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Effect of Fermented Fish waste (Gunapaselam) Application on the Soil Fertility with Special Reference to Trace Elements and the Growth Characteristics of *Vigna radiata*

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Abstract — **The soil fertility largely depends on the nature and the quantity of trace elements. Application of chemical fertilizers to increase the food production slowly robs the soil nutrients, harm the soil fauna and microbes and cause side effects to consumers. Gunapaselam is prepared from fish waste by fermentation. The aim of the present study is to assess the level of trace elements, microbial population and the growth characteristics of *Vigna radiata*, green gram after applying Gunapaselam. The soil was amended with either chemical fertilizer (25kg N_2: 50kg P_2O_5: 25kg K_2O: 20kg S/ha) or Gunapaselam (1:100 diluted) on alternative days. The soil was analyzed for trace elements Iron (Fe), Copper (Cu), Zinc (Zn), Manganese (Mn), Boron (B), Nickel (Ni) before and after applying Gunapaselam. Amendment of Gunapaselam to soil has improved the quantity of trace elements, macro nutrients and plant growth promoting microbial status of soil. Spearman rank correlation test reveals positive correlation with the growth traits and formation of number of nodules. The results suggested that the addition of Gunapaselam may be considered as a novel liquid biofertilizer as it promotes growth and increase nodulation probably by enriching the soil with trace elements and microbial community.**

Key words – **Fermented Fish Waste, Gunapaselam, Nodules, Plant Height, Root Length, *Vigna radiata* (Green gram).**

I. Introduction

Fertile soil is the greatest terrestrial stock that supplies all essential nutrients for the growth of healthy and productive plants. Continuous harvesting of the crops results in depletion of the soil fertility. Addition of chemical fertilizers, supply mainly the macronutrients but not all the essential elements for the proper growth and yield of the plants. Successful and sustained production of crops needs mineral nutrients like carbon (C), hydrogen (H), oxygen (O), nitrogen (N_2), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and Sulphur (S), as well as zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), boron (B), molybdenum (Mo), chlorine (Cl) and nickel (Ni). These micronutrients fulfill the requirements of plants for their growth and development.

Trace elements are essential for physiological and biochemical processes of plants besides the macronutrients. Iron (Fe) has been found to promote legume-rhizobia symbiosis [1] helps in nodule formation [2] and to regulate the activities of enzymes involved in N2 fixation and assimilation. Integrity of the cell membrane, seed formation, elongation of roots and sugar metabolism are governed by Boron [3]. Copper performs vital role in respiration, photosynthesis, N_2 and cell wall metabolism, carbohydrate distribution and seed production [4]. Legumes have very low boron requirements for root growth and root system architecture [5], stem, leaf, and vascular tissue elongation [6] and at the onset of flowering and during seed development [7].

Beneficial plant-associated rhizobacteria also enhances plant growth by providing trace elements (such as iron and zinc), fixing nitrogen, and synthesizing substances including siderophores and indole acetic acid [8] and providing protection against soil pathogens [9]. Manganese has profound influence on electron transport in photosynthesis, photo destruction of chloroplast and chlorophyll, nitrogen fixation [7]. Hussain *et al.* [10] has reported that Nickel is critical for root and shoot growth, photosynthesis, protein synthesis, enzyme activity and maintenance of membrane integrity. Deficiency of zinc results in reduced photosynthesis [11], short internodes formation, decrease in leaf size and delayed maturity [12]. Inadequate availability of the trace elements in the soil not only disturb the plants productivity and yield but also affects the quality of food and feed entering the food chain which causes global burden on animal [13] and human health [14]. The concentration of these trace elements should not exceed the critical limit as they are toxic. Therefore awareness needs to be created and strategies have to be adopted to correct the trace element deficit through natural means.

Legumes are the second largest group of feed crops grown globally contributing 25% of crop production with 61 million tons of grains. Among legumes, *Vigna radiata* (Green gram / Mung bean) is a short duration and cover growing crop. It serves as an excellent intercrop that can add organic matter, cycle the nutrients from depths to soil surface and fix the atmospheric nitrogen by nitrogen-fixing bacteria housed within nodules. The bacteria lodging in the rhizosphere were efficient drivers of nutrient availability and sustainable crop production. Agronomists are currently focused on integrated plant nutrient management system involving plant growth promoting rhizobacteria (PGPRB) towards plant growth. The yield and nodule forming ability of mung bean is influenced by the availability of essential nutrients and interaction with rhizobium species. It was now reported that Indian soils are now facing deficiency of both macro and micro nutrients essential for crop production. Hence there is an urgent need to manage these deficiencies to ensure soil fertility and healthy production of crops. The escalating cost of fertilizers has forced us to recycle the organic waste and reutilize the nutrients.

An organic fertilizer improves soil structure [15], slowly releases the nutrients, and increases beneficial microbial activity [16] and thus serves as a perfect alternative to chemical fertilizers [17]. In recent times, attention has been paid on safer organic foods with high quality. Environmental issues forces the reutilization of animal manure, agricultural waste, animal waste etc., in to a better form of the nutrient as a good waste management practice for sustainable agriculture. Composting and fermentation methods have regained focus as they are accepted ecologically. The obtained products are less expensive and promote plant growth. They are non- polluting and therefore safe to use in agriculture.

More than 70% of fish waste is obtained from the fish processing industry. Environmental problems caused by fish waste can be minimized by transforming them in to useful products like fertilizers as they are rich in essential macro and micronutrients [18]. Fermentation of the fish waste is the right method with many advantages when carbon source, moisture and aeration were provided in right proportion. The effect of fermented fish waste as liquid fertilizer has been investigated on the yield of many vegetables and fruits [19, 20]. Vincent *et al.* [21] has reported the fermentation of fish waste using jaggery and it was used under the trade name "Gunapaselam". The growth promoting effect of Gunapaselam has been known traditionally but the role played in improving the soil micronutrient status was not available. The aim of this study was to evaluate the beneficial role of Gunapaselam in improving the trace elements level in the soil and growth characteristics of *Vigna radiata*.

II. Materials and Methods

2.1. Preparation of Gunapaselam

Gunapaselam was prepared according to the procedure described by Vincent et al. [22]. Fermentation of fish waste was carried out in a covered plastic container. The mixture contained 1.5 kg jaggery, 1 kg chopped fish offal and 5 lit water. The contents were swirled for 14 days followed by filtration. Then the filtrate obtained was Gunapaselam which was used after 1:100 dilution.

2.2 Analysis of Trace Elements and Phosphate Solubilisers in Gunpaselam

The prepared liquid manure was analysed for essential elements like sulphur, zinc, manganese, iron, copper, lead and boron at National Agro Foundation- Laboratory Services Division, Anna University Taramani Campus, Taramani according to the procedure described by Tanden [22] and potential of Gunapaselam to solubilise insoluble phosphate was checked on Pikovskaya's agar medium (PVK) [23]. 1ml of the Gunapaselam was serially diluted with sterile distilled water. 0.1ml of each dilution was spread on PVK containing insoluble tri calcium phosphate and incubated at 30°c for 2 days. Colonies showing halo zones were conferred as phosphate solubilizer and enumerated.

2.3. Site Description and Experimental Setup

Seeds of Mung bean (*Vigna radiata*- VBN GG2) were procured from Agricultural seed store, National Pulses Research Centre, Tamil Nadu Agricultural University, Vamban, Pudukkottai. Experiment was conducted in a field at Porur, Chennai, Tamil Nadu, India. The area of each plot was 3 x 4 $m^{2,}$ keeping plant to plant distance of 10 cm and row to row distance of 30 cm with 3 m row length. The treatment groups were as follows: Group 1- Water control; Group 2- Chemical fertilizer (basal treatment and before flowering); Group 3- 1:100 diluted Gunapaselam (on alternate days). The treatments were arranged in randomized complete block design (RCBD) with three replications. The source of chemical fertilizer includes urea as nitrogen fertilizer, single super phosphate as phosphate, muriate of potash as potassium and gypsum as sulphur. Sowing was done at the end of January. Irrigation was proceeded according to the propose design throughout the growing season. Weeds were manually eradicated whenever observed in the field. The treatment groups received water, chemical and diluted Gunapaselam as per the requirement for a period of 90 days.

2.4. Assessment of Plant, Root Length and Nodulation Efficacy of Vigna Radiata

Ten plants were randomly selected and removed from each plot after 30 days (Flowering stage). The roots were carefully washed to remove the soil particles and the formed nodules were counted manually. The plant height and root length were recorded.

2.5. Physicochemical Analysis of Experimental Soil

Soil samples were taken randomly from the experimental site at a depth of 15 cm or 6 inches for physiochemical analysis. The experimental soil used for the study was analysed at National Agro Foundation-Laboratory Services Division, Anna University Taramani Campus, Taramani before sowing and after the harvest of *Vigna radiata* under different treatment groups. Soil pH, electrical conductivity, moisture, organic matter, organic carbon and content of essential nutrients like nitrogen, potassium, phosphorus, calcium, magnesium, sulphur, zinc, manganese, iron, copper, lead and boron exchangeable cations were determined [22].

2.6. Microbial Analysis of Soil

The rhizosphere soil was collected by uprooting the plants after 45 days of sowing and was serially diluted. The enumeration of total bacteria and rhizobium was done by the spread plate technique. 0.1 ml of the diluted sample was spread on nutrient agar and incubated for 48 hrs for bacteria. 0.1 ml of the sample was spread on Congo red yeast extract mannitol agar medium and incubated for 72 hrs for rhizobium growth. Colonies showing pink colour were counted and expressed as log (CFU/gm). Each plate was replicated 3 times.

2.7. Statistical Analysis

All data are reported as mean ± standard error of mean. Statistical analysis was done using SPSS 12.0 for windows package. The statistical significance of differences between groups was assessed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. All statistical analysis was done using computerized Graph Pad Prism version 5.0, Software package (Graph Pad Software Inc., San Diego, CA, U.S.A.). Spearman rank's correlation was conducted between the trace elements and the number of nodules. $P<0.05$ was considered as statistically significant.

III. Results and Discussion

India is considered to be the center of origin and major producer of green gram. Due to the poor management of crops without proper soil enrichment the average productivity is poor [24]. Indian soil are poor in nutrient status as indicated by the deficit of Zn, Fe, Mn, Cu, B, Mo and S at the rate of 48, 12, 5, 4, 33,13 and 4% [14] It needs fortification in case of inorganic fertilizers but cost and safety are the limiting factors [13]. Hidden hunger of trace elements can be corrected by agronomic rehabilitation practices like organic amendments, biofertilizers, and traditional crop rotation models including legumes, cereals and vegetables to restore the soil fertility. Fish wastes are the important resources with high nutritive value. Fermentation utilizing the microbes break down the complex materials in to simple forms and makes the nutrients available to the soil.

The proximate trace elements present in Gunapaselam (Fermented Fish waste) are shown in Table 3.1. The level of micronutrients in Gunapaselam is Zn (3.21±0.32), Mn (1.34±0.10), Fe (15.69±1.36), Cu (0.55±0.05), Pb (0.69±0.06), Ni (Below detectable limit) and B (1.76±0.14) (mg/Kg). The amount of sulphur and sodium were 0.04±0.00 and 0.07±0.00 (%) respectively. The presence of the above mentioned microelements in Gunapaselam are the essential nutrients recovered from the fish waste by fermentation process. Ghaly *et al.* [25] has reported that fish fillets are excellent sources of Ca, Mg, P, K, Na, Fe, Zn, Mn and Cu. Hence this method would be the best way to recover the plant growth promoting substances from fish waste. Nearly 25 trace elements are found to be the critical components in governing the growth and yield of plants. Moses *et al.* [26] also observed similar results with different poultry manures. The content of trace elements found in Gunapaselam is always within the permissible limits of a fertilizer and they also satisfy the requirement of the plants for their healthy growth [27].

The phosphate solubilising potential of Gunapaselam is shown in Fig. 3.1 and the count was log 7.32 CFU/ml. These phosphate solubilisers when added to soil will mobilize the insoluble phosphates in the soil and hence improve the uptake by the plants and crop growth [28]. Among the beneficial growth promoting microbes, phosphate solubilisers are promising candidates in agriculture as they promote crop production and yield. They play multi-factorial role in improving the soil fertility and plant growth. Phosphate solubilisers improve the soil fertility and plant growth by facilitating mobilization of phosphorus, trace elements , enhancing nitrogen fixation and producing phytohormones [29]. Vincent *et al*. [21] has reported the ammonifying and nitrifying effects of Gunapaselam. Moreover Gunapaselam in its liquid state can be easily absorbed by plants and can also be applied as foliar spray. Thus Gunapaselam, a biological preparation with essential trace elements and inoculants of plant growth promoting microbes may serve as a cost effective and environmental friendly biofertilizer.

The physiochemical parameters of the soil like pH, EC, Organic matter; carbon and CEC, macronutrients and micronutrient levels are found improved after amending the soil with Gunapaselam (Table 3.2.). The pH of Gunapaselam treated group was 7.56±0.47 when compared with chemical (7.89±0.74) and water treated (8.32±0.57). Compared with water control the organic matter and organic carbon were significantly increased in Gunapaselam treatment. Chemical and Gunapaselam treatment have significantly increased the macronutrients N, P, and K when compared with Group I. The content of Zn, Mn and Fe have shown a significant improvement in Group III when compared to other groups. The levels of B and Cu been increased significantly ($P<0.05$) by irrigation with Gunapaselam. Lead remains unaffected in all the treatment groups. Ni level was significantly high ($P<0.01$) in Group III and remains unaltered in Group II and Group I.

Table 3.1. Trace element Composition of Gunapaselam (Fermented Fish Waste)

Parameter	Unit	Results
Iron	mg/Kg	15.69±1.36
Zinc	mg/Kg	3.21±0.32
Manganese	mg/Kg	1.34±0.10
Copper	mg/Kg	0.55±0.05
Boron	mg/Kg	1.76±0.14
Sulphur	%	0.04±0.00
Lead	mg/Kg	(0.69±0.06
Sodium	%	0.07±0.00
Nickel	mg/Kg	BDL

BDL- Below detectable limit; Data represent Mean ± Standard Error Mean

The decrease in soil pH of the Gunapaselam treated plot was due to the decomposition of organic matter in the fish waste in to humus which is further digested by soil microbes to produce organic acids. Slightly acidic soil pH favors the growth of green gram. This property increases the cation exchange capacity of the soil which is responsible for the mobilization of calcium, potassium for plant growth. The variation in the level of trace elements in various treatments may be attributed due to the variation in microbial population caused by changes in the physiochemical properties of the soil like pH, moisture and organic content, different in the concentration of nutrients exuded by mung bean and by the nutrients provided by Gunapaselam. Intensive use of inorganic fertilizers has brought deleterious effects like worsening of soil physical and chemical properties, creating imbalance in soil nutrients, reducing crop yield [30]. The improvement in soil properties shown by Gunapaselam application will restore back the soil fertility otherwise lost due to chemical fertilizers.

The rhizobacterial and rhizobium populations of soil under various treatments are presented in Table 3.3. The total rhizobacterial load in the soil of Group I and Group II plots were significantly lower than Group III (8.30±0.21 log(CFU/gm)). The lowest rhizobium count was recorded in Group I (6.39±0.05) than in Group II and Group III. Microbial populations have major influence on plant growth and productivity [31]. These rhizobacteria contributes to seed germination and influences α- amylase activity that mobilizes the storage reserve and thereby provides energy for development and growth of roots and shoots.

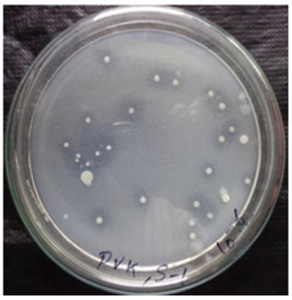

Fig. 3.1. Insoluble Phosphate solubilising potential of Gunapaselam on Pikovskaya's agar

Continuous application of chemical fertilizers may lead to depletion of beneficial microbial community in the soil. Microbial population is controlled by pH, salinity, temperature and organic matter [32] The increase in the rhizobacterial population in Gunapaselam treated soil confirms the favorable atmosphere for multiplication of microbes when compared with chemical and water treatment. They are the chief driving forces in recycling the soil nutrients and make them available for plant growth.

The presence of appreciable amount of symbiotic nitrogen fixing rhizobium species in Gunapaselam treatment establish complex interplay with roots of *Vigna radiata* resulting in the formation of more number of nodules and hence fix atmospheric nitrogen when compared with other treatments. In the present study addition of Gunapaselam with phosphate solubilizers augment plant growth not only by solubilising insoluble inorganic phosphate, also by promoting nitrogen fixation, increasing the availability of trace elements and producing plant hormones [33]. Thus a favourable environment for the beneficial rhizobacteria, phosphate solubilizers and rhizobium species provided by Gunapaselam treatment will be a natural biological approach for plant nutrient management.

Amendment of Gunapaselam has positively influenced the plant length, root length and number of nodules formed (Table. 3.4). Gunapaselam treated plants has recorded significantly longest root length and plant height and more number of nodules per plant. Application of chemical treatment registered the next best results when compared to water treatment. Spearman rank correlation test reveals the nodules formed in Gunapaselam treatment was in positive correlation with the level of trace elements Fe, Zn, Cu, and Mn essential for nodulation (Table 3.5).

The number of nodules produced in chemical fertilizer treatment was lower than Gunapaselam treated plants.

The root system play dynamic role in water and nutrient absorption. The presence of humic substances in organic matter of the soil and availability of nutrients especially ammonium and nitrate modify the root morphology by inducing the proliferation of root cells leading to root elongation, branching of axial roots and elongation of lateral roots [34, 35] . The rhizobacteria also contributes in increasing the root length, number of branches and surface area of roots [36]. Our results prove that Gunapaselam treatment has lead to long and extensive root system which can explore large volume of the soil to acquire water and more nutrients. It might have favored the accumulation of rhizobacteria which adopts the potential mechanism of sorption, precipitation and occultion to mobilize the trace elements to enhance the root growth and proliferation of lateral roots. Similar results are reported with the root growth of nectarine [37], raddish [38] and peach [39] by using cow manure and fish emulsion. The increase in plant height of *Vigna radiata* induced by Gunapaselam was due to the attainment of threshold turgor for stem cell elongation under good moisture condition and the plant will harvest better sunlight for more photosynthates. The increase in plant height was contributed by boron which promotes division and differentiation of cells controlling cellular and metabolic activities [40], copper functions as chlorophyll activator 41]. Sufficient presence of Zinc leads to development of long internodes, therefore increase in plant height and leaf size [12] and nitrogen increases the number and length of the internodes which results in progressive increase in plant height. Similar results were reported by Asha [42] and Brajeshwar [43] on treatment with Kunapajala, a fermented liquid from animal wastes. A healthy root growth is the basis for maintaining a high nutrient status, shoot growth and yield. The well developed root system in Gunapaselam treated plants might have resulted in increased absorption of water and nutrients and hence transportation of the same to the shoot system. In the present study, the changes in the nodule numbers under various treatments might be due to the influence of microbial community and concentration of essential trace elements. The plant growth promoting rhizobes facilitate the available forms of all essential elements in surplus amounts to influence legume root infection and nodulation [44]. Sufficient supply of B ensures formation and upholding of functional sym-biosomes [45]. Our results shows that, lesser concentration of iron content in soil in water treated group limits the root nodule production in *Vigna radiata*, as Fe is essential for symbiotic partnership of root nodule bacteria and multiplication [46], nodule development and their

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functions [47]. Fe is also required for establishing the synthesis of key enzymes involved in N2-fixation (nitrogenase, leghemoglobin) and in nitrogen assimilation [48]. Deficiency of iron has resulted in lesser nodules, bacteriods, symbiosomes and reduced plant growth as evidenced by Balestrasse *et al.* [49]. The increase in the soil nitrogen content after the harvest of *Vigna radiata* was due to increased formation of nodules facilitated by surplus of Fe and Ni essential for nodulation aided transport of ureides to upperparts of the plants, Mn for nitrate reduction and concomitant nitrogen fixation by microbes. This improvement induced by Gunapaselam in the vegetative character and symbiotic relationship might have been due to the improvement in soil properties with enhanced nutrients.

IV. Conclusions

Gunapaselam, the fermented fish waste was found to be a biological preparation epitomizing as a sufficient source of micronutrients and microorganisms. The tangible beneficial role of Gunapaselam was evidenced by providing a proper rhizosphere with beneficial microbes for the growth of *Vigna radiata.* The treatment with Gunapaselam has promoted the plant growth, root length and nodulation when compared with chemical fertilizer and untreated water control. The results suggested that preparation of Gunapaselam by treatment of fish waste will reduce the environmental burden of solid wastes and also it would be an inexpensive alternative to chemical fertilizers to promote the growth of legumes.

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Table 3.2. Soil fertility before the start of the study and after the growth of Mung bean

Parameter	Unit	Before applying	Group I	Group II	Group III
pH	-	8.85±0.63	8.32±0.57 ^{aNS}	7.89±0.74 $^{aNS,\ bNS}$	7.56±0.47 $^{aNS,\ bNS}$
Electrical conductivity	mS/cm	0.24±0.01	0.38±0.03 ^{a*}	0.52±0.02 $^{a***,\ b*}$	0.59±0.04 $^{a***,\ b**}$
Texture		Sandy Clay Loam	Sandy Clay Loam	Sandy Clay Loam	Sandy Clay Loam
Organic matter	%	1.21±0.05	1.27±0.11 ^{aNS}	1.41±0.11 $^{aNS,\ bNS}$	2.04±0.15 $^{a**,\ b**}$
Organic Carbon	%	0.32±0.03	0.35±0.00 ^{aNS}	0.41±0.03 $^{aNS,\ bNS}$	0.53±0.03 $^{a**,\ b**}$
Available Nitrogen	Kg/ha	204±16.11	240±22.80 ^{aNS}	357±21.66 $^{a**,\ b*}$	394±34.34 $^{a**,\ b**}$
Available Phosphorus	Kg/ha	9±0.81	10±0.59 ^{aNS}	15±1.35 ^{a*,b*}	20±1.38 $^{a***,\ b***}$
Available Potassium	Kg/ha	98±7.64	150±14.7 ^{aNS}	219±14.5 $^{a**,\ b*}$	250±19.75 $^{a***,\ b**}$
Available Zinc	ppm	2.15±0.19	2.24±0.19 ^{aNS}	2.81±0.2 $^{aNS,\ bNS}$	5.38±0.36 $^{a***,\ b***}$
Available Manganese	ppm	13.61±1.21	14.05±1.47 ^{aNS}	14.05±1.47 $^{aNS,\ bNS}$	29.99±1.73 $^{a***,\ b***}$
Available Iron	ppm	13.10±1.14	14.24±1.03 ^{aNS}	15.26±1.12 $^{aNS,\ bNS}$	22.37±1.8 $^{a**,\ b*}$
Available Copper	ppm	1.93±0.19	1.99±0.17 ^{aNS}	2.08±0.14 $^{aNS,\ bNS}$	2.87±0.19 $^{a*,\ b*}$
Available Boron	ppm	0.8±0.07	1.0±0.08 ^{aNS}	1.0±0.10 $^{aNS,\ bNS}$	1.37±0.05 $^{a**,\ b*}$
Available Lead	ppm	1.47±0.11	1.34±0.12 ^{aNS}	1.31±0.07 $^{aNS,\ bNS}$	1.67±0.15 $^{aNS,\ bNS}$
Available Nickel	ppm	0.39±0.03	0.37±0.02 ^{aNS}	0.36±0.02 $^{aNS,\ bNS}$	0.68±0.06 $^{a**,\ b**}$
CEC	Meq/100g	22.94±2.24	23.49±1.68 ^{aNS}	24.60±2.23 $^{aNS,\ bNS}$	25.54±2.42 $^{a*,\ b*}$

(Group I- Water; Group II- Chemical Fertilizer (25kg N2: 50kg P2O5: 25kg K2O: 20kg S/ha); Group III- unapaselam (1: 100 dilution)

Mean value between the groups were analysed using one way ANOVA followed by tukey's multiple comparison test in graphpad prism 5.0. a*, a**, a*** indicates p value < 0.05, 0.01 and 0.001, respectively vs before study and b*,b**, b*** indicates p value < 0.05, 0.01 and 0.001, respectively after study vs group I

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Table 3.3. Soil rhizobacterial and rhizobium population influenced by Gunapaselam(GP) on *Vigna radiata*

Group / Treatment	Rhizobacterial load log (CFU/gm)	Rhizobium load log (CFU/gm)
Group I- Water	6.81± 0.15	6.39± 0.05
Group II- Chemical Fertilizer (25kg N_2: 50kg P_2O_5: 25kg K_2O: 20kg S/ha)	7.69±0.23*	7.32± 0.03***
Group III- Gunapaselam (1: 100 dilution)	8.30±0.21**	8.09± 0.04***

Mean value between the groups were analysed using one way ANOVA followed by tukey's multiple comparison test in graph pad prism 5.0. *, **, *** indicates p value < 0.05, 0.01 and 0.001, respectively vs group I

Table. 3.4. Vegetative growth and symbiotic parameter influenced by Gunapaselam(GP) on *Vigna radiata*

Group / Treatment	Plant Length (cm) (30th day)	Root Length (cm) (30th day)	No. of nodules (30th day)
Group I- Water	22.02± 0.75	13.7± 0.85	6±0.1
Group II- Chemical Fertilizer (25kg N_2: 50kg P_2O_5: 25kg K_2O: 20kg S/ha)	25.70±1.12*	17.3± 0.73**	14±0.3***
Group III- Gunapaselam (1: 100 dilution)	27.25±0.75**	19.7± 0.48**	32±0.7***

Mean value between the groups were analysed using one way ANOVA followed by tukey's multiple comparison test in graphpad prism 5.0. *, **, *** indicates p value < 0.05, 0.01 and 0.001, respectively vs group I

Table 3.5. Spearman rank Correlation test

Parameters	rs Value	P value
Iron Vs Number of nodules	1.000	p < 0.0025
Zinc Vs Number of nodules	0.943	p < 0.01
Copper Vs Number of nodules	0.943	p < 0.01
Manganese Vs Number of nodules	1.000	p < 0.01

Based on the critical values of rank correlation (Spearman's rho- rs), null hypothesis of no correlation was rejected and concluded that the aforementioned parameters have correlations

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