SCIREA Journal of Biology
http://www.scirea.org/journal/Biology
March 16, 2022
Volume 7, Issue 1, February 2022
https://doi.org/10.54647/biology18204

BIOSTIMULANT EFFECT OF SPIRULINA (*ARTHROSPIRA PLATENSIS*) ON LETTUCE (*LACTUCA SATIVA*) CULTIVATED UNDER AQUAPONIC SYSTEM.

J.O. Siringi [2*], Losenge Turoop [1], Francis Njonge [2]

[1] Department of Horticulture and Food Security, Jomo Kenyatta University of Agriculture and Technology (JKUAT), P. O. Box 62000, Nairobi, 00200, Kenya.

[2] Department of Animal Sciences Jomo Kenyatta University of Agriculture and Technology (JKUAT), P. O. Box 62000, Nairobi, 00200, Kenya.

*** Corresponding Author:** siringi2007@yahoo.com

ABSTRACT

Indoor experiments were carried out to examine the effect of spirulina (Arthrospira platensis) stimulant on growth performance and biochemical response of lettuce (Lactuca sativa) grown in an aquaponic system. The lettuce plants, 10.45±0.12cm (Mean ± SD), were randomly distributed and transplanted into four treatments consisting of 0 (control), 4.0, 8.0, and 12.0g spirulina/L of water and replicated four times. The treatments were administered once every week in form of a foliar spray for 5 weeks. The effect of spirulina stimulant on Leaf length, leaf width, leaf area, dry matter, and antioxidants content of L. sativa in the aquaponic system was determined.

Analysis with polynomial contrasts showed that the quadratic trend for the number of leaves significantly ($p < 0.05$) increased with a peak at 8 g of spirulina/L of water. The cubic trend for final leaf length, width, and leaf area was significantly ($p < 0.05$) increased with a peak at 8 g of spirulina/L of water. The contrasts analysis showed a significant ($p < 0.05$) linear increment for dry weight with a peak at 12 g of spirulina/L of water. For antioxidant contents, the contrasts analysis showed a linear increase for the FRAP, quadratic increase for the DPPH, cubic increase for the ABTS, and quadratic increase for the Total phenols of L. sativa.

The results above show that the application of spirulina (A. platensis) foliar spray has a positive effect on the growth performance and biochemical properties of lettuce (L. sativa) grown in an aquaponic system.

Keywords: Antioxidants, Lactuca sativa, aquaponic system, spirulina.

1. INTRODUCTION

The Kenyan agricultural sector is under great pressure to increase its productivity to meet the steadily towering population. The output of many crops, including vegetables, has remained stagnant and, in some cases, declined (Ray *et al.,* 2013). The majority of farmers practice rain-fed farming cultivation and, therefore, productivity has been challenged by erratic weather changes due to climate change, land degradation and land fragmentation (Kwena *et al.,* 2015; Osumba and Rioux, 2015). To increase productivity, the adoption of new technologies, practices, and tools will be critical for smallholder farmers. Aquaponics could be one of these new technologies. Aquaponic systems are well known for offering several advantages, more so when food has to be produced innovatively and sustainably. Some of the advantages include the synergistic effects of increased concentration of carbon dioxide (CO_2) for the greenhouse plants and reduction in total heat energy consumption when raising both fish and plants in the same space (Eck *et al.,* 2019; Korner *et al*., 2017). Other advantages include: first, aquaponic eliminates the discharge of aquaculture effluents enriched in nitrates and phosphates into the environment (Buzby and Lin 2014), and second, the soilless plants in the aquaponic system are supplied with organic

nutrients from the fish tanks (Goddek et al. 2015) thereby reducing the use of inorganic fertilizers of mineral origin that are made from non-renewable natural resources.

However, the nutrient concentrations supplied by the fish in the aquaponic system are not sufficient for plant nutrient requirements compared to hydroponic systems. Therefore, certain plants require nutrient supplementation in the aquaponic system (Bittsanszky *et al.,* 2016). Organic nutrient supplementation would be the most beneficial in the aquaponic system for the good health of both plants and fish. Spirulina (*Arthrospira platensis*) biofertilizer can be used as a substitute for organic matter in an aquaponic system. Spirulina is a blue-green microalga that flourishes in warm and alkaline tropical lakes. Spirulina has rich biomass consisting of about 62 % protein, contains vitamins, and the whole spectrum of antioxidants including carotene and Xanthophyll phytopigments considered as the richest natural source of vitamin B_{12} (Abd El-RheemKh *et al.,* 2015). Studies have shown that spirulina biofertilizer increases growth performance and yield of green grams (*Vigna radiata*) in normal soil farming (Aung, 2011).

Lettuce (*L. sativa*) is an annual plant belonging to the family Asteraceae and is the most used species in aquaponic systems (Kim *et al.*, 2018). Its leaves are used in making several varieties of foods including salads, juices, wraps, sandwiches, and processed meals. Lettuce plants are diverse in shape and color, varying from green to red. They also have different surfaces, leaf textures, and margins (Bunning and Kendall, 2012). Lettuce has a rich nutritional profile including vitamins A, C, B complex, and K as well as a substantial number of secondary metabolites such as phenolic acids, flavonoids, carotenoids, and folate which have been found to promote good health (Bunning and Kendall, 2012). The phenolic compounds in lettuce produce antioxidant activity that produce free radicals with scavenging ability. In addition, studies have shown that polyphenols can prevent cardiovascular diseases and cancer (Hooper and Cassidy, 2006; Manach *et al.*, 2004). It has been reported that extracts from lettuce leaves reduce inflammatory and oxidative stress in murine monocyte and macrophage cells by decreasing reactive oxygen species and nitric oxide release (Zapata-Vahos. *et al.*, 2020).

The use of spirulina as a biofertilizer has been limited to conventional farming, mainly legumes and cereals (Jamal Uddin *et al.,* 2019). There is scanty information on the use and influence of spirulina on most crops and, more so, on hydroponic and aquaponic systems (Hegazi *et al.,* 2010). Therefore, the current research was to investigate whether the addition of spirulina biofertilizer

into an aquaponic system increases the growth performance and biochemical properties of lettuce. The level of spirulina biofertilizer that promotes better growth and biochemical responses in terms of antioxidant contents was determined.

2. MATERIALS AND METHODS

The experiments were conducted at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Department of Horticulture and Food Security (at an altitude of 1416 m above sea level, location 1° 10' S, 37° 7' E). Certified lettuce seeds obtained from the Kenya Seed Company were germinated into trays of 66 cells with peat moss as a substrate.

2.1 Experimental design

The research setup consisted of 16 shallow plastic rectangular grow beds (each measuring 90cm by 120cm and 60cm deep) filled with pumice and 16 circular plastic tanks each with a capacity of 1000 L stocked with 20 fish in a closed ebb-flow substrate aquaponic system (Fig. 1). Nile tilapia (*Oreochromis niloticus) fingerlings* with an average initial weight and total length (TL) of 3.90±0.015g and 64.04±0.18mm (Mean ± SD) respectively were randomly distributed into four treatments containing 0.0, 4.0, 8.0 and 12.0 g spirulina/kg of the basal diet of 30.4% crude protein (CP). The fingerlings were fed the diet at 5% body weight twice daily for 16 weeks.

A completely randomized design (CRD) was used in the system layout and each treatment was replicated four times. The lettuce plants, (10.45±0.12cm (Mean ± SD)), were randomly distributed and transplanted into the plastic beds at a spacing of 30cm by 30cm after three weeks from the date of sowing. The treatments were four different levels of spirulina doses consisting of 0 (control), 4.0, 8.0 and 12.0g per/L of water. The doses were administered weekly in a form of foliar feed for 5 weeks from the transplanting date.

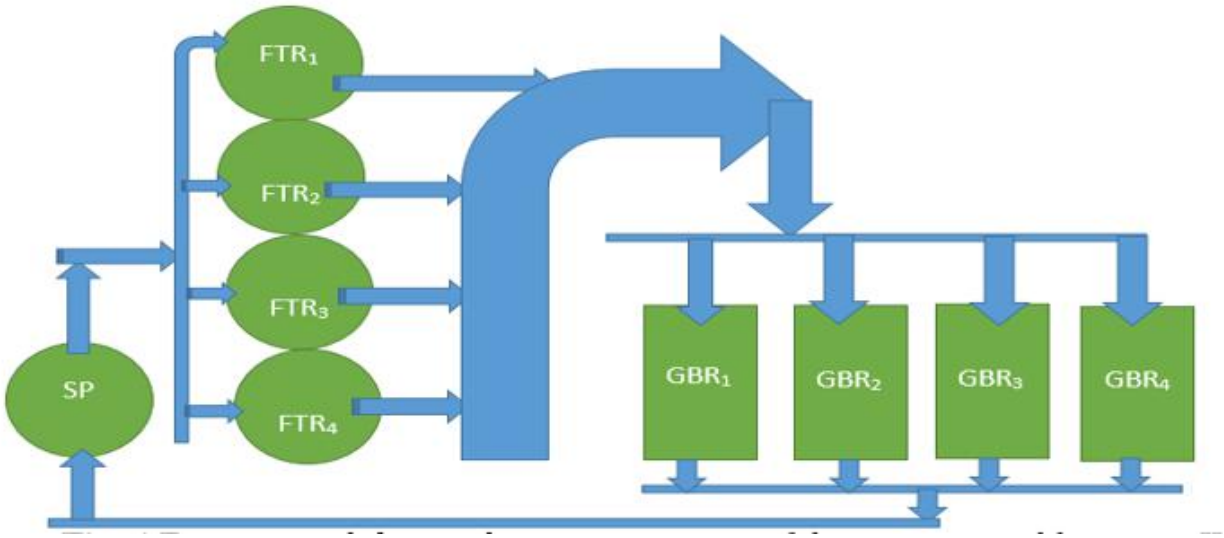

Fig. 1 Experimental design showing one set-up of the experimental layout at JKUAT: FTR= Fish Tank Replication (1, 2, 3, 4); GBR=Grow Bed Replication (1, 2, 3, 4); and SP= Sump and Pump.

2.2 DATA COLLECTION

Data were collected for 5 weeks under a greenhouse operating at a consistent controlled air temperature of 35/20^{o} C (day/night) and 60-80% humidity.

2.2.1 Growth

The first four lower leaves were identified and the dimensions of each leaf (the maximum length (L), maximum width (W), and the total number of leaves per plant) were recorded weekly. Four plants from each replicate at the end of the experimental period were randomly sampled out and the first four lower leaves from each plant were selected for the determination of leaf area (LA) using a LA meter (Licor model 3100 source) (Presnov *et al.,* 2005).

2.2.2 Dry matter content

To evaluate dry matter yield, both shoots and roots were taken to an oven where they remained for 72 hours at 65 °C and then weighed to determine the dry mass. The differences between the initial weight of the sample and that after drying were recorded. Dry Matter (DM) content in the samples was calculated using the formula below:

$$\textbf{Dry matter}(\%) = \frac{\textbf{weight of the dry sample (g)}}{\textbf{weight of sample before drying (g)]}} \text{x}100$$

2.2.3 Antioxidant Contents

This was determined by carrying out an antioxidant activity of the first four lower leaves from each treatment replicate that were randomly sampled out at the end of the experimental period according to Zapata-Vahos *et al.* (2020).

2.2.3.1 Leaf Extract Preparation

The samples from each replicate were rinsed with water, and 1±0.1 g of the samples were placed in an extracting solution consisting of 25 mL of acidulated methanol (HCl 1%). The samples were then homogenized individually using a homogenizer T 25 digital ULTRA-TURRAX-IKA, the extracts were filtered through 0.20 µm filters and immediately stored at -20 °C.

2.2.3.2 Antioxidant Activity

FRAP (Ferric Reducing Ability of Plasma) analysis.

Samples of leaf extract (50 µL) were mixed with 50 µL of acetate buffer solution of pH 3.6 and 900 µL of FRAP solution in HCl 40 mM, at 1:1:10 proportion. The absorbances were measured at 590 nm in a spectrophotometer (PG instruments T80) and FRAP values were expressed as milligrams of Ascorbic Acid Equivalent per 100 g of lettuce (mg AAE $100g^{-1}$).

ABTS (2, 2-azino-bis (3-ethylbenzothiazoline-6- sulphonic acid)) analysis.

This was carried out to examine the radical scavenger activity of the leaf extracts against the stable radical ABTS. Leaf extracts of 10 µL sample and 990 µL of standard ABTS were mixed and absorbance was determined at 720 nm after 30 minutes. The ABTS values were expressed as millimoles of Trolox Equivalents per 100g of lettuce (mmolTE $100g^{-1}$).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) analysis.

This was done to determine the ability of leaf extracts to scavenge the DPPH radical. Samples of leaf extract (10 µL) were added to 990 µL of the DPPH standard solution and thereafter absorbance was determined at 517 nm after 30 minutes. The DPPH values were expressed as millimoles of Trolox Equivalent per 100 g of lettuce (mmolTE $100g^{-1}$).

Total phenols analysis.

Samples of leaf extracts (50 µL) were mixed with 125 µL of Folin-Ciocalteu reagent, 400 µL of $CaCO_3$ (7.1%) and 425 µL of H_2O. The absorbance was read at 760 nm after 30 minutes in the

darkness and the results expressed as milligrams of Gallic Acid Equivalents per 100 g of lettuce (GAE $100g^{-1}$).

2.3 STATISTICAL ANALYSES

A one-way analysis of variance (ANOVA) was used to determine the significant difference ($p < 0.05$) between treatment means and Tukey's test was used to separate treatment means. Polynomial contrasts were further used to determine the linear, quadratic, and cubic effect of different levels of spirulina foliar spray on growth performance and antioxidant contents of lettuce. All results were presented as Mean± Standard Error (SE). All statistical analyses were carried out using SPSS version 20 and data computation and graphical presentations were done by Microsoft Excel.

3. RESULTS

3.1 Growth performance

The weekly gain in the number of leaves was higher in treatment 4, followed closely with treatment 3. Treatment 1 (control) had the lowest gain in the number of leaves (Fig 2). Generally, the rate of increase in the number of leaves was slightly lower in the first week but significantly increased between the third and fourth week for all the treatments. The number of leaves almost remain the same in the fifth week for all the treatments.

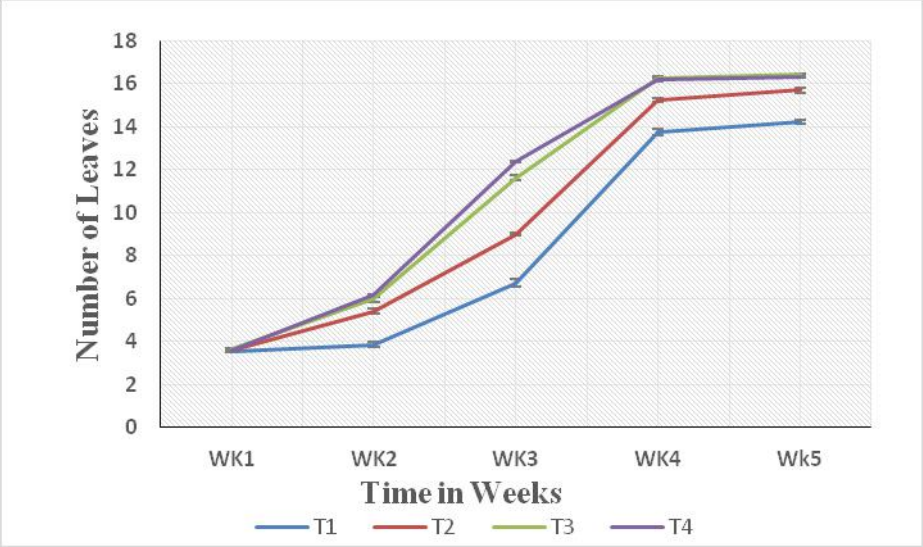

Fig. 2. Mean gain in the number of leaves (Mean ± SE) of L. sativa grown under different levels of spirulina for 5 weeks in spirulina enhanced aquaponic system. Note: T1- No spirulina (control); T2- 4.0g spirulina/L of water; T3- 8.0g spirulina/L of water; T4- 12.0g spirulina/L of water

The same trend was exhibited for mean gain in leaf length and width (Fig 3). The growth parameters of L. *sativa* grown under different levels of spirulina for 5 weeks in a spirulina enhanced aquaponic system increased significantly in all the treatments compared to the control.

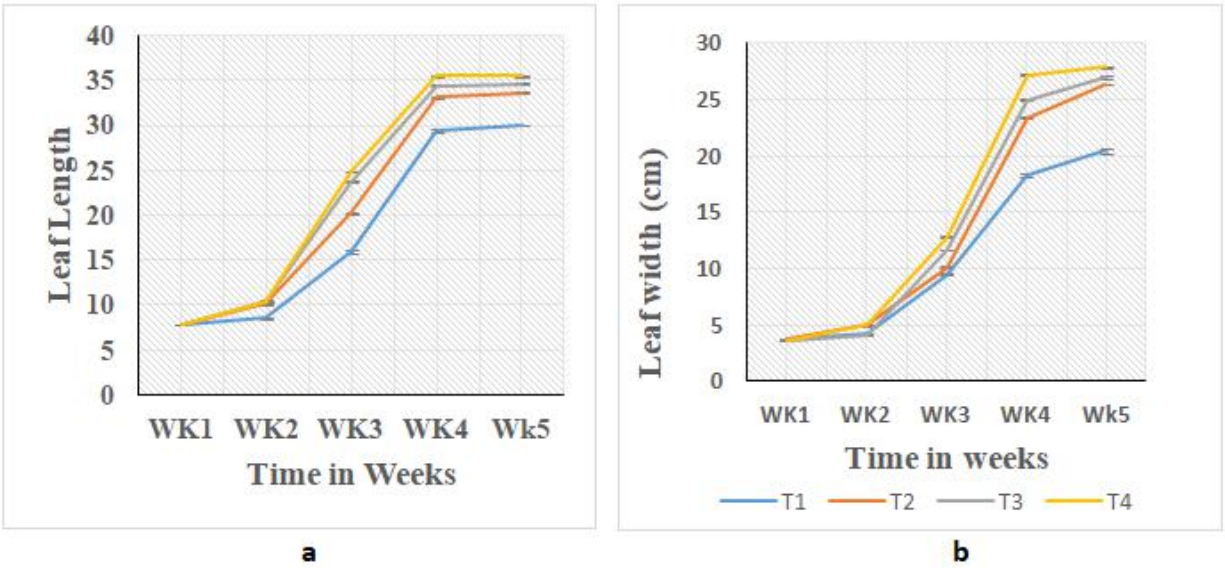

Fig.3. Mean gain in leaf length(a) and width (b) (Mean ± SE) of L. sativa grown under different levels of spirulina for 5 weeks in spirulina enhanced aquaponic system. Note: T1- No spirulina (control); T2- 4.0g spirulina/L of water; T3- 8.0g spirulina/L of water; T4- 12.0g spirulina/L of water

There were significant differences ($p < 0.05$) in the final number of leaves, final leaf length, leaf width Leaf area and dry weight amongst the four levels of spirulina (Table 1). Treatment three with 8 g of spirulina/L of water showed the highest number of leaves while Treatment four with 12 g of spirulina/L of water recorded the highest leaf length, leaf width leaf area and dry weight. The control Treatment recorded the lowest for all the leaf parameters.

There was a significant ($p < 0.05$) quadratic increase for the final number of leaves with peaks at 8 g of spirulina/L of water and cubic increase for the final leaf length, leaf width and leaf area with peaks at 12 g of spirulina/L of water and linear increase for dry weight with a peak at 12 g of spirulina/L of water (Fig 4)

Table 1 Growth performances of L. *sativa* (Mean ± SE) grown under different levels of spirulina for 5 weeks in spirulina enhanced aquaponic system

Parameters	Levels of Spirulina (g/L of water)				Contrast		
	T 1 Control (0.0)	**T 2 (4.0 g/L water)**	**T 3(8.0 g/L water)**	**T 4 (12.0 g/L water)**	**Linear**	**Quadratic**	**cubic**
Number of leaves	14.25 ± 0.1^{a}	15.70 ± 0.13^{b}	16.43 ± 0.05^{c}	16.33 ± 0.05^{c}	*	*	ns
Leaf length (cm)	29.98 ± 0.05^{a}	33.60 ± 0.12^{b}	34.60 ± 0.08^{c}	35.48 ± 0.05^{d}	*	*	*
Leaf width (cm)	19.46 ± 0.21^{a}	26.39 ± 0.05^{b}	27.53 ± 0.13^{c}	28.20 ± 0.07^{d}	*	*	*
Leaf Area (cm^2)	493.25 ± 6.64^{a}	716.57 ± 4.76^{b}	822.07 ± 3.30^{c}	920.56 ± 3.74^{d}	*	*	*
Dry weight (g)	58.80 ± 1.73^{a}	70.86 ± 0.64^{b}	86.43 ± 1.41^{c}	97.51 ± 1.64^{d}	*	ns	ns

*Means with the same letter in the same row are not significantly different at $p < 0.05$, ns= non-significant, *= significant*

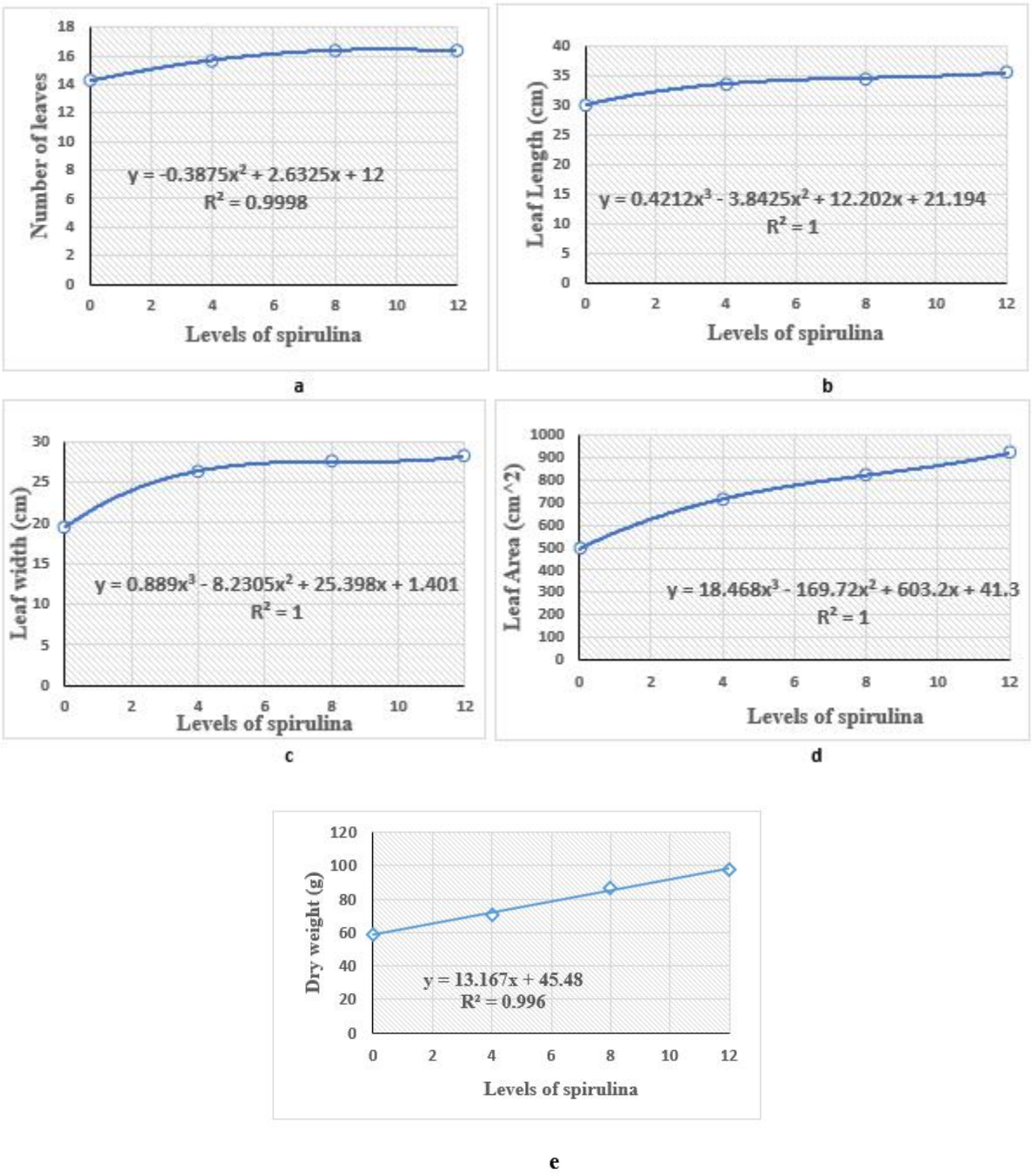

Fig. 4. Quadratic increase of the final number of leaves (a), a cubic increase of the final leaf length (b), Leaf width (c), leaf area (d) and linear increase for the dry weight (e) with an increase in spirulina levels.

3.2 Antioxidant contents

The results of antioxidant contents of L. *sativa* (Mean ± SE) grown under different levels of spirulina for 5 weeks in spirulina enhanced aquaponic system is shown in Table 2

Table 2 Antioxidant contents of L. *sativa* (Mean ± SE) grown under different levels of spirulina for 5 weeks in spirulina enhanced aquaponic system

Parameters	Levels of Spirulina (g/kg of basal diet)				Contrast		
	T 1 Control (0.0)	**T 2 (4.0 g/kg)**	**T 3(8.0 g/kg)**	**T 4 (12.0 g/kg)**	**Linear**	**Quadratic**	**cubic**
FRAP (mgAAE $100g^{-1}$)	391.1 ± 15.54^{a}	425.1 ± 14.38^{a}	557.9 ± 26.57^{b}	673.6 ± 7.07^{c}	*	ns	ns
DPPH (mmolTE $100g^{-1}$)	11.7 ± 0.70^{a}	18.9 ± 1.22^{b}	27.5 ± 0.14^{c}	28.2 ± 0.04^{c}	*	*	ns
ABTS (mmolTE $100g^{-1}$)	9.8 ± 0.16^{a}	11.8 ± 0.34^{b}	18.5 ± 0.38^{d}	15.9 ± 0.28^{c}	*	*	*
Total Phenols (GAE $100g^{-1}$ (cm^{2})	345.8 ± 1.50^{a}	561.9 ± 1.70^{b}	730.9 ± 10.20^{c}	852.9 ± 7.27^{d}	*	*	ns

*Means with the same letter in the same row are not significantly different at $p < 0.05$, ns= non-significant, *= significant. FRAP (Ferric Reducing Ability of Plasma) analysis measured in milligrams of Ascorbic Acid Equivalent per 100 g of lettuce (mgAAE $100g^{-1}$); DPPH (2,2-diphenyl-1-picrylhydrazyl) radical measured in millimoles of Trolox Equivalent per 100 g of lettuce (mmolTE $100g^{-1}$); ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6- sulphonic acid)) analysis expressed as millimoles of Trolox Equivalents per 100g (mmolTE $100g^{-1}$) and total phenols analysis expressed as milligrams of Gallic Acid Equivalents per 100 g of lettuce (GAE $100g^{-1}$).*

Significant differences ($p < 0.05$) in FRAP, DPPH, ABTS and the Total Phenols in the four treatments were observed (Fig.5). Polynomial contrasts analysis indicates that the linear trend for FRAP (Fig 5 a) was significant with the highest level at 12g of spirulina/L of water inclusion in the foliar spray. There was significant ($p < 0.05$) quadratic increase in the DPPH (Fig 5 b) and Total Phenols (Fig 5 d) with the highest level at 12g of spirulina/L of water inclusion in the foliar spray. Cubic increase was significant ($p < 0.05$) in the ABTS **(c)** with the highest level at 8g of spirulina/L of water inclusion in the foliar spray.

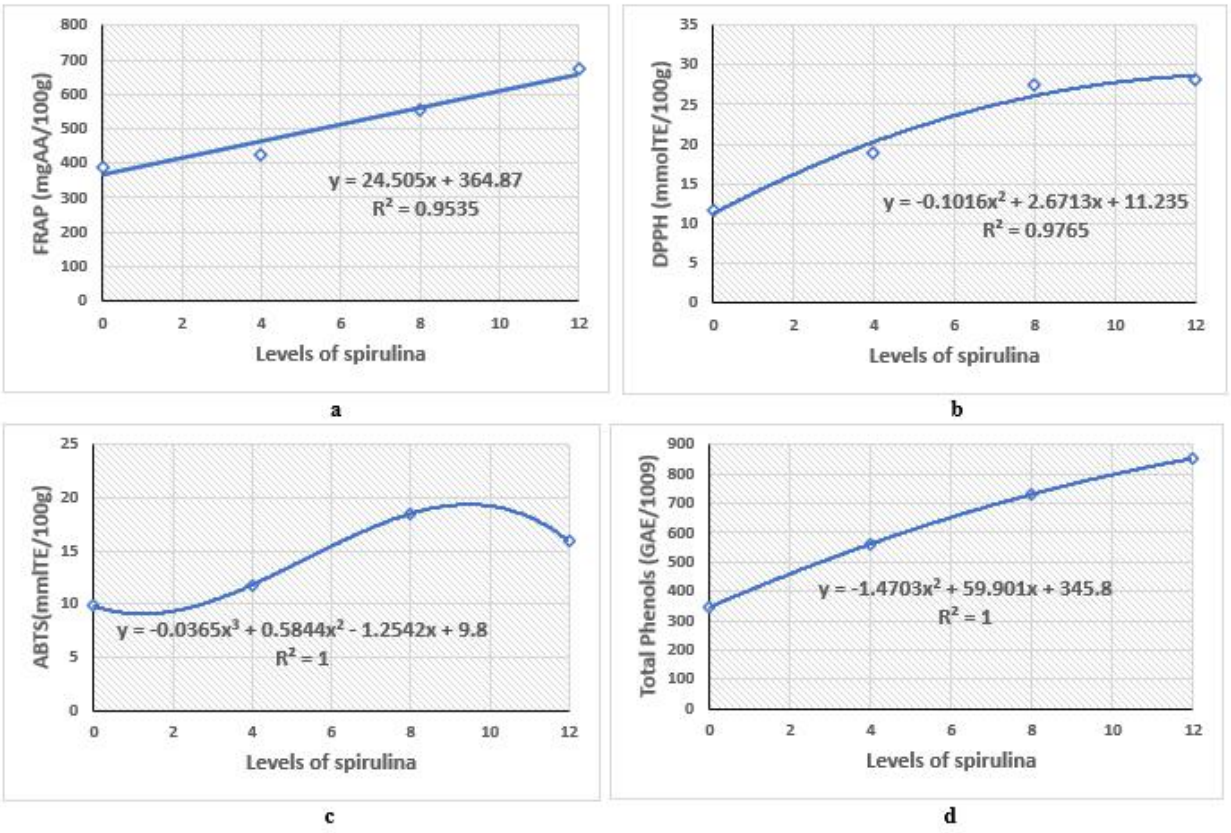

Fig. 5. Linear increase in the FRAP (a); quadratic increase in the DPPH (b); cubic increase in the ABTS (c); and quadratic increase in the Total Phenols (d) of L. sativa grown under different levels of spirulina for 5 weeks in a spirulina enhanced aquaponic system.

4. DISCUSSION

4.1 Growth

The findings of this study show clearly that the application of spirulina as foliar spray to lettuce grown in aquaponic system increases growth performance. Even though both the lettuce under control and lettuce under spirulina foliar application in enhanced aquaponic system showed gradual increase in the number of leaves, leaf length and leaf width over the 5 weeks of the experimental period, the growth performance was significantly greater in lettuce under spirulina treatments. Generally, the growth performance was much higher in the treatment with the highest levels of spirulina (12 g spirulina/L of water). A similar study was conducted to find out the effects of foliar application of spirulina on the growth and yield of okra in the field in which the tallest plants were obtained from the treatment with application of spirulina foliar (Uddin *et al.,* 2019). Plants under spirulina treatment had a maximum number of leaves, a higher number of branches and higher chlorophyll content compared to plants under control treatment. The maximum yield obtained from spirulina treatment was 25.5% higher than the control treatment in terms of dry matter content. This is also in agreement with the findings obtained from a study conducted to determine the effect of spirulina algae and fertilization rates on the yield and growth of wheat plants (Abd El-RheemKh *et al.,* 2015). Increasing levels of spirulina from 25 to 100 ml/L of water under low levels of nitrogen fertilization increased both the growth and yield of wheat. In another study on the effect of spirulina biofertilizer suspension on growth and yield of *Vigna radiata* (L.), spirulina biofertilizer suspension exhibited a positive effect on growth and yield of a green gram while the control recorded the normal conventional growth and yield (Aung, 2011). In this study, the highest stimulation in growth was obtained at 7 g spirulina/L of water.

The current study suggests that spirulina foliar spray in an enhanced aquaponic system is responsible for better growth performance in terms of the number of leaves, leaf length and width, leaf area and dry matter. This is attributed to the fact that spirulina contains macro and micronutrients, amino acids and vitamins which easily get absorbed into plant leaves to promote growth (Uddin *et al.,* 2019; Abd El-RheemKh *et al.,* 2015; Krishnaveni *et al*., 2013).

4.2 Antioxidant contents

DPPH, ABTS and FRAP assays are spectrophotometric methods used to determine the in vitro antioxidant capacity of biological samples. The assays are based on electron transfer reactions

which normally result in the reduction of coloured oxidants (DPPH, ABTS or FRAP as an oxidant). Polynomial contrasts analysis showed that the linear trend for FRAP was significant with a peak at 12 g spirulina/L of water. There was a quadratic increase for DPPH and Total Phenols and cubic increase for ABTS of lettuce grown under different levels of spirulina for 5 weeks in the spirulina enhanced aquaponic system.

Antioxidant analysis may be used to provide useful indices of dietary sufficiency and, therefore, give information that can be used to assess the nutritional status of vegetables and other varieties of food (Thaipong *et al.,* 2006). Antioxidants include carotenoids, flavonoids and related polyphenols which help in neutralizing free radicals in the body. Free radicals are unstable compounds normally produced during normal metabolic reactions in the body or due to the body being exposed to adverse external environmental factors. Studies have shown that free radicals can result in degenerative diseases such as cancer, aging and age-related macular degeneration (Asghari. *et al.*, 2016). Animals obtain antioxidants compounds by feeding on plants, mainly vegetables and fruits.

There are limited studies on the use of spirulina in boosting the antioxidant contents of lettuce in an aquaponic system. Most studies have been focused on antioxidant contents in lettuce grown by conventional methods (Zapata-Vahos. *et al.*, 2020). The results obtained from the current analyses present excellent correlation, confirming that spirulina foliar has a role in boosting antioxidant contents in lettuce plants.

These results are likely to be attributed to the fact that spirulina is a complete nutrient resource of chlorophyll, phycocyanin and Carotenoids (Asghari. *et al.*, 2016). It has also been reported that spirulina has a stimulatory effect on the growth of beneficial soil bacteria in the root zone of plants and this contributes to higher mobilization and uptake of nutrients by plants (Hegazi *et al.,* 2010). In a similar study, in which spirulina was used as biofertilizer for *Amaranthus gangeticus,* the results of leaf analysis showed that there was a significant increase in protein with biofortification of spirulina (Anitha *et al.,* 2016). The study concluded that spirulina can be helpful as a biofortification agent in enhancing plant growth in terms of protein and antioxidant contents.

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusions

The conclusion made from the above results is that foliar application of spirulina in spirulina enhanced aquaponic system significantly improved growth of lettuce in terms of leaf number, leaf length and width leaf area and dry weight. The optimum performance for the growth parameters was at 12.0 g spirulina/L of water in the foliar spray. However, better performance in terms of the number of leaves was at 8.0 g spirulina/L of water in the foliar spray.

The above results also show that foliar application of spirulina in spirulina enhanced aquaponic system significantly improved the quality of lettuce in terms of antioxidant contents. The optimum performance for antioxidant contents was also at 12.0 g spirulina/L of water in the foliar spray.

The use of spirulina biofertilizer significantly increases the yield and improves the quality of lettuce plants in terms of antioxidant contents in a spirulina-enhanced aquaponic system.

5.2 Recommendations

Spirulina biofertilizer can be incorporated in foliar spray to increase yield and improve the quality of lettuce plants grown under the aquaponic system. The optimum level for overall better growth performance and quality of lettuce plants in an aquaponic system should range between 8-12.0 g spirulina/L of water in the foliar spray.

The present study focused on one particular variety of lettuce in Kenya. A similar study should be conducted on other varieties in Kenya and other countries. A comparison of yield and antioxidant contents should also be made for lettuce cultivated in aquaponic systems in greenhouses and conventional soil cultivation using spirulina biofertilizer.

Acknowledgment

This study was financed and supported by the Germany Academic Exchange Service (DAAD), the Department of Horticulture and Food Security and the Department of Animal Sciences, Jomo Kenyatta University of Agriculture and Technology (JKUAT).

REFERENCES

[1] Abd El-RheemKh, M., Zaghloul, S. and Essa, E., 2015. The stimulant effect of the. *Spirulina algae under low levels of nitrogen fertilization on wheat plants grown in sandy soils*, *8*(12), pp.87-91.

[2] Asghari, A., Fazilati, M., Latifi, A.M., Salavati, H. and Choopani, A., 2016. A review on antioxidant properties of Spirulina. *Journal of Applied Biotechnology Reports*, *3*(1), pp.345-351.

[3] Anitha, L., Kalpana, P. and Bramari, G.S., 2016. Evaluation of Spirulina platensis as microbial inoculants to enhanced protein levels in Amaranthus gangeticus. *African Journal of Agricultural Research*, *11*(15), pp.1353-1360.

[4] Aung, K.L.N., 2011. Effect of Spirulina biofertilizer suspension on growth and yield of Vigna radiata (L.) Wilczek. *Univ Res J*, *4*(1), pp.351-363.

[5] Bittsanszky, A., Uzinger, N., Gyulai, G., Mathis, A., Junge, R., Villarroel, M., Kotzen, B. and Kőmíves, T., 2016. Nutrient supply of plants in aquaponic systems. *Ecocycles*, *2*(2), pp.17-20.

[6] Bunning, M. and Kendall, P., 2012. *Salad greens: Health benefits and safe handling* (Doctoral dissertation, Colorado State University. Libraries).

[7] Buzby, K.M. and Lin, L.S., 2014. Scaling aquaponic systems: Balancing plant uptake with fish output. *Aquacultural Engineering*, *63*, pp.39-44.

[8] Eck, M., Körner, O. and Jijakli, M.H., 2019. Nutrient cycling in aquaponics systems. In *Aquaponics Food Production Systems* (pp. 231-246). Springer, Cham.

[9] Goddek, S., Delaide, B., Mankasingh, U., Ragnarsdottir, K.V., Jijakli, H. and Thorarinsdottir, R., 2015. Challenges of sustainable and commercial aquaponics. *Sustainability*, *7*(4), pp.4199-4224.

[10] Hegazi, A.Z., Mostafa, S.S. and Ahmed, H.M., 2010. Influence of different cyanobacterial application methods on growth and seed production of common bean under various levels of mineral nitrogen fertilization. *Nature and Science*, *8*(11), pp.183-194.

[11] Hooper, L. and Cassidy, A., 2006. A review of the health care potential of bioactive compounds. *Journal of the Science of Food and Agriculture*, *86*(12), pp.1805-1813.

[12] Kim, D.E., Shang, X., Assefa, A.D., Keum, Y.S. and Saini, R.K., 2018. Metabolite profiling of green, green/red, and red lettuce cultivars: Variation in health beneficial compounds and antioxidant potential. *Food Research International*, *105*, pp.361-370.

[13] Korner, O., Gutzmann, E. and Kledal, P.R., 2015, July. A dynamic model simulating the symbiotic effects in aquaponic systems. In *International Symposium on New Technologies and Management for Greenhouses-GreenSys2015 1170* (pp. 309-316).

[14] Krishnaveni, R., Palanivelu, K. and Velavan, S., 2013. Effects of probiotics and spirulina supplementation on haemato-immunological function of *Catla catla. International Journal of Research in Fisheries and Aquaculture,* 3:176-181. Available at: http://urpjournals.com/tocjnls/35_13v3i4_10.pdf [Accessed on 11 October2018].

[15] Kwena, K., Ndegwa, W., Esilaba, A.O., Nyamwaro, S.O., Wamae, D.K., Matere, S.J., Kuyiah, J.W., Ruttoh, R.J. and Kibue, A.M., 2015. Climate Change Adaptation Planning in Kenya: Do Scientific Evidences Really Count? In *Adapting African agriculture to climate change* (pp. 35-42). Springer, Cham.

[16] Manach, C., Scalbert, A., Morand, C., Rémésy, C. and Jiménez, L., 2004. Polyphenols: food sources and bioavailability. *The American journal of clinical nutrition*, *79*(5), pp.727-747.

[17] Osumba, J. and Rioux, J., 2015. Scoping study on climate-smart agriculture in Kenya: Smallholder integrated crop-livestock farming systems. *Mitigation of Climate Change in Agriculture (MICCA) Programme Background report*, *8*.

[18] Presnov, E., Albright, L.D. and Dayan, E., 2005, May. Methods to estimate and calculate lettuce growth. In III International Symposium on Applications of Modelling as an Innovative Technology in the Agri-Food Chain; MODEL-IT 674 (pp. 305-312).

[19] Ray, D.K., Mueller, N.D. and Paul, C., 2013. West, and Jonathan A. Foley. *Yield trends are insufficient to double global crop production by*, *2050*, pp.1-8. https://doi.org/10.1371/journal.pone.0066428

[20] Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. and Byrne, D.H., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of food composition and analysis*, *19*(6-7), pp.669-675.

[21] Uddin, A.F.M.J., Rakibuzzaman, M., naher Wasin, E.W., Husna, M.A. and Mahato, A.K., 2019. Foliar application of Spirulina and Oscillatoria on growth and yield of okra as bio-fertilizer. *Journal of Bioscience and Agriculture Research*, *22*(02), pp.1840-1844.

[22] Zapata-Vahos, I.C., Rojas-Rodas, F., David, D., Gutierrez-Monsalve, J.A. and Castro-Restrepo, D., 2020. Comparison of antioxidant contents of green and red leaf lettuce cultivated in hydroponic systems in greenhouses and conventional soil cultivation. *Revista Facultad Nacional de Agronomía Medellín*, *73*(1), pp.9077-9088.