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A biochemical study on the growth traits of *Vigna radiata* (Green gram) influenced by Gunapaselam (Fermented Fish waste) - An approach to marine waste management

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Effective methods are necessary to utilize the discarded waste from the fishery industry. The sustainable agriculture emphasizes the importance of recycling organic wastes to minimize the damages caused by mineral fertilizers and the sky-rocketing price of chemical fertilizers. A study was conducted to assess the fertilizing potential of fermented fish waste - Gunapaselam (GP) on the growth traits of mung bean (*Vigna radiata*). The study groups include Group I (water treatment/ without fertilizer), Group II (chemical fertilizer – NPKS (1:2:1:0.8)) and Group III (GP - 1:100 fold diluted). The performance of the crop was adjudged in terms of growth traits like germination percentage, trifoliate emergence rate, leaf area, number of branches and leaves, number of lateral roots, flowering time and developmental stages of nodules. Except for germination percentage, all the growth traits were significantly improved by GP treatment. GP improved the growth of green gram probably by providing the essential nutrients and enhancing their availability and effective absorption than the chemical fertilizers. The study also revealed that GP influences the beneficial soil microbes and makes the rhizosphere suitable to facilitate nodule formation. It can be concluded from the findings that GP has a pronounced effect on growth traits of *Vigna radiata* and it can replace chemical fertilizers in the future.

[**Keywords**: Growth traits, Gunapaselam (fermented fish waste), Leaf area, Nodule, Organic fertilizer, *Vigna radiata* (green gram)]

Introduction

The rapid population growth requires an additional 70 % of food to meet the demand in 2050 as reported by Food and Agricultural Organisation[1]. Agricultural sector should work towards the production of more food with fewer resources. To meet this greatest challenge, agricultural technologies and activities have expanded widely resulting in increased application of chemical fertilizers and pesticides. They are chemical substances that provide necessary nutrients to the plants to improve the quantity and quality of food products. It was found that several million pounds of toxic chemicals are introduced in the form of fertilizers including radionuclides. Excessive and long-term usage has posed a major threat to the ecosystem. The adverse effects include diminution in the land potential, logging of toxic chemicals in the soil, alarming the survival of beneficial organisms and upsetting the rhizospheric atmosphere of microbes and ultimately the consumer's health. Attenuation of these problems is possible only through the eco-friendly way of the farming system.

The aquaculture industry has always served as an attractive field where there is a great potential for discarded wastes and the by-products derived from them. It was estimated that human fish consumption was around 120 million tonnes in 2010[ref. 2]. In developing countries, fish contributes more to food and nutrition as it was cheap and 17 % of frequently consumed animal food sources in 2013[3]. Only 74 % of fish produced were consumed and the remaining were utilized for non-food products[4]. Fish are known for their high-quality proteins with all essential amino acids, essential fats like omega-3 fatty acids, vitamins (A, B and D) and minerals (including iodine, zinc, calcium, iron, and selenium)[5]. About 70 % of the total weight of the fish is perishable residual wastes like viscera, head, tails, skin, bones and frames of low value[5]. A waste management strategy should be adopted to recover nutrients from the waste for optimal usage. The hydrolyzed fish wastes were used for producing fish fertilizer products like hydrolysates, fish emulsion, fish meal, fish silage and fish soluble nutrients which have benefits over conventional fertilizers. Thus, proper management of fish wastes will serve as an excellent cost-effective organic fertilizer, reducing the environmental damage without any compromise on the quality of food.

Biological fermentation is a cost-effective, and eco-friendly biotechnological tool that converts solid macro waste in to simple molecules by utilizing microbes[6]. Fermentation improves the nutritional value, and digestibility nature and produces stable and humified organic matter[7,8]. Fish waste was fermented by using jaggery[9]. Bio-fermented fish waste contains organic matter, the primary nutrients nitrogen, phosphate and potassium and essential micronutrients which improve the overall growth of plants. Fermented fish waste also promotes plant health indirectly through the action of rhizobacteria that helps in fixing atmospheric nitrogen biologically[10] and increases the accessibility and uptake of essential nutrients[11]. The liquid nature of GP ensures nutrient availability directly to the leaves as a foliar spray and readily absorbed by the roots[12]. Fish emulsion has promoted the growth of raddish[13], chilli[14] and brinjal[15,16] and prevented mold disease in the beans[17]. The effect of fish soluble nutrients on the growth and cultivation of a variety of fruits and vegetables was reported earlier by Aung *et al.*[18].

Mung bean (*Vigna radiata*) is an important legume and short-duration pulse crop to be included in most ethnic foods in Asian countries. India imports around 3 million tonnes and the demand will rise to 27.0 million tonnes by 2015[ref. 19]. The increased consumption was due to its high protein, essential amino acids especially lysine, minerals and vitamins witnessing an ever-increasing demand. The benefits of the mung bean crop include drought-tolerant, lower production costs and usage as animal feed. The nodules fix atmospheric nitrogen by symbiosis and its residues improve the soil quality and hence the productivity of the land. A shift is required to reclaim soil fertility from devastating effects caused by non-renewable chemical fertilizers by organic fertilizers. Hence, an attempt was made to evaluate the growth-promoting activity of fermented fish waste, Gunapaselam (GP) on *Vigna radiata* and to prove the utility of GP as a liquid organic fertilizer.

Materials and Methods

Gunapaselam preparation

Fish waste containing gut, gills, head, fins, trimmings, etc. was collected from a fish market at Vanagaram, Chennai, Tamil Nadu. They were cut into small pieces and minced well. Gunapaselam was prepared by mixing 1 kg of minced fish waste with 1.5 kg of jaggery along with 5 L of water in a plastic container covered by muslin cloth and left for 15 days at 35 °C. The mixture was mixed daily and filtered on the 15th day and stored in a plastic container[9].

Experimental setup

The study was conducted in a field at Porur, Chennai in three replications by following Randomized Complete Block Design (RCBD) method. VBN (Gg2) variety of *Vigna radiata* seeds were obtained from, Vamban-National Pulses Research Centre, TNAU (Tamil Nadu Agricultural University), Pudukkottai. The field was prepared in a 12 m^2 area (3 m x 4 m). The treatment followed was Group I - without fertilizer, Group II - chemical fertilizer (NPKS) (25 Kg N_2: 50 Kg P_2O_5: 25 Kg K_2O: 20 Kg S/ha) and Group III - 1:100 fold diluted Gunapaselam. Recommendations specified for mung bean cultivation by TNAU were followed. Following one pre-sowing irrigation, Group II received the recommended basal dose of chemical fertilizer (25:50:25:20 kg NPKS ha-1). Nitrogen was supplied as urea, phosphorus as diammonium phosphate, potassium as muriate of potash and sulphur as gypsum. It was applied as a foliar spray during the flowering and pod formation stages. GP dilution was chosen based on the phytotoxicity test[20]. Seeds were sown in the last week of January month. The treatment groups (I and III) received water and (1:100) diluted GP (on alternate days) as per the requirement. Weeds were removed from the field when observed.

Germination rate was calculated along with the morphological parameters like trifoliate appearance and flowering time. Mung bean seedlings were uprooted on 5th, 15th and 35th day of seedling emergence to observe the developmental stages of nodules. Calibrated vernier caliper was used to measure the length (L) and width (W) of the leaves after 30 days and area was calculated as the product of width and length including 0.94 as Correction Factor (CF)[21,22]. Number of branches and the total number of trifoliate leaves in *Vigna radiata* were counted on 40th day.

Statistical analysis

Results were reported as mean ± Standard Error of the Mean (SEM). Graph Pad Prism version 5.0, Software package (Graph Pad Software Inc.; San Diego, CA, U.S.A.) was used for statistical analysis.

One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was done to assess the statistical significance of differences between groups.

Results and Discussion

Germination represents the most vital stage in a plant's life cycle. Table 1 shows the germination percentage of all treatment groups. The dry seeds after absorbing water were stimulated to germinate and resulted in the embryonic axis. In *Suaeda salsa* seeds, there was a reduction in the germination rate due to the hindrance effect of heavy metals on water uptake[23]. In the present study, pre-irrigation was done before seeds' sowing. The presence of adequate moisture was utilized for seed germination and embryonic axis elongation and it also confirms that heavy metals are not present in higher concentrations in GP. A reduction in the germination rate of groundnut, cowpea and mung bean was observed due to the presence of allelochemicals in *Celosia argentea* L. leaf extract[24].

The first trifoliate appearance in mung bean was observed on the 5th day. Group I recorded the least percentage (54.58±4.20 %) whereas chemical and GP treatment showed significant improvement (Table 1). Presence of essential nutrients and their imbibitions fastens the seedling emergence rate. Macro and micronutrients fasten the synthesis of DNA, RNA and proteins essential for growth. Micronutrients like Zn, Mn, Mo and B in soil increase the seed vigor[25]. Fishery derived products serve as excellent source of protein and vital nutrients like magnesium, calcium, potassium, phosphorus, sodium, zinc, iron, manganese, copper and boron[26]. Boron assists in transportation and translocation of carbohydrates, and synthesis of uracil thus promoting enhanced cell division, differentiation and meristematic activity leading to better plant growth[27]. Gunapaselam, the fermented fish waste contains all essential nutrients and minerals which improve the emergence rate of trifoliate leaves in green gram when compared to other groups.

Leaf area trait is an important indicator of crop growth, development, and plant health. A significant improvement in leaf area and the number of leaves/ plants was noted in Group III treatment (49.03±1.97 cm^2) and (8.6±0.26 cm^2) respectively when compared with other treatment groups (Table 1). Growth of a plant requires an adequate and continuous supply of the macronutrients nitrogen, phosphorus and potassium. The chlorophyll formation and vegetative growth of the plants are governed by nitrogen. The presence of nitrogen in proteins leads to cell enlargement and division, protein and nucleic acid synthesis and ultimately results in more leaves[28]. Fertile soil is loaded with beneficial microbes. The majority of the rhizobacteria exhibit growth-promoting effects like siderophore and phytohormones production, mobilization of ions and nutrients, fixing up atmospheric nitrogen, etc. Fungal and bacterial species serve as potential phosphate solubilizers. GP was found to possess nitrifying, ammonifying and phosphate solubilizing bacteria[9] and it also serves as the best nutrient base for the microbial community. Thus, the application of GP provides a sufficient amount of nitrogen and also makes the rhizosphere conducive to the multiplication of bacteria that promotes plant growth. This was elicited in the formation of more leaves and with wider leaf area in GP-treated plants.

Better results with more number of branches/ plant were achieved in GP treatment (7.71±0.21) and lowest in control (5.91±0.33) (Table 2). This was

Table 1 — Influence of water, chemical fertilizer and Gunapaselam (GP) on germination percentage, trifoliate appearance and leaf area of *Vigna radiata*

Group/ treatment	Germination %	Trifoliate appearance	Leaf area (cm^2)
Group 1 - Water	70.83±9.50	66.67±9.9	35.65±2.46
Group 2 - Chemical fertilizer (NPKS)	75±4.81^{NS}	70±6.77^{NS}	47.88±4.51*
Group 3 - 1:100 fold diluted GP	75±10.7^{NS}	75.42±8.75^{NS}	49.03±1.97*

(Statistical significance was analyzed by one way ANOVA followed by Tukey's multiple comparison test. p-value < 0.05 (*); NS - Not significant (Group I vs Group II, III)

Table 2 — Effect of Gunapaselam on morphological traits – branches, leaf cluster and flowering ability (40th day) of *Vigna radiata*

Group/ treatment	No. of branches	No. of trifoliate leaf cluster	Flowering rate on 40th day (%)
Group 1 - Water	5.9±0.33	6.30±0.39	8.03±0.33
Group 2 - Chemical fertilizer (NPKS)	7±0.21*	8.08±0.63*	26.13±3.86***
Group 3 - 1:100 fold diluted GP	7.75±0.21***	8.60±0.26**	37.50±2.50***

(Statistical significance was analyzed by one way ANOVA followed by Tukey's multiple comparison test. p-value < 0.05 (*), 0.01(**) and 0.001 (***) , respectively (Group I vs Group II, III) attributed to adequate nutrient availability and its uptake and due to the influence of rhizobacteria that promotes growth especially nitrogen-fixers and phosphate-solubilizers in the organic manure[29]. Application of digested mixture containing bovine, blood and rumen has shown significant increase in growth traits like height, increased leaf area, more leaves and early flowering in brinjal plants[29]. Maximum flowering rate was observed in Group III (37.5±2.5 %) followed by chemical treatment (26.13±3.86 %) and Group I (8.03±0.33 %) (Table 2). Important process like light interception, photosynthesis, growth, and yield of crops were enhanced by an increase in leaf area. Early flowering can be achieved only when plant nutrient such as phosphorus is optimal. The observed increase in leaf area and the number of leaves in gunapaselam treatment enable maximum absorption of sunlight, and better photosynthesis and the translocation of photosynthates from leaves to the reproductive parts and thus induce flowering. Similar results were observed in chillies by Patil & Biradar[30].

In all three groups, the seedlings uprooted after 5 days did not show any visible sign of nodule development (Fig. 1). After 15 days, the maximum number of nodules appeared on the taproot of GP-treated plants than chemical and water treatments (Fig. 2). On the 35th day, the presence of nodules was noted in all three groups, with the maximum in Group III on both tap root and lateral roots (Fig. 3).

The rhizobium species are known to produce auxins that accelerate the processes like cell division, differentiation and vascular bundle formation essential for nodule formation[31]. Low rhizobium populations in Group I and Group II are responsible for the formation of less number of nodules. Thus,

Fig. 1 — Development stages of nodules in Mung beans treated with water (Group I), chemical fertilizer (NPKS) (Group II), Gunapaselam (Group III) on 5th day

Fig. 2 — Development stages of nodules in Mung beans treated with water (Group I), chemical fertilizer (NPKS) (Group II), Gunapaselam (Group III) on15th day

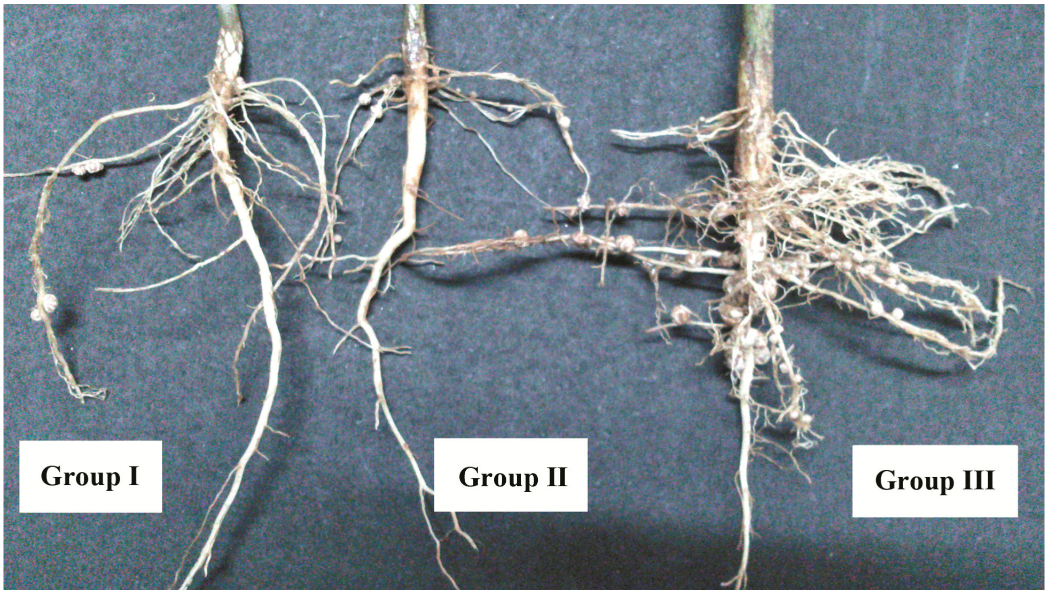

Fig. 3 — Development stages of nodules in Mung beans treated with water (Group I), chemical fertilizer (NPKS) (Group II), Gunapaselam (Group III) on 35th day

treatment with GP supplies an adequate amount of nutrients and maintains good moisture content and microbial community particularly rhizobium species which additively promote the formation of nodules in appreciable amounts. Application of urea results in 60 % reduction of the nodule in green gram[32] while foliar application of humic substances increased the nodulation[33].

Conclusion

In conclusion, fermentation has transformed fish waste into a valuable agronomic resource that can be used as liquid fertilizer. The application of gunapaselam has increased soil fertility by mobilizing the essential nutrients and beneficial microbial community for the growth of mung beans. Because of these changes in the rhizosphere, mung beans produced a larger leaf area, more leaves, branches and nitrogen-fixing nodules and earlier flowering, unlike chemical fertilizer. Therefore, this study strongly proves that gunapaselam will be a good and cheaper alternative to chemical fertilizer in managing soil health. Thus, the synergistic benefit can be achieved by integrating the food production system with less pollution and waste utilization.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Statement

Vigna radiata seeds [VBN- Gg2] were procured from National Pulses Research Centre, TNAU (Tamil Nadu Agricultural University), Vamban, Pudukkottai

Author Contributions

BTH has performed the experiments, analyzed and interpreted the results and wrote the paper. AG has conceived and designed the experiments.

References

1 FAO, *Global agriculture towards 2020*, (Agricultural Development Economics Division Economic and Social Development Department, Rome, Italy), 2009, pp. 1-4.
2 Trine Y, Turid S A & Torbjorn A, Utilisation of feed resources in production of Atlantic salmon (*Salmo salar*) in Norway, *Aquaculture*, 448 (2015) 365–374.
3 FAO, Contributing to food security and nutrition for all, In: *The State of World Fisheries and Aquaculture 2016*, (FAO, Rome), 2016, pp. 4.
4 Arvanitoyannis I S & Aikaterini K, Fish industry waste: treatments, environmental impacts, current and potential uses, *Int J Food Sci Techno*, 43 (2008) 726–745.
5 FAO, *Technologies and practices for small agricultural producers*, (Fisheries and Aquaculture Department in Food and Agriculture Organization, Singapore), 2016.
6 Samaddar A & Kaviraj A, Application of Fermentation Technology to Use Slaughterhouse Blood as Potential Protein Supplement in Fish Feed, *Jordan J Biol Sci*, 8 (1) (2015) 23–30.

7 Singhania R R, Kumar Patel A, Soccol C R & Pandey A, Recent advances in solid-state fermentation, *Biochem Eng J*, 44 (2009) 13-18.

8 Ferrer J, Paez G, Marmol Z, Ramones E, Chandler C, *et al.*, Agronomic use of biotechnologically processed grape wastes, *Biores Technol*, 76 (2001) 39-44.

9 Vincent R, Ismail S A, Dawood S S & Jeyaprakash P, Isolation and Characterization of Microorganisms in the fermented Fish Waste Liquid Foliar Spray-Gunapaselam, *Online J Biosci Informatics*, 2 (2014) 320-324.

10 Whipps J M, Microbial interactions and biocontrol in the rhizosphere, *J Exp Bot*, 52 (2001) 487–511.

11 Kloepper J W, Lifshitz R & Schroth M N, *Pseudomonas* inoculants to benefit plant production, *ISI Atlas Sci Anim Plant Sci*, 1 (1988) 60–64.

12 Narayanamma M, Sal R C, Chiranjeevi C & Prabhakar R I, Influence of water soluble fertilizers on yield of Brinjal, *Veg Sci*, 33 (2006) 94-95.

13 Tarabily K A, Nassar A H, Hardy G E St. J & Sivasithambaram K, Fish emulsion as a food base for rhizobacteria promoting growth of radish (*Raphanus sativus*
L. var. *sativus*) in a sandy soil, *Plant Soil*, 252 (2003) 397–411.

14 Deore G B, Limaye A S, Shinde B M & Laware S L, Effect of Novel Organic Liquid fertilizer on Growth and Yield in Chilli (L.) *Capsicum annum*, *Asian J Exp Biol Sci Spl*, (2009) 15-19.

15 Bhat R V & Vasanthi S, Antiquity of the cultivation and use of Brinjal in India, *Asian Agri-History*, 12 (2008) 169–178.

16 Thendral H B, Sudhalakshmi P & Geetha A, Influence of Gunapaselam, a liquid fermented fish waste on the growth characteristics of *Solanum melongena*, *J Chem Pharm Res*, 6 (2014) 58-66.

17 Huang H C, Erickson R S, Chang C, Moyer J R, Larney F J, *et al.*, Control of white mold of bean caused by *Sclerotinia sclerotiorum* using organic soil amendments and biocontrol agents, *Plant Pathol Bull*, 14 (2005) 183-190.

18 Aung L H, Flick G J, Bluss G R, Aycock H S, Keefer R F, *et al.*, Growth responses of crop plants to fish soluble nutrients fertilization, *Res Div Bull*, 8 (1984) 4–9.

19 Singh J, Present agricultural scenario and future prospects in India: An overview, *Pratiyogita Darpan*, (2011) 1549-1551.

20 Thendral H B & Geetha A, Revealing the non- phytotoxic effect of Gunapaselam (fermented fish waste) by a dose dependent *in vitro* study, *Intl J Pharma Bio Sci*, 8 (2) (2017) 643-648.

21 Kathirvelan P & Kalaiselvan P, Groundnut (*Arachis hypogaea* L.) leaf area estimation using allometric model, *Research J Agric Biol Sci*, 3 (2007) 59-61.

22 Mendoza T C, Light interception and total biomass productivity in sugarcane intercropping, Philipp, *J Crop Sci*, 11 (1986) 181-187.

23 Liu S, Yang C, Xie W, Xia C & Fanc P, The effects of cadmium on germination and seedling growth of *Suaeda salsa*, *Procedia Environ Sci*, 16 (2012) 293–298.

24 Saritha P & Sreeramulu A, Allelopathic effects of *Celosia argentea* L Leaf extracts on crop plant seed germination, *Int J Lifesc Bt Pharm Res*, 2 (2013) 56-64.

25 Harris D, The effects of manure, genotype, seed priming and date of sowing on the emergence and early growth of [*Sorghum bicolor* (L.) Moench] in semi-arid Botswana, *Soil Tillage Res*, 40 (1996) 73-78.

26 Ghaly A E, Ramakrishna V V, Brooks M S, Budge S M & Dave D, Fish Processing Wastes as a Potential Source of Proteins, Amino Acids and Oils: A Critical Review, *J Micro Biochem Technol*, 5 (2013) 107-129.

27 Jones J B J, Agronomic handbook: Management of Crops, Soils, and Their fertility, (CRC Press, Florida, USA), 2003.

28 El-Banna E N, Ashour S A & Abd El-Salam H Z, Effect of foliar application with organic compounds on growth, yield and tubers quality of potato (*Solanum tuberosum* L.), *J Agric Sci Mansoura Uni*, 31 (2006) 1165-1175.

29 Roy M, Karmakar S, Debsarcar A, Sen P K & Mukherjee J, Application Of Rural Slaughterhouse Waste As An Organic Fertilizer For Pot Cultivation Of Solanaceous Vegetables In India, *Int J Recycl Org Waste Agri*, 2 (2013) 2-11.

30 Patil R & Biradar R, Effect of Foliar Application of Essential Nutrients on Chillies, *Agricultura Tecnica Santiago*, 51 (2001) 256–259.

31 Glick B R, Plant Growth-Promoting Bacteria: Mechanisms and Applications, *Scientifica*, 2012 (2012) 1-15.

32 Khalilzadeh R H, Tajbakhsh M J & Jalilian J, Growth Characteristics Of Mung Bean (*Vigna Radiata* L.) Affected By Foliar Application Of Urea And Bio-Organic Fertilizers, *Intl J Agri Crop Sci*, 4 (2012) 637-642.

33 Sangeetha M, Singaram P & Uma Devi, Effect of lignite humic acid and fertilizer on yield of onion and nutrient availability, *Int Union Soil Sci*, 21 (2006) 163-168.