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Fish amino acid application to improve growth performance and yield of Chinese Kale (*Brassica oleracea*)

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Abstract

Chemical fertilizer application is one of the most common practices worldwide due to its fast nutrient release mechanism. Nevertheless, the usage of inorganic fertilizer for vegetable crops is not preferred as it may contribute to the health risk. A field experiment was conducted to evaluate the effect of different concentrations of fish amino acid (FAA) as foliar fertilizer on the growth and yield of kale (*Brassica oleracea* var. alboglabra L). Five treatments of the experiment were; T1 NPK Green (Control), T2 (10% FAA), T3 (20 % FAA), T4 (30 % FAA) and T5 (40 % FAA). The growth performance of kale when treated with T4 showed significantly higher ($P < 0.05$) plant height, number of leaves, number of branches, total leaf area, and plant biomass as compared to other treatments. Fish amino acid at 30 % concentration can be recommended as an effective replacement for NPK green for sustainable kale production.

Key words: *Brassica oleracea,* kale, growth, yield, fish emulsions, foliar fertilizer

Introduction

Brassica oleracea var. *alboglabra* L. is locally known as Kailan or Chinese Kale , is type of vegetable that is under the family of Brassicaceae (Kumar *et al.*, 2019). *B. oleracea* is broadly developed within the Southern China and spread to Southeast Asian nations such as Malaysia, Indonesia, Thailand, Vietnam and Philippines (Qian *et al.,* 2016). The leaves, stem and inflorescence parts are utilized predominantly in Chinese cuisines, including a stir-fry dish, steamed, cooked in soups or eaten as raw greens. It has bitter taste, but sweeter compared to broccoli (Tuquero, 2016).

As the demand for this vegetable is high, farmers are catching up by using inorganic fertilizer. Besides, it is preferable to use inorganic fertilizer as it can hasten the growth in a short time and increased yield. However, the usage of inorganic fertilizer is not highly recommended for vegetables crops due to chemical residue which is not good for human health (Weinert *et al.*, 2014). Tiraieyari *et al.* (2017) found that the reasons of chemicals extensive usage in agriculture are responsible for several negative consequences such as environmental pollution. Therefore, organic farming is one of the well-known agricultural practices that bring benefits specifically for improving high yield and healthy crops production as well as lead to sustainable agriculture.

Organic farming focuses on upgrading crop production with a minimal environmental load with keeping biological balance, comprises the common largescale production and improving agricultural wellbeing of ecosystem (Priyanka *et al.*, 2019). Fish amino acid (FAA) which is product from fermented fish and molasses is an innovative approach in organic farming. FAA is also known as fish emulsions, is one of organic fertilizer which acts as a plant growth promoter (Fahlivi, 2015). According to Radkowski and Radkowska (2018), amino acids are natural compound with a building proteins block, which perform, in structural and metabolic activities in plants. The fish emulsions have been reported to promote seedling germination, fruiting and organism activity in soil (Murray and Anderson, 2003). Priyanka *et al.* (2019) stated that fish amino acid has great value for both plants and microorganisms growth, due to abundant amount of nutrients (nitrogen source for plants) which can be absorbed directly by the crops and stimulates the microorganism activities. Therefore, a study was carried out to evaluate the growth performance and yield of *Brassica oleracea* var. *alboglabra* L. treated with different concentrations of fish amino acid as foliar nutriton.

Materials and methods

Planting materials and maintenance: The experiment was conducted under a rain shelter at Field Unit, Universiti Teknologi MARA, Perlis from December 2019 to March 2020 and daily temperature recorded was 27 to 32 °C. Seeds of Chinese Kale were sown in seedling trays with peat moss as germinating medium. Germinated seedlings with four to six of fully expanded leaf were transplanted into polythene bags size (12 x14 cm). Plants were divided into 5 treatments which were T1 NPK Green (Control), T2 (10 % FAA, T3 (20 % FAA), T4 (30 % FAA) and T5 (40 % FAA). FAA was applied as foliar application once a week. Meanwhile, NPK Green at rate of 10 % was applied on seven days after transplanting. The growing media was a mixture of top-soil, cow dung and fine-sand with a ratio of 3:2:1, respectively. Management practices such as watering was done two times per day while weeding was done manually as needed.

Preparation of fish amino acid: The fresh fish of Indian mackerel were chopped and divided into four parts (head, bones, skin, fins). Then, the fish parts were weighed and mixed with an equal amount of molasses (1:1 kg by weight). The mixture was poured into 5 L container and covered by a breathable cotton cloth to allow aeration. Then the container was stored under a cool dry shady place and allowed to ferment. After approximately three to five days of mixing ingredients, the fish waste began to break down and liquefy through fermentation and the osmotic pressure were generated by the addition of molasses. Fish Amino Acid has a sweet but slightly fish odor when completely fermented (Weinert *et al.*, 2014). Four different concentrations of FAA were prepared in water and NPK green was used as control; T1 liquid NPK Green, T2 (10 % FAA), T3 (20 % FAA), T4 (30 % FAA) and T5 (40 % FAA). The fish amino acid was sprayed (3 mL) once per week after transplanting following the treatment, as foliar fertilizer.

Data collection: Data were divided into which were destructive and non-destructive data. Non-destructive data collected in this study were plant height and number of branches. Those data were collected from week 1 until week 6 with interval of 7 days. As for the destructive data, the plants were harvested on week 6 and total leaf area, fresh weight (FW) and dry weight (DW) were measured.

Plant height was measured manually by using measuring tape and expressed by per plant basis in cm. Number ofbrancheswas measured manually by counting all the visible branches. Total leaf areawas measured by using leaf area meter (LI-3000C, Portable Area Meter). Data was expressed in total of leaf area per plant.As for fresh and dry weight, plants were washed immediately after harvesting to remove soil particles. Each plant was separated into leaf, stem and root according to its treatment. The separated plant parts were weighed using analytical balance(ME303E Mettler Electronic Balance, Japan)and the fresh weight was recorded.The samples were then placed in an oven with 60°C for three days to a constant mass and dry weight were recorded.

Statistical analysis: The experiment was designed in a randomized complete block design (RCBD) with four blocks. Data were analyzed using Analysis of Variance (ANOVA) and mean comparison was done by Tukey test at $P \leq 0.05$ *via.* SPSS software.

Results and discussion

Plant height: The vertical growth of a plant is influence by many physiological mechanisms, especially hormones. The application of differentconcentrations of FAA as foliar fertilizer affected the vertical growth of the plant which is proven by significant different of plant height among the treatments (Table 1). During transplanting, all the plants were uniform. However, one week after FAA application,30 % of FAAshowed rising in plant height with significant different as compared to other treatments. This trend was similar for week 2 until harvesting day. At week 6, the tallest plant was recorded from *B. oleracea* that were treated with 30 % FAA translated into 89.9 % higher than 40 %of FAA. Moreover, there was significant difference between all treatments on week 5 and week 6.

In addition, there were significant differences ($P \leq 0.05$) in plant height between the treatments on week 1, week 2, week 3, week 4, week 5 and week 6. As can be seen on week 1 from Table 1, T4 (30 %) has a significant difference among the other treatments, T1 (NPK Green), T2 (10 %), T3 (20 %) and T5 (40 %). The mean values of plant height by T4 (30 %) is 6.34 cm as compared to the lowest one, T5 (40 %) which had the lowest plant height at 4.25 cm. On the other side, there were no significant difference between T3 (20 %) and T5 (40 %) on week 2 with mean values 4.95 cm and 4.57 cm respectively though there was a significant difference between T1 (NPK Green), T2 (10 %) and T4 (30 %) with mean values 6.34 cm, 5.34 cm and 8.16 cm respectively. Furthermore, T4 (30 %) continuously delivered the highest mean value of plant height among treatments on week 3 until week 6 with mean values 9.64 cm, 11.04 cm, 12.23 cm and 14.05 cm, respectively and has a significant difference compared to other four treatments. Other four treatments T1 (NPK Green), T2 (10 %), T3 (20 %) and T5 (40 %) also had significant difference with each other on week 3 with mean values 7.58 cm, 6.70 cm, 5.71 cm and 5.03 cm, respectively. Effect of the treatments on plant height of Chinese Kale extended on week 4 by showing significant difference among all treatments with mean values 9.10 cm, 7.86 cm, 6.61 cm, 11.04 cm, and 5.85 cm conjointly. Moreover, there was significant difference between all treatments on week 5 followed by week 6.

The range of the plant height (cm) mean value on week 3 were between 4.95 cm to 8.16 cm. Additionally, the all treatments shown a significant difference with each other. The mean averages were increasing in all treatments on week 4 where the highest mean value recorded was by T4 (30 %) which is 11.04 cm followed by T1 (NPK Green), T2 (10 %), T3 (20 %) and T5 (40 %). T4 (30 %) and T5 (40 %) had compete each other to receive the top value of plant height on week 5 with mean values 12.23 cm and 10.19 cm but T4 (30 %) was mentioned to maintain its mean value to be the first ranked but in the same time there was shown a significant difference between both treatments. The final results of plant height were resulted on week 6 where T4 (30 %) reached the highest mean value meanwhile T5 (40 %) was kept being the least number of plant height received. However, T2 (10 %) and T3 (20 %) were also presented the increasing of plant height from week 1 until week 6 but not exceeding than 10 cm which the mean value shown were 8.97 cm and 7.80 cm.

Table 1. Plant height of Chinese Kale as influenced by different concentration of fish amino acid

Treatment	Plant height (cm)						
	Weeks after transplanting						
	0	1	2	3	4	5	6
NPK Green	4.0	5.27^{b}	6.34^{b}	7.58^{a}	9.10^{b}	10.19^{b}	11.35^{b}
10 % FAA	4.0	4.82^{b}	5.34^{bc}	6.70^{bc}	7.86^{bc}	8.36^{c}	8.97^{c}
20 % FAA	4.0	4.57^{b}	4.95^{c}	5.71^{bc}	6.61^{bc}	7.35^{cd}	7.80^{cd}
30 % FAA	4.0	6.34^{a}	8.16^{a}	9.64^{a}	11.04^{a}	12.23^{a}	14.05^{a}
40 % FAA	4.0	4.25^{b}	4.57^{c}	5.03^{c}	5.85^{d}	6.60^{d}	7.40^{d}
$P \leq 0.05$	ns	*	*	*	*	*	*

Note: Mean value within a column followed by the same letter is not significantly different at $P \leq 0.05$ according to Tukey test.

Jasim *et al.* (2013) mentioned that the plant height increases due to nutrients supplement that affecting the physiological processes including cell division and elongation. Sarkar *et al.* (2014) stated that foliar spray helps in expanded the growth development production in cell system resulting in increasing of plant height. In the current study, the suitable amount of fish amino acid was found at 30 %showed better performance than

NPK green. Thus, fish amino acid can be an alternative to replace the use of chemical fertilizer. This is supported by Priyanka *et al.* (2019) where they revealed that fish amino acid has great value to both plants and microorganisms in their growth. This is because it contains abundant amount of nutrients (nitrogen source for plants) which can absorbed directly by the crops and stimulates the microorganism activities, in turns promote plant growth.

Number of branches: The application of different fish amino acid (FAA) concentrations as foliar fertilizer had positive effect on number of branches at $P \leq 0.05$ (Table 2). During transplanting, all the plants were in uniform number of branches. However, one week after FAA application, 30 % of FAA showed increased in number of branches compared to other treatments. This trend was similar for week 2 where plants treated with 30 % FAA produced 6 branches per plant which was the highest among all treatments. As the plants reached the third week after planting, a clear trend was noticed where increased in the concentration of FAA resulted in increased in number of branches until 30 % of FAA which was 9 branches per plant. Control plants showed 7 branches/plant at 3 weeks after transplanting, which was not significantly different with 10 % and 20 % of FAA. However, at 40 % of FAA, the plants seemed to be retarded where it only produced 4 branches per plant, which was same when the plants were at second weeks after transplanting. It is assumed that 40 % of FAA causes the plants to be in stressed condition compared to other treatments.

As the plants reached the fourth week after planting, only one new branch develops in this treatment. However, those treated with 30 % of FAA showed 10 branches per plant. Similar trend occurs where plants treated with 30 % FAA developed more branches compared to other treatment until harvesting time (12 branches per plant).

For this parameter, it is found that the highest mean value the number of branches was recorded by T4 (30 %) whereas the lowest mean value the number of branches brought by T5 (40 %) throughout the weeks. The maximum mean value observed was T4 (30 %) on week 6 which is 12 branches and the minimum mean value the number of branches was T3 (30 %) which is only 5 branches produced.

The application of fish amino acid in different concentrations to all treated plants were shown significantly difference ($P \leq 0.05$) with each other on week 1, week 4 and week 5. However, there was no significant difference ($P > 0.05$) on week 6 between T1 (NPK Green) and T2 (10 %) where the mean value for both treatments recorded was 9 branches. On the same week, there was a significant difference among T3 (20 %), T4 (30 %) and T5 (40 %) with mean values 5, 12 and 6 branches respectively.

Consequently, the average values the number of branches produced by T1 (NPK Green) and T2 (10 %) were similar meaning the treatments were not really affected the number of branches produced in Chinese Kale . Similarly, T3 (20 %) also resulting the least number of branches with T5 (40 %) where there were constantly produced the same average value of branches number on week 3 until week 5 for T2 (10 %) whereas the average value maintained for T5 (40 %) were valued on week 2 until week 5. Although there were increasing the number of branches by T5 (40 %) from week 3 to week 5 but it was still not really affected the parameter as it increases constantly.

Table 2. Number of branches of Chinese Kale as influenced by different concentrations of fish amino acid

Treatment	Number of branches						
	Weeks after transplanting						
	0	1	2	3	4	5	6
NPK Green	3.0^{a}	5.0^{b}	6.0^{b}	7.0^{b}	7.0^{b}	8.0^{b}	9.0^{b}
10 % FAA	3.0^{a}	5.0^{b}	5.0^{bc}	6.0^{b}	7.0^{b}	8.0^{b}	9.0^{b}
20 % FAA	3.0^{a}	4.0^{c}	5.0^{bc}	6.0^{b}	6.0^{c}	6.0^{c}	5.0^{c}
30 % FAA	3.0^{a}	6.0^{a}	8.0^{a}	9.0^{a}	10.0^{a}	11.0^{a}	12.0^{a}
40 % FAA	3.0^{a}	3.0^{c}	4.0^{c}	4.0^{c}	5.0^{d}	5.0^{d}	6.0^{c}
$P \leq 0.05$	ns	*	*	*	*		*

Note: Mean value within a column followed by the same letter is not significantly different at $P \leq 0.05$ according to Tukey test.

As stated by Hartman *et al.* (2018) Moreira and Moraes, (2017), nutrients can be retained by leaves through foliar applications at sufficient levels. The amounts of fertilizer connected through soil can be decreased through foliar fertilizer spray as it can increase the crop production. This can be supported by Weinert *et al.* (2014) fertilizers availability in crops is cost-effective commercialized by supplementing soil with nitrogen for plant growth and high crop yields. Specifically, fish amino acid is a great fertilizer since it increments the crops development during vegetative growth period when applying to the soil and foliage. For instance, fish amino acid will persistently progress the scent and taste in leafy vegetables.

Total leaf area: Results of total leaf area of *B. oleracea* var. *alboglabra* L. is presented in Fig. 1. The application of different Fish Amino Acid (FAA) concentrations had positive effect on total leaf area.

The highest total leaf area was observed from plants treated with 30 % of FAA. It shows that the concentrations of 30 % FAA accelerate the total leaf area of Chinese Kale by 107 % as compared to NPK green. Meanwhile application of 40 % of FAA is not statistical different with 20 % FAA. Concurring to Moreira

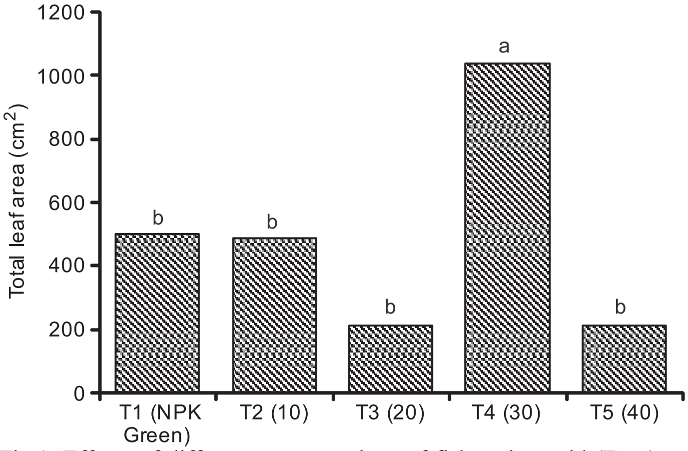

Fig.1. Effects of different concentrations of fish amino acid (FAA) on total leaf area of Chinese Kale after harvesting.

and Moraes (2017), the more FAA utilize as foliar fertilization in agriculture is based on higher saturation when applied through leaf cuticles. The FAA application in the plants nutrients happens resulting in deeper penetration of nutrients through cuticle layer. This is to describe that the concentration of 30 % of FAA resulting in higher diffusion through cuticle layer of Chinese Kale and lead to had superior total leaf area as compared to other concentrations.

Fresh and dry weight: The results of Chinese Kale fresh weight as influenced by different concentrations of FAA is presented in Table 3. There were significant differences ($P \leq 0.05$) observed on leaf, stem, root and total fresh weight of *B. oleracea* as treated with different concentrations of fish amino acid.

Table 3. Effects of different concentrations of fish amino acid on fresh weight (leaf, stem, root) of Chinese Kale after harvesting.

Treatment	Fresh weight per plant (g)			
	Leaf	Stem	Root	Total
NPK Green	16.21[c]	16.18[b]	1.40[ab]	33.79[d]
10 % FAA	12.93[bc]	9.59[ab]	1.34[ab]	23.86[c]
20 % FAA	8.09[ab]	5.63[a]	1.22[ab]	14.94[b]
30 % FAA	25.90[d]	27.89[c]	2.59[b]	56.38e
40 % FAA	5.50[a]	3.31[a]	0.66[a]	9.47[a]

Note: Mean value within a column followed by the same letter is not significantly different at $P \leq 0.05$ according toTukey test.

Results show that application of 30 % of FAA produced the highest total fresh weight per plant, followed by control, and 10 % FAA. meanwhile the lowest total fresh weight was shown by plant treated with 40 % FAA.Regarding on the results given in Table 3, leaf fresh weight had a significant difference among the all treatments where T4 (30 %) display the highest mean value at 25.90 g followed by T1 (NPK Green) at 16.21 g, T2 (10 %) at 12.93 g, T3 (20 %) at 8.09 g and T5 (40 %) at 5.50 g.Identically, there was a significant difference also shown in stem fresh weight between T1 (NPK Green), T2 (10 %) and T4 (30 %) with mean values 16.18 g, 9.59 g and 27.89 g meanwhile there was no significant difference between T3 (20 %) and T5 (40 %) with mean values 5.63 g and 3.1 g respectively. However, statistical analysis exposed that there was no significant difference among T1 (NPK Green), T2 (10 %) and T3 (20 %) in root fresh weight with mean values 1.40 g, 1.34 g and 1.22 g but there was significance value between T4 (30 %) and T5 (40 %) with mean value 2.59 g and 0.66 g.

Treated plants with T4 (30 %) shows a significant difference the mean values of fresh weight in all leaf, stem and root parts compared to other treatments and be the first ranked than other treatments. Based on Table 4.3, the lowest mean values were recorded in T5 (40 %) which is only at 9.47 g. From all the mean values fresh weight given in leaf, stem and roots, the total of fresh weight in all treatments were summed and highlighted in Table 4.3. Thus, the total mean values of fresh weight include 33.79 g (T1), 23.86 g (T2), 14.94 g (T3), 56.38 g (T4) and 9.47 g (T5).

Results in Table 4 show the effects of different rates of fish amino acid on dry weight of *B. oleracea* after harvesting. The dry weight of separated plant parts (leaf, stem and root) and the total dry weight per plants were recorded. There were no significant differences (P>0.05) in dry weight among treatments for leaf, stem and root parts. However, significant difference was observed on total dry weight per plant with the highest dry weight was recorded from the plant treated with 30 % of FAA. Meanwhile, plants treated with 40 %of FAA had significantly lower total dry weight as compared to other treatments, and 74 % lower than plant treated with 30 % FAA.

In this study, 30 % of FAA was found to the best treatment in increasing plant fresh weight and dry weight as well as for substituting the NPK green. Result shows that 30 % of FAA foliar fertilizer has higher total leaf area that resulted in higher fresh weight due to its optimum rate which reduce the water loss compared to 40 % of FAA.Table 4 imply that there was no significantly difference (P>0.05) in all treatments on dry weight of Chinese Kale . However, from the results given, T4 (30 %) had the highest mean value for all plant parts (1.82 g, 2.31 g, 0.52 g) while the lowest mean value of dry weight was shown by T5 (40 %) with value 0.57 g, 0.46 g and 0.17 g respectively. The growth promotion effects by the tested different concentrations treatment on leaves, stem and root were different from the fresh weight recorded effects. This is because dry weight biomass does not contain any moisture or water quantity.

Table 4. Effects of different rates of fish amino acid on dry weight (leaf, stem, root) of Chinese Kale after harvesting

Treatment	Dry weight per plant (g)			
	Leaf	Stem	Root	Total
NPK Green	1.26[a]	1.61[a]	0.29[a]	3.16[d]
10 % FAA	1.27[a]	1.28[a]	0.29[a]	2.84[c]
20 % FAA	0.69[a]	0.83[a]	0.32[a]	1.84[b]
30 % FAA	1.82[a]	2.31[a]	0.52[a]	4.65[e]
40 % FAA	0.57[a]	0.46[a]	0.17[a]	1.20[a]

Note: Mean value within a column followed by the same letter is not significantly different at $P \leq 0.05$ according to Tukey test.

Nowadays, fish amino acids appeared to be the most prominent significance in plant sustenance for getting of higher production and quality also shortening of the profitable cycle with superior dry materials (Wahba, 2015). The higher of plant height and total leaf area were resulting in higher fresh weight of the Chinese Kale . Oda and Tsuji (1992) mentioned that fresh weight is an important indicator among those parameters for monitoring plant growth. It is to examine the vegetables growth which is directly associated with water contents. Hariyadi and Huda (2019) explained that the role of nitrogen as fertilizer is to improve vegetative growth of plants and assist the process of protein formation. On the other hand, Davies (2010) mentioned that foliar fertilizer advancing the plant respiration process, photosynthesis, protein synthesis and reinforcing plant surviving and yield. The growth promotion effects by the tested different rates treatment on leaves, stem and root were different from the fresh weight recorded effects. This is because dry weight biomass does not contain any moisture or water quantity. The finding results as reported by Garba and Oviosa (2019), explained that drying is known to diminish dampness content to improve the foods shelf-life and increment dry matter. The formation and development process of plant organs were essentially impacted by the accessibility of water as well as nutrients in the growing media and are related to the process of growing plant cells (Taofik *et al.*, 2019).

It can be concluded that different rates of FAA had significant effect on plant growth parameters in terms of plant height, number of branches, total leaf area, fresh weight and dry weight. In the present study, the application of 30 % of FAA showed superior effect compared to other treatments. Farmers, home gardener's or beginner in farming are able to use FAA for foliar fertilizer as it offers environmentally friendly, easily available and cheaper compared to inorganic fertilizer.

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