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Influence of Gunapaselam, a liquid fermented fish waste on the growth characteristics of *Solanum melongena*

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ABSTRACT

This study was carried out to evaluate the effect of Gunapaselam – fermented fish waste, on the growth of Solanum melongena (Brinjal) plants. Gunapaselam was prepared by fermenting the fish wastes like head, gut, fins, bones etc., with Jaggery. After 15 days, the fermented liquid fish waste was filtered and used as liquid manure. Brinjal seeds were sown in different pots with only water (control), Urea (reference) and Gunapaselam (test). Application of Gunapaselam decreased the soil pH and enhanced the exchangeable cation levels, organic matter and the essential plant nutrients nitrogen, phosphorus and potassium. Improvement in the growth traits of brinjal plants like leaf area, plant height, stem diameter, root length, fresh plant weight were observed when compared with water control and urea fertilized treatment groups. These results were in synchronization with the anatomical results of root and stem. The thickness of the conducting systems, phloem and xylem were increased which facilitates the translocation, conductance and storage of photosynthates. The results indicated that Gunapaselam is a valuable resource for enhancing the soil fertility and growth of plants like brinjal. This study indicated the potential for the reclamation of fish waste as a useful liquid fertilizer for agricultural purposes.

Key words: Fermented fish waste, Growth traits, Gunapaselam, *Solanum melongena*, Organic liquid fertilizer

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INTRODUCTION

Global population is estimated to reach 8.5 billion by 2020 [1] with parallel demand for food. Agricultural sector is facing the daunting challenge to feed this rapid burst in population by applying chemical fertilizers. Applications of chemical fertilizers have robbed the soil fertility and have resulted in health and environmental hazards. Organic waste does not contain toxins or carcinogenic materials [2] like other chemical fertilizers and are found to improve the soil structure, water holding capacity, microbial biomass, and nutrient availability [3]. Hence, the alternative way is to reduce the use of inorganic fertilizers by recycling the organic wastes as fertilizers. This will pave the way for sustainable solid waste management and agriculture.

Fishery is one of the major sectors of agriculture which could solve this problem but demands a great concern for the management and conservation of environment. India is the second largest supplier of fish in the world after China, with a tremendous 11 fold leap from 0.75 million tonnes in 1950 to 9.6 million tonnes by 2012-13 [4]. Nearly 75% of the total weight of the fish was generated as solid waste in the form of gut, head, skin, bones, fins and frames after processing. The fish wastes rich in nitrogen, potassium, phosphorus and trace minerals [5] can serve as raw material for the production of many nutritive and nonnutritive products.

Fermentation process converts the solid substrates in to simple molecules with the help of microbes. It is one of the promising technologies which convert the fish waste in to useful organic manure, an expensive resource for agriculture without the formation of fusty smell [6]. Utilization of biodegraded fish waste products as liquid fertilizer, "Kunapajala" is an age old practice [7]. Bones, excreta, blood and meat of animals like horses, dead parrot, fish, horns of sheep, goat etc., were used to prepare Kunapajala. Later, the ingredients were modified based on the availability of wastes. Vincent et al. [8] have reported the preparation of Kunapajala / Gunapaselam using fish waste by fermentation and its use as liquid fertilizer and foliar spray. Fermentation has been found to remove anti-nutritional factors [9], increase of nutritive value [10] and digestibility [11].

Dao and Kim [12], has reported the use of fermented fish waste products as liquid fertilizer. The nutrients in the fish emulsion or fish meal stimulates the growth of the plants through growth promoting rhizobacteria, fixing up the atmospheric nitrogen [13] and increasing the uptake of essential nutrients [14]. This fertilizer because of its liquid nature is readily available, releases the nutrients slowly and prevents leaching from the soil. Nutrients can be applied in the form of foliar sprays which immobilize and supplement the nutrients to the leaves [15]. Beneficiary effect of fish emulsion has been proved in the growth of raddish [16], prevention of white mold disease of bean [17], yield of chilli [18] and Brinjal [19]. Aung et al. [20] investigated the various effects of fish soluble nutrients in the cultivation of soybean, corn, peas, radish, lettuce, rice, sorghum, concord grapes, peaches and strawberries.

*Solanum melongena Linn. (*Brinjal) belongs to the family Solanaceae is a popular vegetable grown throughout the year in all states of India. It is a good source of nutrients and possesses therapeutic properties. Brinjal requires high quantity of the major nutrients and organic matter for better growth and yield [21]. A shift from chemical fertilizers to recycled organic waste will represent a sustainable method of agriculture. Hence, the objective of the study is to evaluate the potential effects of Gunapaselam on the growth characteristics of brinjal.

EXPERIMENTAL SECTION

Soil analysis

The experiment was carried out in a Randomized Complete Block Design (RCBD) at Alpha Arts and Science College, Chennai. The experimental soil used for the study was analysed at Vedapuri Krishi Vigyan Kendra (Tamil Nadu Agricultural University - outreach), Kilnelli, Cheyyar (Taluk), Thiruvannamalai (District). Soil pH and Exchangeable cations were determined from the saturated extract (l:5, soil: water) of soils [22,23]. Organic matter and organic carbon were estimated by Walkley and Black method [24, 25]. The levels of available nitrogen by alkaline permanganate method, phosphorus by Olsen's method and potassium by Flame photometer method were estimated by standard procedures [25, 26]. The above said parameters were analysed before and after the application of Gunapaselam.

Preparation of Gunapaselam

Well blended mixture consisting of the head portion, intestines and gills of the fish waste were collected from the local fish market at Vanagaram, Chennai and Gunapaselam was prepared according to the procedure described by Vincent et al. [8]. A clean clay pot of 10 liters capacity was taken and filled with 5 l of water. 2½ kg of powdered native jaggery was added and stir well to dissolve. Then ½ Kg of fish waste was added and mixed thoroughly. The mouth of the pot was covered with a cotton cloth to prevent the entry of flies. The content of the pot was mixed every day. After 14 days, the contents were filtered and the filtrate was used as organic liquid fertilizer, Gunapaselam in the ratio of 1:10 with water.

Experimental Design

A seed bed was raised and healthy seeds obtained from Vedapuri Krishi Vigyan Kendra (Tamil Nadu Agricultural University - outreach) were sown and watered as required. 25 days old healthy plantlets were planted in the clay pots with different groupings. The groupings were Group 1 – Water Control, Group 2 –Chemical fertilizer (Urea) and Group 3 – Gunapaselam. Each treatment group has 15 numbers of replicates. Water / diluted Gunapaselam were added to the respective treatment pots based on the requirement.

Vegetative Growth Characters

The leaf area and plant height were measured for 6 weeks after 15 days of transplantation in to the pot using meter ruler from ten randomly selected plants of each treatment. At the end of 8 weeks the stem diameter was measured using a calibrated digital vernier caliper and root length using meter ruler. The fresh samples were taken to the

laboratory to measure the fresh plant weight using an electronic balance (Shimadzu), and numbers of lateral roots were counted manually.

Anatomy of the root and stem

Collection of specimens

The plant specimens (Stem and root) for the proposed study were collected from each groups. Care was taken to select healthy plants and their parts. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary –Butyl alcohol as per the procedure given by Sass et al. [27]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60ºC) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned to the thickness 10-12 µm. Dewaxing of the sections was by customary procedure [28]. The sections were stained with Toluidine blue as per the method given by O'Brien et al. [29]. Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage and blue to the protein bodies.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. For normal observations bright field was used.

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.). A probability of $P< 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Effect of Gunapaselam on soil nutrients

The physico chemical properties of the soil used in this study before and after the application of Gunapaselam were listed in Table 1. The soil pH was found decreased favourably to support the growth of brinjal in Gunapaselam treatment. After the treatment with Gunapaselam, the amounts of exchangeable cations were increased by 85% (0.24dsm-1). An increase of 3% was observed in the organic carbon, total organic matter and total nitrogen. The phosphorus content was increased to 12 kg/ha (9%) and potassium by 5% (264 kg/ha).

Table 1: Effect of Gunapaselam in the soil properties before and after application

S.NO	PARAMETERS	Before application of Gunapaselam	After application of Gunapaselam
1.	**pH**	8.23	7.98
2.	**Exchangeable Cations (dsm)$^{-1}$**	0.13	0.24
3.	**Organic carbon (%)**	0.30	0.31
4.	**Organic matter (%)**	0.517	0.53
5.	**Nitrogen(kg/ha)**	238	246
6.	**Phosphorous(kg/ha)**	11	12
7.	**Potassium(kg/ha)**	251	264

Fish waste is a great source of minerals, proteins, fat and carbohydrates. Addition of rich carbohydrate source like molasses [30] or jaggery [8] to the fish waste, under enough moisture [31] facilitates the fermentation process as the fish offal or molasses are very good sources of native microbes [30]. The presence of huge microbial population like ammonifiers, nitrifiers, phosphate solubilisers [8] and *Lactobacillus acidophilus* brings out fermentation. The production of acids like lactic acid, acetic acid and carbonic acid decrease the pH of the fermented medium to 4.0 which prevents the fermented product from spoilage [32]. The decrease in the soil pH after Gunapaselam application

was be due to the production of organic acids. It is well known that nutrient uptake and its availability to the crops were influenced by soil pH [33].

Addition of Gunapaselam increases the organic matter, the smallest but dynamic component of the soil. Yin Chan [34] has stated that, addition of organic manure like compost, plant debris and recycled organic waste increases the organic matter of the soil. Sieglinde and Stuart [35], suggested that an amendment rich in nitrogen (protein source) and carbon will result in a good increase of organic matter. Gunapaselam, fermented fish waste manure is a carbon, nitrogen and nutrient enriched amendment which is responsible in increasing the organic matter and hence organic carbon.

According to Takeda et al. [36], the addition of salts and solubilisation of minerals will increase the exchangeable cation content. The cations like magnesium, potassium and calcium which are essential plant nutrients are bound by cation bridges to the negatively charged clay particles and organic matter [37].Water holding capacity is increased and leaching of cations and requirement of nutrients are minimized in soil with high exchangeable cation capacity. Bellaki et al. [38] has observed that a fertile soil will have more exchangeable cations.

Fish waste contains important minerals like calcium, phosphorus, potassium, sodium, magnesium, zinc, manganese and copper [5] similar to the nutritive value as fish. The amount of protein, amino acids, calcium and phosphorus were found to be increased after the fermentation of tauna fish waste [39]. Amino acid is one predominant forms of nitrogen, used by the plants for their growth [40].

Fermentation of the fish waste produces significant amount of organic acids, lactic acid and acetic acid [30] which activates mineral phosphate solubilisation and P- solubilisers [41] like *Rhizobium* [42] and *Bacillus* [43]. Besides increasing the soil phosphorus level, Phosphate solubilisers also increase the nitrogen fixation, trace elements and plant hormones [44].

Plants utilize one of its essential nutrient potassium from the soil, present either in water soluble, exchangeable or non exchangeable forms. Exchangeable cations are held by clay minerals and organic matter in soil. Unavailable potassium can be made available by increasing the microbial activity or by providing acidic environment [45]. This might be the reason for the increase in soil potassium after the application of Gunapaselam. Similar results were observed in the growth of sudan grass by waste mica inoculated with *Bacillus mucilaginosus* [46].

Effect of Gunapaselam on growth characters of brinjal

The effect of Gunapaselam on the leaf area of the brinjal plants are shown in Table 2. The increase in leaf area (Figure 1) could be due to the enhanced availability of the nutrients particularly nitrogen which promotes the leaf area and function, increased cell division and elongation. The results obtained are in accordance with the report in Okra [47], brinjal, chilli and tomato [48].

Table 2: Effect of Water, Urea and Gunapaselam on leaf area of Brinjal plants

Week	Group I (Water) cm^2	Group 2 (Urea) cm^2	Group 3 (Gunapaselam) cm^2
1st Week	15.75±1.23	16.5±1.33	26.61±1.49*
2nd Week	18.87±0.97	21.20±1.70	37.45±2.14*
3rd Week	24.29±1.27	25.83±1.18	44.30±2.31*
4th Week	25.68±1.99	31.67±2.26	63.33±1.75*
5th Week	30.93±2.12	38.22±1.82#	71.82±2.13*
6th Week	36.25±1.53	46.08±1.93#	85.33±1.97*

*Data represent Mean ± Standard Error Mean. #, * indicates p value < 0.05 and 0.001 compared to corresponding control*

Table 3 shows the significant increase in plant height of brinjal after 4th week of gunapaselam application. The increased plant growth is mainly due to the improvement in soil structure, moisture availability and nutrients especially nitrogen (Figure 2). This result is consistent with that of Atakora et al. [49] who has observed higher rate of plant growth in carrot after the application of grass cutter manure. Availability of dry matter from rich nutrients, higher light interception due to increased leaf area and high photosynthetic activity leads to an increase in the plant growth.

Figure 1 Effect of water (a), urea (b) and gunapaselam (c) on the growth of brinjal plant

Table 3: Influence of Gunapaselam on plant height

Week	Group I (Water) cm	Group 2 (Urea) cm	Group 3 (Gunapaselam) cm
1st Week	5.2±0.43	5±0.5	5.28±0.99
2nd Week	6.6±0.58	6.33±0.5	9.86±1.35
3rd Week	9.13±0.71	9±0.89	14±2.37
4th Week	9.53±0.88	11.83±1.32	20.547±2.43#
5th Week	10.5±0.707	12.±0.81	23.73±1.90*
6th Week	11.75±1.70	16.33±1.527	28.75±3.06 #

*Data represent Mean ± Standard Error Mean. #, * indicates p value < 0.05 and 0.001 compared to corresponding control*

Figure 2 Influence of Gunapaselam on leaf area and plant height on Brinjal

Gunapselam treated plants had increased root length, lateral roots, stem diameter, fresh and dry plant weight than urea and water treated controls (Table 4 and Figure 3). The development of the root system is related to the chemical, physical and biological properties of the soil. The organic matter increases the soil porosity and reduces soil bulk density [50] and also on decomposition releases humic and felvic acids that leads to the production of auxin or auxin like substances [51], which enhances the root growth and development of lateral roots. Increased activity of the plant growth promoting rhizoabacteria also enhances the growth and proliferation of the roots by increasing the availability of nitrogen [52] and by the production of auxins [53]. Perez et al. [54] has reported an indirect effect of phosphorus on root growth. Khaled et al. [16] has reported that fish emulsion was found to be the best growth medium for the bacteria and actiomycete isolates that promotes the growth of raddish. The results are in confirmation with the findings of Baldi and Toselli [55] on the root growth of nectarine (*Prunus persica* L.) and peach by using cow manure and compost [56] respectively.

Significant differences in stem diameter and fresh plant weight were detected in Gunapaselam treated when compared with urea fertilized and unfertilized (water) treated group (Table 3). The increase in the stem diameter and fresh plant weight of the whole plant was due to the ample availability of plant nutrients, favorable soil conditions, sufficient water availability which leads to the luxurious growth of the brinjal plant. Similar trends were noted in the findings of many workers [57]. Mathowa et al. [58] observed decrease in the stem diameter when the soil nutrients were depleted.

Table 4: Effect of Gunapaselam on Growth Traits of Brinjal Plants

S.No	Group I (Water)	Group 2 (Urea)	Group 3 (Gunapaselam)
Stem diameter (cm)	0.2± 0.00	0.3±0.01	0.5±0.01*
Fresh plant weight (mg)	100± 5.24	200±4.26*	400±6.31 *
Root length (cm)	14±0.4	13± 0.2	18± 0.5 *
Small root hairs (Numbers)	15±1.2	30±2.1*	45±1.8*

*Data represent Mean ±Standard Error Mean. #, * indicates p value < 0.05 and 0.001 compared to corresponding control*

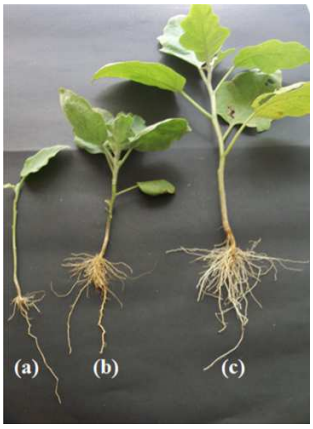


Figure 3 Effect of water (a), urea (b) and Gunapaselam (c) on the development of secondary roots. Gunapaselam treated(c) brinjal plant has developed 45 number of secondary roots when compared with urea(b) and water (a), which has produced 30 and 15 respectively

Effect of Gunapaselam on anatomical characters

The anatomical results show that xylem and phloem tissues of gunapaselam treated brinjal plants had better characteristics features which conduct water and food products respectively. Generally xylem vessels are wide circular for effective diffusion and distribution of water. The size of xylem was found increased (800µm) when compared to urea control (550µm) and water control (less than 300µm). The phloem tissue responsible for conducting of food products and other nutrients are found increased in thickness when compared to that of urea and water controls (Figure 4). The stem xylem and phloem results have also shown similarly improved characteristic features in Gunapaselam treatment when compared to other two groups (Figures 5.1 and 5.2).

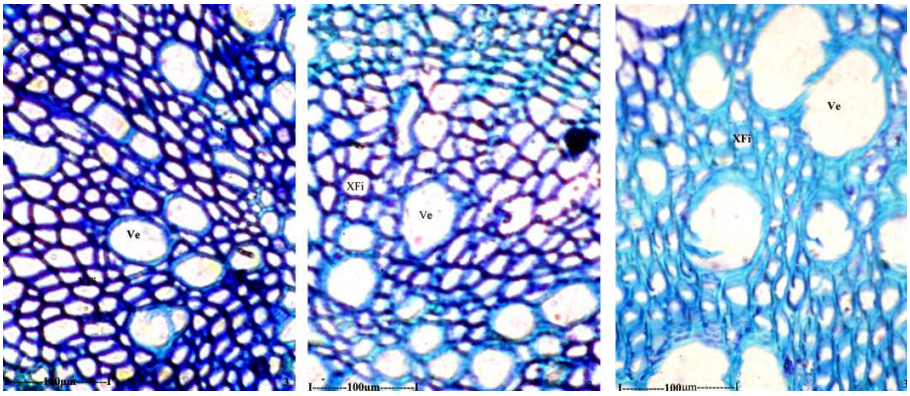


Figure 4. T.S. of root (a) Group 1 (Water) – The phloem elements have undergone shrinkage and compressive; Xylem elements are thin walled (<300µm), (b) Group 2 (Urea) – The phloem elements are large and intact; Xylem elements are 550µm in diameter, (c) Group 3 (Gunapaselam) – The phloem elements are large, thick and intact; Xylem elements are 800µm in diameter. (40x: Co- cortex; Pe – Periderm. Sph- Secondary Pholem; Sx – Secondary Xylem; Ve- Vessels; XFi- Xylem Fibre)

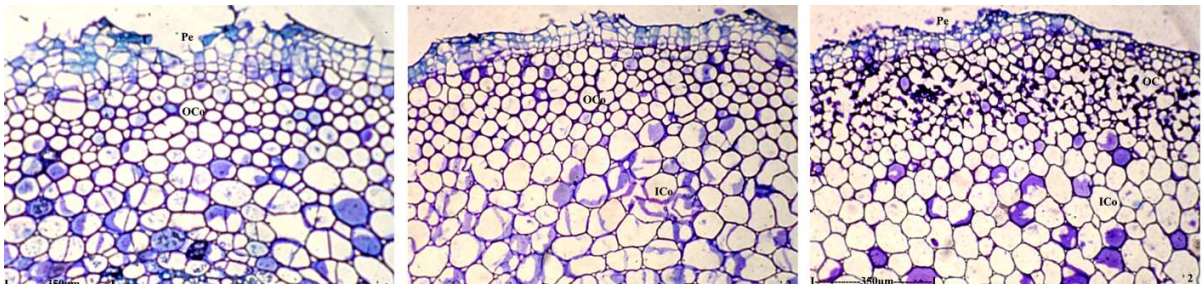
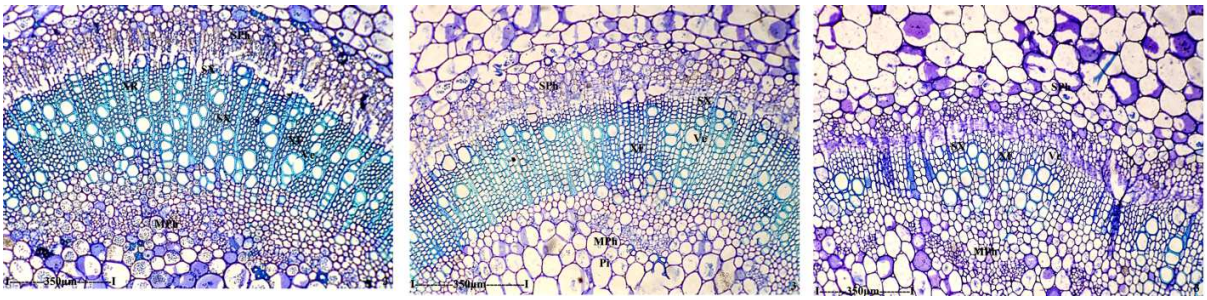


Figure 5.1 T.S. of Stem (a) Group 1 (Water) – Wide well developed periderm and cortical tissues of the stem, (b) Group 2 (Urea) – Prominent periderm well preserved cortex, (c) Group 3 (Gunapaselam) – Well developed periderm and thick zone of collenchymatous cortex and thick parenchymatous inner cortex. (10x: ICo- Inner cortex; OCo- outer cortex; Mph- medullary phloem; Pe- periderm; pi- pith; Sph- Secondary Pholem; Sx – Secondary Xylem; Ve- Vessels; XFi- Xylem Fibre)

From the above mentioned results, the anatomical findings coincide well with the morphological characters of the brinjal plants under various treatments. The two types of conductive tissues in plants are Phloem and Xylem. Growth and developments of the plant is influenced by the phytohormones transported by the phloem whereas the functions of xylem includes transport and storage of water and mineral nutrients and also provide mechanical support to the plants. The increase in the thickness of the secondary phloem in Gunapaselam treated brinjal plants confirms the maximum conduction of photosynthates as explained by Kulkarni and Deshpande [59]. The transport, conductivity and storage of nutrients, photosynthates and water was achieved by increasing the diameter of the secondary xylem in Gunapselam treated brinjal plants. These results are in accordance with the findings of Manoj et al. [60] in chilli, tomato, *Calotropis*, *Solanum* species and grapes. These factors were responsible for the increase in the plant height, leaf area, stem diameter and fresh plant weight. The production of the lateral root growth is directly linked with the number of xylem [61]. Thus the correlation of these anatomical features with the growth traits validates our obtained results.



igure 5.2 T.S. of stem (a) Group 1 (Water) – The cortical zone is 500µm thick and medullary phloem is not well differentiated; Xylem elements are wide, (b) Group 2 (Urea) – The cortical zone is 600µm thick and medullary phloem is also reduced which are small and thin walled masses; Xylem elements are wide and thin walled, (c) Group 3 (Gunapaselam) – The cortical zone is 700µm thick and medullary phloem is prominent; Xylem elements are wide and thin walled. (10x: ICo- Inner cortex; OCo- outer cortex; Mph- medullary phloem; Pe- periderm; pi- pith; Sph- Secondary Pholem; Sx – Secondary Xylem; Ve- Vessels; XFi- Xylem Fibre)

CONCLUSION

The increase in the human consumption of fish results in the production of waste which can be reutilized to reduce their negative impact on the environment. Fish is one of the best animal food rich in proteins, minerals and trace elements. Fermented fish waste, Gunapaselam is found to enrich the soil nutrients required for plant growth and favourably influence the conducting functions of xylem and phloem vessels. Thus Gunapaselam could be used as a valuable organic liquid fertilizer for better yield from crops at lesser cost and also without the harmful effects of chemical fertilizers. However, further study on the mechanism of action in detail is required to support the results of this pilot study.

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