Efficiency of salicylic acid and calcium chloride foliar application on the growth performance of pak choi (*Brassica rapa* subsp. *chinensis*) under nutrient film technique (NFT) hydroponic system

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Efficiency of Salicylic Acid and Calcium Chloride Foliar Application on the Growth Performance of Pak Choi (*Brassica rapa* subsp. *chinensis*) under Nutrient Film Technique (NFT) Hydroponic System

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Abstract. Pak choi is the mustard species which have high consumer in Malaysia, however, it is still insufficient to fulfil the demand of the consumer. Plant hormone and inorganic chemicals such as salicylic acid (SA) and calcium chloride ($CaCl_2$) were used to increase the fresh produce production in agriculture. However, their application as foliar application in the smart planting technique by using hydroponic system not fully studied. Therefore, the study was conducted to investigate the efficiency of SA and $CaCl_2$ foliar application on the growth and yield of pak choi under Nutrient Film Technique (NFT) hydroponic system. The plants were treated with SA and $CaCl_2$ as foliar application at spraying volume of 160 L/ha (equivalent to 45 ml/plant). Plants were treated with a series of concentration: **T0** (0.0 mM SA+ 0.0 mM $CaCl_2$); **T1** (0.5 mM SA + 20 mM $CaCl_2$); **T2** (1.0 mM SA + 20 mM $CaCl_2$) and **T3** (1.5 mM SA + 20 mM $CaCl_2$). The results of this study showed that the plant height of pak choi plants at **T2** and **T3** were significantly increased by 10.5% to 17% and 17% to 23%, respectively as compared to **T1** and **T0.** Similar to plant height, the leaves number and diameter of pak choi plants were also significantly increased at these two concentrations with the percentage value ranging from 12% to 32% and 18% to 30% increment, respectively. For the plant yield, **T3** was found to give greater fresh weight ($P \leq 0.05$) with the mean value of 127 g as compared to the **T1** (78 g) and **T0** (57 g). However, plants that treated with **T2** and **T3** concentration did not give any significant difference in both growth and yield parameters. In term of chlorophyll content, the combination of SA and $CaCl_2$ did not give any effect with the mean values ranging from 40 nm to 50 nm. The results suggest that **T2** was found to be the optimum concentration that can be applied for the production of pak choi plants in economic ways since this low concentration of SA significantly increased the growth and yield of pak choi plants.

INTRODUCTION

Brassica rapa subsp. *chinensis* or 'Pak choi' is the well-known type of leafy vegetables in Malaysia and other Asian countries. This worldwide cultivated vegetable come from cruciferous (Brassicaceae) family which also include with the cabbage, cauliflower, broccoli, and mustard. Pak choi also known as one of the highly versatile vegetables

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due to it uses in many purposes such as for culinary and medicinal which increase the demands of brassica [1]. In 2015, it was reported that the consumption of vegetables increased by 2.6% which is from 55 kilograms to 70 kilograms per annum [2]. Due to the increase of demands, Malaysia had imported around 880,000 tons of vegetables from the temperate area of China, Holland, USA, and Thailand such as cabbage, carrot and cauliflower. In 2020, the demand for vegetables is increased from 1.91 million to 2.4 million tons including the mustard species vegetables [2]. This phenomenon shows that mustard vegetables supply in Malaysia is still not conserving and insufficient for Malaysian local uses. Self-sufficient ratio (SSR) for the mustard species vegetables do not achieve 100% which shows that Malaysia still cannot support the demand for the consumption of mustard vegetables [3].

Most of the mustard species vegetables have been widely cultivated conventionally on the land. However, vegetable growers faced a variety of challenges, including excessive or deficiency of soil nutrients, soil-borne disease, and pollution that hardly to be controlled [4]. Nowadays, several "smart" technologies have been developed for vegetable production for example integrated farming, fertigation, aeroponic, and hydroponic. Hydroponic is one of the methods of growing plants in the water-based which contain nutrients without using soil. This method needs other suitable materials that have same characteristics as soil which can hold the root system and good water retention such as perlite, vermiculite, coco peat, pellet, peat moss and sowing sponge [5]. The basic concept of hydroponic is depending entirely on the nutrient solution which ensures the roots to obtain nutrients and sufficient oxygen rather than planting by using soil. According to Treftz and Omaye [5], hydroponic can increase the yield, growth rate and shorter growth time taken than using the conventional methods. Plants can mature up to 20% faster and produce more yields to 30% more than the plants that cultivated in the soil. Even though the setup costs for hydroponic is quite higher than conventional method, but it could give more profit to the growers for the long-term vegetables production. This in turn would fulfil the consumers demand in Malaysia which consequently ensure the sustainable of food supply [6].

Research on the selected organic and inorganic compound as a biostimulant for enhancing the vegetables growth become popular in agriculture industry. For example, salicylic acid (SA), a natural plant hormone and secondary metabolite was found to increase the growth, photosynthesis and other physiological activities of the plants [7]. Salicylic acid was found significantly enhancing the growth of green mustard (*Brassica juncea*) by stimulating the stomatal activity and pore size of the plants [8]. In addition, this phenolic compound also helps in ion uptake of the nutrients in plant which make it efficient for the nutrient's absorption [9]. Instead of the SA, calcium chloride ($CaCl_2$), an essential nutrient for the plant growth also has an important role in plant physiology, including involvement in the responses to salt stress, and controls numerous processes [10]. According to Mohamed and Basalah [11], salt stressed plants that treated with $CaCl_2$ shows increase in photosynthetic pigments contents.

Several studies have shown the benefits of combination of SA and $CaCl_2$ in selected plant and vegetables species [12, 13]. A study conducted by Lin et al. [14] found that foliar spray comprising SA and $CaCl_2$ on three poinsettia (*Euphorbia pulcherrima* Willd.) cultivars; Noel, Winter Rose, and Ice Punch gave different in appearance, morphological growth patterns, and ability to tolerate high-temperature stress. In addition, Saja and Ammar [15] has investigated the response of three cultivars of *Gladiolus* X *hortulanus* L. with SA concentrations at 0.0 and 250 mg/L as well as treatment with $CaCl_2$ concentrations at 0, 500 and 1000 mg/L. They found that the plants sprayed with SA had a significant effect for almost studied traits, while the plants with $CaCl_2$ with both concentrations resulted in a significant increase in the number of leaves and the spike length. However, to date, there is no study on the efficacy of SA and $CaCl_2$ on *Brassica rapa* subsp. *chinensis* or pak choi under hydroponic culture. Thus, this study was conducted to determine the effect of SA and $CaCl_2$ foliar application on the growth and yield of pak choi under Nutrient Film Technique (NFT) hydroponic system.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The experiment was conducted during October to December 2020 on pak choi plants at a hydroponic greenhouse located in Universiti of Malaysia Kelantan (UMK) Jeli Campus, Kelantan, Malaysia. Pak choi plants (*Brassica rapa* subsp. *chinensis*) were grown under natural light conditions. The humidity during the experiment was 70 to 80% with temperatures ranging from 25 to 30^{o}C.

Treatments

Salicylic acid (SA) at the rate of 0.0, 0.5 1.0 and 1.5 mM, and 20 mM of calcium chloride ($CaCl_2$) were applied as foliar. The SA solution was prepared by dissolving it with absolute ethanol and water (ethanol/water: 1/1000 v/v). Meanwhile, the optimum concentration of $CaCl_2$ was prepared by using distilled water based on the modified method of Youssef et al. [13]. The combination of treatments as shown in Table 1:

TABLE 1. Treatments applied to pak choi plants (*Brassica rapa* subsp. *chinensis)*

Treatment	Application Rate
T0 (Control)	0.0 mM SA + 0.0 mM $CaCl_2$
T1	0.5 mM SA + 20 mM $CaCl_2$
T2	1.0 mM SA + 20 mM $CaCl_2$
T3	1.5 mM SA + 20 mM $CaCl_2$

Note: SA: Salicylic acid; $CaCl_2$: Calcium chloride

Plant Growth Experiment

The pak choi seeds were sown in the nursery by using the sowing sponge. Then, the sponge was immersed in the water to stimulate the seed germination. After 3-7 days of seed germination, the roots of plant seedlings were immersed with the fungicide to avoid contamination. Then, the pak choi seedlings together with the sowing sponge were transplanted into hydropots and growing under the Nutrient Film Technique (NFT) hydroponic system. The plants were fertilized with hydroponic AB fertilizer containing complete nutrient solution of macronutrients and micronutrients: N, P_2O_5, K_2O, Ca, Mg, S, Fe, Mn, Mo and Cu [9]. Before that, the AB fertilizer was diluted into water with the ratio of 1:1 until reach the desired concentration level. At the beginning, the plants were fertilized with AB fertilizer at nutrient concentration of 300 ppm for the first week of planting. The portable EC meter was used to monitor the amount soluble salts content (electrical conductivity) of the water solution. In between 14 days to 30 days of planting, the nutrient solutions were measured and increased to 1200 ppm until harvesting period due to the increase of nutrients requirement for the plant growth. The hydroponic water solution was circulated 24 hours every day by using water pump. The SA and $CaCl_2$ combination treatments at different level of concentration (**T0**: 0.0 mM SA + 0.0 mM $CaCl_2$, **T1**: 0.5 mM SA + 20 mM $CaCl_2$, **T2**: 1.0 mM SA + 20 mM $CaCl_2$, and **T3**: 1.5 mM SA + 20 mM $CaCl_2$) were applied as foliar application with spraying volume of 160 L/ha (equivalent to 45 ml per plant). The treatments were applied at day 10 and day 20 of planting. All the treatments were in three replications. Untreated plants were left as a control and sprayed with distilled water. The NFT hydroponic system and the bioassay plants were monitored continuously to ensure the availability of nutrients solution uptake by the plants.

Measurements

After 30 days of cultivation, the plant height, number of leaves, leaf diameter, chlorophyll content and the fresh weight of pak choi plants were measured. Plant height was measured by using the measuring tape, while vernier caliper was used to measure leaf diameter. The number of pak choi leaves was manually counted and recorded. The chlorophyll content of the plants further was measured by using the SPAD meter (model SPAD-502) and the fresh weight (yield) was measured by using weighing scale.

Statistical Analysis

The experiment was arranged as a completely randomize design (CRD) with three replications. Statistical analysis of the results was performed using SPSS software by one-way analysis of variance (ANOVA) and Tukey test (HSD) to compare the mean among the treatments. Differences were regarded as significant when the p-values are equal or less than 0.05 ($P \leq 0.05$).

RESULTS AND DISCUSSION

The results of the statistical analysis in Figure 1A showed that the pak choi plants treated with SA at **T2 (**1.0 mM SA) and **T3** (1.5 mM SA) show higher mean values of plant height with 11.58 cm and 12.49 cm, respectively. Spraying plants with 20 mM $CaCl_2$ in both concentrations resulted in a significant increase ($P \leq 0.05$) in plant height with increasing concentration from 0.0 mM (**T0**) to 1.0 mM (**T2**) of SA application. Similar trend was also observed for number of leaves and leaf diameter where both concentration of SA at1.0 mM and 1.5 mM exhibit largest significant values with the leaf number of 10 leaf/$plant^{-1}$ and leaf diameter of 15-16 cm, respectively (Figure 1B and 1C). However, in term of chlorophyll content, there is no significant increased among all SA application rates (Figure 1D). It was observed that **T3** possess the highest mean of fresh weight with 126.89 g, followed by **T2** (119.23 g), **T1** (78.54 g) and **T0** (57.29 g). As compared to the control, the fresh weight of pak choi in **T2** and **T3** increased by 53 and 55%, respectively which is two-times higher than control (Figure 1E). However, there is no significant increase in fresh weight between these two treatments. Overall, the values of all the interaction between SA and $CaCl_2$ were significantly different with the control, except chlorophyll content and plant fresh weight.

The results of recent study were in line with the study conducted by Youssef et al. [13] where the combination of SA and $CaCl_2$ significantly increase the vegetative growth parameters (plant height, number of leaves and head/leaves diameter) and macro and micronutrients of romaine lettuce cv. Balady at an application rate of 1.5 mM SA + 20 mM $CaCl_2$. Mirdad [16] reported that the combination of SA with potassium oxide at 200 mgL^{-1} and 1000 mgL^{-1}, respectively shows an increment in plant height and the leaf number of broccoli (*Brassica oleraceae* var. *Italica*) with 31% and 46%, respectively compared to control. In addition, SA foliar application at 10^{-4} M concentration had increased 33-40% of the leaves number of sweet basil (*Ocimum basilicum* L.) and marjoram (*Majorana hortensis*) compared to the control plants [17]. A similar increase in the shoot's growth of soybean (*Glycine max (*L.) Merr. cv. Cajeme) [18] and chickpea plants [19] were also reported in response to SA treatment. The stimulatory effects were also observed on *Satureja khuzistanica* Jamzad herb plant with highest canopy diameter of 41.58 cm at an application rate of 200 mg L^{-1} exogenous SA application [20]. According to a study conducted by Walid et al. [21], the combination of $CaCl_2$ with other nutrient elements which are potassium, boron, and humic acid on the "Anna" apple trees (*Malus domestica* L.) had increased the shoot diameter by 27-30% compared to $CaCl_2$ alone and control. From these previous findings, its show that SA and $CaCl_2$ applications had stimulate the vegetative growth of plants grown under conventional cultivation system.

In the recent study, SA and $CaCl_2$ application does not show any increment in chlorophyll content (Figure 1D). According to Janda et al. [22], the positive effect of SA on photosynthetic activity is not always due to the increased of the chlorophyll content of the plants. However, Altman [23] demonstrated that ethylene might accelerate the chlorophyll loss when the plants exceeded the maturity stage. Thus, the insignificant results on the chlorophyll content in this study might be due to the pak choi plant that has achieved optimum maturity stage. However, Sağlam [24] reported that chlorophyll content might decreased with the increasing of treatment concentration. He found that sunflower that treated with 1000 µM SA possess low chlorophyll content as compared to the plant treated with lower concentration of SA (0.001-10 µM). A similar result also was reported for romaine lettuce cv. Balady where the exogenous applications of SA at different concentrations significantly increased the chlorophyll (a, b and total) content of the bioassay plant [13].

Instead of increasing the plants vegetative growth, SA and $CaCl_2$ application also involved in increased the plant weight (Figure 1E). The combination of SA and Ca in low concentration was found to increase the yield of strawberry by 36% compared to control [9]. In other study, the combination of $CaCl_2$ and paclobutrazol had increased the yield of cucumber plant [12]. In addition, Alyemeni et al. [25] reported that foliar application of SA had induced 26% of the fresh mass per plant of *Cicer arietinum* L. cv. 'Avarodhi compared to control. Moreover, the application of SA helps in alleviated the effect of cadmium (Cd) toxicity at concentration of 25 mg and 50 mg which remained the fresh mass of *C. arietinum.* Sunaina and Singh [26] reported that SA act as protection in ameliorates the oxidative stress that caused by NaCl and maintain the vegetative and yield growth of cabbage (*Brassica oleracea* var. *Capitata*) with the increasing application rate of 0.5mM to 1.5 mM under hydroponic culture.

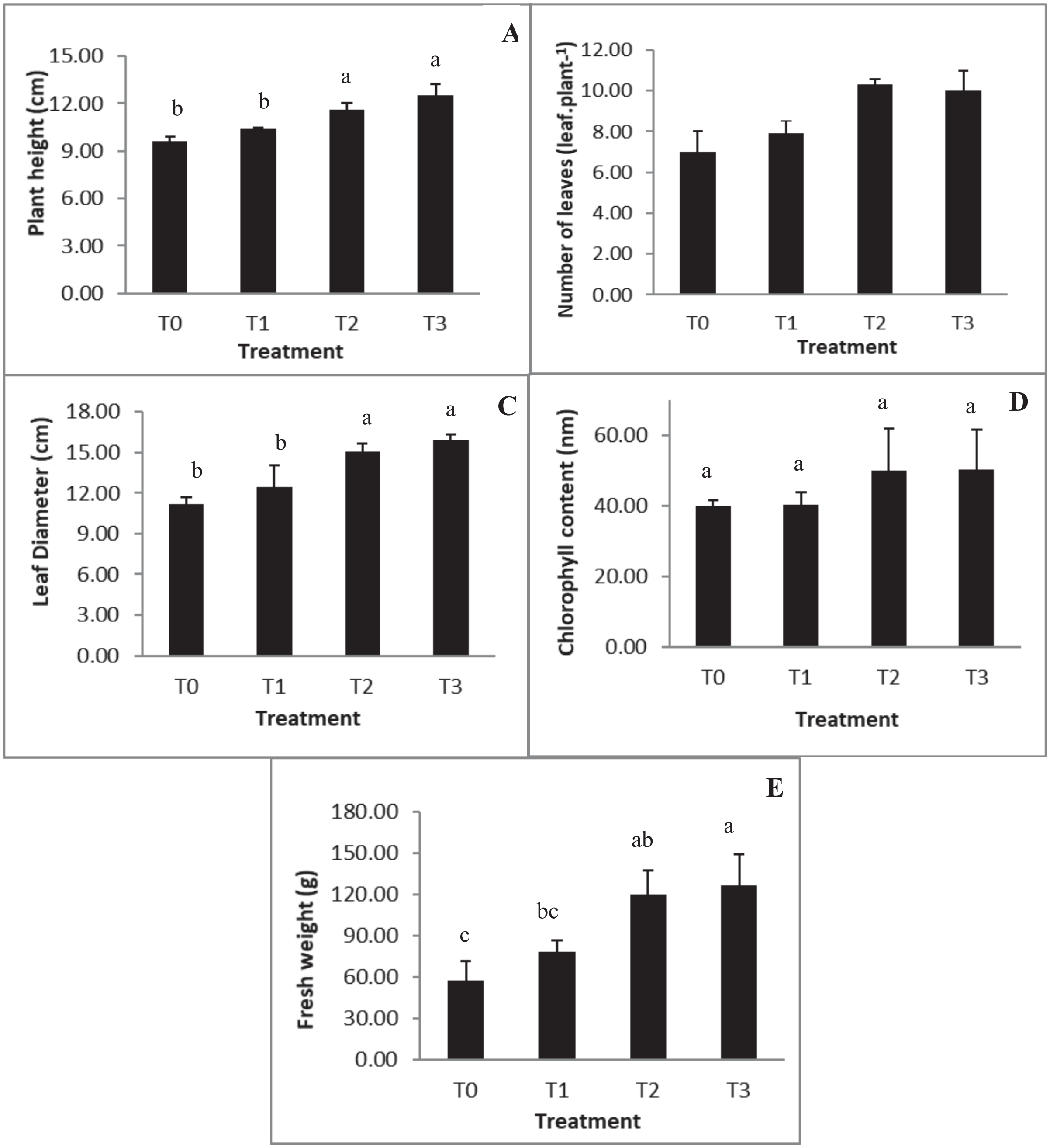

FIGURE 1. The effect of salicylic acid (SA) and calcium chloride ($CaCl_2$) on the plant height (A), number of leaves (B), leaf diameter (C), chlorophyll content (D) and fresh weight (E) of pak choi (*Brassica rapa* subsp. *chinensis*). Vertical error bars represent standard deviation (s.d) of the mean. T0: 0.0 mM salicylic acid (SA) + 0.0 mM calcium chloride ($CaCl_2$), T1: 0.5 mM salicylic acid (SA) + 20 mM calcium chloride ($CaCl_2$), T2: 1.0 mM salicylic acid (SA) + 20 mM calcium chloride ($CaCl_2$), and T3: 1.5 mM salicylic acid (SA) + 20 mM calcium chloride ($CaCl_2$)

CONCLUSION

The results from the present study illustrate that application of SA and $CaCl_2$ had increase the vegetative growth and yield of pak choi plant. The enhancement in the plant's growth could be associated with an optimal selectivity for SA and $CaCl_2$ nutrition. The findings also indicated that combination of SA and $CaCl_2$ could play a synergetic role in regulating several properties conditioning including the efficiency of nutrient uptake for plant growth in hydroponic culture. Further works are required to elucidate the physiological and biochemical activities of the impact of combined SA + $CaCl_2$ treatments on enzymes-related parameters for pak choi plants.

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