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REVEALING THE NON- PHYTOTOXIC EFFECT OF GUNAPASELAM (FERMENTED FISH WASTE) BY A DOSE DEPENDENT *IN VITRO* STUDY

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ABSTRACT

Disposal of fish waste without proper treatment creates an environmental nuisance. Fish waste like head, fins, viscera etc., were collected and degraded by natural fermentation with jaggery. The present study reports the nontoxic nature of fermented fish waste (Gunapaselam) on germination and growth promoting property on *Vigna radiata* at different concentrations (25, 50, 75 and 100 fold dilutions) by Petri plate *in vitro* model. Treatment of green gram seeds with Gunapaselam shows that at dilutions from 50 fold onwards fermented fish waste was able to promote the formation of leaf, shoot, tap root and secondary roots. There is a significant improvement in the concentration of plant pigments (Chlorophyll, carotenoids and xanthophylls) and protein content. This study suggests that the fish waste can be used as fertilizer after diluting the fermented liquid.

KEY WORDS: ***Fish waste, Fermentation, Green gram, Petri plate assay, Pigments, Organic fertilizer***

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INTRODUCTION

The continuous use and rising cost of chemical fertilizers has motivated to switch to an eco friendly sustainable agricultural practice. The re-use of treated solid waste in the field of agriculture has gained considerable importance in current scenario. With the increasing population and rising demand for quality protein more than 1 billion people depend on fish proteins.[1] In India, Sea food industry has taken an enormous leap contributing around 5.68% of global fish production, [2] but due to poor waste management strategies fish wastes are dumped in to the sea or land causing serious environmental problems. Management of fish waste can help to mitigate these problems and it helps to reutilize the nutrients present in fish waste. Fish waste has been found to contain essential nutrients like nitrogen, potassium, phosphorus, calcium, magnesium *etc*. Seed germination process is a key to agriculture as it produces the next generation of plants. Seed germination and further growth involves complex physiological and morphological changes.[3] The seeds will wake up from dormant state only when the conditions are favorable. The dry seeds imbibe water resulting in expansion and elongation of the embryonic axis. The downward pointing radical of the embryonic axis penetrates the seed envelope and the protrusion of the radical thus completes the process of germination.[4] After visible germination, the major storage reserves carbohydrates, lipids and proteins are mobilized through metabolic pathways by enzymes and seedling growth ensues. The radical extends, profusely branched and becomes the primary root system. Root length is a good indicator of germination process and the following metabolic changes.[5] The top of the embryonic axis becomes hypocotyls elongating in to the shoot system. Germination capacity and post germinative events were influenced by hormones, heavy metals, environmental factors, water potential, light and nutrients. [6-9] It was reported that fish waste contains essential nutrients required to support plant growth[10] and this would help in profitable crop production. Seed germination and seedling growth assures the survival of plant growth essential to maintain balance between world population and food production. Fermentation was the most suitable and convenient technique to treat the agro and animal wastes. Domestic waste water after treatment with effective microbes was found to increase the soil fertility and growth of *Vigna mungo*.[11] Fermentation by natural means can be brought out if a good carbohydrate source, sufficient moisture and microbial source are provided. This was found to be more economical without using commercial enzymes or microbial culture. Fish waste mainly the gut was found to be the potential source of many microbes and proteolytic enzymes. Vincent et al.[12] has fermented the fish waste with jaggery as carbohydrate source. The effective usage of this fermented fish waste (Gunapaselam) in agriculture surely will reduce dependence on expensive chemicals. Gunapaselam (GP), due to its liquid nature can be either amended with soil or can also be applied as foliar spray. Although fermented fish waste has growth promoting nutrients it is important to assess its feasibility as fertilizer as imbalance in nutrient content can lead to uneven plant growth and reduce the yields.[13] Fermented fish waste was acidic due to the release of organic acids during fermentation.[14] So exploring the possibility of Gunapaselam for vegetation is essential. Toxicity and maturation of organic manure can be assessed by germination assay. Seed germination[15] and plant growth bioassay or a combination of them[16] gives reliable result to assess the compatibility of treated organic waste on plant growth. Seeds of different plant species like *Amaranthus* sp., *Vigna unguiculata* (L.) Walp and *Abelmoscus esculentus*[17] and *Vigna radiata*[18,] *Lepidium sativ*um[19] were used for germination assay. *Vigna radiata* (Mung bean) is an important pulse crop, fodder and green manure. The demand is high because of its high protein content and low price. To increase the yield of mung bean, the use of synthetic fertilizer (N: P: K: S) has become essential. So the present study examined the effect of Gunapaselam at various dilutions on *Vigna radiata*, green gram by an *in vitro* model.

MATERIALS AND METHODS

Gunapaselam preparation and In vitro Germination assay

Fish waste was collected and fermented according to the procedure described by Vincent et al.[12] Gunapaselam was prepared by mixing finely chopped fish waste (1 kg) of all types with jaggery (1.5 kg) in a plastic container along with 5 L of water. The contents were constantly stirred well and filtered after 15 days. The filtered fermented fish waste (Gunapaselam) was diluted with water to get a dilution ratio of 1:25, 1:50, 1:75 and 1:100 and used for the study. Dry and healthy seeds of *Vigna radiata* of uniform weight (38 ± 2 mg) were surface sterilized with 0.1% Mercuric chloride for 2 minutes and washed thoroughly with distilled water. Each treatment group consists of 10 seeds in completely randomized design with 3 replications. The treatment groups were as follows, Group I – Water control, Group II – 1% Urea, Group III – 1:25 fold diluted Gunapaselam, Group IV - 1:50 fold diluted Gunapaselam, Group V - 1:75 fold diluted Gunapaselam, Group VI - 1:100 fold diluted Gunapaselam. The seeds were then spread equispacially over sterilized whatmann No.1 filter paper kept in sterile Petri-dishes (9 cm). Seeds and filter paper were moistened with 10ml of 1:25/1:50/1:75/1:100 fold diluted GP solutions and maintained at normal room temperature in lab condition for 5 days. The treatment solutions were added regularly to moisten the seeds. On 5th day number of seeds germinated was counted and growth bioassay parameters like leaf area and root development of the plants were recorded. Analysis of data was done for 3 replicates. Leaves were separated from the seedlings and used for estimation of photosynthetic pigments like chlorophyll, carotenoids & xanthophyll[20] and protein content of the germinated seedling was also estimated[21].

STATISTICAL ANALYSIS

Data are reported as Mean ± Standard error of mean. Statistical analysis was done using SPSS Version 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.). P< 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The search for sustainable agriculture has encouraged the use of animal products and wastes as manure. The constant increase in consumption of fish results in enormous amount of solid waste which is usually discarded. Fermentation proves to be a cost effective method to convert the fish waste in to organic fertilizer. The maturity and toxicity of the decomposed products can be assessed by germination assay. Germination and growth are essential indicators that elicit successful plant growth. In the present *in vitro* study, the germination percentage of *Vigna radiata* was highest in all the groups except Group III (Figure -1&2). The might be due to the presence of favorable factors like sufficient amount of moisture, oxygen and temperature. The influence of these factors on seed germination was also observed in *Arabidopsis*.[22] The increased activity of amylase and transportation of sugars to embryo axis and reduced protease activity promotes germination. Reduced activity of amylase and increased protease activity were observed when cowpea was treated with chromium.[23] The reduced germination percentage in Group III (1:25 diluted GP) might be attributed to the high concentration of essential nutrients that exerts a toxic effect on the developing radical. The high osmotic pressure caused by concentrated nutrients reduces the diffusion of water and hence germination. It was reported that treatment with heavy metals reaches the embryo tissues of maize and affect the germination.[24] In seeds of *Vigna radiata* treated with 1:25 fold diluted GP exhibits lowest seedling length (Plumule + radical length), and less number of secondary roots when compared to water treated seeds (Table-1). The stunted growth was due to the impact of heavy metals on radical and hypocotyl extension. Compounds lessen the radical growth by reducing the cell elongation than cell division. Similar results were observed when soyabean was treated with 2,4 dichlorophenoxyacetic acid.[25] Root hairs and lateral roots emerge from the radical. The root hairs become the absorbing structure. Gunapaselam at 50, 75 and 100 fold dilutions was able to promote seedling growth as there was a decrease in concentration of the minerals. When the concentrations of essential biological elements were too high, they are toxic and also cause disturbance in the plants biological processes.[26] This was evidenced in Group III, as the root of the seedlings were in direct contact with concentrated nutrients it causes an inability to absorb the water thereby reducing the plant height or inhibition of cell division in the roots and collapsing the structure which leads to the reduced number of lateral root formation. Similar results were also recorded in *Vigna radiata* due to mercury toxicity[27] and cadmium induced toxicity in *Cucumis sativus.* [28] The leaf extract of *Moringa peregrine* was found to reduce the growth potential of *Hordeum vulgare* and *Trigonella foenumgraecum* by their negative allelopathic effect on radical length. [29] Chlorophyll content is one of the important measures of production capacity of the plant.[30] In Urea treated group significant increase was noted in the content of chlorophyll, carotenoids & xanthophylls and protein (Table-2). At the dilution ratio of 1:25 the amount of chlorophyll, carotenoids & xanthophylls and protein were significantly reduced when compared to control group. However, further dilution of Gunapaselam (1:50, 75, 100) resulted in the increase of chlorophyll and carotenoids, xanthophyll pigments in dose-dependent manner (Table-2). This might be due to the activation of enzymes involved in chlorophyll biosynthetic pathway. Dhanam, [31] has observed an increase in chlorophyll content when *Oryza sativa* L. was treated with 25% diluted dairy effluent. A rapid increase in chlorophyll content was reported after 2 days of germination and the level decreased during dehydration.[32] Presence of sufficient amount of magnesium and iron and its translocation leads to enhanced chlorophyll production.[33] Reduction in chlorophyll content was observed in cow peas exposed to high chromium.[34] The synthesis of proteins resume after imbibitions process. New mRNAs encoding for protein to support germination and protein to support normal cellular metabolism and vegetative growth are transcribed.[35] Changes in mRNA were noted in embryo and in proteins during germination of peas.[36] Germin, a protein specifically produced after germination aids in post cell elongation.[37] In the present study there was a significant increase in protein content with increasing dilution of Gunapaselam (Table-2). This might be due to the production of proteins to support germinative and post germinative process. The protein content of Group I and Group III plants were found to be low when compared to other groups. Plants produce specific change in protein synthesis that enables them to cope up with the induced stress.[38] The decrease in total protein content might have been resulted in the retarded growth rate of the plants with reduced pigment level in Group III.

Table 1
Influence of Gunapaselam(GP) on plant length and lateral root development of *Vigna radiata* after 5 days – Petri-plate Germination Assay

Group / Treatment	Plant Length (cm)	No. of lateral roots
Group I- Water	6.4 ±0.32	3.4 ±0.21
Group II- 1% Urea	8.41 ±0.23***	4 ±0.40NS
Group III- 1:25 diluted GP	5.1± 0.71NS	2.2± 0.63NS
Group IV- 1:50 diluted GP	6.84± 0.32NS	6.3± 0.67*
Group V- 1:75 diluted GP	8.54 ±0.41***	6.5 ±0.93**
Group VI- 1:100 diluted GP	8.81 ±0.43***	6.5 ±0.77**

Data are expressed as Mean ± SEM. 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 1

Table 2
Effect of Gunapaselam (GP) on content of Chlorophyll, Carotenoids & Xanthophylls and Protein content of *Vigna radiata* after 5 days – Petri-plate Germination Assay

Group / Treatment	Total Chlorophyll (mg/gm leaf tissue)	Carotenoids & Xanthophyll (mg/gm leaf tissue)	Protein (mg/gm of seedling tissue)
Group I- Water	0.47 ±0.006	0.17 ±0.01	0.46 ±0.03
Group II- 1% Urea	0.79 ±0.04***	0.12 ±0.02**	0.64 ±0.04*
Group III- 1:25 diluted GP	0.57± 0.03NS	0.21± 0.03NS	0.54± 0.01NS
Group IV- 1:50 diluted GP	0.66± 0.04**	0.39± 0.02***	0.62± 0.03*
Group V- 1:75 diluted GP	1.13 ±0.04***	0.43 ±0.01***	0.64 ±0.04*
Group VI- 1:100 diluted GP	1.22 ±0.06***	0.85 ±0.03***	0.78 ±0.06***

Data are expressed as Mean ± SEM. 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 1

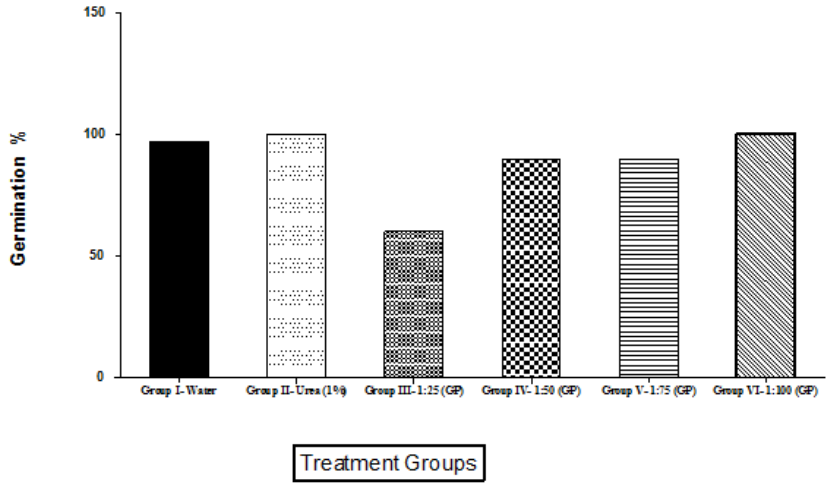

Figure 1
Germination % of *Vigna radiata* after 5 days under different treatments

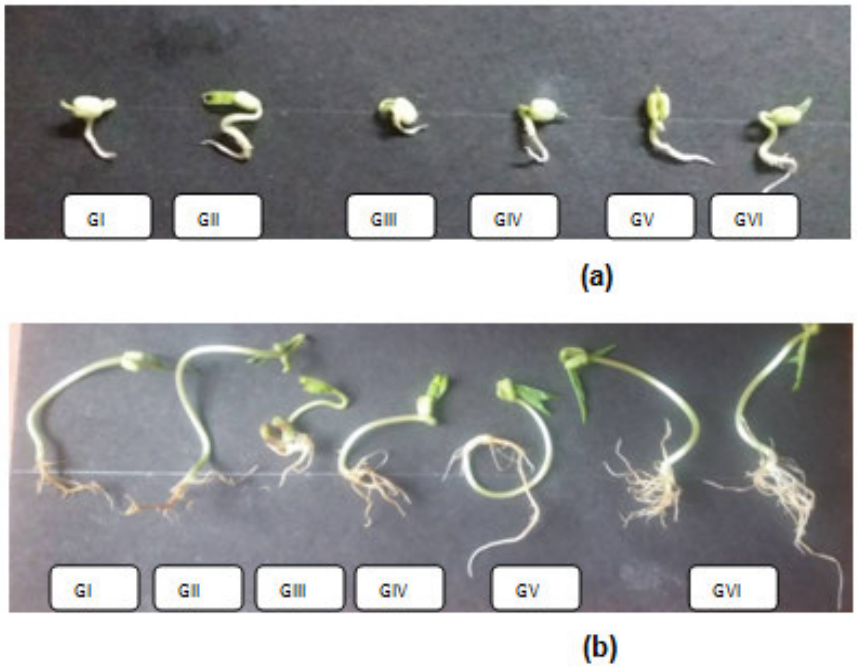

Figure 2
Germination of *Vigna radiata* seeds on (a) 3rd and (b) 5th day

(Group I- Water; Group II- 1% Urea ; Group III- 1:25 diluted GP; Group IV- 1:50 diluted GP; Group V- 1:75 diluted GP ; Group VI- 1:100 diluted GP)

CONCLUSION

It may be concluded from the present study that Gunapaselam at higher concentration is toxic for plant growth and at the dilution of 1:100, it significantly improves the growth parameters of *Vigna radiata* under *in vitro* conditions. Thus this dilution range will be suitable for agricultural use and can be used for field study. Application of this fermented fish waste as liquid organic manure in agriculture would be a better way to reduce the usage and to rectify the hazards caused by chemical fertilizers.

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CONFLICT OF INTEREST

Conflict of Interest declared none.

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