

Fish waste valorisation as biofertilizer: Impact on soil fertility and radish (*Raphanus sativus*) growth

Thendral Hepsibha Balraj*, Dayana Kuppani and Tharani Gopalakrishnan

Department of Biochemistry, Ethiraj College for Women, Chennai, Tamil Nadu, INDIA

*thendralhepsibha_b@ethirajcollege.edu.in

Abstract

The concept of the circular bioeconomy has enabled the conversion of fish waste into a valuable biofertilizer. By using this organic biofertilizer, the harm that chemical fertilizers do to the soil, human health and food security may be mitigated. Because of its high vitamin and nutrient content, Raphanus sativus (Radish; Brassicaceae) is consumed worldwide; yet, for a healthy yield, it needs the right fertilizer. This study attempted to analyse the effect of fermented fish waste biofertilizer (FFWB) on soil properties and growth of radish. The pot experiment has three treatments with 5 replications: Group – I (water control); Group – II (Chemical fertilizer NPK (120-65-100 kg/ha); Group – III (1:100 diluted FFWB).

Among different treatments, FFWB fertilisation has demonstrated significant improvements in soil N, P, K, Ca, Mg, Mn, Zn, S, Cu, Fe, B and CEC on par with chemical fertilizer. Soil microbial analysis indicated that the population of all bacteria, yeast and mold, as well as nitrogen fixers and phosphate solubilizers, improved under the FFWB treatment. A significant improvement in growth traits of radish like plant height, leaf number, leaf area, root length, root diameter and root weight were evidenced in FFWB treatment. The findings indicated that the FFWB treatment has enhanced soil fertility and radish growth. Therefore, chemical fertilizers can be replaced with biofertilizer made from fermented fish waste. As a result, it is possible to develop sustainable organic agricultural practices and recover degraded soil fertility.

Keywords: Fermented fish waste, biofertilizer, soil fertility, radish (*Raphanus sativus*), soil microbes, growth traits.

Introduction

There is an intimidating challenge to feed the growing global population of 9 billion by 2050, along with a concern for food security and food sustainability. With the compounded rise in the middle-class group, increased spending power, concern for a healthy diet and increasing food demand, aquaculture will be a promising field. Aquaculture has made significant progress and is still growing with its sustainable credentials, like greater availability of nutrient-dense marine foods, species diversity, higher revenue and improved employability. Sea foods were preferred over terrestrial

animals due to the superior quality of their protein. FAO²⁰ has reported that global seafood production is 214 million metric tons. Among the sea foods, fish has received considerable attention for its nutritional value and role in ensuring food security.

Fish is a healthy, readily available and affordable food for all groups of people. Fish is a rich source of protein, together with all essential amino acids, long-chain omega-3 fatty acids, vitamins (A, B and D) and minerals (Ca, I, Fe, Zn and Se). Healthy and balanced diet concerns have shifted consumer preferences from red meat to poultry and fish⁴⁸. Global fish consumption is currently 20.4 kg per person and is predicted to rise to 21.2 kg per person by 2032, with Asia having the largest per capita consumption²⁷. With a 7.96% share of the world's fish production, India ranks third in the world. By 2029, 200 million metric tons of fish were predicted to be produced worldwide⁶⁷. According to Hou et al²⁶, 70% of fish that are captured are processed before being sold and every year, around 27 million tons of undesired fish are thrown into the ocean¹⁵. The nutritive value of fish by-products was similar in composition to fish fillets and fish products consumed¹⁵.

Fish waste disposal in landfills produces greenhouse gasses, attracts predators and has a detrimental impact on the environment's health⁴⁵. Additionally, surface slicks (a rise in organic content), oxygen demand and turbidity plumes are caused by marine disposal⁶⁹. Achieving the inflow of organic matter and nutrients from waste and handling fish waste in an eco-friendly and effective manner currently depend on the shift from a linear to a circular economy. The demand for organic and natural biofertilizers for sustainable agricultural farming has resulted in the valorization of fish waste into a value-added fertilizer.

Fish waste can be naturally fermented by adding fish offal comprising of gut portions, muscles, frames and heads along with jaggery⁶⁴ and by utilizing the enzymes available in fish viscera. Enzymes like collagenase, chymotrypsin, pepsin and trypsin are commercially extracted from fish viscera and they are active and stable in a wide pH range^{13,32,70}. Natural fermentation is always advantageous as it uses the enzymes available in the fish parts and does not use commercial enzymes or chemicals and the carbohydrate source (jaggery) added is not expensive.

So, processing by natural fermentation is always an eco-friendly and cost-effective technique. The liquid form of fish biofertilizer was more preferred as it was easily absorbed, readily available and applied as a foliar spray. Liquid-fish-

based biofertilizers have demonstrated promising growth potential in tomatoes⁴, beans²⁸ and chili¹⁷, brinjal⁵⁹ and mung bean⁵⁷.

Radish (*Raphanus sativus* L.) is a tender cruciferous root vegetable that is consumed worldwide for its rich vitamin and nutrient values. Radish extracts have been used since ancient times to treat urinary tract infections, stomach disorders, liver and heart problems and ulcers²⁴. Radishes for their highest yield require optimum nutrients and soil richness. Continuous application of chemical fertilizer without a check on soil status has led to low nutrient use efficiency and low productivity. The beneficial effect of organic manures like farmyard manure, vermicompost, poultry, chicken and pig manure has yielded better quality radish. Hence, this present study aimed to investigate the effect of naturally fermented fish waste biofertilizer (FFWB) on soil fertility and growth of radish.

Material and Methods

Preparation of fermented fish waste biofertilizer (FFWB)⁶⁴: Fish wastes (head, organs, intestines, fins with muscles) collected from the local fish market in Vanagaram, Chennai, Tamil Nadu, regardless of the species. Fish waste (1 kg) was cut into small pieces and minced coarsely using a mechanical grinder. The content was transferred to a plastic container and 1.5 kg of powdered jaggery along with 5 lits of water was added. The mouth of the container was closed with a muslin cloth to avoid the entry of flies and other contamination. The contents were mixed well daily and kept in the shade. After 3rd day, the aromatic smell of alcohol produced confirmed the start of fermentation. After 15 days, the contents were filtered and the filtrate was used as biofertilizer in 1:100 dilution.

Experimental design: Each green bag (pot) was filled with 20 kg of soil. Pot (green bag) experiments were conducted with 3 treatments and each treatment was replicated five times. Group I: Water (No Chemical fertilizer / Fermented fish waste biofertilizer); Group II: Chemical fertilizer (NPK and 120-65-100 kg/ ha (i.e. 2.609 g urea + 3.421 g single super phosphate (SSP) + 2.00 g Sulphate of potash (SOP) / pot⁴⁴); Group III: Fermented fish waste biofertilizer (FFWB) (1: 100 dilution). Urea was applied in two splits 60% as basal application and 40% after a month, 100% of SSP and SOP as single basal application. Fermented fish waste biofertilizer was added as basal application and in 15-day intervals. *Raphanus sativus* L. seeds were procured from Tamil Nadu Agricultural University Extension, Agricultural seed store, Annanagar. Nearly 2 seeds per pot were sown by the hand dibbling method at 1.25 cm to facilitate good root growth. Watering and manual weeding were done as required. Throughout the study, the experimental plants received water and FFWB as recommended.

Physico-chemical parameters of the soil: Soil samples were collected from 3 pots in each treatment from the 0-15 cm plow layer after radish (*Raphanus sativus*) harvest. The

samples were processed for physio-chemical properties and biological analysis. Soil pH, electrical conductivity, organic matter⁶⁵, available nitrogen⁵⁵, available phosphorus⁴⁹, available potassium³⁰, exchangeable Ca and Mg⁵⁶, available S⁵⁴, soil available nutrients (Cu, Fe, Zn)^{37,44} and CEC¹⁰ were determined.

Soil microbial load: The different microorganisms in the soil were enumerated by using specific culture media and conditions: Thornton's agar medium for total bacterial count²⁵ incubated at 37°C ± 1°C for 2 days; Rhizobium identified by yeast mannitol agar with congo red 28 ± 3°C for 2 - 5 days⁶⁰; Azospirillum using azospirillum medium without agar (twin pack) incubated at 36 ± 1°C for 3 - 4 days⁶³; Pseudomonas using King's B medium incubated at 28 ± 1°C for 12 days³³; Jensen's medium for azotobacter incubated at 28 ± 3°C for 4 - 6 days³¹; Martin Rose medium for fungi and yeast incubated at 26 ± 1°C for 5 days in dark⁴¹; Pikovskaya's medium for phosphobacteria⁵⁰ incubated at 28 ± 2°C for 2 weeks. The experiments were done in triplicate and microbial populations were expressed as colony-forming units (CFU) / g soil.

Morphological assessment: Growth and yield parameters of radish (*Raphanus sativus*) like leaf area (length (cm) * breadth (cm)) and number of leaves per plant, plant height (cm), root length (cm), root diameter (cm) and fresh root weight (g) (n=10) were measured after 40 days of germination. Plant height was measured from the base of the radish tuber to the tip of the youngest leaf. The fresh weight of the radish tuber was measured after removing the adhering soil by washing it in water and blotting it with tissue paper. Leaf length was measured from the base to the leaf tip. Leaf and radish width was measured at the widest part. Radish length was measured from the tip to the base of the crown region.

Statistical analysis: Data collected were expressed as mean ± SEM. One-way Analysis of Variance (ANOVA) was performed to assess the statistical significance followed by Tukey's multiple comparison tests using computerized Graph Pad Prism version 5.0, Software package (Graph Pad Software Inc.; San Diego, CA, U.S.A.). p value < 0.05 was considered as statistically significant.

Results and Discussion

Soil properties influenced by FFWB: Plants rely on soil for a critical supply of nutrients. Soil nutrition and crop nutrition are closely linked. Soil is a healthy ecosystem that supports life by transforming and recycling the essential elements of plants, animals and humans in the food chain.

The complex interplay between the physical structures and chemical and biological features of the soil controls and influences the flow of nutrients and the uptake of those nutrients by the plants. The primary factors influencing soil microbial biomass are pH and soil organic carbon.

Table 1
Effect of FFWB on soil fertility status

Parameter	Group I (Water)	Group II (Chemical fertilizer – NPK)	Group III (FFWB) (1:100 dilution)
pH	8.10 ± 0.10	8.00 ± 0.15 ^{NS}	7.8 ± 0.12 ^{NS}
EC (mS/cm)	0.07 ± 0.07	0.15 ± 0.01**	0.16 ± 0.01**
Organic matter (%)	0.69 ± 0.01	0.98 ± 0.02**	1.23 ± 0.03**
Available nitrate Nitrogen (mg/kg)	15.47 ± 0.62	25.05 ± 0.06***	29.36 ± 0.37***
Available Phosphorus (mg/kg)	11.82 ± 0.09	39.54 ± 0.61***	41.67 ± 0.71***
exchangeable Potassium (mg/kg)	96.33 ± 0.88	430.00219 ± 4.93***	528 ± 5.29***
Calcium Exchangeable (ppm)	1106 ± 51.47	1349 ± 25.56**	1864.33 ± 25.1***
Magnesium Exchangeable (ppm)	155 ± 4.58	222.67 ± 5.36***	231 ± 6.35***
Sulphur Exchangeable (ppm)	20.30 ± 0.87	28.73 ± 0.43***	30.77 ± 0.30***
Zinc available (ppm)	2.63 ± 0.32	3.18 ± 0.13 ^{NS}	4.66 ± 0.19**
Manganese available (ppm)	9.86 ± 0.30	18.94 ± 0.14***	21.01 ± 0.15***
Iron available (ppm)	12.67 ± 1.20	16.90 ± 0.06 *	19.59 ± 0.97**
Copper available (ppm)	2.10 ± 0.05	3.10 ± 0.07*	3.77 ± 0.31**
Boron available (ppm)	0.50 ± 0.06	0.80 ± 0.06*	0.80 ± 0.06*
CEC Meq/100g	10.83 ± 0.02	11.33 ± 0.18*	13.01 ± 0.03***

Values are expressed as mean ± S.E.M (n=10). ANOVA followed by Tukey's multiple comparison tests was used to analyse the data. Statistically significant variations are expressed as *p < 0.05, **p < 0.01, *** p < 0.001, NS- non-significant. Group I vs Group II and Group III.

The addition of biofertilizer made from fermented fish waste in this study has dynamically altered the physical, chemical and biological characteristics of the soil. The soil properties, pH, EC, organic matter, nitrate- N, P, K, Ca, Mg, S, Zn, Fe, Cu, B and CEC influenced by chemical fertilizer and fermented fish waste biofertilizer amendment were presented in table 1. The lowest pH (7.8 ± 0.12) was recorded in FFWB treatment though not significant, a 3.7% reduction was observed than control. The electrical conductivity (EC) of the soil was significantly increased (p<0.01) in both chemical fertilizer and FFWB treatments in comparison to the control. The highest soil organic matter (SOM) content was recorded in group III (1.23 ± 0.03 %; p<0.01) followed by group II (0.98 ± 0.02 %; p<0.05). The lowest SOM was recorded in the control group (0.69 ± 0.01 %).

The treatments using chemical fertilizer (p<0.001) and organic biofertilizer (FFWB) had more available nitrate nitrogen, potassium and phosphorus than the control (p<0.001). Group - II soil had exchangeable calcium levels of 1349 ± 25.56 mg kg⁻¹ (p<0.01). The FFWB treatment had the highest exchangeable magnesium content (231 ± 6.35 mg kg⁻¹) (p<0.001). The control soil had the lowest amount of accessible sulfur (20.30 ± 0.87 mg kg⁻¹). Comparing the application of FFWB to the control, the available Zn, Mn, Fe and Cu increased by 77%, 114.08%, 54.61% and 79.52 % respectively. Zn, Mn, Fe and Cu levels have increased by 20.9%, 92.98%, 33.38% and 47.61 % respectively, following chemical fertilizer application compared to the control.

The boron (B) content has increased by 60% (0.80 ± 0.06 mg kg⁻¹ soil) compared to the control group with both FFWB and

chemical treatment. The cation exchange capacity trended as follows under different treatments: control (10.83 ± 0.08 meq 100 g⁻¹ soil) < chemical fertilizer (11.33 ± 0.18 meq 100 g⁻¹ soil; p<0.05) < FFWB (13.01 ± 0.03 meq 100 g⁻¹ soil; p<0.001). The reduction in pH in FFWB treatment was due to the acidic nature of the biofertilizer⁵⁸ and it might also be attributed to CO₂, the aliphatic hydroxyl-organic acids and aromatic acids released by the decomposition of organic matter by microbes^{43,53}. The acidic pH of FFWB will be a suitable additive that suits radish (*Raphanus sativus*) cultivation⁴².

This was due to the increase in organic carbon content and decrease in soil density in group III which has increased soil porosity, water filtration and water holding capacity. As a result, an improvement in soil moisture was achieved. This soil condition, due to FFWB, provides suitable conditions for nutrient transformation and nutrient availability influenced by soil microbes and thus improves soil quality. Wang et al⁶⁶ have reported similar improvements in soil properties and maize yield when ox manure was applied. In Group - II, the application of chemical fertilizer has led to soil compaction as a result impedes water penetration, moisture content and hence less soil nutrient status.

The increase in nitrate form of N₂ in FFWB treatment was due to the fractions of N present in FFWB and microbial-mediated transformation of organic to inorganic form. The organic acids produced during microbial metabolism solubilize the insoluble phosphate and potassium⁶⁸. The sesquioxide layer produced by organic matter reduces P and K binding and thus makes these nutrients available in the soil³⁵.

An increment in NPK soil content was observed when farm yard manure was added for pearl millet-wheat crops³⁴ and vermicompost in onion and okra⁵². The high Fe content might be contributed by jaggery and fish wastes, sources of iron in FFWB. The increment in Zn content in group III was due to the addition of Zn to the soil present in FFWB and also due to the biochemical process that makes inaccessible zinc available to plants. Organic matter forms complexes with metals thereby reducing the fixation, adsorption and precipitation of micronutrients and making them available¹⁸. Zn and Cu easily form complexes with organic matter that cannot be adsorbed or migrated and are found easily available³⁸.

The overall improvement in soil macronutrient and micronutrient availability was due to the synergistic effect of added liquid FFWB and microbial metabolic effects. The effect of liquid biofertilizers (panchagavya and jeevamrut) along with organic manure has improved the soil nutrient status and brinjal yield⁵¹.

Soil Microbiology: The effects of chemical fertilizer and FFWB on viable microbial counts are shown in table 2. A significant improvement in total bacterial count was noted in group III (FFWB treatment) ($52.67 \pm 2.72 \times 10^5$ CFU/gm soil; $p < 0.001$) and group II (Chemical fertilizer) ($42.62 \pm 1.76 \times 10^5$ CFU/gm soil; $p < 0.01$) (Table 2). The yeast and mould count were recorded in the following order FFWB > Chemical fertilizer > Water control. The highest rhizobium and azotobacter counts were recorded under FFWB treatment ($p < 0.001$). A significant increase in phosphobacteria count was observed only in FFWB

treatment. The lowest count of pseudomonas fluorescens was noted in water treatment ($10.66 \pm 0.88 \times 10^5$ CFU /gm soil).

The increased microbial population of N_2 fixers (Rhizobium, Azotobacter, Azospirillum), phospho bacteria and pseudomonas, yeast and mold (Table 2) in group III was due to the increase in soil organic carbon level and alkaline pH when compared to chemical fertilizer. The increase in rhizobacterial count in group III (FFWB) confirms that, their endurance in the rhizosphere will help radish to gain the plant growth-promoting potential.

Naveed et al⁴⁷ and Bellabarba et al⁹ have reported that consortia of bacteria and fungi as biofertilizer inoculants will improve the growth of plants than a single inoculant. FAO²¹ has defined biofertilizer as a product with one or more microorganisms either in a live or dormant state²¹. Biofertilizer, when amended to soil, will help to solubilize or mobilize the nutrients and will increase the nutrient utilization efficacy of plants. In par with this definition, in the present study, the biofertilizer prepared from fish waste was reported to have a consortium of bacterial population (nitrogen fixers and phosphate solubilizers) along with fungi and also has a load of nutrients required for plant growth⁵⁸.

So, this could serve as a natural biofertilizer thereby eliminating the cost of commercial microbial formulations. The addition of fermented fish waste biofertilizer adds organic matter and minerals to the soil and provides conditions for the proliferation of its microbes and native soil microbes.

Table 2
Soil microbial population influenced by FFWB

Group	Group I (Water)	Group II (Chemical fertilizer – NPK)	Group III (FFWB) (1:100 dilution)
Total bacterial load (CFU/ml) $\times 10^5$	28.33 ± 0.88	$42.66 \pm 1.76^{**}$	$52.67 \pm 2.72^{***}$
Yeast and Mold (CFU/ml) $\times 10^4$	5 ± 0.57	7.66 ± 0.78^{NS}	8.33 ± 0.80^{NS}
Rhizobacterial load (CFU/ml) $\times 10^5$	12 ± 0.57	$22.33 \pm 2.02^{**}$	$29.33 \pm 1.45^{***}$
Azospirillum (CFU/ml) $\times 10^4$	1.66 ± 0.32	$4.66 \pm 0.53^{**}$	2.00 ± 0.21^{NS}
Phosphobacteria (CFU/ml) $\times 10^4$	4.33 ± 0.88	6.33 ± 0.60^{NS}	$11 \pm 0.57^{**}$
Azotobacter (CFU/ml) $\times 10^5$	8.33 ± 1.20	$21.33 \pm 2.08^{**}$	$28.33 \pm 1.20^{***}$
<i>Pseudomonas fluorescens</i> (CFU/ml) $\times 10^5$	10.66 ± 0.88	$16.66 \pm 1.20^*$	$20 \pm 1.15^{**}$

Values are expressed as mean \pm S.E.M (n=3). ANOVA followed by Tukey's multiple comparison tests was used to analyse the data. Statistically significant variations are expressed as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS- non-significant. Group I vs Group II and Group III.

The results obtained by the addition of biofertilizers like panchagavya and jeevamrut are in line with the current results and their effects on paddy, maize and beans were evidenced^{5,62}. Rhizobia promotes plant growth through several mechanisms like fixing up of nitrogen, solubilizing phosphates, producing plant hormones (cytokinin, auxin, gibberellins) and protecting the plants from stress, pathogens and systemic resistance^{8,61}.

Co-inoculation with rhizobium and another PGPR has improved the nodulation and nitrogen content of *Phaseolus vulgaris*²² and rhizobium–bacillus has improved nodulation and root morphology of soybean, pigeon pea and bean⁹, Bradyrhizobium, on dry matter of radish³. Pseudomonas, azotobacter and azospirillum are highly efficient extracellular PGPR¹. Pseudomonas promotes plant growth by fixing up nitrogen, solubilizing potassium and phosphate, phytohormones and controlling diseases by producing organic acids, lytic enzymes, antibiotics and secondary metabolites^{16,19}.

As most of the agricultural lands are deficient in nitrogen and phosphorus, this novel approach of amending FFWB, a polymicrobial biofertilizer will benefit the agricultural sector, as an eco-friendly substitute for chemical fertilizer which could reduce the use of inorganic fertilizers or pesticides. Bargaz et al⁷ have also confirmed the positive effect of polymicrobial inoculation on agricultural production. In the present study, the presence of a microbial consortium of rhizobium, azospirillum, phosphobacteria, azotobacter, pseudomonas fluorescens, yeast and mold in soil will surely increase and supplement each other's efficacy in improving soil fertility.

Growth traits of radish: The results revealed that application of recommended chemical fertilizer (49.75 ± 1.72 cm; $p < 0.001$) and FFWB (45.56 ± 1.56 cm; $p < 0.001$) have resulted in significant tallest radish (*Raphanus sativus*)

plants (Table 3; Fig. 1 and Fig. 2) when compared with control (21.18 ± 2.04 cm). A similar trend was obtained with the length of radish as control < chemical fertilizer < FFWB. The increment in plant height and root length might be due to the availability of N, P and K along with micronutrients which have enhanced the assimilation of protoplasm leading to a greater number of cells and tissues, increased cell division and vigor of radish.

The addition of FFWB has resulted in improved soil nutrient status incremented by soil microbial activity which has made the nutrients easily available and absorbable. The macronutrient nitrogen plays an important role in cell expansion, synthesis of enzymes, chlorophyll and enzymes and increases the root length. The present results are incongruent with the findings of Kushwah et al³⁶ who observed an improvement in radish (*Raphanus sativus*) growth after the addition of organic manure and biofertilizers.

Radish requires a higher amount of nitrogen with a constant amount of P and K as it is a fast-growing vegetable⁶. Phosphorus plays an indirect role in increasing the ability of the plant to absorb nitrogen N². K is essential to induce taproot formation and for cell expansion, photosynthesis, translocation and protein synthesis^{12,29}. The root structure of rice was improved by the addition of Azospirillum, Azotobacter and Rhizobium biofertilizers¹⁴. In the present study, amended FFWB adds all essential nutrients and it enhances the activity of native rhizomicrobes facilitating radish (*Raphanus sativus*) growth.

A maximum number of leaves and leaf areas were observed in FFWB and chemical treatment (Table 3 and Fig. 2 and Fig. 3) with the least count in control. This increase in leaf area and leaf number was mainly due to the nitrogen supplied as inorganic NPK in group II and attributed by FFWB in group III.

Table 3
Effect of FFWB and chemical fertilizer on plant height, leaf count and leaf area of *Raphanus sativus*

Group	Plant height (cm)	No. of leaves	Leaf area (cm ²)
Group – I (Water control)	21.18 ± 2.04	7.28 ± 0.5	139.27 ± 2.36
Group - II Chemical Fertilizer (NPK)	$49.75 \pm 1.72^{***}$	$12.57 \pm 0.30^{***}$	$167 \pm 5.7^{***}$
Group – III (1:100 diluted FFWB)	$45.56 \pm 1.56^{***}$	$12.42 \pm 0.4^{***}$	$161.25 \pm 2.03^{***}$

Values are expressed as mean \pm S.E.M (n=10). ANOVA followed by Tukey's multiple comparison tests was used to analyse the data. Statistically significant variations are expressed as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS- non-significant. Group I vs Group II and Group III.

Table 4
Radish root parameters influenced by FFWB

Group	Radish length (cm)	Radish Diameter (cm)	Radish weight (gm)
Group – I (Water control)	11.25 ± 1.32	2.9 ± 0.31	97.22 ± 9.6
Group - II Chemical Fertilizer (NPK)	$22 \pm 1.21^{***}$	$4.17 \pm 0.30^*$	$198.33 \pm 19.77^{***}$
Group – III (1:100 diluted FFWB)	$23 \pm 1.52^{***}$	$4.09 \pm 0.36^*$	$199.29 \pm 15.96^{***}$

Values are expressed as mean \pm S.E.M (n=10). ANOVA followed by Tukey's multiple comparison tests was used to analyse the data. Statistically significant variations are expressed as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS- non-significant. Group I vs Group II and Group III.



Group - I (water control)

Group - II (NPK)

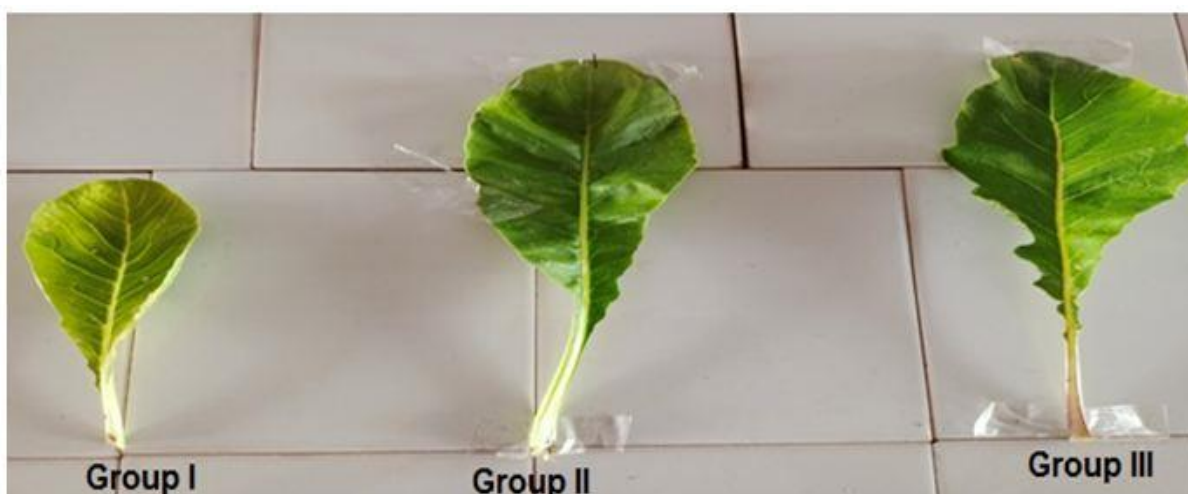
Group - III (1:100 FFWB)

Figure 1: Growth of *Raphanus sativus* on Day 30 under various treatments

Group I

Group II

Group III

Figure 2: Length of *Raphanus sativus* influenced by FFWB

Group I

Group II

Group III

Figure 3: Leaf area of *Raphanus sativus* on Day 40 under various treatments

Nitrogen is a vital component of DNA, amino acids, proteins, coenzymes, phytohormones and alkaloids, promotes cell growth, cell division and cell expansion.

This might have resulted in increased leaf area. Along with the nutrient N, soil microbes produce and promote the activity of growth-promoting substances, making the nutrients available and leading to an increase in leaf area. The findings made by Nandish et al⁴⁶ were consistent with the aforementioned results when chicken manure and biofertilizer consortium of azotobacter, phosphate and potassium solubilizers were added with NPK. Potassium also leads to an increase in leaf area and plant growth²³.

The observed increase in leaf area of radish (*Raphanus sativus*) in FFWB treatment might also contribute to the increase in radish weight as they distribute the photosynthates to the roots. Mahsa et al³⁹ observed an increase in the fresh weight of radish using tea waste compost due to an increase in leaf area.

Significant improvements in root attributes like root length, root weight and root diameter were noted in group II (NPK) and group III (Table 4; Fig. 2) while the control group recorded the minimum root weight and diameter. The addition of FFWB has shown improvement in soil organic matter; hence, an increase in porosity and water-holding capacity and a decrease in bulk density have contributed to the space and volume of radish (*Raphanus sativus*) roots. Bloom¹¹ has demonstrated the notable influence of nitrogen on cytokinin production which increases the cell elasticity, cell growth and number of meristematic cells. An increase in metabolic activities and auxin synthesis will also increase the root length, weight and yield.

Radish (*Raphanus sativus*) root being the storage organ the increase in weight would be the result of nitrogen assimilated in the amino acids which combine to form complex proteins responsible for the better growth of radish. Mali et al⁴⁰ got similar results in radish length and weight when phosphate-solubilizing bacteria and vermicompost were added.

Conclusion

This study demonstrated that the strategy of sustainable fish waste management and utilization for biofertilizer preparation achieves a circular bioeconomy with the nutrient influx and prevents the negative impact on the environment and human health. The results of the conducted study confirm that fermented fish waste biofertilizer improves the soil nutrient status and increases the soil microbial population. Application of fermented fish waste biofertilizer has shown a significant improvement in the growth traits of Radish (*Raphanus sativus*) on par with chemical fertilizer.

Hence, the burden caused by chemical fertilizer on health and the environment could be reduced or removed by the application of fermented fish waste. This is an economical

and eco-friendly approach towards an alternative sustainable agricultural practice to ensure food security and availability.

References

- Ahemad M. and Kibret M., Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective, *Journal of King Saud University - Science*, **26**, 1–20 (2014)
- Anasrullah A., Sajjaphan K., Rattanapichai W., Kasemsap P., Nouvellon Y., Chura D. and Chotiphan R., The Dynamics of Immature Rubber Photosynthetic Capacities under Macronutrients Deficiencies, *Trends in Sciences*, **20**(5), 4527–4527 (2023)
- Antoun Rani, Chantal J. Beauchamp, Nadia Goussard, Rock Chabot and Roger Lalande, Potential of Rhizobium and Bradyrhizobium species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.), *Plant and Soil*, **204**, 57–67 (1998)
- Aranganathan L. and Rajasree R.S.R., Bioconversion of marine trash fish (MTF) to organic liquid fertilizer for effective solid waste management and its efficacy on tomato growth, *Management of Environmental Quality*, **27**, 93–103 (2016)
- Aulakh C.S., Singh H., Walia S.S., Phutela R.P. and Singh G., Evaluation of microbial culture (jeevamrit) preparation and its effect on productivity of field crops, *Indian Journal of Agronomy*, **58**, 182–186 (2013)
- Baloch P.A., Uddin R., Nizami F.R., Solan A.H. and Siddiqui A.A., Effect of nitrogen, phosphorus and potassium fertilizers on growth and yield characteristics of radish (*Raphanus sativus* L.), *Environmental Science*, **14**(6), 565–569 (2014)
- Bargaz A., Lyamlouli K., Chtouki M., Zeroual Y. and Dhiba D., Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system, *Frontiers in Microbiology*, **9**, 1606 (2018)
- Barquero M., Poveda J., Laureano-Marín A.M., Ortiz-Liebana N., Brañas J. and González-Andrés F., Mechanisms involved in drought stress tolerance triggered by rhizobia strains in wheat, *Frontiers in Plant Science*, **13**, 1036973 (2022)
- Bellabarba A., Fagorzi C., Diczko G.C., Pini F., Viti C. and Checcucci A., Deciphering the Symbiotic Plant Microbiome: Translating the Most Recent Discoveries on Rhizobia for the Improvement of Agricultural Practices in Metal-Contaminated and High Saline Lands, *Agronomy*, **9**, 529 (2019)
- Berger K.C. and Truog E., Boron determination in Soils and Plants, *Industrial and Engineering Chemistry Analytical Edition*, **11**, 540–545 (1939)
- Bloom A.J., Influence of Inorganic Nitrogen and pH on the elongation of maize seminal roots, *Annals of Botany*, **97**(5), 867–873 (2006)
- Brintha I. and Seran T.H., Effect of paired row planting of radish (*Raphanus sativus* L.) intercropped with vegetable amaranthus (*Amaranthus tricolor* L.) on yield components of radish in sandy regosol, *Journal of Agricultural Sciences*, **4**(1), 19–28 (2009)

13. Byun H.G., Kim Y.T., Park P.J., Lin X. and Kim S.K., Chitooligosaccharides as a novel β -secretase inhibitor, *Carbohydrate Polymers*, **61**, 198-202 (2005)
14. Choudhury M.A. and Kennedy I.R., Prospects and potentials for system of biological nitrogen fixation in sustainable rice production, *Biology and Fertility of Soils*, **39**, 219–227 (2004)
15. Coppola D. et al, Fish Waste: From Problem to Valuable Resource, *Marine Drugs*, **19**, 116 (2021)
16. Da Silva Faccioli Y.E., Da Silva G.O., Da Silva R.D. and Sarubbo L.A., Application of a biosurfactant from *Pseudomonas cepacia* CCT 6659 in bioremediation and metallic corrosion inhibition processes, *Journal of Biotechnology*, **351**, 109–121 (2022)
17. Deore G.B., Limaye A.S., Shinde B.M. and Laware S.L., Effect of Novel Organic Liquid Fertilizer on Growth and Yield in Chilli (*L.*) *Capsicum annum*, *Asian Journal of Experimental Biological Sciences*, **1**, 15-19 (2010)
18. Dhaliwal S.S., Naresh R.K., Mandal B.A., Singh R. and Dhaliwal M.K., Dynamics and transformations of micronutrients in agricultural soils as influenced by organic matter build-up: A review, *Environmental and Sustainability Indicators*, **1–2**, 100007 (2019)
19. Dimkic I., Janakiev T., Petrovic M., Degrassi G. and Fira D., Plant-associated *Bacillus* and *Pseudomonas* antimicrobial activities in plant disease suppression via biological control mechanisms-A review, *Physiological and Molecular Plant Pathology*, **117**, 101754 (2022)
20. FAO, The state of world fisheries and aquaculture, 1-266 (2022)
21. FAO, The international Code of Conduct for the sustainable use and management of fertilizers, Rome, Italy, FAO, 56, <https://doi.org/10.4060/CA5253EN> (2019)
22. Figueiredo M.V.B., Martinez C.R., Burity H.A. and Chanway C.P., Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.), *World Journal of Microbiology and Biotechnology*, **24**, 1187–1193 (2008)
23. Gardner F.P., Pearce R.B. and Mitchell R.L., Fisiologi Tanaman Budidaya H Susilo, Jakarta, UI-Press, 428 (1991)
24. Goyeneche R., Roura S., Ponce A., Vega-Galvez A., Quispe-Fuentes I., Uribe E. and Di Scala K., Chemical characterization and antioxidant capacity of red radish (*Raphanus sativus* L.) leaves and roots, *Journal of Functional Foods*, **16**, 256–264 (2015)
25. Hamza M.A., Understanding soil analysis data, Department of Agriculture and Food, Western Australia, Report, 327 (2008)
26. Hou Y. et al, Marine shells: Potential opportunities for extraction of functional and health-promoting materials, *Critical Reviews in Environmental Science and Technology*, **46**, 1047–1116 (2016)
27. <https://www.oecd-ilibrary.org/sites/4033fea6-en/index.html?itemId=/content/component/4033fea6-en#endnote142ccbce5e3>
28. Huang H.C. et al, Control of white mold of bean caused by *Sclerotinia sclerotiorum* using organic soil amendments and biocontrol agents, *Plant Pathology Bulletin*, **14**, 183-190 (2005)
29. Inam A., Sahay S. and Mohammad F., Studies on Potassium content in two root crops under Nitrogen fertilization, *International Journal of Environmental Sciences*, **2(2)**, 1030–1038 (2011)
30. Jackson M.L., Soil Chemical Analysis, Prentice-Hall, India Private Limited, New Delhi (1973)
31. Jensen H.L., Note on Biology of *Azotobacter*, Proceedings of the Society for Applied Bacteriology, **14**, 89 (1940)
32. Kim S.K. and Mendis E., Bioactive compounds from marine processing byproducts: A review, *Food Research International*, **39**, 383-393 (2005)
33. Kreig N.R. and Holt J.G., eds., Bergey's Manual of Systematic Bacteriology, Williams and Wilkins, London, **1**, 100-103 (1984)
34. Kumar S., Dahiya R., Kumar P., Jhorar B.S. and Phogat V.K., Long-term effect of organic materials and fertilizers on soil properties in pearl millet-wheat cropping system, *Indian Journal of Agricultural Sciences*, **46**, 161–166 (2012)
35. Kumawat A. et al, Impact of levels of residue retention on soil properties under conservation agriculture in vertisols of central India, *Archives of Agronomy and Soil Science*, **68(3)**, 368–382 (2022)
36. Kushwah L., Sharma R.K., Kushwah S.S. and Singh O.P., Influence of organic manures and inorganic fertilizers on growth, yield and profitability of radish (*Raphanus sativus* L.), *Annals of Plant and Soil Research*, **22(1)**, 14-18 (2020)
37. Lindsay W.L. and Norvell W.A., Development of DTPA soil test for zinc, iron, manganese and copper, *Soil Science Society of America Journal*, **42**, 421-428 (1978)
38. Liu Z., Zou B.J., Zhu, Q.Q. and Cai Z.C., Agricultural Chemistry of Microelements, Agriculture Press, Beijing, China (1991)
39. Mahsa Tarashkar, Mansour Matloobi, Salman Qureshi B. and Akbar Rahimi, Assessing the growth-stimulating effect of tea waste compost in urban agriculture while identifying the benefits of household waste carbon dioxide, *Ecological Indicators*, **151**, 110292 (2023)
40. Mali D.L., Singh Virendra, Sarolia D.K., Hittora Suresh and Dhakar Rajendra, Effect of organic manures and bio-fertilizers on growth and yield of radish (*Raphanus sativus* L.) cv, Japanese white *International Journal of Chemical Studies*, **6(2)**, 1095-1098 (2018)
41. Martin J.P., Use of acid, rose Bengal and streptomycin in plate method for estimating soil fungi, *Soil Science*, **69**, 215 (1950)
42. Masabini, Easy Gardening, The Texas A&M University System, U.S. Department of Agriculture and the County Commissioners Courts of Texas Cooperating, Texas A&M, AgriLife Communications, 1-3 (2014)
43. Meena A.L. et al, Impact of 12-year-long rice based organic farming on soil quality in terms of soil physical properties, available micronutrients and rice yield in A typic Ustochrept soil of India,

Communications in Soil Science and Plant Analysis, **51(18)**, 2331–2348 (2020)

44. Mehwish Kiran, Muhammad Saleem Jilani, Kashif Waseem and Muhammad Sohail, Effect of Organic Manures and Inorganic Fertilizers on Growth and Yield of Radish (*Raphanus sativus*), *Pakistan Journal of Agricultural Research*, **29**, 4 (2016)

45. Mo W.Y., Man Y.B. and Wong M.H., Use of food waste, fish waste and food processing waste for China's aquaculture industry: Needs and challenge, *Science of the Total Environment*, **1**, 613–643 (2018)

46. Nandish M.S., Vidya M.D. and Suchitha Y., Studies on performance of radish (*Raphanussativus* var. *Longipinnatus*) as influenced by liquid plant growth promoting Rhizomicrobial consortia, *The Pharma Innovation Journal*, **10(11)**, 09-20 (2021)

47. Naveed M., Hussain M.B., Mehboob I. and Zahir Z.A., Rhizobial Amelioration of Drought Stress in Legumes, In *Microbes for Legume Improvement*, Zaidi A., Khan M.S. and Musarrat J., Eds., Springer International Publishing, Cham, Germany, 341–365 (2017)

48. OECD/FAO, OECD-FAO Agricultural Outlook (2023-2032) OECD Publishing, Paris, <https://doi.org/10.1787/08801ab7-en> (2023)

49. Olsen S.R., Estimation of available phosphorus in soil by extraction with sodium bicarbonate, Circular No. 939, US, Department of Agriculture, Washington DC, USA (1954)

50. Pikovskaya R.I., Mobilization of phosphorus in soil in connection with vital activity of some microbial species, *Microbiology*, **17**, 362-70 (1948)

51. Rathore G., Kaushal R., Sharma V., Sharma G., Chaudhary S., Dhaliwal S.S., Alsuhaibani A.M., Gaber A. and Hossain A., Evaluation of the Usefulness of Fermented Liquid Organic Formulations and Manures for Improving the Soil Fertility and Productivity of Brinjal (*Solanummelongena* L.), *Agriculture*, **13**, 417 (2023)

52. Sharma R.P. and Datt N., Effect of vermicompost, farmyard manure and chemical fertilizers on yield, nutrient uptake and soil fertility in okra (*Abelmoschusesculentus*)—Onion (*Allium cepa*) sequence in wet temperate zone of Himachal Pradesh, *Journal of the Indian Society of Soil Science*, **57**, 357–361 (2009)

53. Srinivasarao C. et al, Effect of 22-year-long conjunctive use of organic and chemical sources of nutrients on crop yield, soil properties and nutrient balance in post-monsoon sorghum (*sorghum bicolor* L.) in peninsular vertisols of India, *Communications in Soil Science and Plant Analysis*, **49**, 1-16 (2018)

54. Subba Rao A. and Sammi Reddy K., Analysis of soils for pH, EC and available major nutrients plant materials for macro and micro nutrients, *Methods of analysis of soils, plants, water, fertilizers and organic manures*, edited, Tandon H.L.S., FDCO, New Delhi, 21- 59 (2013)

55. Subbiah B.V. and Asija G.L., A rapid procedure for the determination of available nitrogen in soils, *Current Science*, **25**, 259-60 (1956)

56. Tandon H.L.S., *Methods of analysis of soils, plants, water, fertilizers and organic manures*, FDCO, New Delhi, 44-154 (2009)

57. Thendral Hepsibha B. and Geetha A., A biochemical study on the growth traits of *Vigna radiata* (Green gram) influenced by Gunapaselam (Fermented Fish waste) An approach to marine waste management, *Indian Journal of Geo Marine Sciences*, **51(05)**, 496-501 (2022)

58. Thendral Hepsibha B. and Geetha A., A Physicochemical characterization of traditionally fermented liquid manure from fish waste (Gunapaselam), *Indian Journal of Traditional Knowledge*, **18(4)**, 830-836 (2019)

59. Thendral Hepsibha B., Sudhalakshmi P. and Geetha A., Influence of Gunapaselam, a liquid fermented fish waste on the growth characteristics of *Solanum melongena*, *Journal of Chemical and Pharmaceutical Research*, **6(12)**, 58-66 (2014)

60. Thronton H.G., On the development of standardised agar medium for counting soil bacteria with special regard to repression of spreading colonies, *Annals of Applied Biology*, **9**, 241–274 (1922)

61. Tran Le Khanh-Linh, Le Thi Thanh Mai, Vo N. Hong-Tham and Ngo Dai-Nghiep, Optimization of the polyphenol extract conditions from Xao Tam Phan leaves (*Paramignya trimera*), *Res. J. Chem. Environ.*, **27(7)**, 40-44 (2023)

62. Vaish S., Garg N. and Ahmad I.Z., Microbial basis of organic farming systems with special reference to biodynamic preparations, *The Indian Journal of Agricultural Sciences*, **90(7)**, 1219–1225 (2020)

63. Vincent J.M., *A Manual of the Practical study of the root Nodule Bacteria International Biological Programme London, Handbook*, **15**, 164 (1970)

64. Vincent R., Ismail S.A., Sharief S.D. and Jeyaprakash P., Isolation and characterization of microorganisms in the fermented fish waste liquid foliar spray-Gunapaselam, *Online Journal of Biosciences and Informatics*, **23**, 320–324 (2014)

65. Walkley A. and Black C.A., An examination of different methods for determing soil organic matter and a proposed modification of chromic acid titration methods, *Soil Science*, **37**, 29-38 (1934)

66. Wang X., Yan J., Zhang X., Zhang S. and Chen Y., Organic manure input improves soil water and nutrients use for sustainable maize (*Zea mays*. L) productivity on the Loess Plateau, *PLoS ONE*, **15(8)**, 0238042 (2020)

67. Wisniewsk A., Wiggers V., Simionatto E., Meier H., Barros A. and Madureira L., Biofuels from waste fish oil pyrolysis, *Chemical Composition Fuel*, **89**, 563–568 (2010)

68. Yadav D. et al, Sustaining the properties of Black soil in central India through crop residue management in A conservation-agriculture-based soybean–wheat system, *Land Degradation Development*, **32(10)**, 2906–2921 (2021)

69. Zhang R. and El-Mashad H.M., Bio-diesel and bio-gas production from seafood processing by-products, In *Maximising*

the Value of Marine By-Products, Elsevier Ltd., Amsterdam, The Netherlands, 460–485 **(2006)**

A Review, *American Journal of Biochemistry and Biotechnology*, **7**, 104-123 **(2011)**.

70. Zhou L., Budge S.M., Ghaly A.E., Brooks M.S. and Dave D., Extraction, purification and characterization of fish chymotrypsin,

(Received 26th July 2024, accepted 04th October 2024)