Biofortification of Iodine Concentration in the Leaves of *Amaranthus* Sp and *Ipomea reptan Poir* Growing in Hydroponic Culture

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Sitasi: Munandar M, Toumae V, Ammar M, Gustiar F. 2019. Biofortification of Iodine Concentration in the Leaves of *Amaranthus* Sp and *Ipomea reptan Poir* Growing in Hydroponic Culture. *In*: Herlinda S *et al.* (*Eds.*), Prosiding Seminar Nasional Lahan Suboptimal 2019, Palembang 4-5 September 2019. pp. 360-369. Palembang: Unsri Press.

ABSTRACT

Biofortification of *Amaranthus* sp and *Ipomea Reptan Poir* with iodine is one of the alternative strategies for the enrichment of iodine in vegetable plants. The leafy green vegetables are categorized as horticultural crops, these are easy to cultivate, cheap and affordable for people in rural areas. The aim of this research was to increase iodine concentration in the leaves of Kangkung (*Ipomea reptan poir*) and spinach (*Amaranthus* sp) grown in hydroponic culture and evaluate its effect on plant growth. The experiment was conducted from November 2018 to February 2019 at the Hydroponic Shade House, Department of Agronomy, Faculty of Agriculture, Sriwijaya University.The design method used for this research was Completely Randomized Design (CDR) with 4 treatment of Iodine concentration in culture solution and 4 replications. The treatments were: T_0 = 0 ppm, T_1 = 25 ppm KI, T_2 = 50 ppm KI and T_3 = 75 ppm KI, which comprises of 16 experimental units. Each unit of experiment consisted of a container filled with 6 liters of A&B mix culture solution and 6 plants, i.e. 3 kangkung and 3 spinach were planted. Based on the results, it showed that the analysis of variance for plant height, leaves number, and chlorophyll content were not significantly different for both kangkung and spinach. While the ANOVA for fresh weight of kangkung leaves and stalks were highly significant, and the dry weight of kangkung leaves was significant. The iodine in leaves of kangkung (*Ipomea reptan poir*) increased with the increasing the amount of iodine concentration supplementation in culture solution. The iodine content in leaves of T_1 plant (25ppm) was between the range of 7-15ppm, for T_2 plant (50ppm) while in T_3 plant (75 ppm) the iodine content in leaves was between the range of 20-27 ppm. Spinach plant (*Amaranthus* sp) grow well only until the Iodine concentration, treatment of 25 ppm (T_1), and the iodine content in leaves reached the range of 7-15ppm. At the treatment of 50 ppm KI (T_2) and 75 ppm (T_3) leaves of spinach plants showed heavy necrosis as a symptom of high iodine toxicity, and plants was dry and died at three weeks after the Iodine treatment. In general, it is concluded that biofortification of Iodine in the leafy green vegetables by applying hydroponic culture is possible.The highest concentration supplementation of Iodine in culture solution for Iodine biofortification in Spinach plant (*Amaranthus* sp) was 25 ppm, while for kangkung (*Ipomea reptan poir*) was 50 - 75ppm.

Keywords: biofortification, iodine supplementation, hydroponic culture. kangkung, spinach

INTRODUCTION

There has been an issue that half of the world's population suffers from malnutrition and other micronutrients including I (Iodine) (Mao *et al*., 2014). Iodine deficiency is a micronutrient malnutrition crisis that arises due to the inadequate iodine intake leading to health problems that are generally regarded as iodine deficiency disorders (IDDs) (Zimmermann, 2011). These IDDs affect all age groups resulting in the increase pregnancy loss, infant mortality, and growth impairment. However there are solution made such as adding salt in food to help reduce the problem but on the contrary too much salt intake is capable of generating other health problems like hypertension. According to the study of Gonzali *et al.*(2017) the implementation of iodized salt is the most common iodine deficiency prophylaxis that has been successful in several countries. However it is still excessively inadequate. In addition many countries are reducing their salt intake to prevent diseases such as hypertension and cardio vascular.

Therefore introducing bio fortification of crops with iodine has been seen as an alternative approach that could be effective in a way that can suppress and control iodine deficiency (Bouis and Saltzman, 2017). The supplementation of iodine can be through irrigated water, foliar spray or in hydroponic solutions.Although iodine is well known as an essential nutrient for human health, it is not essential for plant, regardless studies that proof plants can accumulate iodine (Dai *et al*., 2006). Some of the plants that can take up iodine nutrients are spinach, caisin, lettuce and kangkung.

Leafy green vegetables such as *Amaranthussp and Ipomeareptanpoir*, has been chosen as the best option for iodine bio fortification because they are affordable, easy to cultivate and source of nutrients for communities in rural areas. These plants grew well in hydroponic system, because their growth pace from seedling until harvesting time is relatively short compared to other horticultural plants. The aim of the research was to enrich leafy vegetable plants with iodine and to determine the concentration of iodine in the leaves of spinach and kangkung plants. Another objective is to evaluate the effect of KI on selected parameters: Length, chlorophyll content, number of leaf, plants fresh weight and dry weight.

RESEARCH METHODOLOGY

This research was conducted at the hydroponic shade house, Department of Agronomy, Agriculture Faculty, University of Sriwijaya, starting from late November 2018 to mid-February 2019

This research used Completely Randomized Design, with 4 treatments and 4 replications, which comprises to 16 experimental units. Each unit consists of 6 plants, 3 kangkung and 3 spinach. The treatments for this experiment were the fertigation of 4 different level of iodine concentration KI. Hydroponic nutrients A&B mix was also added as basal fertilizer. (30ml of A Mix + 30ml of B Mix per 6L of water.)

The treatment of KI:
T0 = Control (0 ppm).
T1 = 25 ppm (KI), 25mlKI Stock Solution /1L water = 150ml KI/6L Freshwater
T2 = 50 ppm (KI), 50mlKI Stock Solution /1Lwater = 300ml KI/6LFreshwater
T3 = 75 ppm (KI), 75 mlKI Stock Solution /1Lwater = 450ml KI/6LFreshwater

All data or results were subjected to One Way analysis of variance (ANOVA), with a level of significance of LSD$p < 0.05$.

ISBN : 978-979-587-821-6

RESULT

The analysis of variance for all the parameters observed was tabulated in Table 1. The table of ANOVA shows that plant height, leaf number and chlorophyll content was not significant for both kangkung and spinach. Fresh weight of leaves and stems of kangkung was highly significant, while it was not significant for the roots. Spinach leaves, stems and roots fresh weight was also not significant. In dry weight it was slightly different, for kangkung the stem and roots are not significant, whereas for the leaves it was significant, for dry weight of spinach leaves, stems and roots was not significant, based on the Least Significant Difference P < 0.05.

Table1. Analysis of variance for all the parameters observed

Parameters	Kangkung		Spinach	
	F Value	CV (%)	F Value	CV (%)
Plants Height	2.90 ns	12. 5	1.69 ns	64.58
Leaf Number	2.36 ns	12.1	2.91 ns	9.58
Chlorophyll Content	1.31 ns	7.87	0.02 ns	32.15
Fresh Weight				
✓ Leaf	10.8 **	12.3	1.15 ns	137.21
✓ Stalk	10.9 **	19.6	1.54 ns	130.22
✓ Root	0.87 ns	30.2	1.13 ns	106.91
Dry Weight				
✓ Leaf	5.54 *	28.9	1.41 ns	125.05
✓ Stalk	2.92 ns	35.5	1.58 ns	160.03
✓ Root	0.81 ns	23.2	1.03 ns	151.69
F Table 5%	3.49		3.49	
F Table 1%	5.95		5.95	

Key: **= Highly significant *= Significant; ns= Not significant; CV= Coefficient of Variance

Plants Height (cm)

The measuring of plant height of kangkung and spinach plants was done and recorded after every five days observation when the hydroponic solution was recycled. Plant height data was collected accordingly to the different treatments and replications of the experiment, which was illustrated in Figure 1.

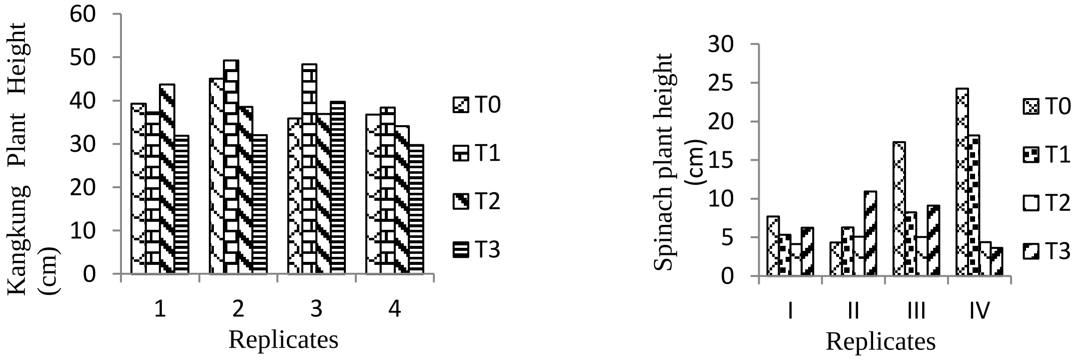

Figure 1. Plant Height of kangkung and spinach with 4 treatments and 4 replications

According to the above graphs, the treatment with the highest plant height was T_1 for kangkung and T_0 for spinach, whilst the least plant height was found on treatment T_3kangkung and T_2 spinach for each of the observations and replicates. In addition the supplementation of iodine has not affect plant height of kangkung, however it does affect spinach growth and height as can be seen from the two graphs. The Analysis of variance for plant height of kangkung and spinach according to the F value, plant height was not significantly different for both kangkung and spinach.

Number of leaves

The observation and counting of leaves number for kangkung and spinach were recorded alongside the other parameters for all the treatments and replicates, figure 2.

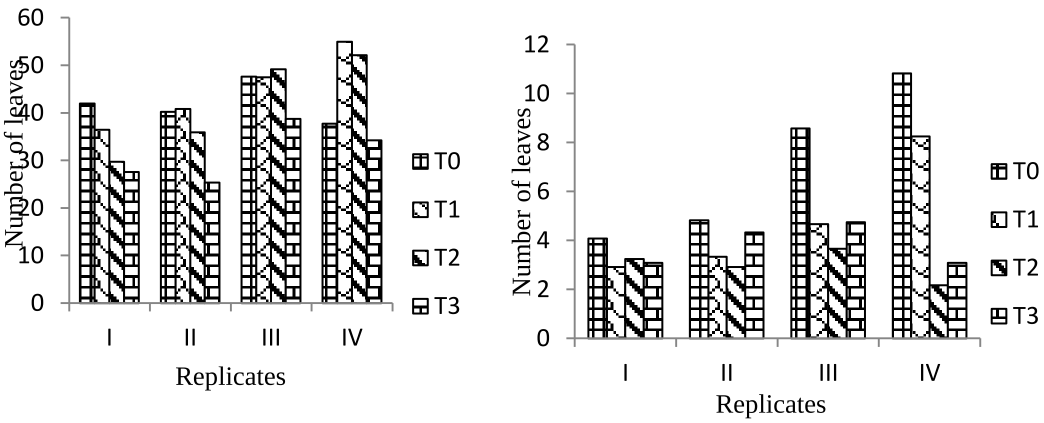

Figure 2. Number of leaves of kangkung and spinach for 4 treatments and 4 replications

Figure 2 display the number of leaves of kangkung and spinach plants for 4 different treatments and replications. The treatment with the highest number of leaves was T_1 for kangkung and T_0 (control) for spinach, the least number of leaves was found at the second replicates for kangkung which was treatment T_3, and T_2 for spinach at the fourth replication. The analysis of variance for the number of leaves based on the F value was not significant for both kangkung and spinach plants.

Chlorophyll Content

The chlorophyll content of kangkung and spinach can be seen in Figure 3, two separate column graphs for kangkung and spinach with 4 treatments and 4 replications.

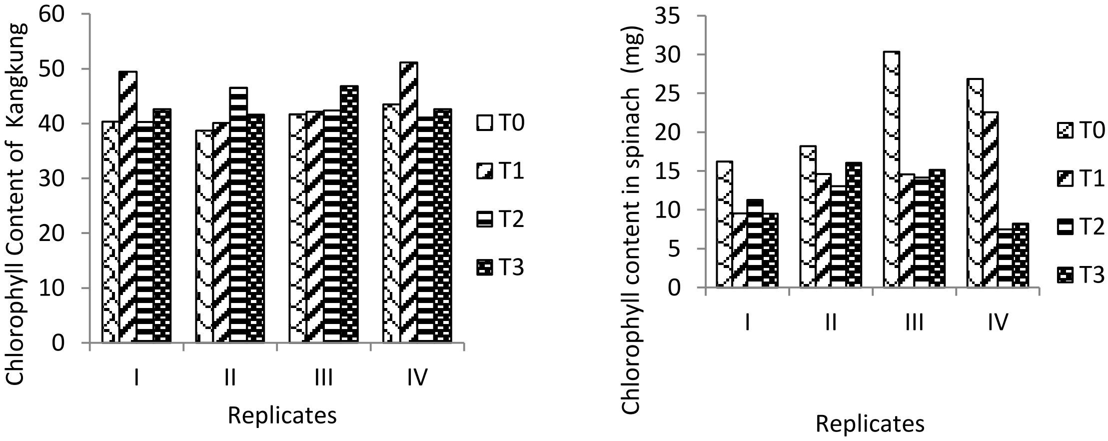

Figure 3. Chlorophyll content of kangkung and spinach for 4 treatments and replications, measured using a SPAD meter

Figure 3 depict how much chlorophyll was present in the leaves of the plants at 4 different treatments and 4 replicates or tests. The treatment with the highest level of chlorophyll content was T_1kangkung and T_0 spinach plants, whereas the lowest level of chlorophyll content was found in treatment T_0 for kangkung and T_2 for spinach.

The analysis of variance for chlorophyll content in the leaves of kangkung and spinach was not significantly different because the F value was smaller than the F ($p<0.05$) T table.

Fresh Weight

The plants fresh weights were divided into leaves, stalks and roots and were weighted separately on an analytic scale. Thus the mean fresh weights of kangkung and spinach were illustrated in Figure 4 below.

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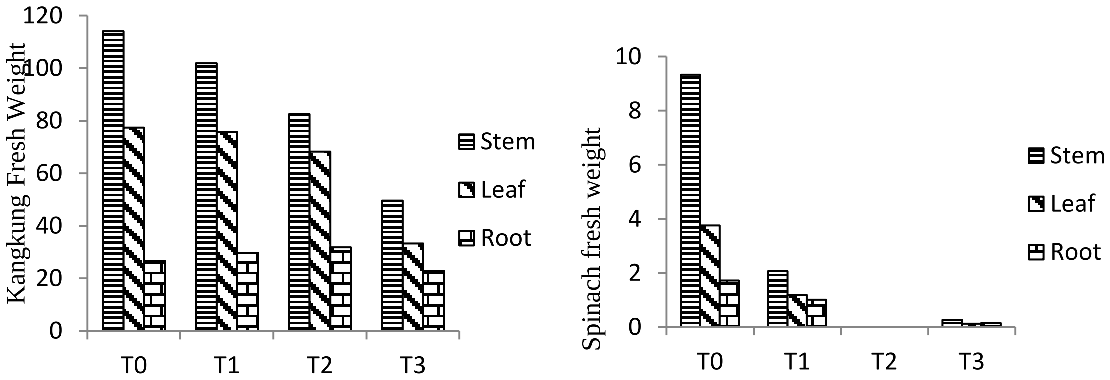

Figure 4. Fresh weight for leaves, stem and roots of kangkung and spinach

The fresh weight of stalks, leaves and roots of kangkung and spinach shows that for stalks, the highest fresh weight was at T_0 (114g) Kangkung and T_0 (9g) Spinach, while the least fresh weight was at T_3 (50g) Kangkung and T_3 (0.3g) Spinach. For leaves fresh weight, the highest value was at T_0 (77g) kandkung and T_0 (3.8g) spinach, the lowest was at T_3 (33g) Kangkung and T_3 (0.1g) spinach. Finally for roots, the highest fresh weight was recorded at T_2 (32g) kangkung and T_0 (1.7g) spinach, while the least was at T_3 (23g) kangkung, and T_3 (0.4g) spinach. The analysis of variance for kangkung and spinach fresh weights of leaves and stalks are tabulated in Table 2.

Table 2. The analysis of variance for kangkung plant fresh weight

	Mean Average	
Treatment	Leaf	Stalk
T_0	77.42_b	114.09_c
T_1	75.65_b	101.85_b
T_2	68.27_b	82.48_b
T_3	33.26_a	49.57_a
LSD 5%	12.1	26.2

Note: Means followed by same letters are not significantly different at $P < 0.05$

Based on the analysis of variance, the fresh weight of kangkung leaves and stalks was highly significantly different, based on the Least Significant Difference 5% test.

Dry Weight

The dry weight of kangkung and spinach was scaled in parts of stalks, leaves and roots Figure 5.

ISBN : 978-979-587-821-6

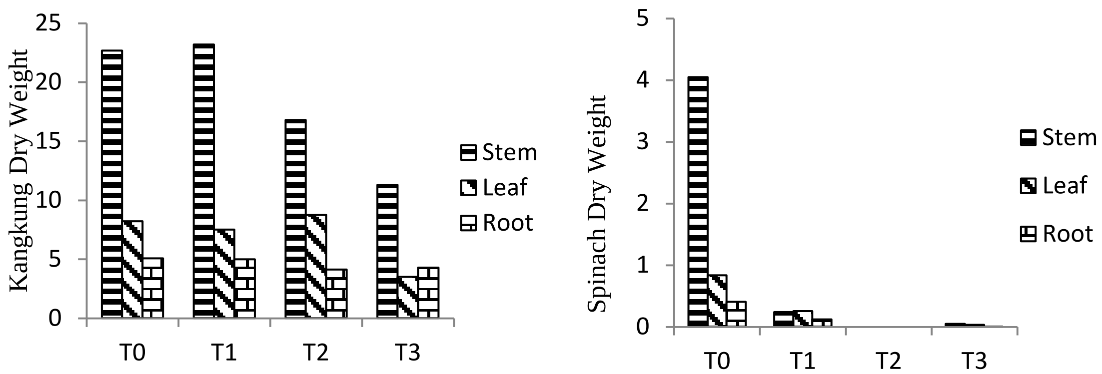

Figure 5. Dry weight of stalks, leaves, and roots of kangkung and spinach

For plant stalks, the treatment with the highest dry weight was T_1 (23g)for kangkung and T_0 (4g) for spinach, while the least dry weight was recorded at T_3 (11.3g) kangkung and T_3 (0.05g) spinach. The highest dry weight for leaves was at T_0 (8.7g) kangkung, and T_0 (0.8g) spinach, and the lowest dry weight was found at T_3 (3.5g) kangkung, and T_3 (0.03g) spinach. Lastly the highest dry weight for roots was at T_0 (5.1g) kangkung and T_0 (0.4g) spinach while the lowest dry weight was recorded at T_2 (4.1 g) kangkung and T_3 (0.01 g) spinach.

Table 3: Analysis of variance for dry weight of kangkung

	Mean Average	
Treatment	Leaf	Stalk
T_0	8.21	b
T_1	7.51	b
T_2	8.74	b
T_3	3.50	a
LSD 5%	3.12	

Note: Means followed by same letters are not significantly different at P < 0.05

The analysis of variance for Dry weight of kangkung leaves was tabulated in Table 7. Based on the T table the F value was greater than the F (p<0.05), hence dry weight of leaves was significantly different at the LSD 5% test.

Iodine Determination

Iodine determination was done using simple test-kit method, provided with standard color photo for iodine in salt and its estimated iodine concentration range, The standard colors determine different range of iodine concentration. 0ppm correspond to a clear light color, 7 – 15ppm have a brownish purple color, 20 – 27ppm as light purple, 28 – 30ppm dark purple and >30ppm dark blue in color. Iodine content in the leaves of kangkung and spinach determined by Test-kit method can be seen below with code numbers, Table 4.

Table 4. Iodine determination result of the sample leaves

Treatment (Ppm)	Code number	Kangkung	Spinach	Test-Kit Color
T_0(0ppm)	1(0ppm)	1	1	Light clear to yellowish
T_1(25ppm)	2(7-15ppm)	2	2	Brownish purple
T_2(50ppm)	3(20-27ppm)	3	-	light purple
T_3(75ppm)	4(28-30 ppm) 5(30>ppm)	3	-	light purple

ISBN : 978-979-587-821-6

The result for iodine determination in the leaves of kangkung and spinach varies, in treatment T_0 or control treatment, kangkung and spinach were in the same level or range which was coded with number 1 (0ppm), after the reagent solutions were added to the sample the color remains clear to yellowish which signifies lack of iodine. For T_1 the iodine level was code number 2 (7 – 15 ppm) for both plants with an outcome color of brownish purple. Treatment T_2 and T_3 falls into the estimated iodine range or category between 20 – 27 ppm coded with number 3 with a light purple color.

Figure 7 was the color result when conducting the simple test-kit method on samples to determine the estimated iodine content in the samples, and comparing it to the standard colors below.

Necrosis Symptoms

Necrosis and chlorosis are some of the symptoms of high iodine toxicity level in plants, especially in sensitive vegetable plants such as spinach and kangkung; both plants have different levels of toxicity. For instance, when supplying different concentration of iodine KI to the plants, it was observed that kangkung and spinach have different levels of toxicity.

Kangkung have a high toxicity level since it has the ability to grow well despite the increasing level of iodine added, like in T_2 50 ppm it experience abscission of leaves and T_3 75 ppm, the plant leaves have curled brown edges, while for spinach it has a low toxicity level, the observation revealed that the leaves were curled in, brown, wilted and stunted stem, also their growth was hampered since most died.

DISCUSSION

The analysis of variance for all the parameters shown in Table 1, exhibit that plant height, number of leaves, chlorophyll, fresh weight of roots, and dry weights of stalks and roots were not significantly different, whereas for fresh weight of leaves and stalks and dry weight of leaves were highly significantly different based on the Least Significant Difference $P < 0.05$ Test.

Although plants height and number of leaves were not significantly different based on the analysis of variance, the treatment with the highest plant height was T_1 (49.2 cm) for kangkung and T_0 (24.3cm) for spinach, whilst the lowest plant height was measured at treatment T_3 (31 cm) kangkung and T_2 (4.1 cm) spinach. The weekly observation of plants height was carried out at 5 days interval routinely and it was observed that unlike kangkung, spinach was very sensitive to KI. When iodine KI was supplied in large quantities it shows negative effects for spinach growth and height, however, in Kangkung plant, the growth response to KI in high concentration was not dramatic in comparison to spinach. This is because different vegetable plants have different toxicity level to certain elements that enables them to grow and tolerate in different conditions. According to Hong, Weng *et al*. (2008). Excessive iodine concentration may be detrimental and impair normal plant growth; on the contrary, iodine overdose greatly reduces the efficiency of iodized fertilizer usage. Furthermore, for the number of leaves, the treatment with the highest leaves number was T_1 (55) for kangkung and T_0 (control) (11) for spinach, while less number of leaves was found at T_3 (25) kangkung and T_2 (2) for spinach. The illustration in Figure 2 shows that spinach has the lowest number of leaves compared to kangkung of the same treatment. Weekly observation shows that, spinach plants experience a very slow growth and have less number of leaves compared to kankung.

This occurs as a result of iodine toxicity, although plants nutrients such as A and B Mix were administered into the hydroponic solution to help with the plant vegetative growth, High iodine concentration can be dangerous for sensitive plants, in fact, spinach plant growth undergo leaves abscission and wilting at an early stage. On the other hand kangkung plant show signs of leaves abscission and brown leaves spots at the latter weeks of experiment, yet it tolerate the effect of iodine and still grow, this proof that some plant can still tolerate high iodine concentration, while some are not. Conforming to a pot-culture experiment, four vegetables seedlings were injured when iodine supplementation in soil was higher than 50 mg kg, resulting in chlorosis in young leaves brown, stunted and coralloidroots (Hong *et al*., 2008.)

Chlorophyll content measurements were recorded using SPAD meter. The analysis of variance for the effect of iodine concentration to chlorophyll was not significantly different. Based on Figure 3, the treatment with the highest level of chlorophyll content was T_1 (51.1) for kangkung and T_0 (30.3) for spinach, whereas the lowest level of chlorophyll content was found in treatment T_0 (38.7) for kangkung and T_2 (7.4) for spinach, hence the chlorophyll of the plants was not affected. By observation, the chlorophyll value of kangkung and spinach was significant and consistently for each of the treatment during the four observation period. Which explained that Iodine doses of KI addition does not affect the biomass or chlorophyll content of the seedlings but decreased their length (Krzepiłko A *et al*., 2016)

The analysis of variance for Plants fresh weights (Table 2) describe the mean fresh weight of leaves and stalks weighing separately was highly significantly different at LSD $P < 0.05$, while the roots fresh weight was not significantly different. The weight of the plant stalks was the highest at T_0 with the mean of 114 g, followed by the fresh weight of leaves at T_0 77 g and the roots had the lowest fresh weight of (23 g) at T_3.

For dry weight, the analysis of variance, Table 3 shows that the dry weight of leaves was significantly different at LSD $P < 0.05$, but it was not significant for the stalks and the roots, based on ANOVA. In Figure 5, kangkung stalks has the highest dry weight at T_1 (23.2 g), and the lowest was at T_3 (11.3 g) while the leaves highest dry weight was recorded at T_2 (8.7 g), and lowest was at T_3 (3.5 g). Roots highest dry weight was at T_0 (5.1 g) and the lowest dry weight was at T_2 (4.1 g). For spinach, the highest dry weight for stalks, leaves and roots was at T_0 (0 ppm) and the lowest dry weight was at T_3 (75 ppm). In addition both the fresh and dry weights were not significantly influenced by the iodine concentration; however it depends on the external factors such as the environment and temperature also including plant growth and maintenance does influence fresh weight and dry weight. This study however shows that fresh weight and dry weight were not influenced by iodine. This is similar to the study of Krzepiłko., (2016), which stated that the enrichment of iodine to lettuce plant did not affect plant biomass.

The analysis of iodine was determined through an iodine test-kit method for salt, the determination results of the samples (Table 4) shows the treatments and the iodine content of the sample leaves that ranges from 0ppm for zero iodine to < 30 ppm the highest. Figure 7 display the resulted colors of the iodine determination conducted on the grounded leaf samples. The result shows that at T_0 both kangkung and spinach had (0 ppm) of iodine, then in T_1 both vegetable plants had iodine concentration between the range of (7-15 ppm), in T_2 and T_3 kangkung iodine concentration falls in the range (20-27 ppm). It can be concluded that kangkung plant is more tolerant to iodine than spinach, since in treatment T_2 and T_3 spinach suffered injured leaves and impairment growth that reduces its height. Thus Increase iodine supplementation increases the iodine content in leaves and roots of kangkung but affect spinach growth.Iodine transportation in plants takes place in the xylem

ISBN : 978-979-587-821-6

most, which explained the increase iodine concentration in the leaves of the vegetable plants. A previous study by Weng H X *et al*. (2013) stated that the absorption of iodine by the vegetable increases with increasing amount of the algal-based iodized organic fertilizer in general. And the uptake of iodine by leaf vegetable is significantly greater than that by fruit vegetable.

Necrosis symptoms were spotted on the leaves of spinach and kangkung because the different concentration of iodine KI influences their growth metabolism, resulting in iodine toxicity in plants organs causing plants to wilt shade leaves and dye. High concentration of iodine can lead to iodine toxicity in sensitive plants especially spinach, in contrast kangkung plant was tolerant to iodine. It was observed that kangkung grew well despite the high iodine supplementation compared to spinach. Spinach show symptoms of wilting and brown sports after the first observation and most plants died at the following observation, however for kangkung plant the symptoms was visible at Treatment T_3 (75 ppm) yet kangkung still capable to grow until harvesting.

These symptoms and effects can be seen in Figure 8. During the first weeks of observation spinach plants already experience signs of wilting at T_2 50 ppm and T_3 75 ppm, then in the second week the spinach leaves starts to curled in and have brown edges and spots, leaves abscission and their length decreases. Week 3 and 4, most of these spinach plants became stunted and many eventually died, while for Kangkung the symptoms surfaced in the final weeks of experiment, especially on treatment T_3 (75 ppm), this may due to the high iodine concentration accumulated in the plants leaves and roots resulting in iodine toxicity. According to Gonda*et al*. (2007).Spinach grown with a higher I (-) concentration showed symptoms of necrosis and leaf abscission on plants cultivated in hydroponic culture, and inhibition in vegetable growth (Hong *et al*., 2008).

The pH value of the solution culture decreases in the last pH observation, because all the nutrients or fertilizers were used up by the plants, for producing new leave, increasing plants height and plant growth causing the pH level of the solution to reduce at the last observation done after harvesting. However the decrease was not significant since the pH values was optimal and in a consistent close range. Islam *et al.* (1980) reported that a pH range of 5.5 to 6.5 is optimal for the availability of nutrients from most nutrient solutions for most species.

Electrical Conductivity (EC) value of the culture solution was decreases at the end of observation compared to the beginning. The increase value of EC at the beginning 1.8mS was due to the fact that, the nutrients were just added and were not yet accumulated by the plants. Conversely, the end observation of the EC value decreased because there were fewer nutrients available in the solution culture as a result of increasing uptake of nutrients by the plants. EC values for hydroponic systems range from 1.5 to 2.5 ds m-1. Higher EC hinders nutrient uptake by increasing osmotic pressure, whereas lower EC may severely affect plant health and yield (Samarakoon *et al*., 2006).

CONCLUSION

The best Treatment for spinach in this research was T_1(25ppm), while for kangkung regardless of the high iodine concentration in T_2 and T_3Kangkung plants still capable to grow and tolerant to iodine KI toxicity. Kangkung plant growth response to iodine was quite significant compared to spinach in the sense that apart from the necrosis effect visible in T_2 and T_3, there was no other striking effect that triggers plant dying, whereas iodine toxicity in spinach plant was obviously noticeable in the first week, since it is sensitive to iodine. In general, the bio-fortification of iodine to kangkung was successful, and for spinach due to its sensitivity to iodine, supplying 25ppm and less would be best.

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ISBN : 978-979-587-821-6