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Analysis of fermented liquid fertilizer from marine crab waste

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Abstract— *Soil is the main source of supplying essential nutrients for plant growth. In agricultural practice, when a particular nutrient or a group of nutrients are absent in the soil, it may affect the growth of the plant. Thus, most of the farmers practicing to apply chemical fertilizers to overcome the soil nutrient deficiency. Chemical fertilizers enhance the soil fertility on one hand whereas on the other hand it cause environmental pollution. Therefore alternative methods of soil nutrition practice must be considered. Hence this work focused to prepare fermented liquid fertilizer to enhance the plant growth. In the present study the fermented liquid fertilizer was prepared by fermenting the* Portunussanguinolentus *(Herbst, 1783) crab wastes with jaggery. After 15 days, the fermented liquid from crab waste was filtered and used for further study. The harvested liquid subjected to physico chemical and microbial analysis. The functional group and active compounds present in the fermented liquid was analyzed through FT-IR and GC-MS study. Phytotoxicity of the fermented liquid was determined through seed germination assay by using TMV-7 ground nut seeds. The result showed that the fermented liquid was diluted in water in the ratio of 1:100 exhibited higher seed germination when compared to other test dilutions and control. Thus the present work strongly supports the view that the traditionally fermented crab waste liquid contain high nutrients and active compounds and that may support the ground nut plant growth*.

Keywords— ***Liquid fertilizer, Fermented crab waste, FT-IR, GC-MS, seed germination assay.***

I. INTRODUCTION

Nutrition is one of the most important factors to control agricultural productivity. Continuous agricultural practices diminish the nutrient content of the soil. Soil is the main source of supplying essential elements to plants. When a particular mineral or a group of minerals are absent in the soil, the plant will show deficiency symptoms and affect the physiological processes in plants including reproduction and growth(Sandip, 2011). To overcome the mineral deficiency and to increase the yield, the soil can be enriched by supplying these nutrients from external sources. The major compounds which are added into the soil to improve its fertility are grouped under two broad categories: (a) Chemical fertilizers and (b) Organic fertilizers. Most of the farmers fertilize the soil by adding chemical fertilizers due to their quick action in soil (Ann McCauley, 2009). Chemical fertilizers contains a large amount of the heavy metals like Mercury (Hg), Cadmium (Cd), Arsenic (As), Lead (Pb), Copper (Cu) and Nickel (Ni); natural radionuclide like Uranium (^{238}U), Thorium (^{232}Th), and Polonium (^{210}Po). Thus, application of chemical fertilizer leads to water, soil and air pollution. (Sönmez*et al*., 2007). Chemical fertilizers enhance the crop yield on one hand whereas on the other hand act as environmental hazards. Therefore alternative methods of agricultural practice must be considered. Under present conditions, application of organic farming is much promising (Matson *et al.*, 1997; Drinkwater *et al.*, 1998; Tillman 1999; Zhu *et al.*, 2000; Reganold*et al.*, 2001; Xie*et al.*, 2003). Organic farming is a method of agriculture practice in which farmers not use synthetic pesticides, fertilizers, genetically modified organisms and growth hormones. Organic farming will increase the agriculture production and makes a pollution free environment (Ramesh *et al*., 2005). Large quantities of wastes are generated in the processing of aqua foods from crustaceans and fishes. These materials contain appreciable amounts of nutrients to plants which may be useful for agriculture practices (MacLeod *et al*., 2006). Most aqua food processing waste was disposed of in landfill sites or applied haphazardly to land (Swanson *et al.*, 1980; Murado*et al.*, 1994). Land application of crustacean waste is problematic and cause soil pollution. Hence these waste must be processed in alternate way.Dao & Kim (2011), has reported the use of fermented fish waste products as liquid fertilizer and the nutrients in the fermented liquid fish waste may stimulates the growth of the plants through beneficial soil microorganisms present in it and increasing the uptake of essential nutrients. This fertilizer because of its liquid nature is readily available, releases the nutrients slowly and prevents leaching from the soil. Nutrients can be applied in the form of foliar sprays which immobilize and supplement the nutrients to the leaves. From the foregoing literature, it can be clearly understood that in order to manage the high amount of crustacean waste and it may be fermented and used as a liquid fertilizer for various agriculture practices and this would paves the way for sustainable development in agriculture.

II. MATERIALS AND METHODS

2.1. Raw material collection & processing

The discarded part of the processed marine crabs were collected from the fish market at Kelambakkam -603103. The collected waste mainly consisting of the carapace, intestines and other discarded part of the three-spot swimming crab, *Portunussanguinolentus* (Herbst, 1783). The collected crab waste was washed in chlorine free water for fermentation process.

2.2. Preparation of fermented liquid fertilizer

Liquid fertilizer was prepared in traditional way based on the procedure described by ThendralHepsibha B. &Geetha A. (2019). According to that procedure 1.5 kg of native jaggery, 1 kg of crab waste and 5 liters of chlorine free water were added inside the clay pot and mixed thoroughly. The mouth of the pot was covered with a cotton cloth to prevent the entry of flies. After 14 days, the fermented materials were filtered by using cotton cloth and the filtrate was subjected to further analysis.

2.3. Analysis of physico-chemical parameters

The filtered liquid fertilizer was subjected to physical parameters like Color, Odor, pH, Electrical Conductivity. The chemical parameters such as Total Nitrogen (Kjeldahl), Total Phosphorous, Potassium, Sodium, Calcium, Magnesium, Sulphur, Zinc, Manganese, Iron and Copper were tested at NAF Research & Development Centre, Anna University Taramani Campus, Chennai - 600 113, according to the procedure described by Tanden (1993). Heavy metals such as Mercury, Cadmium and Lead were determined by Atomic Absorption Spectroscopy method.

2.3. Microbial analysis

The heterotrophic bacterial count, total coliform and fecal coliforms in fermented liquid were determined. For heterotrophic plate count, 1 ml of the fermented liquid was diluted with 9 ml of sterilized distilled water, then serially diluted and analyzed by standard plate count method. 0.5 ml of serially diluted samples were poured on petri plate contain nutrient agar medium and incubated at 35°C for 48 hours. Total bacterial counts are represented as log of colony forming units per mL of the fermented liquid (CFU/ml). Total coliform bacteria and fecal coliform bacteria were determined through multiple-tube fermentation technique and the results were expressed as log (MPN/ml) (Thendral Hepsibha & Geetha 2017). All the experiments were replicated three times.

2.4. FT-IR analysis

Fourier Transform Infrared Spectrophotometer (FT-IR) is the most powerful tool for identifying the functional groups present in any compounds. In the present work functional group of the liquid fertilizer was analyzed in the range from 400 to 4000cm^{-1} with a resolution of 4 cm^{-1} by using FT-IR spectrophotometer (Shimadzu, IR Affinity 1, Japan) (Sugumar*et al.*, 2015).

2.5. GC-MS analysis

GC-MS analysis is performed to identify the active biomolecule present in the liquid fertilizer from fermented crab waste. For that the liquid fertilizer was injected into the instrument GC and MS JEOL GC mate equipped with secondary electron multiplier (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m×0.25 mm I.D. The study conditions were 20 min. at 100°C, 235°C for column temperature at 3 min and 240°C for injector temperature, carrier gas was helium, and split ratio was 5:4. The 1 µl of the sample was evaporated in a split-less injector at 300°C and the run time was 22 min. The active biomolecule of the liquid fertilizer was identified by Gas Chromatography coupled with Mass Spectrometry and the result spectrum was analyzed using the NIST08 library (Radhakrishnan*et al.*, 2017).

2.6. Seed germination assay

Seed germination assay was performed to determine the phyto-toxicity of the fermented liquid fertilizer. For this experiment the fermented liquid fertilizer was diluted in chlorine free water with the ratio of 1:200 (5 ml / 1000 ml), 1:100 (10 ml / 1000 ml), 1:50 (20 ml / 1000 ml), 1:25 (40 ml / 1000 ml) and control (water alone). The ground nut seed (TMV-7) was soaked in liquid fertilizer for 24 hours. After the soaking period, 10 numbers of each ground nut seeds were placed in tissue paper sprinkle with liquid fertilizer. Now the seeds were incubated in room temperature for 72 hours(Muthezhilan*et al.*, 2012& 2014; Thendral Hepsibha & Geetha 2019). After the incubation the germinated seeds were identified and the percentage of germination and percentage of Relative Seed Germination (RSG) were calculated by the following formula,

$$\text{Germination \%} = \frac{\text{Number of Seeds Germinated}}{\text{Total Number of Seeds}} \times 100$$

$$\text{RSG \%} = \frac{\text{Number of seeds germinated in test sample}}{\text{Number of seeds germinated in control}} \times 100$$

III. RESULTS & DISCUSSION

3.1. Analysis of physico-chemical parameters

The objective of physico-chemical analysis was to identify the nutrient content of the fermented liquid and to determine its suitability for agriculture purpose. The color of the fermented liquid was light golden yellow color and it produced fruity odor at the time of harvest. The results of physico-chemical analysis were presented in Table 1. Heavy metals such as Mercury (Hg), Cadmium (Cd) and Lead (Pb) content of the organic fermented liquid was analyzed (Table 1).

Table.1: Physico-chemical characterization of fermented liquid on day 15

S. No	Parameters	Unit	Result
Physical parameters			
1.	pH		5 ± 0.01
2.	Electrical conductivity	µS/cm	11623 ± 1.45
Chemical parameters			
3.	Total Nitrogen (Kjeldahl)	%	0.21 ± 0.008
4.	Total Phosphate as P_2O_5 #	%	0.41 ± 0.01
5.	Potassium as K_2O #	%	0.27 ± 0.003
6.	Sodium as Na #	mg/kg	1500 ± 1.15
7.	Calcium as Ca #	mg/kg	5830 ± 0.57
8.	Magnesium as Mg #	mg/kg	1283 ± 0.57
9.	Sulphur as S #	mg/kg	282 ± 0.88
10.	Zinc as Zn #	mg/kg	9.64 ± 0.008
11.	Manganese as Mn #	mg/kg	17.33 ± 0.01
12.	Iron as Fe #	mg/kg	57.30 ± 0.34
13.	Copper as Cu	mg/kg	2.31 ± 0.01
Heavy metal analysis			
14.	Mercury (Hg)	ppm	BDL
15.	Cadmium (Cd)	ppm	BDL
16.	Lead (Pb)	ppm	BDL

Data are expressed as Mean ± SEM (Standard Error Mean)

BDL: Below Detectable Level

Crab waste may contain high amount of protein, lipid, carbohydrate, calcium and sodium and these components starts to degrade and liquefy due to the action of microorganism, protease enzyme and acid. In the present work, 230 g (23%) of delicate crab shell residue remained after filtration and this clearly indicating that 77% of crab waste is degraded and liquefied by microorganism and its enzymes. The results of physical parameter analysis revealed that there was a higher range of pH and Electrical conductivity were recorded in fermented liquid fertilizer. The increased amount of pH and EC may affect plant growth (Jwan J Abdullah *et al.*, 2020), but in the present work it was optimum and may not affect plant growth. The chemical parameters such as N, P_2O_5, K_2O, Na, Ca, Mg, S, Zn, Mn, Fe and Cu were high in fermented liquid. These findings are similar with the results of Thendral Hepsibha & Geetha (2019). Since the substrate for the fermentation was from marine source the fermented liquid was subjected to heavy metal analysis (Hg, Cd and Pb). The results revealed that the heavy metal content of the fermented liquid was below detectable level.

3.2. Microbial analysis

The results of total bacterial load of the fermented liquid was 7.54 ± 0.02 Log (CFU / ml) and total coliform count was 0.41 ± 0.01 Log (MPN / ml).There was no fecal coliforms identified in the fermented liquid (Table 2).

Table 2: Microbial population of fermented liquid on day 15

S. No.	Test	Unit	Result
1.	Total bacterial load	Log (CFU / ml)	7.54 ±0.01
2.	Total Coliforms	Log (MPN / ml)	0.40±0.01
3.	Fecal Coliforms	Log (MPN / ml)	No Detectable

Data are expressed as Mean ± SEM (Standard Error Mean)

Similar results were reported in Gunapaselam, that the total heterotrophic bacterial load was found to be 6.77 ± 0.02 (Thendral Hepsibha & Geetha 2019), where as in the present work total bacterial load was recorded little high (7.54 ± 0.02). The total coliform count was recorded in the present work was 0.41 ± 0.01 log (MPN / ml) and this count was little higher than total coliform count identified in Gunapaselam (0.30 ± 0.01 log MPN /mL) by Thendral Hepsibha & Geetha 2019. There was no fecal coliforms identified in the fermented liquid and from these results, it understood that the fermented liquid harvested marine crab waste at 14th day was free of harmful and pathogenic microorganisms.

3.3. FT-IR analysis

The FT-IR results of the fermented liquid was presented in Table 3 and Figure 1.The graph showed the strong peaks for Alkynes (C=C), Amides (N-H), Carboxylic derivatives &Arenes (C=C) and weak & medium peaks for amines.

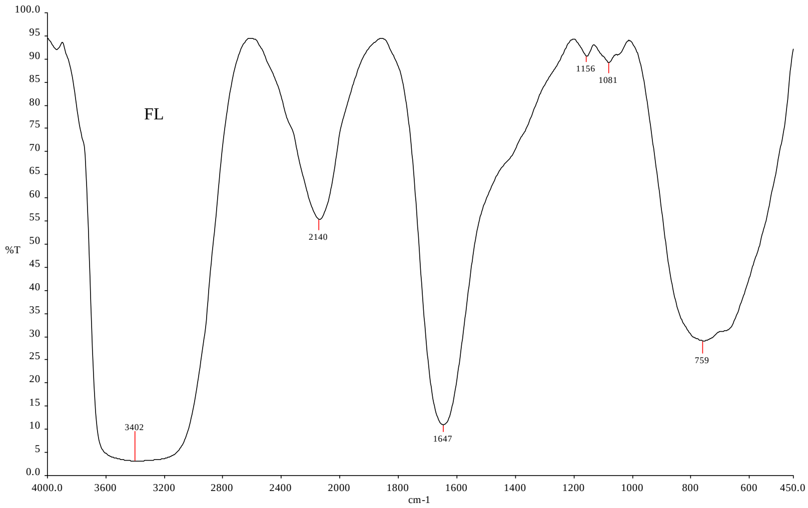

Fig.1: FT-IR graph of fermented liquid on day 15

Table 3: FT-IR interpretation of fermented liquid on day 15

S. No	**Transmittance % -T**	**Functional group**	**Intensity**	**IR frequency Range cm^{-1}**	**Types of bond**
1.	3402	Amine (N-H)	Weak	3400– 3500	Stretching vibrations
2.	2140	Alkynes (C=C)	Strong	2100– 2250	
3.	1647	Amides (N-H)	Strong	1630– 1695	Stretching vibrations
4.	1156	Carboxylic derivatives	Strong	1100– 1735	Stretching vibrations
5.	1081	Amines (N-H)	Medium	1000– 1250	Stretching vibrations
6.	759	Arenes (C=C)	Strong	690– 900	Bending vibration

The FT-IR spectroscopic technique is used to determine the degradation of polysaccharides, polypeptides, aliphatic, phenolic and carboxylic groups. Ravindra n*et al*., (2013) reported that the FT-IR spectroscopic analysis of fermented animal fleshing composted with vermicomposting revealed that the appearance of Carboxylic groups and relative reduction in CH_3 and CH_2 groups which indicated the organic waste mineralization. In the present study reported that there was an appearance of carboxylic groups which could confirm the mineralization process took place completely during fermentation.

3.4. GC-MS analysis

The GC-MS analysis of fermented liquid fertilizer exhibit the presence of nine bioactive compounds (Table 4 & Figure 2). Based on the retention time and peak value the biomolecules present in the fermented liquid was confirmed. The bioactive compounds present in the fermented liquid fertilizer were 3-Methylene-1,6-heptadiene Phenol, 3-methyl, Phenol,2- ethyl, 3-Pentadecanone, E,E-6,8-Tridecadien-2-ol, acetate, Undecane, Benzeneacetic acid, alpha,3-bis(acetyloxy)-5-methoxy-, methyl ester, Furfural and 2-Heptanone.

Table 4: Compounds identified in the fermented liquid by using GC-MS

S. No	Retention time	Compound name	Molecular formula	Molecular weight
1.	15.93	3-Methylene-1,6- heptadiene	$C_{10}H_{16}$	136.238 g/mol
2.	16.55	Phenol, 3-methyl	$C_{17}H_{21}NO_2$	271.36 g/mol
3.	17.52	Phenol,2- ethyl	$C_9H_{13}NO$	151.209 g/mol
4.	18.32	3-Pentadecanone	$C_{15}H_{30}O$	226.404 g/mol
5.	18.95	E,E-6,8-Tridecadien-2-ol, acetate	$C_{15}H_{26}O_2$	238.37 g/mol
6.	20.15	Undecane	$C_{11}H_{24}$	156.313 g/mol
7.	23.33	Benzeneacetic acid, alpha,3-bis(acetyloxy)-5-methoxy-, methyl ester	$C_{14}H_{16}O_7$	296.27 g/mol
8.	13.07	Furfural	$C_5H_4O_2$	96.085 g/mol
9.	14.85	2-Heptanone	$C_7H_{14}O$	114.188 g/mol

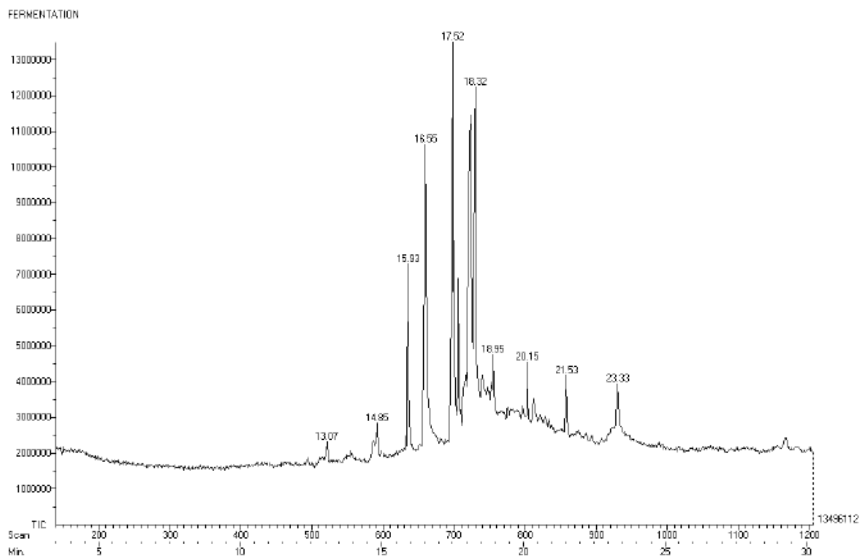

Fig.2: GC-MS graph of fermented liquid on day 15

Furfural (C_4H_3OCHO) is anorganic, colorless liquid and it is a dehydrated sugar product. The hydrogenated furfural products are used as a solvent in herbicide formulations in agriculture. The chemical compound 2-Heptanone is a colorless or white liquid ketone. It has banana-like fruity odor (https://pubchem.ncbi.nlm.nih.gov/compound/8051).

This may be the reason that the liquid fertilizer at 14th day exhibit fruity smell. The other products are the by-product of the mineralization and polymerization of crab waste.

3.5. Seed germination assay

Fermented liquid fertilizer from marine crab waste is found to be rich in minerals and plant nutrients, but application of this fermented liquid in soil or plants has some limitations. Because, some of the products like ammonia, amino acids and organic acids etc., released during fermentation may act as a toxic to plants and native microorganisms of the soil. Zucconi*et al*., (1981) also reported that the application of partially degraded organic waste in to soil leads to high microbial activity and cause the soil oxygen depletion and arrest the nitrogen availability of the soil. In view of that the present work the phytotoxic effect of the fermented liquid was determined at different dilutions and compared with control (Table 5). In this work percentage of seed germination and Relative Seed Germination (RSG) were recorded high in 1:100 dilution (80%) when compared to control (70%) whereas percentage of germination and RSG were equal to control at lowest dilution (1:200). In contrast the percentage of germination and RSG were decreased by increasing concentrations of fermented liquid, it may due to higher pH and mineral content of the fermented liquid. Thus the idea of reusing the marine crab waste as a liquid fertilizer through simple conventional fermentation technique used to mitigate the environmental pollution and it may help to prevent the loss of soil nutrients.

Table 5: Results of seed germination assay

S. No.	Test	Total seed	Germinated seed	Seed germination (%)	RSG (%)
1.	Test 1 (1:200)	10	7 ± 0.33	70	100
2.	Test 2 (1:100)	10	8 ± 0.57	80	114.28
3.	Test 3 (1:50)	10	4 ± 0.33	40	57.14
4.	Test 4 (1:25)	10	3 ± 0.33	30	42.85
5.	Control (H_2O)	10	7 ± 0.33	70	

Data are expressed as Mean ± SEM (Standard Error Mean)

RSG: Relative Seed Germination

IV. CONCLUSION

The present work proves that marine crab waste can be fermented conventionally by using native jaggery without adding catalytic enzymes and it also confirm that conventional fermentation is a suitable method to bio-transform the marine crab waste into nutrient rich liquid fertilizer. The bacterial load of the fermented liquid revealed that the bio conversion is carried out by their action. The maturity and goodness of the fermented liquid is confirmed through FT-IR and GC-MS analysis. The *in vitro* seed germination assay confirms that the fermented liquid from marine crab waste (ratio of 1:100) is suitable for agriculture use.

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