BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2014. Vol. **11**(3), 1515-1519

Production, Formulation and Application of Seaweed Liquid Fertilizer using Humic Acid on Growth of *Arachis hypogaea*

P. Prakash[1], Sudipta Medhi[1], Swaraj Saikia[1], G. Narendrakumar[1], Thirugnanasambandam[2] and L. Stanley Abraham[2]*

[1]Department of Biotechnology, [2]Centre for Ocean Research,
Sathyabama University, Rajiv Gandhi Salai, Chennai - 600 119, India.

doi: http://dx.doi.org/10.13005/bbra/1546

(Received: 04 August 2014; accepted: 06 September 2014)

The aim of the present investigation was to determine the influence of Seaweed Liquid Fertilizer (SLF), Humic acid (HA) and combinations of SLF: HA in various concentrations on the growth of Groundnut (*Arachis hypogaea*). The above plant growth promoters are applied as foliar spray at an interval of twice in a month with the following concentrations such as SLF (1 to 10%), HA (0.1 to 1%) and SLF:HA (1:0.9% to 9:0.1%). The SLF:HA (6:0.4%) was found to be the most effective formulation with increasing growth of shoots, number of branches, total carbohydrate content, total protein content and total chlorophyll content followed by SLF 5% and HA 0.6%. This study helps to formulate an efficient chemical free fertilizer for maximum Agriculture produce.

Key words: Growth promoters, Humic acid, Seaweed liquid fertilizer, *Arachis hypogae* L.

Seaweeds are the macroscopic marine algae found attached to the bottom in relatively shallow coastal waters. They grow in the intertidal, shallow and deep sea areas up to 180 meter depth and also in estuaries and backwaters on the solid substrate such as rocks, dead corals and pebbles[1].Seaweeds constitute the most essential live organisms used commercial products wide scale commercially, and extracts from seaweeds are commonly called seaweed liquid fertilizer (SLF)[2]. Applied as organic farm input the seaweed fertilizer application will be useful now for achieving higher production. Recently, Seaweed liquid fertilizers (SLF) get hold on the market for a simple reason that they contain many growth promoting hormones like auxin, gibberellins, trace elements, vitamins, amino acids and micronutrients[3]. They represent an alternative to conventional chemical fertilizers. Many report recommended that commercial use of liquid extracts, obtained from seaweeds successfully used as foliar sprays for several crops[4]. Seaweeds have been used as manure, cattle feed, food for human consumption and as a source of phycocolloids such as agar, alginic acid and carrageenan[5]. In plants it is used to develop tolerance to environment stress[6] and increase nutrient uptake from soil[7].

Humic acid is an organic molecule that is formed by the breakdown of coal, soil, peat and dystrophies lakes. Recently Gutierrez-Miceli *et al.* (2007)[7] developed liquid formulation using vermicompost leachate having high concentration of humic acid which enhanced the growth of sorghum (*Sorghum bicolor* (L.) Moench). Prakash

* To whom all correspondence should be addressed.
Mob.: +91-9841658827;
E-mail: stanleyabraham313@gmail.com

et al. (2010)[8] have reported that *Trichoderma viridi* has the ability to convert lignite into humic acid. Many methods have been used to produce humic acid. The most commonly used method is extraction of humic components from soft brown coal deposits with an alkaline solution. The extracted product will be alkaline pH, when the cation exchange sites are filled primarily with hydrogen (H) ions. The material is considered an acid and is known as humic acid. They exhibit both hydrophobic and hydrophilic characteristics and can bind to soil mineral surfaces. The constituents in humic acid contain carboxyl groups and weakly acidic phenolic groups, which contribute to their complexation and ion-exchange properties[9]. Humic substance helps to supply nutrients to growing plants, makes soil fertile and productive, increases water holding capacity and seed germination. Humic acid also reduces the other fertilizer requirements, increases aeration of the soil, increase the protein and mineral contents of most crops[10]. The aim of the present study is to formulate SLF using with humic acid for enhancing growth of Groundnut plant.

MATERIALS AND METHODS

Collection of Seaweed Liquid Fertilizer

Seaweed liquid fertilizer collected from "Jeppiar Maritime Private Ltd", Chennai. Tamilnadu, India.

Preparation of Humic Acid from Lignite

Humic acid was extracted by following the procedures of International Humic Substances Society (IHSS). Crude Humic acid was used for all trials. Ten grams of coal (leonardite) was weighed and ground. It was then passed through a mesh sieve to get the fine particles and the coal sample was treated with 100 ml of 4% Potassium Hydroxide and mixed thoroughly. Water soluble salt of humic acid thus formed was filtered through a Whatman No. 42 filter paper to separate it from insoluble residues and 1ml of concentrated hydrochloric acid was added to bring the pH < 2. The humic acid will get precipitated at the bottom of the beaker and the precipitate thus obtained was Humic acid.

Formulation of slf using humic acid

The collected Jeppiaar seaweed fertilizer was prepared as 3 groups. Group -1. SLF Humic acid (9:1,8:2,7:3,6:4,5:5,4:6,3:7,2:8,1:9),Group-2. SLF (1 to 10%), Group- 3, Humic acid (1% to 10%) and control trials were also separately maintained.

Effect of formulated SLF on Groundnut

Garden soil was collected from the premises of University garden and mixed with 10% of cow dung and filled in 25×10 cm size black colour polythene bags. 1.5 kg of soil added in 40 bags. Each bag 3 Groundnut seeds were sown. After germination first treatments were started as per the formulation. Bags were watered regularly and every 15 days monitoring plant height, no of branches and biochemical analysis such as total carbohydrates, total proteins, and total chlorophylls contents were observed.

Estimation of carbohydrate contents

Carbohydrates content in different Groundnut leaves samples from apical, middle and bottom portion of the Groundnut leaves were quantitatively measured by Anthrone Reagent method[11].

Estimation of Total Protein

Protein content of the above sample was quantitatively measured by Lowry's method (1951)[12]. The results were expressed in µg/gm.

Analysis of chlorophylls

One gram of fresh leaves was weighed and minced well with scissors. 5 ml of water was added and the sample was homogenized in a blender. The final volume was made to 10 ml. an aliquot (0.5ml) was taken and mixed with 4.5 ml of 80% acetone. The acetone extracts the pigments. The supernatant was collected by centrifugation and the O.D at three wavelengths 480, 638, 645 and 663 was measured total chlorophyll content was calculated using the following formula.

Total Chlorophyll (g/lit) = (0.0202) (O.D645) + (0.00802) (O.D665)

Statistical analysis

All the samples were maintained in triplicates and Mean square error (MSE) was calculated using IBM SPSS Statistics v20.

RESULTS AND DISCUSSION

In our experiments, use of seaweed liquid Fertilizer (SLF) significantly increased the rate of growth and physiology of Groundnut. There was a noticeable increase in growth, biochemical and yield characters when seaweed liquid fertilizer was

Table 1. Effect of humic acid on Groundnut

S. No.	Humic Acid (%)	Shoot length of Plant (cm)	No of Branches	Total Proteins (µg/g)	Total carbohydrates (µg/g)	Total Chlorophylls (g/l)
1	0.1	32 ± 0.24	7	98 ± 0.02	310 ± 0.06	0.0049 ± 0.002
2	0.2	34 ± 0.19	7	100 ± 0.04	320 ± 0.07	0.0051 ± 0.009
3	0.3	38 ± 0.21	7	100 ± 0.01	325 ± 0.09	0.0056 ± 0.007
4	0.4	39 ± 0.14	8 ± 0.42	115 ± 0.08	340 ± 0.18	0.0056 ± 0.002
5	0.5	40 ± 0.17	8 ± 0.42	120 ± 0.06	340 ± 0.18	0.0061 ± 0.005
6	0.6	43 ± 0.22	8 ± 0.42	140 ± 0.02	360 ± 0.14	0.0072 ± 0.002
7	0.7	36 ± 0.27	7	120 ± 0.24	330 ± 0.32	0.0067 ± 0.003
8	0.8	31 ± 0.09	6 ± 0.33	110 ± 0.12	330 ± 0.34	0.0059 ± 0.004
9	0.9	32 ± 0.14	6 ± 0.33	110 ± 0.07	320 ± 0.31	0.0056 ± 0.005
10	1.0	32 ± 0.17	6 ± 0.33	100 ± 0.02	310 ± 0.25	0.0051 ± 0.003
11	Control	29 ± 0.19	6 ± 0.33	85 ± 0.03	295 ± 0.14	0.0045 ± 0.001

*Mean Square Error

Table 2. Effect of SLF on Groundnut

S. No	SLF %	Height of the plant (cm)	No of branches	Total proteins µg/g	Total sugars µg/g	Total chlorophylls g/l
1	1	29 ± 0.17	6 ± 0.33	105 ± 0.04	310 ± 0.21	0.0059 ± 0.008
2	2	29 ± 0.21	6 ± 0.33	110 ± 0.09	315 ± 0.09	0.0062 ± 0.003
3	3	30 ± 0.29	7 ± 0.33	120 ± 0.01	330 ± 0.15	0.0064 ± 0.001
4	4	34 ± 0.14	8 ± 0.33	120 ± 0.01	335 ± 0.19	0.0073 ± 0.005
5	5	39 ± 0.19	8 ± 0.33	130 ± 0.07	360 ± 0.01	0.0075 ± 0.006
6	6	34 ± 0.11	8 ± 0.33	110 ± 0.09	320 ± 0.02	0.0072 ± 0.008
7	7	33 ± 0.09	8 ± 0.33	100 ± 0.02	310 ± 0.09	0.0060 ± 0.001
8	8	29 ± 0.19	7	100 ± 0.02	310 ± 0.14	0.0060 ± 0.002
9	9	30 ± 0.11	7	90 ± 0.05	300 ± 0.07	0.0060 ± 0.09
10	10	32 ± 0.12	6 ± 0.33	90 ± 0.01	300 ± 0.12	0.0054 ± 0.007
11	Control	29 ± 0.14	5 ± 0.33	75 ± 0.009	285 ± 0.17	0.0049 ± 0.005

*Mean Square Error

Table 3. Effect of SLF:Humic acid on Groundnut

S. No.	SLF + HA%	Height of the plant(cm)	No of branches	Total proteins µg/g	Total sugarsµg/g	Total chlorophylls g/l
1	1:0.9	34 ± 0.21	7	110 ± 0.9	420 ± 0.14	0.0070 ± 0.002
2	2:0.8	35 ± 0.17	7	120 ± 0.03	430 ± 0.15	0.0073 ± 0.006
3	3:0.7	37 ± 0.19	7	130 ± 0.01	440 ± 0.19	0.0074 ± 0.008
4	4:0.8	36 ± 0.08	7	136 ± 0.02	440 ± 0.04	0.0079 ± 0.007
5	5:0.5	39 ± 0.15	8 ± 0.33	140 ± 0.03	450 ± 0.09	0.0085 ± 0.09
6	6:0.4	48 ± 0.21	10 ± 0.33	145 ± 0.01	460 ± 0.23	0.0091 ± 0.008
7	7:0.3	40 ± 0.18	9 ± 0.33	140 ± 0.03	450 ± 0.19	0.0087 ± 0.001
8	8:0.2	39 ± 0.11	8 ± 0.33	130 ± 0.07	440 ± 0.10	0.0068 ± 0.002
9	9:0.1	38 ± 0.12	8 ± 0.33	110 ± 0.04	420 ± 0.21	0.0064 ± 0.005
10	Control	29 ± 0.24	5 ± 0.33	75 ± 0.02	285 ± 0.17	0.0055 ± 0.003

*Mean Square Error

applied to the test plant.

Various concentration of Humic acid was administered to the Groundnut plant and effects were observed. Among the concentration, maximum growth was observed in 0.6% of humic acid (Table-1). Prakash et al, 2013[13,14] reported that maximum growth rate of Morus alba was recorded in 4% of Potassium humate and also enhances the growth of Stevia rebaudiana , 60% of Potassium Humate for Mushroom growth and 0.2% of Humic acid enhancing the growth of Spirulina plantasis. Potassium humate was extracted from lignite treating with high alkaline solution like NaOH and KOH. These extracts contain humic acid and fulvic acid. Humic acid consists of nitrogen, potassium and other plant growth promoting micronutrients and minerals. The role of humic acid is to increase the plant growth and enhance the uptake of nutrients from the soil, also it act as a chelating agent and the fulvic acid helps to transfer the Nutrient content through the plant[19]. In this study various parameters like physical parameters, biochemical parameters and insect mortality effects were recorded. There was substantial increase of nutrient content by treating the plant with humic acid. 0.6% of Humic acid treated plants were healthy (height and branch) and they are rich in nutrient like total carbohydrates (360µg/g) and (the control total carbohydrates 250µg/g), total proteins (140µg/g) and (control 85µg/g) and total chlorophyll content 0.0072mg/l (control 0.0045 mg/l). The present investigation has been undertaken to achieve high growth rate of Groundnut with Humic acid and Seaweed Liquid Fertilizer (Table-3). In this study, the Seaweed Liquid Fertilizer formulated with various concentration of Humic acid such as 9:0.1, 8:0.2, 7:0.3, 6:0.4, 5:0.5, 4:0.6, 3:0.7, 2:0.8, 1:0.9. Among the concentration, it is quite interesting to mention here that 6:0.4 % of concentration of Seaweed Liquid Fertilizer and Humic acid has showed remarkable influence on all the growth parameters such as height 48 cm. Similarly the Seaweed Liquid Fertilizer: Humic acid increases the total sugar, total protein and chlorophyll content. The seaweed extract applied as foliar spray enchases the leaf chlorophyll level in plants[6]. Kinga Matysiak *et al.*[15] well reported that Seaweed Liquid Fertilizer and mixture of Humic acid enhancing the seed germination and growth rate of maize. Recently Gutierrez-Miceli *et al*. (2007)[7] developed liquid formulation using vermicompost leachate having high concentration of humic acid which enhanced the growth of sorghum (Sorghum bicolor (L.) Moench). Ray Tucher in 1999[16], reported that carbon is highly essential nutrients for plant growth and potassium and nitrogen is secondary nutrients in plants i.e. essential for photosynthesis activates the enzyme facilitates cell division and increases diseases resistance. Kowalski *et al.* 1982[17], Humic acid enhances the level of organic matter solubilize silica associated with higher concentration of silica in plants improving plant resistance to pest and diseases. Prakash *et al*. 2009[18], reported that *Trichoderma verendi* has the ability to convert lignite in Humic acid. This Humic acid is it acts as fertilizer as well as biocontrol agent. Sridhar *et al*., 2010 reported that maximum growth was observed in the plant treated with 1.0% of Seaweed Liquid Fertilizer and 1.0% Seaweed Liquid Fertilizer plus recommended rate of chemical fertilizer indicating the synergistic influence of the seaweeds and chemical fertilizer.

CONCLUSION

Our present investigations revealed that improvement on the growth, biochemical and yield characteristics of Groundnut might be due to the presence of micro and macro elements, growth hormones, trace elements, vitamins etc. in our SLF and Humic acid. Hormone and important constituent in chlorophyll biosynthesis might have played a vital role in enhancement in growth. Increase in crop growth may vary according to chemical constituents of seaweed extracts. Further, the study also highlights that seaweed extracts can be valuable used as organic bio stimulants to the plants and also an eco-friendly growth enhancer in organic farming.

REFERENCES

1. Thirumaran,G., Arumugam, M., Arumugam, R., Anantharaman, P. Effect of Seaweed Liquid Fertilizer on Growth and Pigment Concentration of *Cyamopsis tetrogonolaba (L.)* Taub. Am. Euras. J. Agron., 2008; **2**: 50-56.
2. Bai, N. R., Banu, N. R.L., Prakash J.W., Goldi, S.J.,. Effects of *Asparagopsis taxiformis* extract on the growth and yield of *Phaseolus aureus*. *J. Basic Appl. Biol.*, 2007; **1**(1), 6-11.

3. Rajkumar Immanuel, S., Subramanian, S.K. Effect of fresh extracts and seaweed liquid fertilizers on some cereals and millets. *Seaweed Res.Utiln.*, 1999; **21**: 91.
4. Bokil, K.K., Mehta, V.C. and Datar, D.S.,Seaweeds as manure: II pot culture manorial experiments on wheat, Phykos, 1974; **13**(1): 1–5.
5. Chapmam, G.J., Seaweeds and their uses. Methuen and Co.ltd. London, 66 .1970
6. Blunden, G.T., Jenkins and Liu-yan-Wen,Enhanced leaf chlorophyll levels in plants treated with seaweed extract. *Jornal of Applied Phycology*, 1997; **8**: 535-543.
7. Gutierrez, MFA. Roberto, CGG. Reiner RR. Vermicomposting leachate (Worm tea) as liquid fertilizer for Maize (Zea Maize.L) forage production, 2007; 13-18.
8. Prakash P, Karthik Raja Namasivayam N. Niveditha, K. Vishnu Tejaswini. Optimization of humic acid by *Trichoderma viridi* and it's effect on sorghum plant. *Journal of Biopesticides*, 2010; **3**: 155 – 157.
9. Mikkelsen, R.I., Humic materials for agriculture. *Better crops,* 2005; **89**: 6-10.
10. Salman, S.R., Abou-hussein, S.D., Abdel-Mawgoud, A.M.R., ElNemr, M,A., Fruit Yield and Quality of Watermelon as Affected by Hybrids and Humic Acid Application. *Journal of Applied Sciences Research*, 2005; **1**: 51-58.
11. Trevelyan, W. E., Forrest, R.S; Harrison, J.S., "Determination of Yeast Carbohydrates with the Anthrone Reagent". *Nature*, 1952; **170** (4328): 626–627.
12. Lowry, O.H, Rosenbrough, N.J, Farr, A.L, Randall, R.J, Protein measurement with the Folin *Phenol Reagent J Biol Chem*, 1951; **193**; 265-275.
13. Prakash P, Dhanalakshmi P,K, Anusha, B., Effect of Humic Acid on *Spirulina plantensis* Production and Analysis of Nutrient Contents. *Recent Research in Science and Technology,* 2011; **3**: 87-89.
14. Prakash P, RajaKumari P , Aishwarya V, Thanuja Polani , Archana Priya Venugopal, Thirumurugan A. The influence of potassium humate on *Stevia rebaudiana*. *International Journal of Agricultural and Food Science*, 2012; **2**: 30-31.
15. Kinga Matysiak, Sylwia Kaczmarek, Roman Krawczyk, 2011. Influence of seaweed extracts and mixture of humic and fulvic acids on germination and growth of *zea mays* L. *Acta Sci. Pol., Agricultura,* 2011; **10**(1) 33-45.
16. Ray Tucker M, Essential Plant Nutrients : Their presence in North Carolina soils and role in plant nutrition.(1999).
17. Kowalski. R. and Davies, G.E. *Plant Soil*, 1982; 68: 139-141.
18. Prakash P., Samundeeswari, R., Vivek, C., Chitra Devi, A. The influence of potassium humate on *Pleurotus florida*. *World Journal of Science and Technology*, 2011; **1**: 28-31.
19. Robert E Pettit, organic matter, humus, humate, humic acid, Fulvic acid, and humin. 2004