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BIOCONVERSION OF FISH WASTE INTO A LIQUID FERTILIZER AND ITS IMPACT ON SEMI- ARID TROPICAL CROPS

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ABSTRACT: Large amounts of fish wastes are being generated, mostly from the industrial processing of fish. There are many ways to utilize this fish waste for the production of many value added products. The main theme of this work is to convert fish waste into an excellent organic fertilizer and study its effect on growth of some semi-arid crops of India. This is the first report on the growth studies of crops *Pennisetum glaucum (Pearl millet) and Trigonella foenum-graecum (Fenu greek)* subjected with fertilizer made out of fish waste. Fish wastes of different Indian carps like *Catla catla, Labeo rohita* and mixed fish waste was collected and different liquid fertilizers with these three different fish wastes were prepared using bacterial cultures. *Pennisetum glaucum and Trigonella foenum- graecum* crops growth was tested with these fertilizers. Crops treated with chemical fertilizer Saphal-19 were kept as control. 120 small cups dispersed with *Pearl millet* seeds and 120 small cups dispersed with *Fenu greek* seeds were maintained for the growth studies. Triplicates were maintained for each type of fertilizer. Length of leaf, shoot and root were measured in time intervals of 5 days. Statistical data was obtained by DMR (Duncan's mean range) test ($p < 0.05$) using Statistical Package for the Social Sciences (SPSS) software (version 16.0).

KEYWORDS: Organic fertilizer; *Catla catla;, Labeo rohita*; Semi-arid crops; *Pennisetum glaucum* (*Pearl millet)*; *Trigonella foenum- graecum (Fenu greek)*

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1.INTRODUCTION

In India, industrial fish processing generates 302,750 tons of waste. Annually over 100million tons of fish are harvested worldwide and about half of the total catch is discarded as processing waste [1, 2]. Fish processing generates solid wastes that can be as high as 50 - 80% of the original raw material

[3-5]. An important waste reduction strategy for the industry is the recovery of marketable byproducts from fish wastes. The utilization of byproducts is an important cleaner production opportunity for the industry, as it can potentially generate additional revenue as well as reduce disposal costs for these materials [6]. The transportation of fish residues and offal without the use of water is an important factor for the effective collection and utilization of these byproducts. Many useful products can be obtained from fish waste if we process it in a right way [7]. Organic fertilizers are the fertilizers derived from animal matter, human excreta or vegetable matter. Bone meal, hoofs, horns, fish meal are included under animal matter [8]. Organic fertilizers can have very good results on crops and on the environment. Fish waste can be converted to an excellent organic fertilizer using some methodology [9]. Fish waste is an abundant source for Nitrogen, Phosphorous, and Potassium. Nitrogen promotes leaf growth and forms proteins and chlorophyll. Phosphorous contributes to root, flower and fruit development. Potassium contributes to stem and root growth and the synthesis of proteins [10]. Digestion of biomass in order to produce biogas and bio-fertilizer is an attractive mode of treating waste biomass [11-13]. The waste of dry matter contain large variety of macro and micro minerals whose composition was reported to be 6% Na, 0.2% Mg, 100ppm of iron, 62ppm of Zn, 6ppm of copper in dry matter of different fish wastes [14-16]. In this work we developed biofertilizer from fish wastes of *Catla catla, Labeo rohita* and mixed fish waste and used to grow *Pennisetum glaucum (Pearl millet)* and *Trigonella foenum-graecum (Fenu greek*) plants along with control.

2. MATERIALS AND METHODS

2.1 Collection of Fish Waste:

Fish waste of major Indian Carps like *Catla catla, Labeo rohita* and mixed fish waste was collected from fish industries in and around of Anantapur district in clean polytheine covers bags under iced conditions and stored at 4^0C for further use. The Fish Waste was cleaned with distilled water to remove dirt particles and dried for a month in sunlight. The dried fish waste was powdered in a blender and fish waste powder was stored in air-tight containers.

2.2 Isolation of Microorganisms:

One gram of fish waste was weighed and serially diluted with 9ml of distilled water as 10^{-1}, 10^{-2}, 10^{-3}… $10^{-9.}$ Nutrient agar medium was prepared with peptone: 5gms, Beef extract: 3 grams, NaCl: 5grams, Agar: 20 grams and Distilled water: 1000ml. The medium was autoclaved at 121° C temperature, at 15 lbs pressure for 20minutes. After autoclaving the medium was allowed to cool and transferred into Petri-plates and allowed to solidify. 1ml of each dilution was transferred into respective petri plate and spreader on agar medium. The plates were incubated at 37°C for 24hrs and observed for growth [17]. The isolated colonies were sub-cultured on fresh NAM and sent for 16s rRNA analysis. 16s rRNA analysis of microorganisms was done and submitted to NCBI for sequence deposition and got accession numbers LC052333 and LC052631. The organisms were identified as *Pseudomonas stutzeri* and Bacillus *flexus.*

2.3 Conversion of Fish Waste into Liquid Fertilizer:

100grams of *Catla catla* fish waste, 100 grams of *Labeo rohita* fish waste, 100 grams of mixed fish waste was weighed and transferred into three 500ml conical flasks. Distilled water was added in 1:2 ratios with respect to fish waste to make it into slurry. Initial pH of Fish Waste fertilizer was recorded. The slurry was autoclaved at 121° C temperature, 15 lbs pressure for 15 minutes. Once autoclaving was done the fish slurry was allowed to cool. After cooling, bacterial culture was introduced into the conical flasks under aseptic conditions to the slurry containing conical flasks were put into shaking incubator at 45± 2° C, 75±2 rpm for 4 days. Final pH was taken after 96 hrs were recorded. After 96 hrs the fermented Fish Waste was filtered through Whatmann No.1 filter paper and supernatant was stored for further growth studies on semi-arid crops [18].

2.4 Plantation of Semi-Arid Crops:

Semi-arid crops of India like *Pennisetum glaucum (Pearl millet)* and *Trigonella foenum-graecum (Fenugreek*) were selected for the growth studies. These are the important semi- arid crops of India.

2.4.1 Plantation of *Pennisetum glaeucum* (*Pearl millet)*:

100mg of red soil was taken in small cups and to each cup seeds of *Pearl millet* were sown and grown for 30 days. Three replicates were maintained for each type of fertilizer. Cups with chemical fertilizer namely Saphal-19 were treated as control. Length of each leaf, shoot and root were measured after 5, 10, 15, 20, 25, 30 days [19].

2.4.2 Plantation of *Trigonella foenum- graceum (Fenu greek):*

100mg of red soil was taken in small cups and to each cup seeds of *Fenu greek* were sown and grown for 30 days. Three replicates were maintained for each type of fertilizer. Cups with chemical fertilizer 19-19-19 NPK were treated as control. Length of each leaf, shoot and root were measured after 5, 10, 15, 20, 25, 30 days.

2.5 Application of Fertilizers to Crops and Measurement of Growth:

Small pots were maintained for each type of crop, out of which triplicates were maintained for *Catla catla* fish waste fertilizer, triplicates were maintained for *Labeo rohita* fish waste fertilizer, triplicates were maintained for mixed fish waste fertilizer and triplicates were maintained for chemical fertilizer as control. 1ml of each type of fish waste fertilizers was transferred into respective cups. Length of leaf, shoot and root of *Pearl millet* and *Fenu greek* were measured with scale on 2^{nd}, 5^{th}, 10^{th}, 15^{th}, 20^{th}, 25^{th}, 30^{th} days were shown in Statistical analysis was done for this data by DMR test using SPSS software.

2.6 Statistical Analysis:

For statistical analyses, the SPSS Statistics Base software package (version 16) was used. Significant differences were evaluated using one-way ANOVA and Duncan's test at $P \leq 0.05$. All the reported data are the means of 3 replicates (n=3). Mean standard deviation and level of significance was calculated for data obtained. Data were analyzed using Duncan's multiple range (DMR) test. P values <0.05 were considered significant. All statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software (version 16.0).

3. RESULTS AND DISCUSSION

Bacteria which can degrade the fish waste meal were isolated and were sent for 16s rRNA analysis and were identified as *Bacillus flexus* and *Pseudomonas stutzeri.* These two isolates were used for conversion of fish waste solids into liquid organic fertilizer (Fig 1). The cultural characteristics of fish wastes were observed in 500ml flask for 96 h. pH steadily decreased (Fig 2). This drop in pH was also seen in other studies involving fish wastes [20-24]. The weight of the fish waste was decreased from 100 grams to 25 grams within 96hrs, which means that the suspended solid wastes were degraded by the isolated microorganisms. The fishy smell also decreased towards the end of degradation. A pleasant smell was also reported during fish meal waste water treatment [25-27].

Fig 1: The three different liquid fertilizers made out of three different fish wastes. **a.** liquid fish fertilizer made from fish waste of *Catla catla*. **b.** liquid fish fertilizer made from fish waste of *Labeo rohita* **c.** liquid fish fertilizer made from fish waste of mixed fish waste

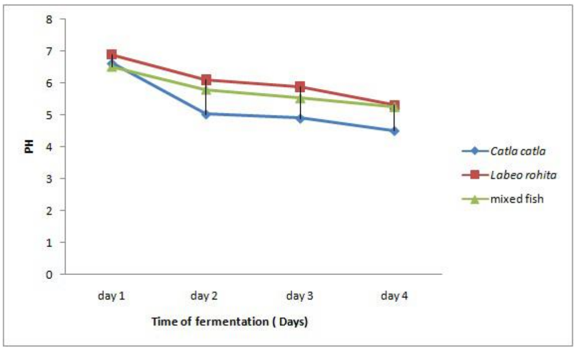

Fig 2: shows the graph of decline in the pH values of the different fish waste liquid fertilizers made out of *Catla catla, Labeo rohita* and mixed fish waste

In general fish waste is a rich source of minerals when compared to other animal wastes. The dry matter of different type of fish waste contains a large variety of macro and micro minerals with concentration of 6% Calcium, 2% of Phosphorous, 0.7% of Potassium, 0.6% of Sodium, 0.2% of Magnesium, 100 ppm of Iron, 62 ppm of Zinc, 6 ppm of Manganese, 1 ppm of Copper (Fig 3 and 4). Fertilizers formulated in appropriate concentrations and the combinations supply three main nutrients; nitrogen, phosphorous and potassium (N, P, K). Nitrogen promotes leaf growth and forms proteins and chlorophyll [28-30]. Phosphorous contributes to root, flower and fruit development. Potassium contributes to stem and root growth and the synthesis of proteins [31]. Chemical fertilizers are harmful to mankind when we intake them and also they are environmentally hazardous. Since the biodegraded broth of fish waste contained compounds potentially useful for plant growth, it could be used as a fertilizer [32-36].

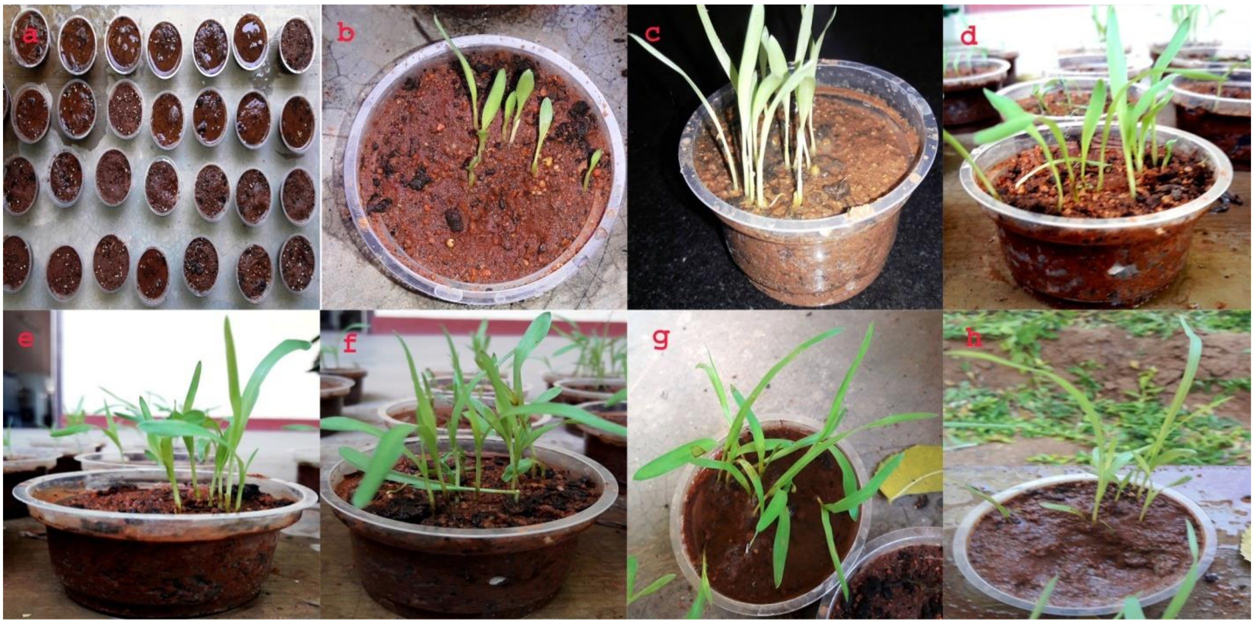

Fig 3: Growth of *Pearl millet* plants. **a.** growth of *Pearl millet* plant on day 0 **b**. growth of *Pearl millet* plant on 2nd day, **c.** growth of *Pearl millet* plant on 5th day, **d.** growth of *Pearl millet* plant on 10th day, **e.** growth of *Pearl millet* plant on 15th day, **f.** growth of *Pearl millet* plant on 20th day, **g.** growth of *Pearl millet* plant on 25th day, **h.** growth of *Pearl millet* plant on 30th day

Fig 4: Growth of *Fenu greek* plants. **a.** growth of *Fenu greek* plant on day 0 **b**. growth of *Fenu greek* plant on 2nd day, **c.** growth of *Fenu greek* plant on 5th day, **d.** growth of *Fenu greek* plant on 10th day, **e.** growth of *Fenu greek* plant on 15th day, **f.** growth of *Fenu greek* plant on 20th day, **g.** growth of *Fenu greek* plant on 25th day, **h.** growth of *Fenu greek* plant on 30th day

Measurement of Plant Growth:

The data of leaf shoot and root length of *Pearl millet* and *Fenu greek* were collected at every 5 days interval of time i.e., initial length was taken on 2nd day of growth of sprouted seeds. Length of leaf, shoot and root of *Pearl millet* (F**ig 5**) and *Fenu greek* were collected on 5th, 10th, 15th, 20th, 25th, 30th day.(F**ig 6**) DMR (Duncan's mean range) test (P < 0.05) was done for the data of parameters. Graphs were drawn for the data individually for leaf, shoot and root of *Pearl millet* and *Fenu greek* comparing the growth for all 30 days (**Fig 7**).

Fig 5: Growth of *Pearl millet* in different time intervals. **a.** length of control *Pearl millet* plant **b.** length of *Pearl millet* plant on day 2. **c.** length of *Pearl millet* plant on day 5. **d.** length of Pearl *millet* plant on day 10. **e.** length of *Pearl millet* plant on day 15. **f.** length of Pearl *millet* plant on day 20. **g.** length of *Pearl millet* plant on day 25. **h.** length of *Pearl millet* plant on day 30.

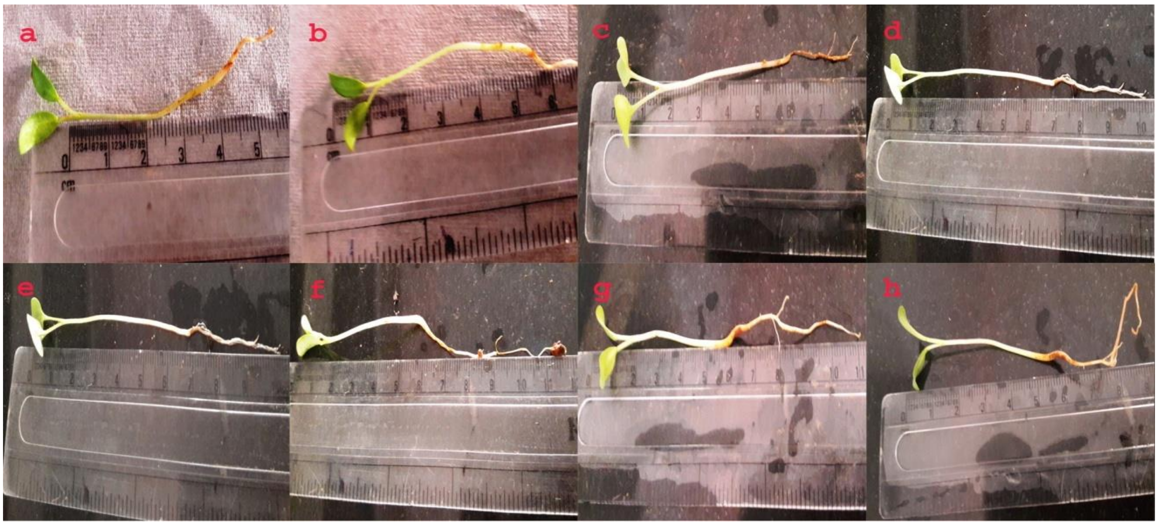

Fig 6: a. length of control *Fenu greek* plant. **b.** length of *Fenu greek* plant on day 2. **c.** length of *Fenu greek* plant on day 5. **d.** length of *Fenu greek* plant on day 10. **e**. length of *Fenu greek* plant on day 15**. f.** length of *Fenu greek* plant on day 20**. g.** length of *Fenu greek* plant on day 25. **h.** length of *Fenu greek* plant on day 30

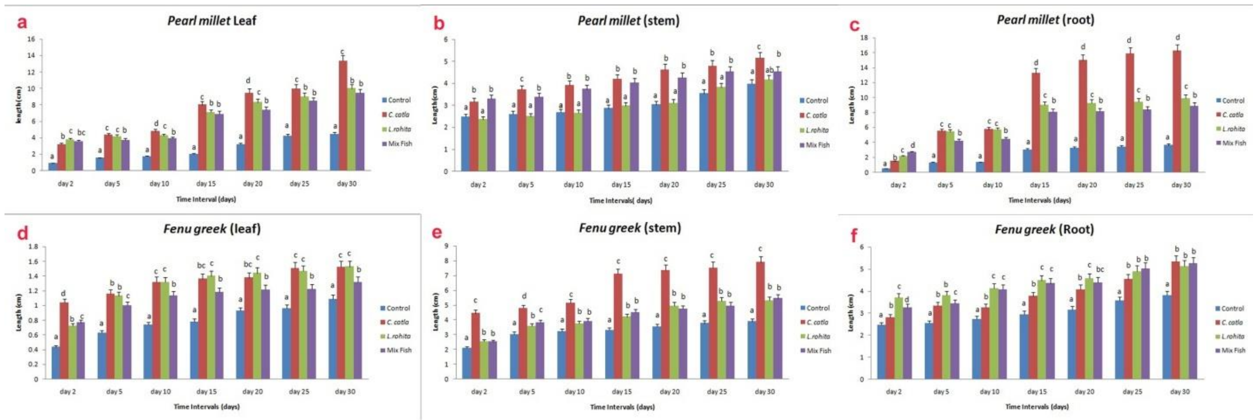

Fig 7: a. graph shows comparative studies of *Pearl millet* leaf in different time intervals. **b.** graph shows comparative studies of *Pearl millet* stem in different time intervals. **c.** graph shows comparative studies of *Pearl millet* root in different time intervals. **d.** graph shows comparative studies of *Fenu greek* leaf in different time intervals. **e.** graph shows comparative studies of *Fenu greek* stem in different time intervals. **f.** graph shows comparative studies of *Fenu greek* root in different time intervals.

4. CONCLUSION

Conversion of fish waste into organic liquid fertilizer and its effect on plant growth has been demonstrated in the present study. Bacteria which can degrade the fish waste were isolated and were analyzed, identified as *Bacillus flexus* and *Pseudomonas stutzeri. Pennisetum glaucum and Trigonella foenum- graecum* crops growth was tested with these fertilizers for the growth studies confirmed. However scale up, preservation and cost effectiveness of this fertilizer still have to be demonstrated.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

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