Rwanda journal of agricultural sciences Vol.2,No.1

Effect of foliar spraying Mixed with Fish Amino Acids (FAA) and Oriental Herbal Nutrient (OHN) extract on Growth, Yield and Quality of watermelon (*Citrullus lanatus*)

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Abstract

Foliar spraying with mixed Fish Amino Acids (FAA) and Oriental Herbal Nutrients (OHN) extract are essential elements for watermelon (*Citrullus lanatus*) growth and development, production and significantly increase lycopene and total soluble solids content without degrading the natural resources. The mixture of F_3O_3 (3ml.l^{-1} of FAA + 3ml.l^{-1} of OHN) shows the highest vine length, internode length and number of leaves with 601.6 cm; 27.4 cm and 129.3 at 60 DAT respectively,while the control (F_0O_0) was 402.8 cm; 23.4 cm and 97.2 respectively. Highest yield (ton.ha^{-1}) and fruit length (cm) were recorded under F_3O_3 with 82.0 ton.ha-1 and 42.1 cm respectively, where the control (F_0O_0)was 53.3 ton.ha^{-1} and 33.6 cm respectively. The high concentration of FAA in a mixture had negative effect on total soluble solids content but their effects were positive in lycopene content while the high concentration of OHN had a positive effect on the total soluble solids content in brix percentage and less effect on Lycopene content. The highest brix content were 14.2 in F_1O_3 followed by 14.1 in F_1O_2, the least found in F_3O_1 with 12.6. The lycopene content were high in F_3O_3 (105.91µg.g^{-1}) followed by F_3O_2 (105.04µg.g^{-1}) where the least were (38.26µg.g^{-1}) F_3O_3 fresh weight.

Keywords: *Watermelon; Fish Amino Acids; Oriental Herbal Nutrients and Bio stimulant*

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Introduction

The children of Israel during their sojourn in the Sinai desert, with hunger and thirsty remembered the watermelon they knew from the land of Egypt (Numbers 5:11). Thefruit is highly nutritious, sweet and thirst-quenching(Sylvestre*et al.*, 2014). It is a good source of pure water and serves as excellent diuretic with about 93% of water(Gul *et al.,* 2014). Watermelon is a good fruit liked by many and available in all local markets of Rwanda(Sylvestre*et al.*, 2015).It is also a very important crop in Korea(Huh *et al.*, 2008). Watermelon is relatively new in Rwanda and now gradually gaining ground in the Eastern part of the country and high levels of economic importance both in generation of income and provision of nutritional value (Sylvestre *et al.,* 2015). It is a good source of lycopene which makes fundamental contributions to human health(Naz*et al.*, 2014). It is a popular fruit in the world characterized by soluble solids content (SSC) used for assessing its quality (Tian *et al.*, 2007).

The application of bio stimulants allowed a reduction in fertilizers without affecting yield and quality (Bulgari *et al.*, 2015). However, bio stimulants are plant extracts and contain a wide range of bioactive compounds that are mostly still unknown (Bulgari *et al.*, 2015). The development of plant bio stimulants has become the focus of much research interest. Plant bio stimulants are used to enhance plant growth (GU *et al.*, 2014).The aim of the present research is to reduce inputs like Chemical fertilizers and Pesticides without reducing the yield and quality and adopting agricultural growing practices that evolving towards sustainable and environmental friendly systems.

Materials and Methods

The study was carried out at the Agriculture Research Center in Paju City, South Korea beginning from March till November, 2018. The latitude of study area is 37.7647744, and the longitude is126.787376with the GPS coordinates of 37° 45' 53.0784" N and 126° 47' 14.5536" E.The soil physical property shows that pH of soil was 6.1, Organic matter 18g.kg^{-1}), Phosphorus (366mg.kg^{-1}), K (0.62cmol^{+}.kg^{-1}), Ca (4.6cmol^{+}.kg^{-1}), Mg (1.2 cmol^{+}.kg^{-1}) and EC (0.6Ds.m^{-1}). The annual rainfall of the study area is 1300 and the annual average temperature is 11.5^{o}C. The materials used are Clay jar, cedar bucket, container, fish bones, Porous paper for covering, Garlic and Ginger and other Medicinal Plant, Electronic balance HW 200KGL, Hand held Brix refractometer RHB-32 ATC and UV-VIS spectroscopy.

The design of an experiment was completely randomized factorial design with two factors, Fish Amino Acid (FAA) and Oriental Herbal Nutrient (OHN). Each treatment required three (3) replications, which made thirty (30) experimental units in total. Each experimental unit comprised of five (5) watermelon plants which make 150 plants for the whole experiment. The seedlings were transplanted on 14th May, 2018 maintaining the spacing of 45cm from plant to plant and 250 cm between rows giving a planting density of 8889plants per hectare. The sprayed mixture were: Control (no FAA or OHN dose sprayed); F_1O_1; F_1O_2; F_1O_3; F_2O_1; F_2O_2; F_2 O_3; F_3O_1;F_3O_2 and F_3O_3 where indices indicate number of Milliliters per liter(ml/L) sprayed.The seedlings materials were bought from Farm Hannong Co. Lt. Grafted sambokkul cultivar were used as seedlings, where rootstocks were bottle gourd (*Lagenaria siceraria* Standl). To control pest, water-pepper and tobacco solution, 1milliliter of extract per liter of solutionwere used and applied directly to the plant leaves.Spoiled fish and oyster fish (bones, head, internal organs and skin) was collected andpounded using mortar and pestle into small pieces. 2 kg of crude sugar was added to4 kg of pounded materials, and thenthe mixture was mixed thoroughly using a wooden ladle. After all fish parts are coated with sugar. Juice of the fish was extracted after storing for a thirty-day period. Four (4) kilograms of crushed garlic and four (4) kilogramsof Ginger filled in the jar and brown sugar (2kg) covered with porous paper. The amount of mixture occupied 2/3 of the space of the jar for good fermentation and left it for six days. After the fermenting process threeliters of 'Soju' was poured into the remaining 1/3 of the jar and covered it with vinyl film. Diligently, the mixture was stirred clockwise every early morning for two weeks.

Data collection and analysis

The variables measured included: vine length (cm), length of internodes (cm) and Number of leaves per plant at 15; 30; 45 and 60 days after transplanting. Three middle plantsfromeachplotweretaggedfromwhichgrowthofthevine, Internodes length and number of leaves recorded from chosen plant. Vine length (cm) measured from the soil surface to the end tip of the plant using the ruleranda rope. The length of internodes was recorded using a ruler and rope from holding fruit stalk and numberofleavesper plant fromthree middle plantsofeachplotwascountedat 15, 30,45and60daysaftertransplanting from the stalk chosen.

Fruit weight per plant (kg), Fruit length (cm), fruit diameter (cm) and yield (tons/hectare) were recorded after harvesting at 90 DAT and analyzed. Fruitsfromthethree middle plantsineachplotwereweightedone byone using the electronic balance (HW 200KGL, A and D Platform Scale, Japan). The watermelon fruits'diameter was determined by digital Vernier calipers. Watermelon was placed vertically at its most stable position. Length of the fruit was recorded for analysis by measuring the distance between the top portion and the bottom portion. The top portion is the tendril end, whereas the bottom portion is the crown surface. The total numbers of watermelon used for this experiment was 90 pieces. The average values of three replications were reported. Data collected from each variable was subjected to analysis of variance using IBM SPSS statistical software (version 24, Korea) using Turkey testat5%probability level.

The Three (3) fruits from each treatment were subjected to percentage of Total soluble solids using a Hand held Brix refractometer RHB-32 ATC and the results expressed as ^{o}Brix. Three replications of all of the treatment were carried out. The fruit were cut lengthwise from stem end to calyx-end a piece of fruit flesh at Centre were cut off and squeezed, an equal number of drops are placed onto the refractometer prism plate. The prism lid was closed to get proper readings, the instrument turned towards the light and the eye piece was focused until a clear image appears. The position at which the demarcation line between the light and dark regions crosses the vertical scale gives the percentage soluble solids reading. The reading on the prism scale was noted to one decimal place. After each test the prism plate was cleaned with distilled water and wiped dry with a soft tissue.

Lycopene was extracted according to a method of Rodriguez-Amaya, (2001)and Saini *et al.*, (2018)with some modifications. All the extractions were performed under low light conditions (LL) due to the light-sensitive properties of lycopene. Briefly, 5 g of watermelon pulp sample was transferred into a 50 ml falcon tube and homogenized with 30 ml of acetone: hexane (1:1). After homogenization, samples were centrifuged at 5000g (5 min at 4°C temperature), and the supernatant was recovered. The extraction was repeated until the pallets became colorless. Supernatant from all extractions was pooled, partitioned with water, and upper hexane layer containing lycopene was recovered. The recovered hexane was vacuum-dried in a rotary evaporator and, the extract was recovered with 5ml of light petroleum ether.

The lycopene content was measured by UV-VIS spectroscopy using the specific absorption coefficient of lycopene. Briefly, the 50µl of sample (in light petroleum ether) was mixed with 2950 µl of light petroleum ether, to obtain the 60x dilution. The absorbance (Abs) was recorded at 470 nm using a Shimadzu UV-1600 spectrophotometer. The content of lycopene was calculated using the following equation:

$$\textbf{Lycopene (µg/g) of sample} = \frac{\mathbf{10000}\,x\ Abs\ x\ Dilution\ factor\ x\ volume\ of\ sample\ (ml)}{Specific\ adoption\ coefficient\ x\ fresh\ weight\ of\ sample}$$

Where, Specific adoption coefficient of lycopene= 3450 (in light petroleum ether)
Dilution factor =60, volume of sample (ml) = 5, and fresh weight of sample (g) = 5

Table 1: Soil physical properties before Transplanting and after harvesting

Soil properties	Before Transplanting	After Harvesting	Sufficient Range for watermelon
pH (1:5)	6.1	6.4	6.0 – 6.5
Soil Organic matter (SOM) (g.kg^{-1})	18	25	20.0-30.0
Phosphorus (mg.kg^{-1})	366	751	250 – 350
K (cmol^{+}.kg^{-1})	0.62	1.58	0.6 – 0.7
Ca (cmol^{+}.kg^{-1})	4.6	4.9	5.0 – 6.0
Mg (cmol^{+}.kg^{-1})	1.2	2.2	1.5 – 2.0
EC (Ds.m^{-1})	0.6	1.9	0.0 – 2.0

Results and Discussion

Growth and Development

The collected and analyzed data on growth and development, vine length, internodes length and number of leaves showed non-significant difference at 15 DAT(Table 2). The significant differences occur at 30, 45 and 60 DAT, the mixture of F_3O_3 (3ml.l^{-1} of FAA + 3ml.l^{-1} of OHN) had the highest vine length and internodes lengthwith 601.6 and 27.4 cm, followed by F_2O_2 (2ml.l^{-1} of FAA+ 2ml.l^{-1} of OHN) with 590.9 and 27.2 cm, while the shortest were recorded in the control (F_0O_0) with 402.8 and 23.4 cm respectively, (Table 2 and 3). 3ml.l^{-1} of FAA in each mixture showed the highest vine and internodes length at different growth stage. Therewas significant improvement in the growth and development compared to the findings ofRao *et al.*, (2016)who reported that a vine length of 136.4cm. Lyngdoh *et al.*, (2017) reported that the highest pod length (36.84 cm) of cowpea was observed in the mixture of Vermicompost + Fish Amino Acid + Panchagavya + Bio-fertilizers.

Furthermore,plant growth and yield increase linearly with the increase of Nitrogen rate as confirmed by Dhillon *et al.*, (2011). The results obtained are consistent with previous findings where Nitrogen is found to be major macro nutrient of FAA and is up to 90% in FAA(Benedict C. *et al.*, 2011).Amino acid has positive effects on plant growth and yield, Amino acid application as foliar spray significantly improved all the reduced parameters, the highest level of amino acid exerted the strongest effect (Abdelhamid *et al.*,2014). Vine length increased with an increase of internodes length (Table 2 and 3). However, the application of equal proportion of FAA and OHNhad positive effect on number of leaves (Table 4). The plant absorb amino acids rapidly, accounting at least 60% of total nitrogen absorbed (El-Aal *et al.*, 2010) and OHN provide plants and soil microorganisms with micro-nutrients, which may optimize their resilience to environmental stresses (Chang *et al.*, 2014). Therefore, FAA and OHN are good elements for plant growth and development. However, OHN concentration in a mixture had less effect on Plant growth and development compared to FAA, but it saves plant from stress since it works as natural fertilizer and medicine as well(Chang *et al.*, 2014).

Equal proportion of FAA and OHN bio-stimulant has shown influence on number of leaves compared to others mixtures at 60 DAT. The highest number of leaves was found in mixture of F_3O_3 (3ml.l^{-1} of FAA + 3ml.l^{-1} of OHN) with 129.3 followed by F_2O_2 (2ml.l^{-1} of FAA+ 2ml.l^{-1} of OHN) with 117.0 and F_1O_1 (1ml.l^{-1} of FAA + 1ml.l^{-1} of OHN) with 117.0 while the lowest was under control with 97.2 cam (Tab.4).

Table 2. ***Effect of foliar spraying with Mixed Fish Amino Acid (FAA) and Oriental Herbal Nutrient (OHN) extract on Vine length of watermelon***

Treatment doses	Vine length (cm) at			
	15 DAT	30 DAT	45 DAT	60 DAT
F_0O_0	27.6	102.7^{b}	230.0^{c}	402.8^{c}
F_1O_1	24.9	153.8^{a}	266.8^{bc}	464.0^{b}
F_1O_2	25.7	165.9^{a}	267.0$_{bc}$	460.8^{bc}
F_1O_3	24.7	158.2^{a}	260.1^{bc}	469.6^{b}
F_2O_1	25.6	181.9^{a}	287.9^{abc}	543.9^{a}
F_2O_2	26.6	179.9^{a}	288.7^{abc}	563.3^{a}
F_2O_3	26.9	178.3^{a}	289.0^{abc}	559.9^{a}
F_3O_1	26.8	187.6^{a}	302.1^{ab}	594.1a
F_3O_2	31.2	196.8^{a}	295.2^{ab}	590.9^{a}
F_3O_3	32.1	198.6^{a}	332.0^{a}	601.6^{a}
F-test	Ns	*	*	*

Table 3. ***Effect of foliar spraying with Mixed Fish Amino Acid (FAA) and Oriental Herbal Nutrient (OHN) extract on internodes length of watermelon.***

Treatment doses	Internode length (cm) at			
	15 DAT	30 DAT	45 DAT	60 DAT
F_0O_0	4.5	9.2^{c}	16.4^{c}	23.4^{d}
F_1O_1	5.1	12.4^{b}	18.6^{b}	25.6^{bc}
F_1O_2	4.7	13.5^{ab}	18.7^{bc}	25.7^{bc}
F_1O_3	5.6	13.3^{ab}	18.7^{bc}	25.7^{bc}
F_2O_1	5.2	13.3^{ab}	19.5^{abc}	26.5^{abc}
F_2O_2	4.9	13.6^{ab}	19.8^{ab}	26.8^{ab}
F_2O_3	5.2	13.7^{a}	19.9^{ab}	26.9^{ab}
F_3O_1	5.0	13.7^{a}	20.1^{a}	27.1^{a}
F_3O_2	5.2	14.4^{a}	20.2^{a}	27.2^{a}
F_3O_3	5.0	13.8^{a}	20.4^{a}	27.4^{a}
F-test	Ns	*	*	*

Table 4. ***Effect of foliar spraying with Mixed Fish Amino Acid (FAA) and Oriental Herbal Nutrient (OHN) extract on number of leaves of watermelon***

Treatment doses	Number of leaves at			
	15 DAT	30 DAT	45 DAT	60 DAT
F_0O_0	12.3	29.4^{c}	61.8^{b}	97.2^{d}
F_1O_1	12.9	32.4^{bc}	69.9^{ab}	117.0^{abc}
F_1O_2	12.9	29.4^{c}	72.0^{ab}	114.6^{bc}
F_1O_3	12.3	31.2^{bc}	73.2^{ab}	112.2^{c}
F_2O_1	14.1	33.3^{abc}	78.0^{a}	113.6^{abc}
F_2O_2	15.9	37.2^{abc}	75.0^{ab}	117.0^{abc}
F_2O_3	15.9	38.1^{abc}	75.6^{ab}	114.6^{bc}
F_3O_1	12.3	31.2^{bc}	73.2^{ab}	112.2^{c}
F_3O_2	15.9	38.1^{abc}	75.6^{ab}	114.0^{bc}
F_3O_3	18.3	45.0^{a}	82.8^{a}	129.3^{a}
F-test	Ns	*	*	*

Table 5. ***Effect of foliar spraying with Mixed Oriental Herbal Nutrient and Fish Amino Acid extract at different doses on Yield and Yield components parameters of watermelon***

Treatment doses	Yield Parameters at Harvesting stage 90 DAT			
	Fruit Weight(kg)	Fruit height(cm)	Fruit Diameter(cm)	Yield(ton/ha)
F_0O_0	7.2^{c}	33.6^{c}	19.5^{b}	53.3^{c}
F_1O_1	8.6^{bc}	38.0^{b}	22.2^{ab}	63.7^{bc}
F_1O_2	10.0^{ab}	38.6^{b}	21.8^{ab}	73.8^{ab}
F_1O_3	10.1^{ab}	38.5^{b}	23.0^{a}	74.6^{ab}
F_2O_1	10.0^{ab}	39.4^{bc}	22.0^{ab}	74.1^{ab}
F_2O_2	10.4^{ab}	39.4^{bc}	22.9^{a}	76.8^{ab}
F_2O_3	10.5^{ab}	39.3^{bc}	22.4^{a}	77.5^{ab}
F_3O_1	10.6^{ab}	39.8^{bc}	22.6^{a}	78.3^{ab}
F_3O_2	10.8^{a}	42.0^{a}	22.7^{a}	80.3^{a}
F_3O_3	11.1^{a}	42.1^{a}	23.0^{a}	82.0^{a}
F-test	*	*	*	*

Means with different letters in the column differ by Tukey test at 5%;
*Ns = not significant by Tukey test at 5%; * = significant by Tukey test at 5%.*
DAT= Days after transplanting; F_0O_0= control (No FAA and OHN dose sprayed).

F_1O_1; F_1O_2; F_1O_3; F_2O_1; F_2O_2; F_2O_3; F_3O_1; F_3O_2 and F_3O_3= mixed spraying solution of FAA and OHN where number of indices indicate number of Milliliters per liter(ml.l^{-1}) sprayed respectively.
FAA: Fish amino acid and OHN: oriental herbal nutrient.

The treatment with the highest number of leaves F_3O_3 had the highest weight of 11.1kg as shown in (Table 5), as reported by (Talukder *et al.*, 2015)that aqueous extract of Belericmyrobalan (*Terminalia belerica*) increase germination and growth of spinach. (El-Naggar & Swedan, 2009) reported that the amino acid tryptophan concentrations, they significantly increased leaves number, leaf length and width compared to the control. The leaves number were also encouraged by foliar spray, plant absorbs amino acids immediately. Amino acids is well known bio stimulant which has positive effects on plant growth and yield, and significantly mitigates the injuries caused by abiotic stresses (Abdelhamid *et al.,* 2014). Watermelon grown under NO_3^- NH_4^+ receiving the high ammonium treatment expressed symptoms of NH_4^+toxicity and declined rapidly after bloom and The challenge is that reducing nitrate to ammonium significantly reduce growth; water use; fruit yield; soluble solids and uptake of nutrients(Simonne*et al.*, 1992). The NO_3^- is the most available form of Nitrogen to plant, it is very mobile and easy to leach(Liu & Lee, 2012)and this is the reason why plant under mixture of FAA and OHN has the highest length, internodes and number of leaves compared to others because the plant absorb nitrogen in form of amino acids without any transformation.

Yield and its Components

The application of FAA and OHN had a positive effect not only on watermelon growth but also on plant yield and yield components. The fruit weight mean ranges from 7.2 to 11.1 kg.There were significant differences between treatments at 5% level of significant using Turkey Test. The highest concentration of mixture F_3O_3 (3ml.l^{-1} of FAA + 3ml.l^{-1} of OHN) showed the highest yield with 82 ton.ha^{-1} followed by F_3O_2 (3ml.l^{-1} of FAA + 2ml.l^{-1} of OHN) with 80.3 ton.ha^{-1} while the least yield was recorded under the control (0 dose of Mixture of FAA and OHN) with 53.3 ton.ha^{-1} (table.5). The results are higher compared to (Gichimu *et al.*, 2009) reported weights of 2-3 kg, while fruit weights of 1.5 to 2.1 kg have been reported by(A.S. Adeyeye *et al.*, 2016) and (Sylvestre *et al.*, 2015) reported 1.71kg to 2.861kg while (Rao*et al.*, 2016) reported 37.57 ton.ha^{-1}. The yield was high because of use of Bio-stimulant which enhances vegetative and growing stage as reported by (Sadak*et al*, 2015) that Amino acids has positive effects on plant growth and yield, Amino acid application as foliar spray significantly improved all the

reduced parameters, the highest level of amino acid exerted the strongest effect.They also encourage and stimulate plant metabolism, stress reduction, etc. (Parrado *et al.*, 2008). The amino acids is essential quantities increase yield and quality of crop.(El-Aal*et al.*, 2010) reported that all morphological characters parameters of eggplant plants (plant length, number of leaves and number of branches and fresh and dry weight of leaves per plant) were improved by using all different of biostimulators treatments compared to non-treated plants (control); Yield and its components of eggplant plants followed also the same trend. The results are similar to watermelon, the use of FAA and OHN bio stimulant improve all yield parameters and size of fruit compared to control treatment.

Total Soluble Solids Content (TSSC)

Total Soluble Solids content (°Brix) analysis was carried out using a Hand held refractometer, Thisindicate that the higher the concentration of OHN in a mixture, the higher Brix percentage (fig.1). Where the highest brix content mean was recorded under F_1O_3 (1ml.l^{-1} of FAA + 3ml.l^{-1} of OHN) with 14.2 and the least was recorded in F_3O_1 with 12.6.

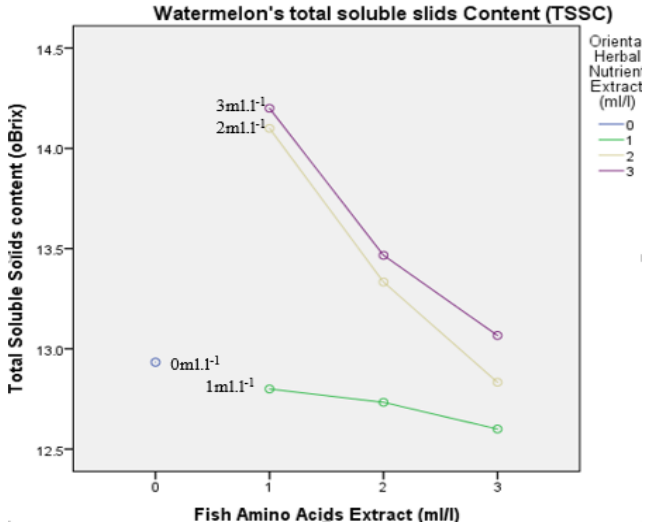

Figure1. Total Soluble Solids contents

The results are higher compared to the findings of Quek *et al.*, (2007)who reported 12.1°Brix Total Soluble Solids. Previously, Okur & Yagmur, (2004)found 11.40 °Brix TSS. OHN is a good bio stimulant to increase sweetness of fruit. However; the increase of concentration of FAA in mixture decreases the total soluble solids.

Lycopene Content

The obtained range of lycopene content among treatment was ranged from 38.26 to 105.91 µg.g^{-1}fresh weight. The highest lycopene content (105.91 µg.g^{-1}) was recorded underF_3O_3 (3ml.l^{-1} of FAA + 3ml.l^{-1} of OHN) treatment with highest concentration of FAA and OHN followed by F_3O_2 with 105.04; F_3O_1 with 67.83; F_2O_3 with 57.57; F2O2with 57.04; F_2O_1 with 54.78; F_1O_3 with 50.87; F_1O_2 with 44.7; F_1O_1 with 39.13 and the least was found to be 38.26 µg.g^{-1}Fresh Weigh in F_0O_0 Control treatment.The lycopene content mean in treatment with mixed Fish Amino Acids and Oriental Herbal Nutrient Extract has strongly significant difference compared to the treatment not applied with FAA and OHN (Control). The lycopene content increased with the increase of mixture of FAA and OHN applied (fig.3). The results are consistent with previous findings of Perkins *et al.*, (2001) which showed that depending on the cultivar and growing conditions, lycopene can vary from 34 to 112 µg.g^{-1}fresh-weight; thus, red-fleshed watermelon is a rich source of readily bio-available lycopene.

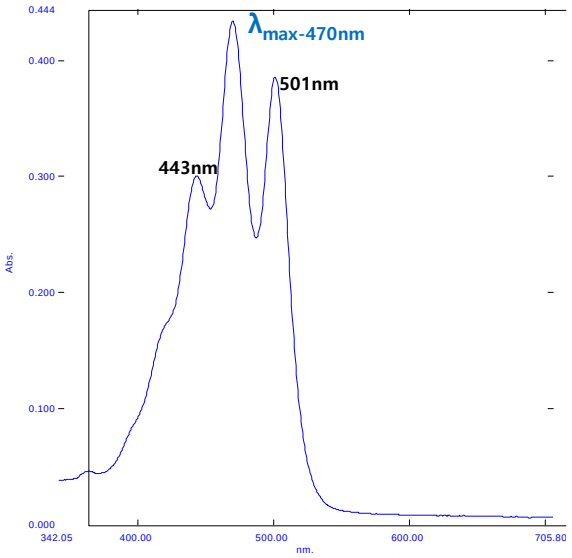

Figure2. UV visible Absorption Spectrum of lycopene, Showing the λmax at 470nm

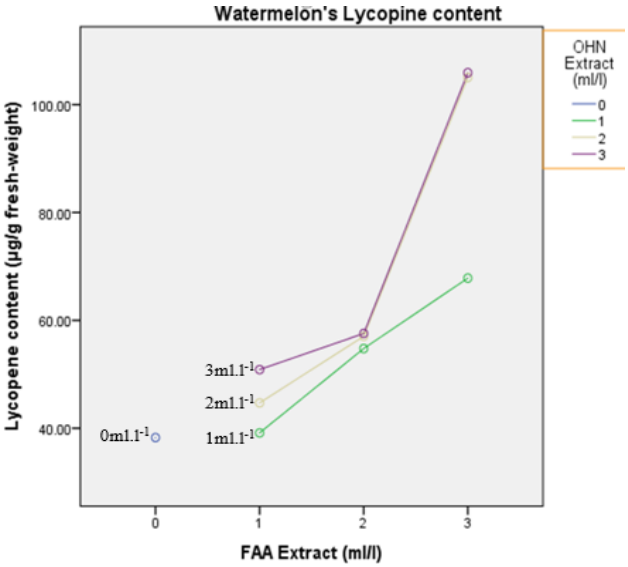

Figure 3. Lycopene content

Conclusion

The study results indicated that Mixture of Fish Amino Acids and Oriental Herbal Nutrient Foliar sprayed produce food and fiber without degrading the natural resources and the ability of future generations. Since new technologies, Mechanization, Chemical use and Government Policies applied in Agriculture has maximized production and reducing food prices. These developments have had many positive effects. They also generated negative effects on human health, like pesticides residues raises serious health concern for consumers and the environment such as topsoil depletion, food contamination, spread of new pathogens, groundwater contamination, air pollution, contamination of environment, greenhouse gas emissions, etc… Referring to the results obtained and literature review cited; watermelon under Mixture of FAA and OHN foliar sprayed has the highest vine length; internodes length; number of leaves; fruit weight; fruit sweetness and Lycopene content. Thus, Mixture of FAA and OHN are essential components for plant growth and development; yield and fruit quality as well as for economic and environmental reasons .

Acknowledgment

The authors express their gratitude to Professor Doo Hwan Kim and KOICA as well as University of Rwanda for the of research fellowship. Special thanks go to Dr Saini Ramesh for his support and Laboratory analysis.

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