97) and NPKOZ Verti (M = 21.20, SE = 1.88) exceeded more than 20 leaves, which is higher than other systems. The results are not the same for the hydroponic system where the observed number of leaves was found similarly lower in both control and NPKOZ. Furthermore, petiole length for the plant after treated with NPKOZ Pot and Verti systems showed the highest length as compared to other systems. Moreover, the measured leaf area signified the highest value for NPKOZ Verti and Pot systems, where it is almost doubled compared to the control system. The root length of the plant did not differ in the verticulture and pot systems whereby it is higher in the hydroponic system due to its liquid nature based or Deep Water Culture (DWC) bed (Okemwa, 2015). Besides that, the TFB and TDB results for NPKOZ systems clearly outnumbered the control systems. For instance, NPKOZ Verti (M = 11.29, SE = 1.96) weighted in almost doubled for its fresh biomass than the control Verti (M = 7.41, SE = 0.96). Apart from the morphological parameters, the biochemical properties for the second cultivation were also recorded as positive results in chlorophyll contents and nutrient contents in *C. asiatica*. Among all NPKOZ systems, NPKOZ Verti is observed as the highest recorded data for the combined chlorophyll a and b. Besides that, it is noticeable that phosphate content in the three systems containing NPKOZ contributed to the highest value of P in *C. asiatica*. Initial conclusion showed that the verticulture systems contributed more positive parameters than other cultivation systems, based on either morphological or biochemical growth parameters.

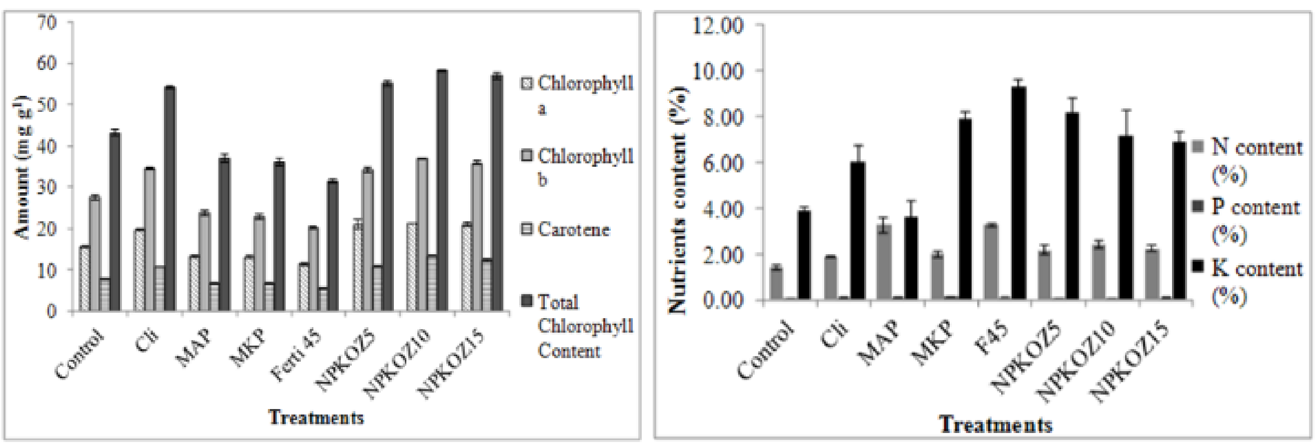

Figure 3:

Table - 3: Means comparisons with morphological growth parameters in eight treatments towards the growth of *C. asiatica* after 70 days. For each parameter, significant difference between means among different treatments are indicated by different letters (Duncan test, α= 0.05).

Treatments	No. of leaves	No. of branches	No. of flowers	TFB (g)	TDB (g)	BLA (cm^2)	BSLA (cm^2/g)
Control	$2.33^{a} \pm 0.33$	$1.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$1.28^{a} \pm 0.45$	$0.10^{a} \pm 0.03$	$7.33^{a} \pm 0.33$	$383.33^{a} \pm 16.67$
Zeo	$4.33^{ab} \pm 0.88$	$1.33^{ab} \pm 0.33$	$0.33^{a} \pm 0.33$	$3.64^{ab} \pm 0.24$	$0.47^{ab} \pm 0.05$	$12.33a^{b} \pm 1.09$	$316.11^{a} \pm 33.89$
MAP	$6.00^{abc} \pm 1.53$	$2.33^{ab} \pm 0.67$	$0.67^{a} \pm 0.33$	$4.58^{ab} \pm 1.88$	$0.51^{ab} \pm 0.20$	$14.67a^{bc} \pm 3.72$	$330.24^{a} \pm 25.78$
MKP	$5.00^{abc} \pm 1.00$	$2.33^{ab} \pm 1.33$	$1.33^{a} \pm 1.33$	$3.35^{ab} \pm 0.60$	$0.37^{ab} \pm 0.08$	$13.83^{abc} \pm 0.73$	$422.22^{a} \pm 43.39$
F45	$11.00^{bc} \pm 4.04$	$4.33^{ab} \pm 1.76$	$1.67^{a} \pm 1.67$	$8.91^{b} \pm 2.80$	$0.77^{b} \pm 0.28$	$21.33^{c} \pm 3.81$	$452.50^{a} \pm 58.86$
NPKOZ5	$5.33^{abc} \pm 1.45$	$2.00^{ab} \pm 1.00$	$1.33^{a} \pm 0.88$	$3.67^{ab} \pm 0.46$	$0.39^{ab} \pm 0.46$	$15.67^{abc} \pm 2.09$	$428.33^{a} \pm 73.39$
NPKOZ10	$12.00^{bc} \pm 4.36$	$5.00^{ab} \pm 2.31$	$3.33^{a} \pm 1.76$	$6.40^{ab} \pm 2.30$	$0.69^{ab} \pm 0.24$	$14.67^{abc} \pm 1.96$	$346.67^{a} \pm 39.30$
NPKOZ15	$12.67^{c} \pm 1.45$	$5.33^{b} \pm 0.88$	$3.00^{a} \pm 1.53$	$8.13^{b} \pm 2.12$	$0.91^{b} \pm 0.30$	$19.33^{bc} \pm 4.10$	$348.33^{a} \pm 53.26$

Notes: (Included Standard Error Mean (SEM), Total Fresh Biomass (TFB), Total Dry Biomass (TDB), Biggest Leaf Area (BLA), and Biggest Specific Leaf Area (BSLA))

Table - 4: Pearson correlation coefficients comparisons with morphological growth parameters in eight different systems towards *C. asiatica* growth at significance level Sig (2-tailed). Noted that * represent significant (p<0.05) and ** represent highly significant correlation (p<0.01).

Parameters	No. of leaves	No. of branches	No. of flowers	TFB (g)	TDB (g)	BLA (cm^2)	BSLA (cm^2/g)
No. of leaves	1						
No. of branches	.948**	1					
No. of flowers	.817**	.874**	1				
TFB (g)	.908**	.814**	.759**	1			
TDB (g)	.866**	.768**	.785**	.969**	1		
BLA (cm^2)	.522**	.398	.443*	.688**	.663**	1	
BSLA (cm^2/g)	-.095	-.105	-.108	.018	-.105	.070	1

Notes: (Total Fresh Biomass (TFB), Total Dry Biomass (TDB), Biggest Leaf Area (BLA), Biggest Specific Leaf Area (BSLA))

Table - 5: Means comparisons with morphological growth parameters in three systems towards the growth of *C. asiatica* after 70 days. For each parameter, significant difference between means among different treatments are indicated by different letters (Duncan test, α= 0.05).

Systems	No. of leaves	TPL (cm)	Root length (cm)	TFB (g)	TDB (g)	Leaf area (cm^2)	BSLA (cm^2/g)
Control P	$18.80^{bc} \pm 2.35$	$8.38^{a} \pm 0.77$	$14.59^{a} \pm 0.82$	$7.28^{ab} \pm 0.61$	$0.83^{b} \pm 0.11$	$10.56^{bc} \pm 0.89$	$655.93^{bc} \pm 86.00$
NPKOZ P	$20.00^{c} \pm 2.97$	$12.18^{bc} \pm 1.68$	$14.53^{a} \pm 4.49$	$8.85^{bc} \pm 1.27$	$1.15^{c} \pm 0.12$	$19.53^{c} \pm 2.14$	$491.25^{ab} \pm 20.12$
Control H	$10.60^{a} \pm 0.93$	$8.24^{a} \pm 1.24$	$16.21^{a} \pm 2.41$	$4.47^{a} \pm 0.38$	$0.53^{a} \pm 0.04$	$8.21^{a} \pm 1.00$	$623.00^{bc} \pm 86.54$
NPKOZ H	$10.60^{a} \pm 1.21$	$8.02^{a} \pm 0.41$	$11.66^{a} \pm 1.45$	$4.27^{a} \pm 0.31$	$0.51^{a} \pm 0.04$	$9.21^{bc} \pm 1.37$	$780.60^{c} \pm 69.79$
Control V	$13.40^{ab} \pm 0.93$	$10.60^{ab} \pm 0.97$	$12.09^{a} \pm 0.95$	$7.41^{ab} \pm 0.65$	$0.98^{bc} \pm 0.09$	$13.05^{b} \pm 0.83$	$401.43^{a} \pm 61.09$
NPKOZ V	$21.20^{c} \pm 1.88$	$15.04^{c} \pm 1.24$	$14.95^{a} \pm 1.27$	$11.29^{c} \pm 1.96$	$1.50^{d} \pm 0.14$	$21.51^{c} \pm 1.83$	$486.38^{ab} \pm 46.47$

Notes: (Included Standard Error Mean (SEM), Total Fresh Biomass (TFB), Total Dry Biomass (TDB), Tallest Petiole Length (TPL), and Biggest Specific Leaf Area (BSLA))

Table - 6: Pearson correlation coefficients comparisons with morphological growth parameters in three systems towards *C. asiatica* growth at significance level Sig (2-tailed). Noted that * represent significant (p<0.05) and ** represent highly significant correlation (p<0.01).

Parameters	No. of leaves	TPL (cm)	Leaf area (cm^2)	Root length (cm)	TFB (g)	TDB (g)	BSLA (cm^2/g)
No. of leaves	1						
TPL (cm)	.493*	1					
Leaf area (cm^2)	.685**	.884**	1				
Root length (cm)	.595**	.429	.404	1			
TFB (g)	.729**	.773**	.917**	.524*	1		
TDB (g)	.697**	.827**	.930**	.506*	.901**	1	
BSLA (cm^2/g)	-.049	-.605**	-.505*	-.261	-.501*	-.479*	1

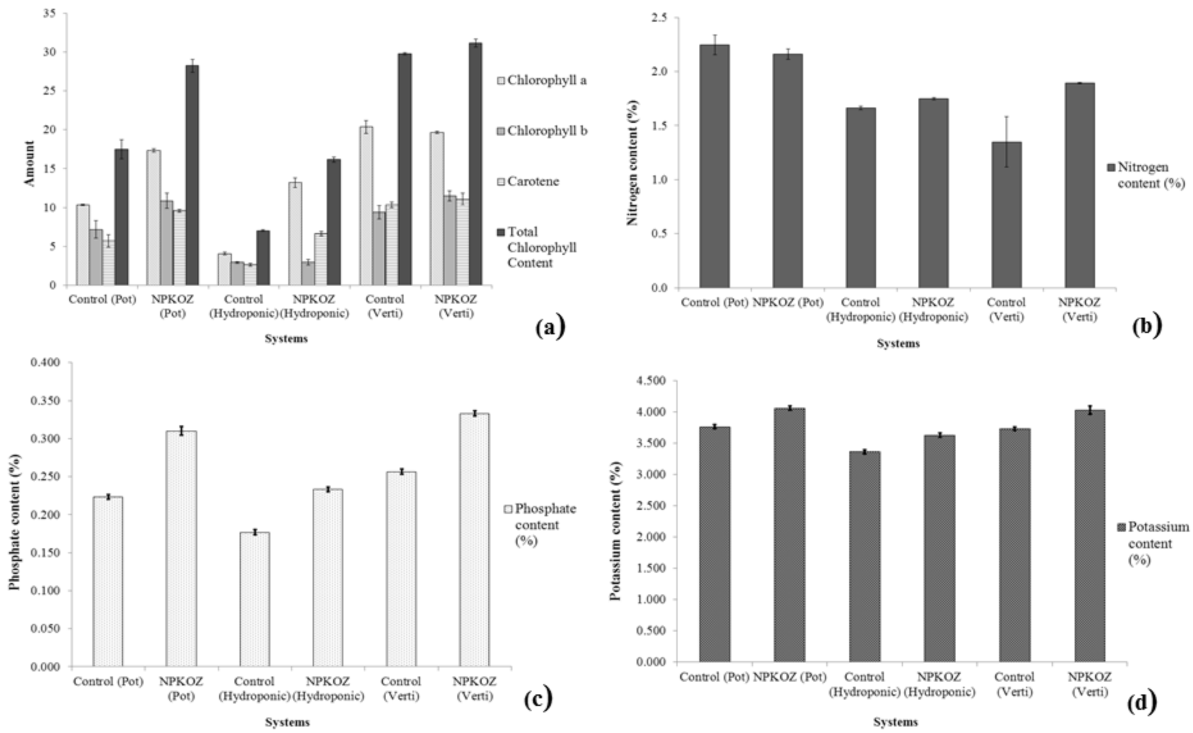

Figure 4

The second cultivation of *C. asiatica* by using three systems applied with NPKOZ also recorded significant results, especially the high number of leaves. Nitrogen source plays an important role in the development of plant leaves, which is the main reason of the highest number of leaves. Ohyama (2010) briefly described that nitrogen (N) element not only provide a shoot growth in excess, but also preferred the young leaves if the N source is depleted. In contrast, NPKOZ application in the hydroponic system provided only slightly positive results in overall parameters compared to control. The hydroponic system used in this study was based on Deep Water Culture (DWC) bed which needs small plant pots with coco peat media (Okemwa, 2015). The little pots keep afloat in water based bed where it is usually filled or replace with liquid organic fertilizers that provide nutrients in liquid formed. In this experiment, no solution fertilizer based was used and furthermore, NPKOZ is insoluble in water. For this reason, NPKOZ in teabag method is subjected to the loss of nutrients before being taken by the plant. For the chlorophyll parameter part, NPKOZ application on each system of the cultivation showed higher values as compared to the control system in term of TCC. The greener the tone colour of the plant itself, the higher the chances of chlorophyll content piling up. Limantara *et al* (2015) studied the comparison between ten green leafy vegetables where they found that the darker green colour contributed to the total peak of chlorophyll content using *Brassica rapa L.* (Chinese flowering cabbage). In this study, almost all morphological and biochemical growth parameters recorded either significant or highly significant correlation. For example in signification correlation, there was a positive correlation between tallest petiole length ($r = .493$, $n = 18$, $P<0.05$), total chlorophyll content ($r = .518$, $n = 18$, $P<0.05$) and N content ($r = .572$, $n = 18$, $P<0.05$) towards the number of leaves. In broad, NPKOZ Verti took two out of four morphological growth parameters highest assessments. Based on the positive results of the verticulture system in BGP (chlorophyll and phosphate content) and high in four MGP parameters (NOL, TPL, leaf area, TFB and TDB), the initial conclusion clarified that the verticulture is the best system among other systems.

Conclusion

From characterization of the prepared materials by XRD, FTIR and leaching study, it was proven that the cationic surfactant HDTMA molecules were attached on the zeolite framework without changing the framework structure of the zeolite. In addition, these surfactant molecules assist zeolite in adsorbing phosphate anions. Chemical fertilizers resulted in a dramatic loss of major nutrients in leachate, especially F45 but, NPKOZ fertilizers able to attain the highest number of leaves, flowers and branches as well as overall chlorophyll contents because of the slowly release of the plant nutrients. Furthermore, the verticulture method applied with NPKOZ is selected as the best cultivation system and it is a potential sustainable fertilizer for the high yield of *C. asiatica*.

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