FORMULATION AND CHARACTERIZATION OF BIO FERTILIZER PREPARED FROM FISH WASTE

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ABSTRACT

The bio-stimulant root system has enabled the crop to have a rapid and balanced nutritional intake, since the presence of polysaccharides, phosphorus and other essential trace elements in combination with amino acids allowed to have a well-developed root system, increase the size and length of the primary and secondary roots, stimulate the physiological functions of plants during periods of high activity. In addition, the effect of application of the bio-stimulant on the root part made it possible to detect a development and a growth of the root biomass in comparison with the control maize which was not treated with this bio-stimulant.

The effective utilization of biofertilizer from the fish processing waste would not only provide economic benefits to the farmers but also improve and maintain soil fertility and sustainability in natural ecosystem. The biofertilizer which we prepared by using fish waste was tested in our gardens by spraying it to the plants. The results we got were excellent. The biofertilizer acts as a good manure, it is purely organic .The yield is doubled after using this bio fertilizer. The biofertilizer not only acts as an excellent manure for plants, but also it is a good biopesticide. It is noted that, the land containing the plants is spoiled with some pests but after spraying the biofertilizer for the second time to the plants there were no pests at all. From the biofertilizer various physico chemical parameters and biochemical analysis were tested. From the results obtained, we conclude that the biofertilizer prepared by using fish waste is a pure organic manure. At the same time it is harmless to all the organisms, pollution free and safe to use in our environment.

Keywords: Biofertilizer, Fish waste, Biochemical analysis, plant growth, germination

INTRODUCTION

Fish waste biomasses are locally available resources which contain nutrients. Fermentation of the biomass produces slurries used for plankton production. Bio-refinery of fish waste material can be converted into value added biological products such as biofuels, industrial chemicals, animal and fish feed, human food, neutraceuticals and organic fertilizer, etc. Fish hydrolysate generally shows a beneficial effect on growth performances an feed utilization at low inclusion levels. The performance is postulated to be due to the balance of free amino acids, peptides and proteins in digestion, absorption and utilization. Fish processing industry and fish markets produce more than 60% by-products as waste, which includes skin,

heart, viscera, trimmings, liver, frames, bones and roes. These by-products contain good amount of protein rich nutrient material.

The fish waste contain nutrients and all the essential cofactor for the growth of plants and comparing with the chemical fertilizer and organic fertilizer. Biofertilizer are very popular amoung the farmer to produce quality product. These are eco-friendly and the cheapest source of nutrients. Recently, some studies have examined the reutilization of biodegrade fisheries waste product as liquid fertilizer (Kim and Lee, 2009: Kim *et al*.,2010:Dao and Kim, 2011)

Biofertilizers or biostimulants for the plant are substances and materials which when applied to plants, seeds or growth substrates in a specific formulation have the ability to alter the physiological processes of plants in order to provide potential growth benefits, development and / or stress response. Fish processing generates considerable quantities of solid waste in the form of head and body carapace. These body parts comprise 48-56% depending on the species (Sachindra *et.al*., 2005). Jaggery or molasses are cheap source of sucrose and sucrose is reported to be an ideal carbohydrate source for lactic acid bacterial fermentation (Cira *et al*., 2002). This technique and material should be used to economically present shrimp waste and made into fertilizer and feed for aquaculture and agriculture uses. Sugarcane molasses/Jaggery was provided by local grocery market. Tilapia wastes were chopped with a mineer and sugarcane molasses were added to have a mixture ratio of fish waste: molasses of 50:50(w/w). Although, the analyses of these fish by-products showed a high load of pathogenic microorganisms, they also showed a wealth of organic material including proteins and minerals. These components and others are capitalizing to be used for agricultural or agro-food, hence the need for the establishment of an industrial process of their treatments and their valuations. That's why our laboratory, has made a biotechnological method based on the use of a fermentation leaven and carbon-rich source of carbohydrates, A hydrolyzate rich in amino acids and trace elements was obtained with excellent hygienic quality. It will be used as a fertilizer and soil improver. Its use will be as factors for fertilizer and soil amendment. We produced two biostimulants necessary for plant growth: Rooting- development, elongation-production.

A hydrolyzate rich in amino acids and trace elements was obtained with excellent hygienic quality. Numerous long-term experiments carried out in the last decade of the twentieth century showed that national management of mineral fertilizers and organic amendments made it possible to increase crop yields and to sustain soil fertility sustainably. (Bado *et al.*, 1997; Bationo and Makwuney, 1999; Berger *et al.*, 1987;Pichotétal.1981; Piéri, 1989; Sédogo, 1981, 1993). Bio-refinery of fish waste material can be converted into value added biological products such as biofuels, industrial chemicals, animal and fish feed, human food, neutraceuticals and organic fertilizer, etc. Fish processing waste could be regarded as a promising renewable biomass resource for biorefineries.

MATERIALS AND METHODS

Collection of fish waste: 4kg of fish wastes were collected from various fish markets at Tirunelveli.They were split into half kg and filled in 8 plastic bottles.

Collection of sugar: Two types of sugars were collected. Jaggerry (2kg) and Udankudi Jaggerry (2kg).2kg of Jaggerry were split into half kg and filled in 4 plastic bottles.2 kg of Udankudi Jaggerry were split into half kg and filled in another 4 plastic bottles.

Mixing and incubation: Both the fish waste and jaggery were mixed in equal proportions and filled in 4 plastic bottles.Similarly the fish waste and udankudi jaggery were also mixed in equal proportions and filled in 4 plastic bottles.The 8 bottles were kept undisturbed for about 50 days.

Analysis time periods: In 30 days 2 bottles were opened one from jaggery and one from udankudi jaggery. The colour and odour were noted. In 40 days another 2 bottles were opened and the colour and odour were noted. In 50 days another 2 bottles were opened and the colour and odour were noted. As the days goes on colour of mixture becomes darker. Similarly the odour of the mixture becomes lesser.

Biochemical analysis: For biochemical analysis the fish waste sample were collected from local markets. Catalase test, Indole test, Oxidase test, Urea test, Gelatin test, **Nitrate reduction test**, **Starch test**, **Yoges proskauer** were performed.

Growth in nutrient agar: Nutrient agar is used as a general purpose medium for the growth of a wide variety of non- fastidious microorganisms.

NPK analysis: The nutrients that are required by crops in the largest amounts are N,P and K. For that reason they are often considered as the most important nutrients. The important nutrient available in the organic fertilizer are nitrogen (N), phosphorous (P) and potassium (K),micro and macro elements which are important for plant and animal growth.

Nitrogen analysis: Nitrogen is a necessary component which is used for the growth of the plant. Plants need a limited amount of nitrogen for their growth. Some crops need more nitrogen for their growth. In recent years biofertilizer have emerged as a promising component of integrating nutrient supply system in agriculture.(Mishra and Dadhich, 2010). For soils that are nitrogen deficient, treat, manure,wheats, grass clippings, and other garden waste with CBPA and add the resulting compost to the soil. This will result in a simultaneous improvement in humus and nitrogen content. During processing, the total Kjeldahl nitrogen content marks stabilization for M1 composition. Unlike other tests, such rates increased during the 15 days of biotransformation.

Phosphorus analysis: A natural phosphate rock product can be the best sourse of phosphorus. Phosphate rock is most effective when applied in combination with manure. Treat the manure with CBPA and work into the soil. A richer formula in fish waste and molasses allows phosphorus rates to be improved.

Potassium analysis: Among the three, potassium is the third important macronutrient element of plant nutrient that plays a significant role in the activation of several metabolic processes, including photosynthesis, protein synthesis and ezymes as well as in resistance to diseases, insects, etc (Rehm and Schmitt 2002) potassium though is present as an abundant element in the soil are is applied to filled as natural are synthetic fertilizers, only 1-2% of this available to plants, the rest being bound to other minerals and therefore unavailable to plants. Soil

microorganisms influence the availability of soil minerals, playing a central role in ion cycling and soil fertility (Lian *et al.* 2008)

Chromatography analysis: High performance liquid chromatography is a specific form of column chromatography generaly used in biochemistry and analysis to separated, identify, quantity the active compounds.

Total ion chromatogram (tic): A TIC (Total Ion Chromatogram) is a chromatogram created by summing up intensities of all mass spectral peaks belonging to the same scan. The TIC is compared to a GC chromatogram. Note that the TIC includes background noise as well as sample components.

Isolation of 27kDa protein from fertilizer: The nitrogen in CAN is made by the slow ammonium nitrogen and the fast nitrate nitrogen at equal ratio, therefore it can be applied for both top dressing and basic fertilization for any kind of soil types and vegetation.

The dolomite content with the nitrogen reduces the source of soil. So it is specifically recommended for treating sour soils, calcium (magnesium)improves the soil structure, as its clay/humin complexes make it crumbly (water, heat, and air retaining capacity of the soil will be better due to porosity), and increases its fertility. It increases the absorption and utilization of other nutrient through improving the ion balance.

Treatment: concentration exposure germination and growth: To evaluate the phytotoxicity of the mixture culture (which is based on fish waste) a seed germination test was carried out according to Kyun Kim (2010) . For tests of seed germination and root length, 10 ml of filtrate was pipetted into a sterile Petri dish lined with Whatman filter paper. Seed germination and root length in each plate were measured. It is very important to improve the utilization of fertilizer nutrients, since the growth of plants and their quality are mainly a function of the quantity of fertilizer.

Germination: To evaluate the phytotoxicity of the mixture culture, a seed germination test was carried out according to Kyun Kim (2010).For tests of seed germination, 5 ml of filtrate was pipetted into a sterile petri dish lined with Whatman filter paper.

Plant growth: Plant growth promoting bacteria reduce susceptibility to diseases caused by plant pathogens, and improve tolerance of stress conditions such as drought, salinity and nutrient deficiency.

RESULT AND DISCUSSION:

The sugars were made into small pieces and mixed with fish waste.The fish waste along with the sugars were mixed in equal proportions and kept in anaerobic condition for about 50-60 days.Then the fish waste with two types of sugars were converted into biofertilizer.

Odour At Different Time Intervals: As the time goes on, the odour of biofertilizer gets lessened. First interval: At 30 days the odour of bio fertilizer is heavier. Second interval: At 40 days the odour of biofertilizer becomes lesser. Third interval: At 50 days there is no odour. AND Physico Chemical analysis of Jaggery were interpreted in Table.1. Figure .1 represents the diagrammatic representation of Table.1.

Physical Nature of Compost At Different Time Intervals: As the time passes on, the physical structure changes. First interval: At 30 days the appearanc of biofertilizer is light in

colour. Second interval: At 40 days the appearance of biofertilizer becomes thick in colour. Third interval: At 50 days the appearance of biofertilizer thick brown in colour.

Fish waste is a potentially valuable resource from which high value protein can be obtained. Anaerobic digestion of the original fish waste and the fish sludge remaining after enzymatic pre treatment to extract fish oil and fish protein hydrolysate was evaluated regarding the potential for methane production. Physico Chemical analysis of Udankudi Jaggery were interpreted in Table.2. Figure.2 represents the Diagrammatic representation of Table.2.

Table-1 Physico Chemical Parameters of Jaggery

EXPRIMENTAL VALUE	Initial	30 days	40 days	50 days
pH	8.77	3.41	3.82	3.92
Acidity	0	0.57	0.32	0.21
Colour at 430nm in EBC	4.25	2.4	4.1	3.02
Bitterness at 275 in IBU	18.7	12.5	11.5	12.8
Growth rate at 600 nm	0.03	0.07	0.35	0.26
Antioxidant activity	10%	28%	32%	41%

Figure 1: Diagrammatic representation of Table.1

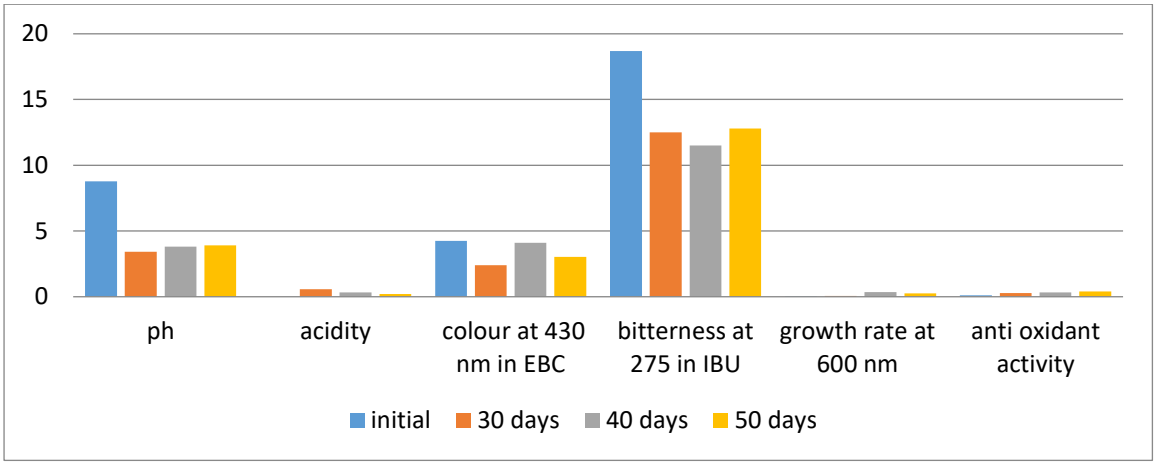
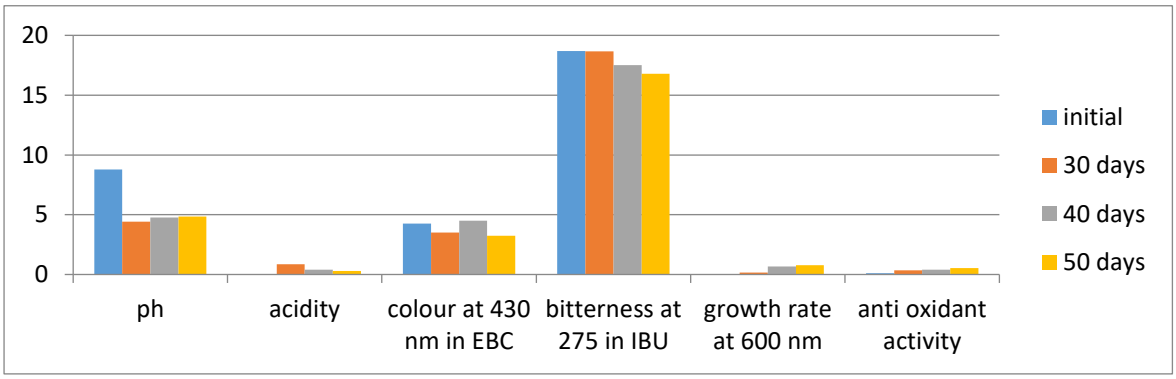


Table 2. Physico- Chemical parameters of Udankudi Jaggery

EXPRIMENTAL VALUE	Initial	20days	30 days	40 days
pH	8.77	4.41	4.77	4.84
Acidity	0	0.87	0.39	0.30
Colour at 430nm in EBC	4.25	3.5	4.5	3.25
Bitterness at 275 in IBU	18.7	18.65	17.5	16.8
Growth rate at 600 nm	0.03	0.165	0.675	0.766
Antioxidant activity	10%	35%	40%	54%

Figure :2 Diagrammatic representation of Table.2

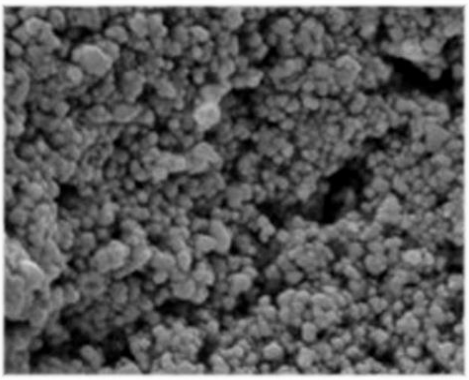


Biochemical Analysis: The result of analysis reveal that the quality of fish feed formulation. The availability of good quality raw material at an affordable price must ensured for achieving high yields and optimum growth of fish stalk in public and private sectors fish farms. For biochemical analysis the fish waste sample were collected from local markets. The result obtained indicated that the nutritional values of the commercial available fish waste ingredients was comparable with the standard values. Bio chemical analysis of fish waste were interpreted in Table 3.

Table 3: **Bio-chemical analysis of fish waste**

S.NO	TEST	RESULT
1	Catalase	Positive
2	Indole	Negative
3	Oxidase	Positive
4	Urea	Negative
5	Gelatin	Positive
6	Growth in Nutrient Agar	Positive
7	Nitrate reduction	Positive
8	Starch	Positive
9	Voges Proskauer	Positive

Final product **Manure**



Characterization of the biofertilizer from fish waste using chromatogram / plant germination and growth. Figure 4 represents the Isolation of Protein from bio fertilizer. Table.4 represents the peak report of TIC.

Figure 4 27 kDa protein isolated from fertilizer

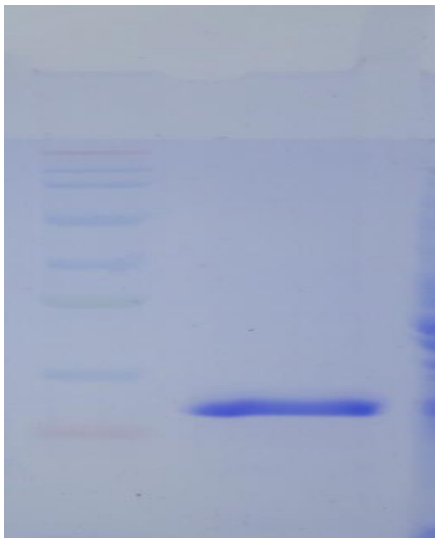
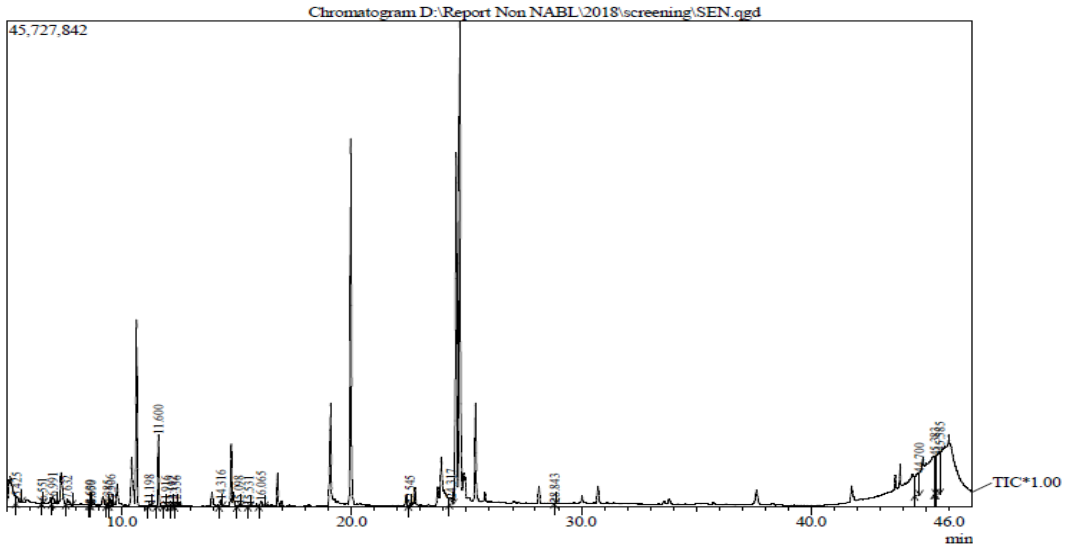


Table : 4 Peak Report Of TIC

Peak Report TIC

Peak#	R.Time	Name	Area	Area%	Height	Height%
1	5.425	4-Ethoxystyrene	4635275	3.45	596655	2.75
2	6.551	Ninhydrin	339723	0.25	184052	0.85
3	6.991	1,3-Benzodioxole	4170441	3.11	673602	3.11
4	7.632	4-Hydroxy-3-nitrobenzaldehyde	3185698	2.37	490485	2.26
5	8.609	Ethyl hydrogen suberate	199285	0.15	62612	0.29
6	8.650	Solasonine	66151	0.05	43908	0.20
7	9.385	2-Aminobenzophenone	613876	0.46	140898	0.65
8	9.506	Phenol, 4-nitro-	3225458	2.40	626097	2.89
9	11.198	Chloromethyl phenyl sulfide	690989	0.51	169825	0.78
10	11.600	Tributyl phosphate	22273629	16.59	6736563	31.09
11	11.916	l-Tyrosyl-l-alanyl-l-phenylalanine	165347	0.12	52187	0.24
12	12.192	Salicylidene aniline	391138	0.29	105208	0.49
13	12.356	Corymbolone	384195	0.29	79211	0.37
14	14.316	Thiophanate	2394958	1.78	646208	2.98
15	15.098	Heptafluorobutyric acid, n-octadecyl ester	833249	0.62	129922	0.60
16	15.531	Isopropyl Myristate	169241	0.13	64704	0.30
17	16.065	Carbonodithioic acid, S,S-diphenyl ester	2310937	1.72	503207	2.32
18	22.545	Isopropyl Palmitate	513403	0.38	105998	0.49

Figure: 5 Chromatogram Analysis



Germination of plants

4 th day **7 th day**


Comparison of the control and treated plants.

Growth of Ladies Finger on first week

Growth of Indian Broad Beans on third week

The final product, compost, can be used as a soil amendment that improves soil texture and fertility and thus reduces the use of synthetic fertilizers applied to the soil. In this case, the conversion should be performed by implementation of novel technologies for the recycling of waste in the form of compost for their use in agriculture. In addition to the usage of compost as a fertilizer, applying compost to the soil may increase the carbon storage capacity within the soil, which reduces greenhouse gas (GHG) emissions into the atmosphere . Biofertilizer are very popular among the farmer to produce quality product. These are eco-friendly and the cheapest source of nutrients. Recently, some studies have examined the reutilization of biodegrade fisheries waste product as liquid fertilizer (Kim and Lee, 2009: Kim *et al.*,2010:Dao and Kim, 2011). The above-mentioned material is of great potential use in agriculture. Numerous long-term experiments carried out in the last decade of the twentieth century showed that national management of mineral fertilizers and organic amendments made it possible to

increase crop yields and to sustain soil fertility sustainably. (Bado *et al.,* 1997; Bationo and Makwuney, 1999; Berger *et al.,* 1987; Pichotétal.1981;

The difference in physico chemical parameters of both Jaggery and Udankudi Jaggery are clearly noted. As the days goes on ,The pH level of both becomes reduced. The acid content is low at the initial period ,at 30 th day it gets increases ,but at the 40 th and 50 th day there is a reduction in acid content.

The bitterness level gets reduced . The growth rate of the plants gets increases. The percentage of anti oxidant activity becomes increases. The biochemical analysis of certain amino acids shows both Positive and Negative values. The amino acids like Catalase, Oxidase, Gelatin, Growth in Nutrient Agar, Nitrate reduction, Starch, Voges Proskauer shows positive effects. The amino acids Indole and Urea shows negative effects

CONCLUSION: Thus we can conclude that bio fertilizer have positive effects on plant growth in all organs. It also increases the photosynthesis, lengthens the vegetative cycle, promotes pollination, improves the size and early maturity of the fruit and improves the over all condition of the plant. Bio fertilizer are very popular among the farmers to produce quality products.

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