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Physicochemical characterization of traditionally fermented liquid manure from fish waste (Gunapaselam)

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Kunapajala, a fermented liquid from animal wastes has been used as manure since ancient times. Using this indigenous knowledge, fermented manure, Gunapaselam (in Tamil, Indian language), was prepared using fish waste and jaggery. An attempt was made to characterize Gunapaselam (GP) by measuring biological oxygen demand (BOD), chemical oxygen demand (COD), crude protein, lipid, minerals and amino acid content. The microbial status of the fermented manure was also enumerated. Phytotoxicity analysis was performed to scientifically validate its manurial potential. Changes in the pH, glucose, lactic acid, ethanol and acetic acid contents were recorded for first 5 days till the pH dropped to 4.0. The results show that during the course of fermentation, glucose concentration decreases with concomitant increase in the concentration of lactic acid, ethanol, and acetic acid. The analysis reveals the presence of macro, micronutrients and essential amino acids in Gunapaselam that could promote plant growth. Gunapaselam preserved permissible limits of BOD and COD levels. Microbial analysis proved the preparation was free from spoiling microbes. The highest germination index achieved from 50 fold diluted Gunapaselam. The data obtained confirmed the manurial potential of Gunapaselam which could restore the fertility of the soil deteriorated by chemical fertilizers.

Keywords: Fish waste, Gunapaselam, Natural fermentation, Organic fertilizer, Phytotoxicity
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The increased demand for food, land scarcity and poor farming practices have compelled intensive agriculture requiring the use of synthetic fertilizers. All over the world, the use of chemical fertilizers has become essential to enrich the soil with nutrients and this has helped to combat hunger and death but leaving dangerous imprints. Organic farming by recycling the organic waste is the only suitable alternative strategy to promote agro-ecosystem health. The fishery is one among the vibrant commercial food export sectors in India and source of income and livelihood for the underprivileged community. India occupies the second place in the global production of fish with 5.68%. Currently, the consumption of fish was on rising accounting for 140.4 million tons of global production. Out of which nearly 25% (34.8 million) are generally discarded without use[1] due to the low consumption, small size, and appearance.

From Vedic times we were taught how to yoke with nature to improve the quality of food and human health. Many chemists, expert scholars and soil scientists from western countries have acknowledged the traditional agricultural practices followed by Indian farmers. Traditionally, farmers are producing organic manure to increase their crop yield by using the available resources like cow dung, cow urine, farmyard manure, crop residues, dried leaves, weed residues and biofertilizers. Vrikshayurveda which speaks about better yielding healthy plants compiled by Surapala around 1000 AD in Eastern India and in Lokopakara compiled by a poet Chavundaraya in 1025 AD in Karnataka, Southern India[2] there was a mention about a liquid manure 'Kunapajala'. Flesh, blood, and wastes of animals were fermented and applied as fertilizer. This was practiced since ancient times in India and well documented as 'Kunapajala' (in Sanskrit - Indian language).

The immense fertilizing potential as evident by the improved growth, reproductive traits, and yield of many crops were seen after Kunapajala application. The raw materials used for Kunapajala were flesh, blood, marrow, skin, fat, brain and excreta of animals including sesame oil cake, ghee, honey, husk and black gram[2]. Later based on the availability of the raw

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materials and associated legal issues, considerable flexibility was made in the composition and proportion of Kunapajala preparation.

Despite rich agricultural applications, the importance of Kunapajala does not come into limelight until Valmiki Sreenivasa Ayangarya experimented and documented the effects on various trees and plants. He prepared different Kunapa products like herbal Kunapa using mango & soapnut, manujala with human urine, sasyagavya by using herbs, indsafari with safari fish, kukkutakunapa using chicken and mushina kunapa using rats[2]. The manurial potential of Kunapajala was proved in senna, langali, mango, coconut, chilly, kiwi, brinjal and paddy[3] but the proper characterization was not done.

With the rich indigenous knowledge on Kunapajala, a similar type of liquid manure, Gunapaselam was prepared using fish waste and jaggery[4]. Even though it has been used for the cultivation of vegetables, still no records were found about the complete characterization of Gunapaselam. The present investigation focuses on the natural fermentation of fish waste by using jaggery as the carbohydrate source and to scientifically prove the validation of fermented product Gunapaselam, as fertilizer by phytotoxicity test.

Methodology

Raw material and fermentation of fish waste

Fish waste (irrespective of species type) comprising of the gut, head, scales etc collected from fish market at Vanagaram, Chennai, Tamil Nadu, India. Fermentation of non sterilized fish waste was carried out in a 7.5 L plastic container without the addition of any commercial starter microbial culture or enzymes. Fish wastes (1 kg) were minced well and mixed with finely powdered jaggery (1.5 kg) and distilled water (5 L) and maintained at room temperature of 25°C[4]. Three replicates were maintained. Every day the fermentation mixture was measured for pH using digital pH meter (Elico make), glucose, ethanol, lactic acid and acetic acid content till the pH reached 4. Proper mixing ensured to supply oxygen and to vent out the released CO_2. The preparation was left for 15 days and filtered through gauze (1 – mm^2 mesh) to separate the solid particles. The filtrate was stored in a closed container and subjected to biochemical and microbiological analyses.

Physiochemical analysis

The fermented fish broth sample was collected for 5 days and the pH was measured using digital pH meter. The sample was centrifuged at 13,000 g for 10 min at 4°C to separate suspended particles. Then 2 mL of the clear liquid was dissolved with HPLC grade milli-Q water and made up to 25 mL in a volumetric flask and sonicated for 2 min. The sample was then filtered by Whatman nylon filter (0.45 mm) to remove the insoluble particles and used for the assay of fermented products. The sample was analyzed for glucose, lactic acid and acetic acid by HPLC and ethanol by GC for 5 days from the day 0. Lactic acid and acetic acid content in the fermented fish broth was determined by using a mixture of Acetonitrile: 25 mM phosphate buffer buffer (adjusted to pH 2.5 by H_3PO_4) (12:88) as mobile phase with a flow rate of 1 mL/min[5]. The analyses were carried out isocratically at a flow rate of 1 mL/min, injection volume of 20 µL and employing acetonitrile: water (79:21) as mobile phase for estimation of glucose content[6].

Ethanol content in the fermented fish broth was analyzed by Gas Chromatography (GC) method. Separation of compounds was conducted on a fused silica column as stationary phase using helium as carrier gas. The flow rate was 2 mL/min, the injector temperature kept at 200°C with a split ratio of 1:20 whilst the GC–MS detector temperature was 280°C. The oven temperature was programmed as follows: the column was held initially at 40°C for 12 min, then increased to 240°C till 32 min and held at that temperature for 10 min[7] more.

Proximate Analysis

The fermented fish waste evaluated for BOD[8], COD[9] and constituents like crude protein[10] and lipid[11]. The levels of nitrogen, phosphorus, potassium, calcium and magnesium were determined[12]. The ash content was also estimated[13].

The total amino acids in the fermented fish waste on 15th day was analyzed by HPLC using a mixture of 20: 80 (Acetonitrile: 25 Mm Potassium Phosphate pH 3.3) as mobile phase at the flow rate of 1 mL/min, temperature 38ºC and detection wavelength of 220 nm[14].

All the samples were run in triplicate. All the chromatographic data of all the analysis were recorded and calibrated with the respective standard graphs.

Microbial analysis

Microbial counts for total bacteria, lactic acid bacteria (LAB), yeast, mold and hazardous microbe fecal coliforms in Gunapaselam were determined. For microbial count, 1 mL of the sample was diluted with 9 mL of autoclaved distilled water, then serially diluted and analyzed by plate count method. Aliquots of

serially diluted samples were plated on nutrient agar medium and incubated at 30ºC for 48 h to determine the total bacterial count[8]. Lactic acid bacteria were enumerated by incubating the sample in Lactobacillus MRS agar at 30ºC for 48 h[15]. The counts of mold and yeast were enumerated by spreading 0.1 mL of the sample on potato dextrose agar maintained at 25ºC for 5 days. Microbial counts are expressed as log of colony-forming units per mL of the fermented fish waste (log (CFU/mL))[16]. The multiple-tube fermentation (MTF) technique includes three tests – presumptive (lauryl tryptose broth), confirmatory (brilliant green bile broth) and completed test (eosin-methylene blue agar) for enumeration of total coliform bacteria and fecal coliform bacteria[8] was followed and the results were expressed as log (MPN/mL).

Phytotoxicity test

The *in vitro* phytotoxicity test was performed by seed germination method using *Vigna radiata* (mung bean). The experimental groupings were: Group I – Water control; Group II – 1:25 diluted Gunapaselam; Group III – 1:50 diluted Gunapaselam; Group IV – 1:75 diluted Gunapaselam; Group V – 1:100 diluted Gunapaselam; Group VI – 1:150 diluted Gunapaselam and Group VII- 1% Urea (commercial fertilizer). 10 mL of the water/diluted Gunapaselam/1% urea was added to sterile petri plates lined with Whatman #1 filter paper. Then ten seeds of *Vigna radiata* were placed equidistant in the petri plate. The plates were incubated at laboratory condition for 72 h. The percentages of germination index (GI) of the treatment groups (3 replicates) was calculated with reference to relative seed germination (RSG) and relative root growth (RRG) and) by using the following formula[17].

RSG (%) = (Number of seeds germinated in test sample/Number of seeds germinated in control)*100

RRG (%) = (Mean root length in the test sample/Mean root length in control)*100

GI (%) = (RSG × RRG)*100

Statistical analysis

All data are reported as a mean ± standard error of the mean. Statistical analysis was done using SPSS 12.0 for windows package.

Results and Discussion

Production of liquid fertilizer

Fish waste in the fermented mixture starts to liquefy due to the action of proteases present in the fish and also due to acidity[18]. In the present study maximum liquefaction of solid waste was attained as evidenced by the presence of only 7.12% of residues remaining after filtration (Table 1). This shows that the proportion of fish waste: jaggery added was efficient for optimal fermentation. A similar reduction in solid residues was obtained after ensiling fish waste with molasses (1:1 w/w)[19]. The pH profile was used as the indicator of the course of fermentation. In our study, the pH was 6.98±0.03 on the day 0, decreased slowly and reached a stable value of 4.15±0.01 on day 4, indicating complete fermentation probably by the natural acidifying microbes in the fish offal/jaggery (Fig. 1). Similar pH reduction to 4.5 from 6.5 was obtained when non-inoculated fish waste was fermented with 50% sugarcane molasses[20]. Fermentation at ambient temperature also plays a vital role in pH reduction. It was observed that fermentation carried out at 25ºC or 35ºC significantly increased the lactic acid bacteria count[18].

In our study, effective fermentation by LAB species was evidenced by the increase in the consumption of glucose along with a proportionate decrease in glucose

Table 1 — Characterization of Gunapaselam on day 15

Parameter	Character/Amount
pH	4.15±0.01
Colour	Light Brown
Odor	Pleasant Smell
Solid residues	7.12%
Biological Oxygen Demand (3 days @27ºC) BOD(mg/L)	52±0.13
Chemical Oxygen Demand (3 days @27ºC) COD (mg/L)	216±1.4
Crude Protein (%w/w)	9.34±0.10
Crude Lipid (%w/w)	0.993±0.08

Data are expressed as mean±SEM (n=3)

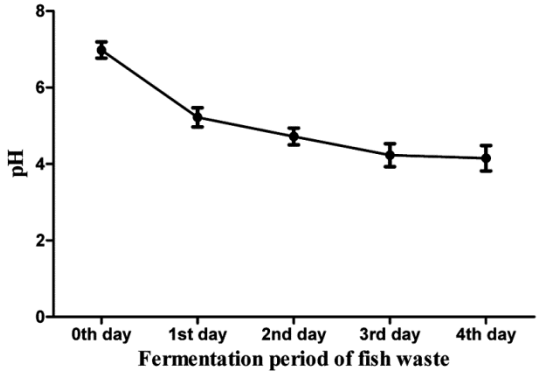

Fig. 1 — pH profile of fish waste during fermentation period (0 to 4 days)

level from day 0 to day 4 (115.6±2.31 mg/mL) as represented in Fig. 2. This report is further supported by a concomitant increase in the production of lactic acid, acetic acid and ethanol (Fig. 2). Heterofermentative LAB breaks down glucose to produce lactate, ethanol, and CO_2 as end products. Fermentation of lactate and citrate or metabolism of amino acids by bacteria leads to acetic acid production. The hexose was utilized by 6-phosphogluconate pathway producing a higher amount of lactic acid (356.7±1.4 mg/mL), acetic acid (21.9±0.91 mg/mL) and ethanol (404.6±1.43 mg/mL) (Fig. 2). The appearance of bubbles on the surface was noticed by 15 h due to the release of CO_2 and production of ethanol converts the stinky fish odor to pleasant sensory smell. The production of acetic acid may be due to another AA-producing microorganism in the fish waste or conversion of lactic acid to acetic acid during glucose exhaustion as observed in *L. mesenteroides* and *L. plantarum* species[21]. Mixing the fermentation broth daily ensures an adequate supply of oxygen which was found to be a critical factor in aerobic degradation and oxidative metabolism of cells[22]. A similar increase in the organic acid content was produced by lactobacillus during fermentation[23].

Chemical analysis

BOD and COD indicate the organic matter and nutrient content of waste materials. BOD is a measure of the oxygen consumed by microbes to decompose the organic content. Nitrates, phosphates and other organic nutrients may contribute to the high BOD levels. The amount of oxygen required to digest the organic matter using oxidizing agents instead of microbes is COD. The BOD and COD of Gunapaselam were 52±0.13 mg/L and 216±1.4 mg/L, respectively as listed in Table 1. Untreated organic wastes are rich in organic matter and nutrients. Such untreated waste when applied to soil will have adverse effects like increase of pCO_2, temperature and organic acid formed during decomposition will lead to immobilization of plant nutrients[24]. Hence treatment like fermentation and storage for a time period is essential for stabilizing the product. In the present study, BOD and COD of Gunapaselam are within the permissible limits of 40–500 mg/L and 80–600 mg/L of wastewater for agricultural reuse[25]. A mild increase in BOD and COD level will not affect soil property as they are easily degraded by soil microbes[24].

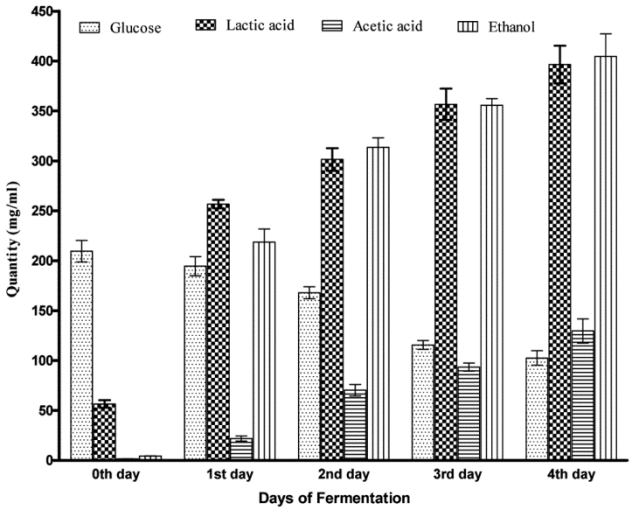

Fig. 2 — Concentration of glucose, lactic acid, acetic acid and ethanol detected for 5 days (From 0th to 4th day) during the fermentation fish waste

Besides organic matter, fish waste contains an appreciable amount of nutrients like N, P, K, Ca, Mg which can be utilized by the plants. The Gunapaselam is rich in nitrogen (1.49±0.02%), phosphorus (0.52±0.01%), potassium (0.48±0.02%), calcium (0.40±0.03%) and magnesium (0.28±0.01%). These macro and micronutrients are essential for the vegetative growth, fruit and flower development and disease resistance of plants. Dilution of treated waste is essential as they are toxic in concentrated form. This will surely reduce the dependence or completely replace the need for inorganic fertilizer. Thus this will be the best eco-friendly way to harness the full potential benefits from fish waste.

Moisture, crude protein (CP), crude lipid (CL) and ash content of the Gunapaselam (% wet weight basis) are shown in Table 1 & Table 2 respectively. The composition of the fermented fish waste will vary based on the source and species, sex, nutritional status, age, and health. The composition of most of the fish shows 15-30% protein, 0-25% fat and 50-80% moisture[26]. Loss of soluble nitrogen was prevented by using unsterilized fish waste[18]. The ash and mineral content was due to the degradation of fish bones[27].

Table 2 — Mineral content of Gunapaselam on day 15

Parameter	Amount
Moisture (%)	73.60±1.8
Nitrogen (Kjeldahl) (%)	1.49±0.02
Phosphorus (%)	0.52±0.01
Potassium (%)	0.48±0.02
Calcium (%)	0.40±0.03
Magnesium (%)	0.28±0.01
Ash (%)	5.83±0.01

Data are expressed as mean±SEM (n=3)

A suitable balance of amino acids is essential for the growth of the plants. The amino acid profile of Gunapaselam at the end of the experimental period is presented in Table 3. The result shows the presence of essential amino acids like arginine, threonine, valine, isoleucine, methionine, leucine, lysine and tryptophan in the fermented fish waste. The fish protein contains nearly 16-18 amino acids in a well-balanced form which are derived by microbial proteolysis. The composition of alanine, cysteine, and serine was found low. Reduced amount of serine while fermenting wool waste[28] and alanine[29] during fermentation of fish meal waste water, have been reported. The amino acid forms the active fraction of organic matter in a fertilizer and amino acids like methionine serve as the precursor of many plant growth regulators[30]. Plants could take up amino acids directly through the transporters during limited nutrient supply. The composition of nitrogen, phosphorus, potassium and essential minerals like calcium & magnesium and amino acids makes it suitable to be utilized as bio-fertilizer in agriculture, horticulture and aquaculture fields.

Microbial count

The microbial populations of total bacteria, LAB, yeast and mold in Gunapaselam was found to be 6.77±0.02, 4.14±0.01 and 2.24±0.01 (log (CFU/mL)) respectively (Table 4). The total and fecal coliforms counts were 0.30±0.01 log (MPN /mL) and nil (Table 4). Presence of harmful microbes was reduced/removed after fermentation when fish waste was treated with *L. acidophilus*[20] and slaughterhouse blood[31]. Products of LAB metabolism like hydrogen peroxide, diacetyl and bacteriocins contribute to the preservation property of Fish silage[32]. The reduction in the coliform count after fermentation was due to the acidic pH generated which prevents pathogenic microbes like *Salmonella* and *Clostridium botulinum*[33].

The increase in the total bacterial and LAB count after ensiling process showed that the condition of the fermentation broth favors their growth. Fish waste serves as a potential source for many microbes particularly LAB, enterobacteria, enterococci, yeast and molds and the supplemented carbohydrate source; jaggery might be responsible for the increase in the total bacterial load in the fermented broth. Similar results were reported in Panchakavya, that the total microbial load was 1.08 X 10^4 CFU/g[34]. From the results, it is understood that without the addition of any commercial inoculums or enzymes, natural fermentation can be achieved by LAB species that persisted throughout and were found to be the dominant species at the end and Gunapaselam produced is free of pathogenic and spoilage microbes.

Phytotoxicity assay

Improperly decomposed waste materials when applied to soil induce high microbial activity thereby reducing the soil oxygen content and also limit the availability of nitrogen[17]. Phytotoxicity of treated waste can be determined by *in vitro* seed germination test by using different plant species and by assessing the effect on seed germination %, plant length, root length and plant biomass. Gunapaselam is found to be rich in nutrients and minerals favorable to stimulate the plant growth and improve soil characteristics, but it has some limitations due to some of the products

Table 3 — The amino acid composition of Gunapaselam on day 1

Parameter	Amount (mg/100 g sample)
Aspartic acid	124.2±1.7
Glutamic acid	247.8±0.98
Asparagine	330.69±0.51
Serine	2.3±0.01
Glutamine	145.6±0.13
Glycine	203.5±0.17
Threonine	34.6±0.03
Arginine	129.7±0.14
Tyrosine	306.7±0.73
Histidine	129.5±0.51
Valine	356.7±0.75
Methionine	297.4±0.37
Isoleucine	705.7±0.64
Phenylalanine	291.7±0.55
Leucine	394.8±0.92
Lysine	703.7±0.46
Proline	298.8±0.57
Tryptophan	123.6±0.79
Alanine	In traces
Cysteine	In traces

Data are expressed as mean±SEM (n=3)

Table 4 — Microbial population dynamics of Gunapaselam on day 15

Microorganisms	Day 15
Total bacterial load Log (CFU / mL)	6.77±0.02
Lactic acid bacteria Log (CFU / mL)	4.14±0.01
Yeast and Mould Log (CFU / mL)	2.24±0.01
Total Coliforms Log (MPN / mL)	0.30±0.01
Fecal Coliforms Log (MPN / mL)	Not Detected

Data are expressed as mean±SEM (n=3)

released during fermentation like organic acids, ammonia, amino acids, and peptides. So the phytotoxicity of Gunapaselam was examined at various dilutions and compared with that of commercial fertilizer, urea. In this study germination index (GI) of the fermented fish, waste was 41.77±2.06% at 25 fold dilution (Table 5 & Fig. 3). This shows the adverse effect of fermented waste on root growth attributed to the high concentration of ammonia and organic acids[35]. Similar sensitiveness was observed in *Lepidium sativum* when treated with sewage sludge[36].

At 50, 75, 100 and 150 fold dilutions the GI value was found to be increased as 71.34±0.62, 120.34±1.2, 142.68±0.68, and 143.36±2.75% respectively. GI value below 50% reveals the phytotoxic nature of the treated waste and above 125% the recycled waste can be considered as phytonutrient and phytostimulant[37]. Grape marc after microaerobic treatment has proved to be a stimulator of ryegrass growth with GI value>125%[38]. It was confirmed from the results that Gunapaselam is not phytotoxic from 50 fold dilution onwards and hence it can be used as a liquid fertilizer. Since the GI value exceeds 125% (at 100 and 150 fold dilution) can also be used as bio-stimulant. Thus the idea of recycling the fish waste through efficient fermentation technique as fertilizer will reduce the pollution effects, prevents the loss of nutrients and curtail the escalating price of fertilizer production.

Table 5 — RSG (%) & RRG (%) of *Vigna radiata* seeds in Phytotoxicity assay

Group	No. of Seeds germinated : 9; Root length : 4.03±0.03 cm	
Group I (Water Control)	RSG%	RRG%
Group II (1:25 diluted Gunapaselam)	66.66±0.00	62.65±3.10
Group III (1:50 diluted Gunapaselam)	100±0.00	71.34±0.62
Group IV (1:75 diluted Gunapaselam)	100±0.00	120.34±1.24
Group V (1:100 diluted Gunapaselam)	111.11±0.00	128.41±0.62
Group VI (1:150 diluted Gunapaselam)	111.11±0.00	129.03±2.48
Group VII (Urea 1%)	111.11±0.00	105.86±2.48

Data are expressed as mean±SEM (n=6)

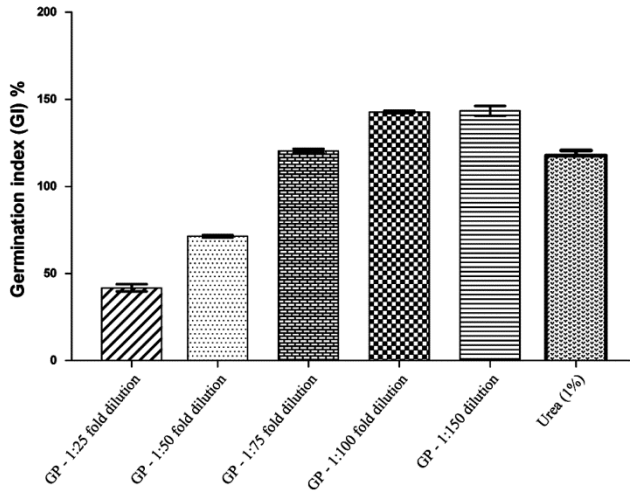

Fig. 3 — Germination index % (GI %) of *Vigna radiata* influenced by Gunapaselam by petriplate *in vitro* method

Conclusion

The present study proves that fish waste can be naturally fermented by using jaggery without adding commercial enzymes and microbes. It also revealed that fermentation is an appropriate technique to transform the fish waste into nutrient-rich liquid fertilizer. The level of glucose, lactic acid, acetic acid and ethanol confirms the major role of lactic acid bacteria to ferment fish waste. The goodness of Gunapaselam is characterized by low BOD, COD levels and also free from spoilage microbes. It is found to contain proteins and amino acids along with important plant nutrients like nitrogen, potassium, phosphorus, calcium, and magnesium. Phytotoxicity study by petri plate assay method confirms the manurial potential of Gunapaselam from 50% dilution onwards. The results confirm that Gunapaselam is suitable for agricultural use. Thus recycling and reusing of fish waste may result in converting a waste into a value-added product.

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References

1 FAO (Food and Agriculture Organization of United Nations), The state of world fisheries and aquaculture 2006, Food and Aquaculture Department, (Rome, Italy), 2007.

2 Nene YL, Kunapajala a liquid organic manure of antiquity, *Asian Agri- History*, 10 (2006) 315–321.

3 Bhat Savitha D, Ashok BK, Acharya R & Ravishankar B, Importance of *Kunapajala* (Traditional Liquid Organic Manure) of *Vrikshayurveda* in medicinal plant cultivation, *Global Journal of Research on Medicinal Plants & Indigenous Medicine*, 1 (2012) 272–279.

4 Vincent R, Ismail SA, Dawood Sharief S & Jeyaprakash P, Isolation and Characterization of Microorganisms in the fermented Fish Waste Liquid Foliar Spray-Gunapaselam, *Online Journal of Biosciences and Informatics*, 3 (2014) 320-324.

5 Sanchez-Machado DI, Lopez-Cervantes J & Martinez-Cruz O, Quantification of organic acids in fermented shrimp waste by HPLC, *Food Technology and Biotechnology,* 46 (2008) 456–460.

6 Hernandez JL, Gonzalez-Castro MJ, Alba IN & Garcia CC, High-Performance Liquid Chromatographic determination of mono- and oligosaccharides in vegetables with evaporative light-scattering detection and refractive index detection, *Journal of Chromatographic Science,* 36 (1998) 293-298.

7 Shannon C & David R, Analysis of bio-ethanol by gas chromatography, In: *Agilent Technologies Inc*, (USA), 2012, 1-4.

8 Rajan S & Selvi Christy R, Experiments in Microbiology, (Anjanaa Book House, Chennai), 2015, 216-217.

9 Yadav BR and Dyan Singh, Analysis of irrigation waters for quality assessment, In: Methods of analysis of soils, plants, water, fertilizers and organic manures, edited by Tandon HLS, (FDCO, New Delhi), 2009, 112-152.

10 AOAC (Association of Official Analytical Chemists), Standard Official Methods of Analysis of the Association of Analytical Chemists, 2nd edition, edited by S.W. Williams, (Washington DC), 1975.

11 AOAC (Association of Official Analytical Chemists), Standard Official Methods of Analysis of the Association of Analytical Chemists, 15th edition, (Arlington, VA, USA), 1990.

12 Tandon HLS, Methods of analysis of soils, plants, water and fertilizers, (FDCO, New Delhi), 2009.

13 Gaur AC, Methods of Analysis of organic manures, In: Methods of analysis of soils, plants, water, fertilizers and organic manures, edited by Tandon HLS, (FDCO, New Delhi), 2009, 183-201.

14 Bhandare P, Madhavan P, Rao BM & Someswar Rao N, Determination of amino acid without derivatization by using HPLC-HILIC column. *Journal of Chemical and Pharmaceutical Research,* 2 (2010) 372-380.

15 De Man JD, Rogosa, M & Sharpe ME, A Medium for the Cultivation of Lactobacilli, *Journal of Applied Bacteriology,* 23 (1960) 130-135.

16 Downes FP & Ito K, Compendium of Methods for the Microbiological Examination of Foods, 4th Edition, (APHA, Washington, DC), 2001.

17 Zucconi F, Pera A, Forte M & De Bertoldi M, Evaluating toxicity of immature compost, *Bio Cycle,* (1981) 54-57.

18 Hassan TE & Heath JL, Biological Fermentation of fish waste for potential use in animal and poultry Feeds, *Agricultural Wastes*, 15 (1986) 1-15.

19 Sahu BB, Barik NK, Mohapatra BC, Sahu BN, Sahu H *et al.*, Valorization of Fish Processing Waste through Natural Fermentation with Molasses for Preparation of Bio Fertilizer and Bio Supplement, *Journal of Environmental Science, Computer Science and Engineering & Technology*, 3 (2014) 1849-1856.

20 Samaddar A & Kaviraj A, Processing of fish offal waste through fermentation utilizing whey as inoculums, *International Journal of Recycling of Organic Waste in Agriculture*, 3 (2014) 45-52.

21 Goffin P, Muscariello L, Lorquet F, Stukkens A, Prozzi D *et al.,* Involvement of pyruvate oxidase activity and acetate production in the survival of *Lactobacillus plantarum* during the stationary phase of aerobic growth, *Allied and Environmental Microbiology*, 72 (2016) 7933-7940.

22 Hickert LR, Da Cunha-Pereira F, De Souza-Cruz PB, Rosa CA & Ayub MAZ, Ethanogenic fermentation of co-cultures of *Candida shehatae* HM 52.2 and *Saccharomyces cerevisiae* ICV D254 in synthetic medium and rice hull hydrolysate, *Bioresource Technology*, 131 (2013) 508–514.

23 Mathivanan R, Edwin SC, Viswanathan K & Chandrasekaran D, Chemical, Microbial composition and antibacterial activity of modified panchagavya, *International Journal of Cow Science*, 2(2) (2006) 23-26.

24 Chhonkar PK, Datta SP, Joshi HC & Pathak H, Impact of industrial effluents on soil health and agriculture – Indian experience: part I – distillery and paper mill effluents, *Journal of Scientific and industrial research*, 59 (2000) 350 – 361.

25 FAO (Food and Agriculture Organization of United Nations), Wastewater treatment and use in agriculture, In: *Irrigation and Drainage*, edited by Pescod MB, Paper 47, (FAO, Rome), 1992.

26 Ghaedian R, Coupland JN, Decker EA & McClements, JD, Ultrasonic determination of fish composition, *Journal of Food Engineering*, 35 (1998) 323-337.

27 Bogard JR, Thilsted SH, Marks GC, Wahab MA, Hossain MAR, Jakobsen J & Stangoulis J, Nutrient composition of important fish species in Bangladesh and potential contribution to recommended nutrient intakes, *Journal of Food Composition and Analysis*, 42 (2015) 120–133.

28 Fang Z, Zhang J, Liu B, Du G & Chen J, Biodegradation of wool waste and keratinase production in scale-up fermenter with different strategies by *Stenotrophomonas maltophilia* BBE11-1, *Bioresource Technology*, 140 (2013) 286–291.

29 Kim JK & Lee G, Aerobically biodegraded fish-meal wastewater as a fertilizer, *Environmental Research Journal*, 9 (2009) 219-236.

30 Amir R, Hacham Y & Galili G, Cystathionine γ-synthase and threonine synthase operate in concert to regulate carbon flow towards methionine in plants, *Trends in Plant Science*, 7 (2002) 153-156.

31 Samaddar A & Kaviraj A, Application of Fermentation Technology to Use Slaughterhouse Blood as Potential Protein Supplement in Fish Feed, *Jordan Journal of Biological Sciences*, 8 (2015) 23 – 30.

32 Sven Lindgren E & Walter J, Antagonistic activities of lactic acid bacteria in food and feed fermentations, *FEMS Microbiology Reviews*, 7 (1990) 149-163.

33 Tatterson IN & Windsor ML, Fish silage, *Journal of the Science of Food and Agriculture*, 25 (1974) 369-379.

34 Ganesh Kumar K, Kumaravelu N, Sivakumar T & Gajendran K, Study on Panchakavya - an indigenous formulation and its effect on the growth promotion of crossbred pigs, *Indian J Anim Res*, 40 (2) (2006) 158 – 160.

35 Fang M & Wong JWC, Effects of lime amendment on availability of heavy metals and maturation in sewage sludge composting, *Environmental Pollution*, 106 (1999) 83-89.

36 Fuentes A, Llorens M, Saez J, Aguilar M, Ortuno J & Meseguer V, Phytotoxicity and heavy metals speciation of stabilized sewage sludges, *Journal of Hazardous Materials*, A108 (2004) 161-169.

37 Emino ER & Warman PR, Biological assay for compost quality, *Compost Science and Utilization*, 12 (2004) 342–348.

38 Moldes AB, Vazquez M, Domínguez JM, Díaz-Fierros F & Barral MT, Evaluation of mesophilic biodegraded grape marc as soil fertilizer, *Applied Biochemistry and Biotechnology*, 141 (2007) 27–36.