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Full Length Research Paper

The Effect of Calcium and Magnesium on the Growth and Yield of Okra (*Abelmoschus esulentus* L.) through Foliar Application

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ABSTRACT: Foliar fertilization is a popular practice for supplying mineral nutrients to the crops, especially under conditions of limited availability of soil nutrients. This study hypothesized that the foliar nutrients application can enhance the growth and yield of okra. The experiment was conducted at Complex Field 10, Faculty of Agriculture, Universiti Putra Malaysia, and laid out as Randomized Completely Block Design (RCBD) with four treatments and six replications. The treatments consist of Treatment 1 (T1): Foliar (calcium and magnesium); Treatment 2 (T2): NPK 15:15:15; Treatment 3 (T3): Foliar (calcium and magnesium plus NPK 15:15:15), and Treatment 4: Control. The foliar were sprayed with 2% on the leaf every 2 weeks and the fertilizer was spread around the crop. The data were recorded by measuring (i) the plant height (4 weeks interval); (ii) fruit yield (until 120 DAP); (iii) days of flowering (from 1 DAP); (iv) number of fruits and (v) branches per plant (until 120 DAP). Soil analyses were conducted before and after the experiment. All the parameters measured were analyzed using ANOVA and mean separations of LSD test using SAS 9.0 with $p < 0.05$. This study concluded that T3 showed the highest growth rate and yield as compared to other treatments. There was a significant difference between T1 and T2, where T2 showed prominent results. Therefore, it was concluded that fertilizer (NPK) cannot be substituted with foliar nutrients (calcium and magnesium), it can only supplement the current application.

Keywords: foliar nutrient, calcium, magnesium, *Abelmoschus esulentu* L., growth, yield

INTRODUCTION

Foliar application of mineral nutrients has been long practice to correct plant chlorosis with foliar sprays of iron (Gris, 1844). Foliar nutrients improved the deficiencies, strengthen weak or damaged crops and accelerate the growth of strong plants. According to the study, nutrients up-take through the leaves is about 10 to 100 times faster than the roots (Oosterhuis, 2007). However, aerial plant parts are designed to minimize exchange of water with the environment rather than to absorb mineral nutrients.
For this purpose, leaf surfaces are equipped with a water repellent lipophilic cuticle which presents a high resistance against the penetration of hydrophilic solutes such as mineral nutrients. Moreover, stomata are protected against the infiltration of liquid water (Schonherr and Bukovac, 1972). Foliar-applied nutrients thus have to overcome the barrier properties of leaf surfaces to be absorbed by leaves (Eichert and Fernandez, 2012). Foliar containing calcium and magnesium which are macronutrient that lacking in common NPK fertilizer and several other micronutrients that can be easily used by the plants as it is in the form solution.

Calcium (Ca) is an important element that is found in 3% of the earth's crust (White and Broadley, 2003; Campbell, 1983). Calcium is needed not only for human but also for plant to grow. Plants exhibit better growth and development include stronger cell walls (Anghileri, 1982), and increased disease resistance (Starkey and Pederson, 1997). Gerasopoulos and Drogoudi, (2005) stated that calcium plays a major role in cell wall stability and in regulating plant senescence and fruit ripening. Calcium has a vast role in order to serve a good platform for others plant nutrients availability. White and Broadley (2003) mentioned that calcium is an extremely important in maintaining the strength of stems and stalks of plants due to its divalent cation characteristic. This nutrient also regulates the absorption of nutrients across plasma membranes, plant cell elongation and division, structure and permeability of cell membranes, nitrogen metabolism and carbohydrate translocation. The presence of calcium also can reduce the risk of disease infection. Magnesium is also commonly found minerals in the earth crust, comprising of 2%. However, most soil Mg cannot be directly available for the plant to absorb as it is incorporated in the crystal lattice structure of minerals. The total content of Mg in soils varies considerably, between 0.05% and 0.5% (Grimme, 1991; Maguire and Cowan, 2002; Gransee and Führs, 2013). Magnesium is an essential element for plant growth and plant defense mechanisms as well in abiotic stress situations. The involvement in these physiological and biochemical processes; make it as key role element in plant development (Cakmak and Kirkby, 2008; Cakmak and Yazici, 2010; Cakmak, 2013; Gransee and Führs, 2013; Huber and Jones, 2013; Mengutay et al., 2013).

Okra (*Abelmoschus esculentus* L.) is a flowering plant in the mallow family. It is a short-day plant, well growth in full sun and resist to drought and waterlogging, but slightly not suitable on cold temperature (Rédei, 2008). The normal growth and production of okra need temperature between 24ºC to 28ºC, but at higher temperature approximately between 40ºC to 42ºC, flowers may dry up and drop, causing yield losses (Choudhary et al., 2015). It is one of the main warm season fruit vegetables grown on the tropical countries, which has been identified as one of the world's oldest cultivated crops (Paththinige et al., 2008). Okra was growing for its fibrous fruits or pods contain round and white seeds. The soil used for growing okra is loam soil which is the main soil for this plant and mix with sandy loam. Okra grow well in the most soil types which is sandy loam, loam, and clay soils with pH around 5.8 to 8.0 (Gajete, 2004). The okra seed germination starts at 2 to 12 days after seedling process. During this period, the seed will become very sensitive to the weeds because weeds can disturb the nutrient update from the plants.

Currently the production of okra is quite low, and it needs more fertilizer to overcome this problem. In Malaysia, production of okra decreased every year because almost all farmers depend on natural fertility of the soil and invest huge amount of money to reach a high yield of okra. Higher production of okra needs intensive management practices that can manage the soil and nutrients required. Natural mineral fertilizers are the next potential fertilizer although it is quite limited as it considered as non-renewable resources. Due to its properties in improving physical and chemicals structures of the soil and the plants, it can be the substitution of common compound fertilizer NPK (Akintoye et al., 2011). In Malaysia, the foliar practice and research were still in a low and unsatisfied stage. This study hypothesized that the foliar nutrients application can enhance the growth and yield of okra and eventually substitute the usage of solid granule NPK fertilizer application.

MATERIALS AND METHODS

Location of the experiment and research design

The experiment was carried out at Complex Field 10, Faculty of Agriculture, Universiti Putra Malaysia where okra (*Abelmoschus esculentus* L.) was planted as a test crop. There were four treatments including in this study and each treatment consists of six replicates. The treatments used in this study were as follows: (i) Treatment 1 (T1): Foliar nutrient (calcium and magnesium); Treatment 2 (T2): Straight fertilizer (NPK 15:15:15); Treatment 3 (T3): Foliar nutrient (calcium and magnesium) plus straight fertilizer (NPK 15:15:15), and Treatment 4: Control (without foliar nutrient or fertilizer). A total of 24 plants were planted and the experiment was conducted using Randomized Complete Block Design (RCBD) with six replications.

Land preparation

The experimental site was ploughed once a week before planting using rotovator to loosen the soil followed by bed preparation using rotor-ridger. Beds were built up with size of 0.7 m width x 6.0 m length and height of 0.3 m with distance between rows of 0.5 m. There is no herbicide application (pre-emergence nor post emergence) as we need to maintain a natural environment for the treatment to success.

Fertilizer application

According to Department of Agriculture (DOA), State of Perak (n.d) split fertilizer application helps to optimize nutrients absorb by the okra's root and prevent losses of nutrients through leaching. About 3.9 g of urea were applied at Day 7. On the other hand, about NPK Green (15:15:15) were applied 2 g at Day 21 and another 2 g at

Table1: Amount of fertilizer applied on okra.

Fertilizer type	Amount applied to the plant
Urea	3.9 g X 24 = 93.6 g
NPK (15:15:15)	4.0 g X 12 = 48 g
Foliar nutrient (Ca and Mg)	2 g/L X 2 = 4 g/2L

Day 35. Amount of fertilizer applied as follow the (Table 1). The system management of planting was monitored accordingly.

Harvesting

The fruit yield was plucked at 3-4 days after flowering. The data were only taken until 120 DAPS. The plants were kept and not destroyed after 120 DAPS for marketing the yield purposes. Parameters such as plant height, yield weight, days to flowering, number of branches and number of fruits were recorded accordingly.

Soil sample analysis

Soil analysis such as bulk density and moisture content, pH, soil electrical conductivity (EC), NPK and trace elements were carried out before and after the experiment.

Bulk density and moisture content

Bulk density can be defined as the ratio of dry solids mass to the bulk volume of the media. The core method was used to measure bulk density. Through this method, a core ring is pressed into the soil to the desired depth and then carefully removed to maintain the volume sample. The sample was dried to 105°C and weighed to determine the field moisture content. The calculation of bulk density as shown below:

$$\text{Bulk Density} = \frac{\text{weight of soil, dried at } 105^{\circ}\text{C}}{\text{volume of soil}}$$

The amount of water associated with a given volume or mass of soil ("soil water" or "soil moisture") is a highly variable property. It can change on time scales of minutes to years. However, most soil properties are more stable, and should be referenced to dry soil weight. In this experiment, the researchers focused on dry weight basis. The formula is as follow:

$$\text{Moisture Content} = \frac{\text{Weight of wet soil} - \text{Weight of dry soil at } 105^{\circ}\text{C}}{\text{Weight of dry soil at } 105^{\circ}\text{C}}$$

Soil pH

The pH of the soil was measured using pH meter at Ratio 1:2 (W/V) soil: water. The pH meter was calibrated with a proper pH buffer solution before using.

Soil electrical conductivity

The EC of the soil was measured using EC meter at Ratio 1:2 (W/V) soil: water. The EC meter was calibrated with a proper buffer solution before using.

C and N analysis

The C and N concentration in the soil sample was determined using CNS Trumac Determinator (Leco, USA).

Soil P analysis

Total soil phosphorus is determined using Brey II Method. First, ammonium fluoride (1M NH_4F) was prepared by weighed 37 g of ammonium fluoride NH_4F into 1000 ml volumetric flask and brings to volume with water. Next, hydrochloric acid (0.5M HCL) was prepared by diluted 20.4 ml of concentrated HCL with 500 ml water. Then, extraction reagent was prepared by mixed 30 ml 1M NH_4F with 200 ml 0.5M HCL in 1000 ml volumetric flask and dilute to volume with water. This solution is 0.03M NH_4F and 0.1M in HCL (known as extraction reagent), has pH of 2.6. After the extraction reagent was prepared, 2.0 g of soil sample were weighed into a plastic vial. 14 ml extraction reagent was added and shaked for 5 minutes on a reciprocating shaker. Then, the mixture was filtered through filter paper No.2. The filtrate was sent to Auto Analyzer (AA) Machine for P determination.

K, Ca, Mg analysis

The total K, Ca and Mg concentration in the soil was determined using shaking method. Shaking method also known as neutral normal ammonium acetate as describe by Chapman (1965). First, the extraction needs to be prepared. 77.1 g of ammonium acetate is weighed in 900 ml water in 1 L volumetric flask, and the pH is adjusted to

7.0. After that, 10 g of air-dried soil was weighed into 50 ml plastic vial. 50 ml of the extraction was added and shaked well for 5 – 10 minutes. The mixture was then filtered, and the filtrate was sent to Auto Analyzer (AA) Machine for K, Ca, and Mg determination.

Statistical analysis

The experiment was conducted as a complete random design. The analysis of variance (ANOVA) was carried out by submitted the result by using software SAS 9.1 System. The differences at $p<0.05$ were considered significant using LSD (Least Significant Different) Test.

RESULTS AND DISCUSSION

Plant growth study

The application of calcium and magnesium through foliar on okra could be an alternative way of fertilization. Certain parameters of the study were recorded and analyzed based on plant height, fruit yield, days of flowering, number of branches and fruits per plant. Based on plant height of okra, Treatment 2 and Treatment 3 were significantly higher than Treatment 1 (Figure 1). The overall plant height in Treatment 2 was recorded in the range of 34.25 ± 2.40 cm. Plant height in Treatment 3 was 38.43 ± 1.79 cm and Treatment 1 only 28.23 ± 2.22 cm. The extremely difference may be due to the combination of foliar nutrients plus fertilizer which supplied to the plants in Treatment 3 as compared to single application in Treatment 1 and Treatment 2. However, all the treatments showed some positive growth after application even though shorter than expected in the previous study which is 210-240 cm tall (Tripathi *et al.*, 2011). This is properly due to different variety of okra in this study. Besides that, the soil physical, chemical properties and environment factors may also contribute to differentiation in the plant's height.

The fruit yield from Treatment 3 was significantly higher than Treatment 1 and Treatment 2 (Figure 2). The yield is calculated by weighing total fruit yield per plant of okra for each treatment until 120 days after planting (DAPS). The weight of fruit yield is highly contributed by the nutrient absorb by the plant. The highest yield was obtained from Treatment 3 with 11.47 t/ha, while the lowest yield was 3.25 t/ha from Treatment 4 which served as control. However, that the yield obtained through this experiment were slightly low than the expected yield from recommendation of DOA. According to DOA (n.d), the expected yield ranges from 14 – 18 t/ha for common NPK fertilizer practice. However, it is considerably as the yield was higher than that the world average production. The total world production of okra was calculated roughly at 4.99 million metric tons, planted in area of 0.78 million hectares with an average yield of 6.39 t/ha (Kumar *et al.*, 2010). Treatment 2 showed only 10.39 t/ha yield indicates that the soil poor condition that inhibit the full potential yield to be unleashed from the plant. Besides, during the experiment, the weather was drought and haze, causing the plant easily lose water and as a result reduce the yield. From the graph (Figure 2), Treatment 3 showed the yield of at 11.47 t/ha followed by Treatment 2 at 10.39 t/ha, Treatment 1 at 8.26 t/ha and T4 at lowest which is 3.25 t/ha. There is a clear significant difference of fruit size from each treatment as showed in (Figure 3).

Days of flowering could be the indicator of the nutrient availability to the plant. The data recorded that Treatment 3 and Treatment 2 have no significant difference in the days of flowering in okra. Days of flowering for both treatments are in the range of 47-48 days as showed in (Figure 4). However, okra from Treatment 4 showed the shortest days of flowering as compared to the other treatments. This may be due the physiological response of plants towards its stressed environment, and thus having to reproduce as soon as possible (Ahanger et al., 2017). In the comparison of foliar application, there are some significant difference between Treatment 1 and 2. Foliar nutrients did not show any significant effect on days of flowering in okra plant.

The combination of NPK (15:15:15) and foliar nutrients in Treatment 3 showed the highest branches number with 32.50 branches, followed by treatment NPK (15:15:15) alone Treatment 2 which achieved 24.17 branches number, followed by treatment foliar fertilizer at T1 which gained 22.67 branches number. The control treatment only success to get 14 branches over 120 DAPS of no pruning (Figure 5). Treatment 1 and Treatment 2 showed no significant differences at $p<0.05$ which presumably foliar nutrients are competitive to the NPK usage. The branches number indicated that the number of leaves and thus represent the photosynthesis activity of the plant. The higher the number of branches, the more leaves the plant have to do photosynthesis. This may be the result of the magnesium effect of the foliar fertilizer as mention earlier. The number of fruits per plant in Treatment 3 was significantly higher than Treatment 1 and Treatment 2 (Figure 6). The highest number of fruits per plant was recorded Treatment 3 as 93.17; Treatment 1 and Treatment 2 has 61.83 and 66.17. The control treatment has the lowest number of fruits which is 40.67. As mentioned earlier, Treatment 3 was applied with foliar nutrients and NPK (15:15:15) that contributed to the highest number of fruits per plant as compared to other treatments. In comparison between Treatment 1 and Treatment 2, although the number of fruits were slightly different, but there were a significant different for both treatment at $p<0.05$.

Soil analysis

Soils samples also collected randomly for analysis before

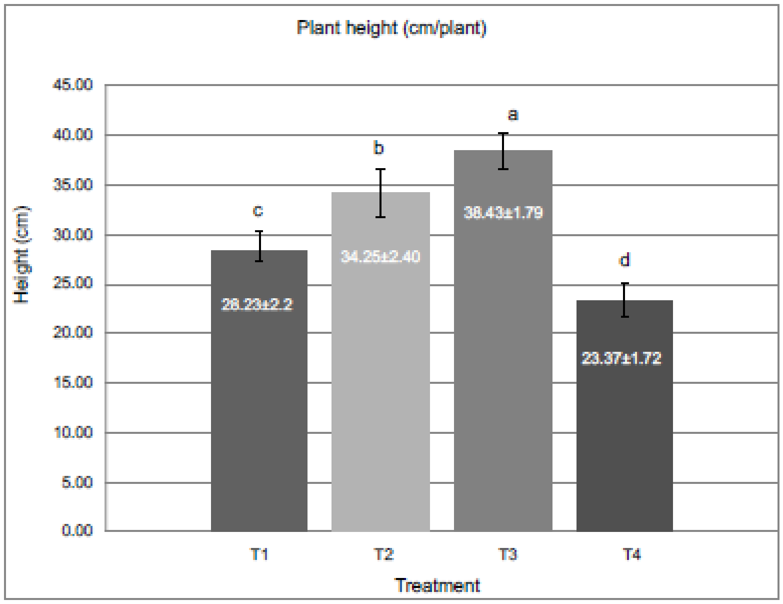

Figure 1: The effect of different type of treatments on the plant height of okra.

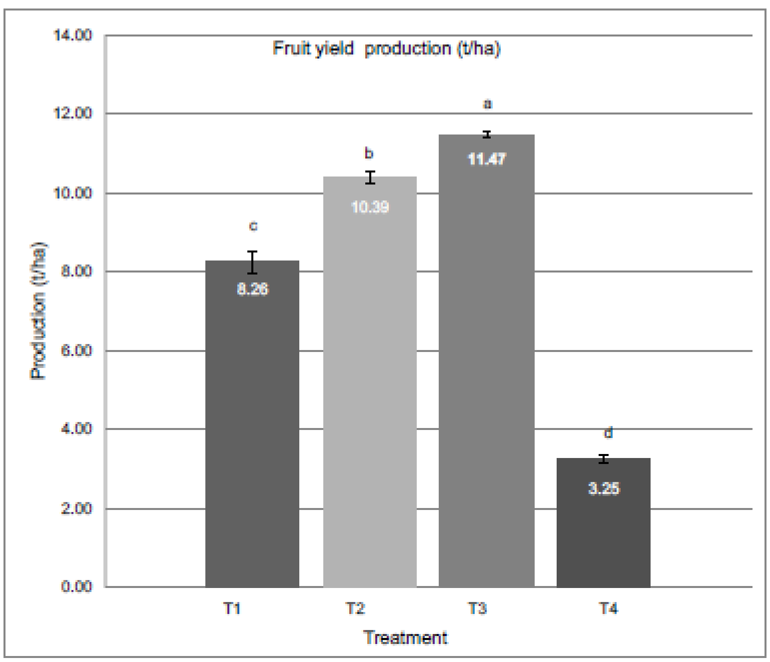

Figure 2: The effect of different type of treatment on the fruit yield of okra.

the implementation of this study. The bulk density and soil moisture content were determined and tabulated in (Table 2). Soil moisture content is very dependent on soil type. From the study, saturated coarse, sandy soil can hold far less water than saturated heavy silty clay. Sand has large particles which take up a lot of physical space, and also, as sand particles do not bind water, a lot of water will drain out of the sand due to gravity before field capacity is reached. For these two reasons, sand has much lower maximum and minimum water content than a clay soil does. The mean bulk density is 1.299 g/cm^3 with moisture content of 30.5% gave good indicator about the

Table 2: Bulk density and moisture content of the soil.

Sample	Bulk Density (g/cm^3)	Moisture Content (%)
S1	1.136	40.0
S2	1.353	26.9
S3	1.399	25.2
S4	1.309	29.9
Mean	1.299	30.5

Figure 3: The variety of size of okra fruit according to respective treatment.

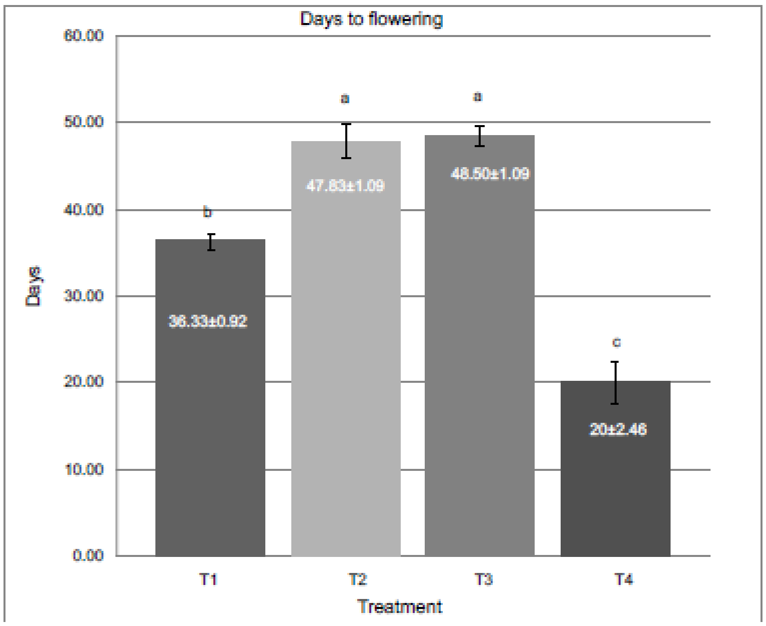

Figure 4: The effect of different type of treatment on the number of days to flowering of okra.

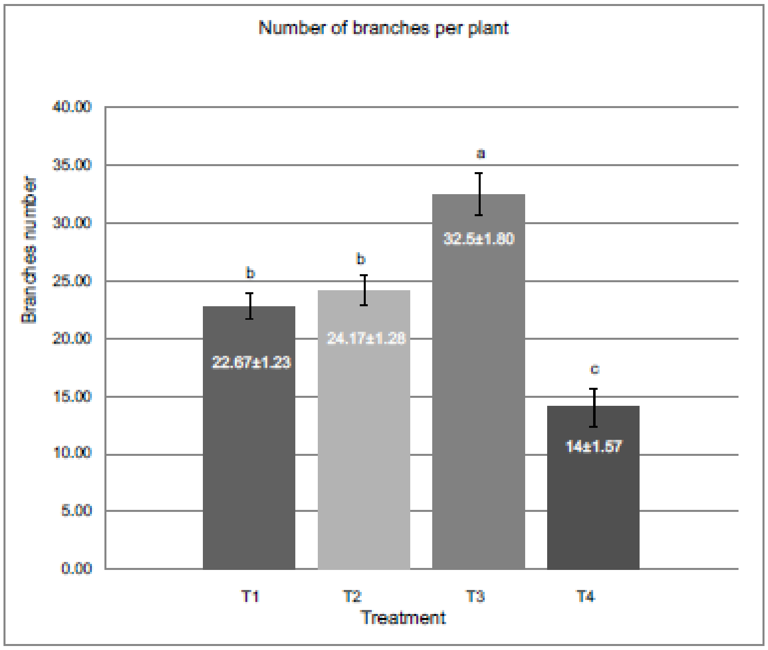

Figure 5: The effect of different type of treatment on the number of branches per plant of okra.

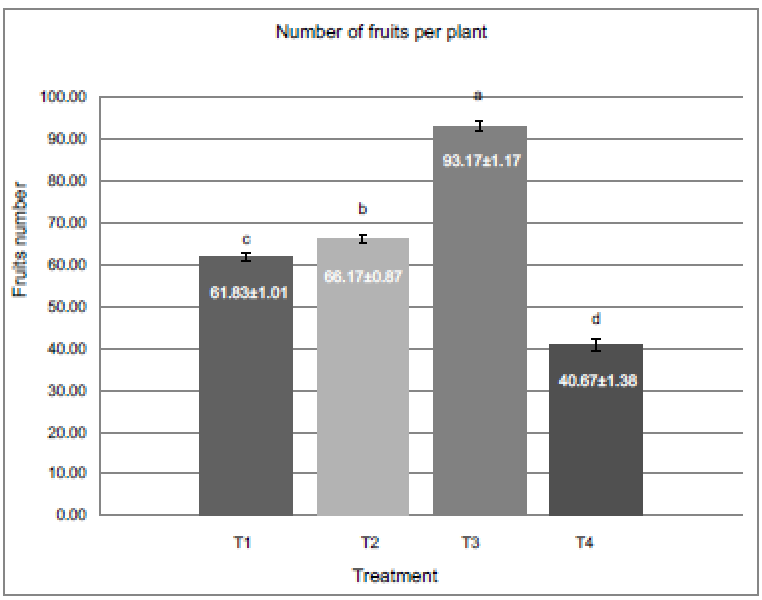

Figure 6: The effect of different type of treatment on the number of fruits per plant of okra.

soil physical condition. It is generally desirable to have soil with a low BD (<1.5 g/cm^3) (Hunt and Gilkes, 1992) for optimum movement of air and water through the soil. However, the growth of the plant in term of plant height, fruit yield was still low. This is most probably due to the unpredictable weather and environment. Besides that, soil pH also considered important element in this study. There are some differences of mean between the soil pH before and after the treatments. The pH of soil at treatments T1, T2, T3 and T4 were 4.31, 4.82, 4.33, and 4.51 respectively (Table 3). After applying the treatment, there was a significant different on the mean pH of T1, T2 and T3 at $p<0.05$. The soil pH for T1 and T3 was significantly higher than T2 (Table 3) but there is a significant drop in pH for T2. The pH for T4 is maintained with no significant different throughout the experiment.

Table 3: The effect of different type of treatment on pH value before and after fertilization.

Nutrient	Treatment			
	T1	T2	T3	T4
pH before fertilization	4.31b SE±0.111	4.82a SE±0.054	4.33b SE±0.030	4.51b SE±0.052
pH after fertilization	5.84a SE±0.264	4.41b SE±0.055	6.15a SE±0.027	4.44b SE±0.079

Table 4: The fertilizer efficiency (%) at varying soil pH levels (Jones, 2001).

Soil acidity	pH	Nitrogen (%)	Phosphate (%)	Potash (%)
Extreme	4.5	30	23	33
Very strong	5.0	53	34	52
Strong	5.5	77	46	77
Medium	6.0	89	52	100
Neutral	7.0	100	100	100

Table 5: The effect of different type of treatment on EC before and after fertilization.

Nutrient	Treatment			
	T1	T2	T3	T4
EC before fertilization	440.2a SE±11.86	432.3a SE±7.84	464.6a SE±7.28	453.5a SE±10.08
EC after fertilization	412.9a SE±4.99	38.2b SE±2.89	401.5a SE±25.41	46.6b SE±0.93

According to classification systems based on water pH, the mean soil pH after treatment for T2 falls under pH extremely acidic category while for T1 and T3 falls under pH strong and medium category respectively (Jones, 2001). The soil pH gave impacts on plant growth by affecting the nutrient availability in soil, such as phosphorus that is directly affected. Phosphate ions tend to react faster with calcium and magnesium to form less soluble compounds at alkaline pH. In contrast, phosphate ions will react with aluminium and iron at acidic pH to form less soluble compounds. In fact, most of the nutrients especially micronutrients tend to be less available when soil pH is higher than 7.5 (Vineyard, 2010). The most essential plant nutrients were available at pH 6.5 to pH 7.5. On the other hand, soil pH also affecting the solubility of fertilizer to be absorbed by the plant root (Jones, 2001). According to (Table 4), it showed that the fertilizer efficiency of nitrogen, phosphate, and potash was affected by the pH of the soil. Soil pH also associates to percentages of risk attack by pest or diseases contamination and deficiency problems like shortage of minerals or ion that crucial for better growth to achieve susceptible level.

There are no significant different of EC among all treatments (Table 5). The EC inside the soil has been worn out during the growing period of okra at T2 and T4. This is not shown in T1 and T3 as the minerals and ions that replenished inside the soil is replace by the foliar treatment. This showed that the foliar fertilizer containing calcium and magnesium is suitable to maintain a sustainable environment of planting crop without replenishing the soil nutrients and minerals ion. The main objective of this study is to observe the potential of calcium and magnesium as foliar nutrient on the okra based on growth and yield production. Theoretically, calcium is an essential plant nutrient. It is required for structural roles in the cell wall and membranes, as a counter cation for inorganic and organic anions in the vacuole, and as an intracellular messenger in the cytosol (White and Broadley, 2003; Marschner, 1995). These values reflect both Ca availability in the environment and the contrasting Ca requirements of different plant species. Calcium deficiency is rare in nature but may occur on soils with low base saturation and/or high levels of acidic deposition (McLaughlin and Wimmer, 1999).

On the other hand, magnesium (Mg) seemed to be neglected and was even termed “a forgotten element in crop production” (Cakmak and Yazici, 2010). People keep thinking of NPK (nitrogen, phosphorus, and potassium) in plant production. Actually, magnesium has a dominant role in photosynthesis and associated processes in the chloroplast, where up to 35% of leaf Mg is located (Cakmak and Yazici, 2010). The chloroplast ultrastructure, i.e., grana formation, requires Mg. The divalent Mg cation screens negative surface charges on the thylakoid membranes and thus, allows the thylakoid membranes to stack (Puthiyaveetil et al., 2017). Magnesium is required for the synthesis of chlorophyll (Masuda, 2008) and is involved in both Ribulose1,5-bisphosphat-carboxylase/-oxygenase (Rubisco) activation by forming a complex with Rubisco activase, and for Rubisco activity by binding to the carbamate group of Rubisco (Portis, 2003). Application of magnesium by spraying on the leave parts could be supplementary to the fertilization application. Thus, from the study, Treatment 3 seems to have the highest growth rate as compared to the other treatments because beside the standard NPK which applied through the root part, okra also absorbed magnesium and calcium through the leave.

Conclusion

The effectiveness of calcium and magnesium through foliar application on okra could be determined by plant height, total yield production, days of flowering, number of branches and fruits per plant. Calcium and magnesium can only be used as supplementary to the plants, not substitution of current fertilizer application. Combination of both application proven to enhance the vegetative growth and fruits production in overall in okra.

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Conflict of interest

The authors declare no conflicts of interest for this work. Authors also aware of the plagiarism policy of the organization and all are our original research manuscript.

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