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Evaluation of the biostimulant properties of scale gelatin from Protonibea diacanthus on Vigna radiata

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Abstract : Biostimulants are environmentally safe compounds which work by stimulating plant growth to boost agricultural yield. Animal protein hydrolysates are generally by-products obtained from food industry. Gelatin derived from fish scale waste is a protein hydrolysate which has the potential to be used as biostimulants. After pre-treating the waste scales of *Protonibea diacanthus* (Ghol) gelatin was extracted by thermal hydrolysis using an autoclave. Extracted gelatin was brownish yellow in colour with fishy odour. Scale gelatin was used as a biostimulant to study its effects on plant growth, percentage seed germination, root and shoot length, leaf area, chlorophyll content, and protein concentration of *Vigna radiata* (mung). The gelatin treatment to mung seeds showed positive influence on all the plant growth parameters. Increase in the chlorophyll content and protein content of the plant was observed which may lead to better yield. Hence scale gelatin was found to be an effective biostimulant.

Index Terms **-** Biostimulant, gelatin, *Protonibea diacanthus*, *Vigna radiata*, scale, chlorophyll

INTRODUCTION

In the present-day world agriculture sector is facing challenges to increase the crop production to sustain the expanding population (Rouphael and Colla, 2020). With increasing advancement in technologies several methods have been used to increase the crop production and to reduce the use of fertilizers and pesticides (Calvo, *et al.*, 2014). Seed enhancement technology is one of the eco-friendly, effective, and reliable way to improve crop production. This technique is used to improve the properties of seed like breaking of dormancy, stress tolerance, synchronization of germination etc. The treatment of seeds is performed prior to sowing (Taylor, *et al.*, 1998).

Organic material derived from animal, plant waste or microorganisms can be employed for the improvement of plant productivity and protection against unfavourable conditions. Such substances are known as biostimulants. Kauffman, *et al.*, (2007) described biostimulants as materials other than fertilizers to promote plant growth when applied in low quantities to roots. Baltazar, *et al.*, (2021) described different categories of biostimulants which are bioactive compounds like humic and fulvic acids, protein hydrolysates, seaweed extract, chitosan, beneficial fungi and beneficial bacteria. Animal derived biostimulant have a higher nitrogen content ranging from 9 to 16% of total dry matter (Polo, *et al.*, 2006). Animal based protein hydrolysates have been found to have a positive effect on yield in tomato, maize and lettuce (Parrado, *et al.*, 2008, Ertani, *et al.*, 2009, Xu and Mou, 2017).

Gelatin, which is derived from hydrolysis of collagen, is an example of an animal hydrolysate. Gelatin is obtained from alkali and acid hydrolysis of animal connective tissues, bone and skin (GMIA, 2012). It is rich in amino acids like proline and hydroxyproline. According to Kieliszewski and Shpak, (2001) hydroxyproline is found in plant cell wall. Hydroxyproline rich glycoproteins are found in abundance in plant reproductive organs like pistil, pollen tube etc and they play an important role in maintaining the structure of cell and also facilitate in communication among the cells (Wu, et al., 2001). Gelatin and hydrolysed collagen are rich in hydroxyproline which may act as source for these hydroxyproline rich glycoproteins. Hydrolysed collagen and gelatin capsules have been used for the study of its biostimulant effect by Wilson, *et al.*, (2018) on cucumber plant. Use of gelatin capsules have also been described by Trias, and Takahashi, (2014) which increased the growth of plants from seeds in greenhouse studies. Fish scale waste is discarded from fish markets and fish processing industry regularly. Hence the primary goal of the present study was to utilize the waste scales to obtain gelatin rich in hydroxy proline. Secondly to characterize the action of waste generated gelatin on plant growth parameters, leaf area and protein content.

MATERIALS AND METHOD

- **Sample Collection and Storage:**

Scales of *Protonibea diacanthus*, Figure 1A. commonly called the black-spotted croaker fish (Ghol) were collected from the Mohane road, Dhakate, Shahad Figure 1B. Fish scales were cleaned and rinsed with tap water to remove blood and detritus material. The scales were dried under sunlight and stored in the plastic zip lock bag until used.

Figure 1A: Scales of Ghol fish

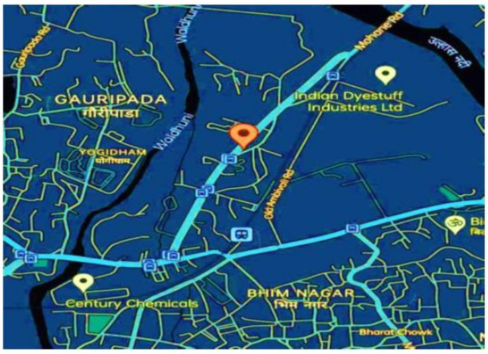


Figure 1B: Sample collection Site (Shahad)

- **Extraction of Gelatin**

Extraction of gelatin was performed using the method of Zakaria and Bakar (2015) with slight modifications. 50 gm scales were treated with 0.4 (W/V) sodium hydroxide solution for 2 hours. Following the treatment scales were washed in running tap water to neutralize the pH for 1 hour. Scales were then soaked in 0.4(v/v) hydrochloric acid (100ml) for two hours. HCl treated scales were washed in running tap water to remove all the acid and bring the pH to normal. Double the volume of distilled water was added to the treated scales and autoclaved at 15 psi for 3 hours. The supernatant was filtered in sterilized trays using two layers of sterile muslin cloth. The tray was covered properly to avoid contamination, and gelatin film was dried in oven at 50°C for 24 hours and stored in sterile ziplock pouch till further use.

Organoleptic characteristic and percentage yield of gelatin

Colour, texture, form and odour of gelatin was recorded. Percentage yield of gelatin was calculated using the formula:

$$\% \, yield = \frac{\text{weight of gelatin}}{\text{wt of dried scales}} \times 100$$

- **Seed treatment and measurement of plant growth parameters**

The seeds of green mung bean (*Vigna radiata*) were purchased from WeNatural Organic food store, Thane. Undamaged seeds were surface sterilised with $HgCl_2$ for 2 minutes (Hanif, *et al.*, 2019). In order to maintain their sterility, seeds were rinsed in sterile distilled water and dried. Sterile seeds (40) were soaked in 0.5% sterile gelatin solution for 12 hours. The seeds (40) soaked in sterile distilled water served as control. Percentage germination was estimated using paper towel method. Following germination seeds were potted in pots filled with 1kg of sterile dried soil. The seeds were grown at the room temperature under full sunlight. As the plant is very susceptible to the fungus, moderate amount of water was sprayed onto the plant. Triplicates were maintained for both control and treated seeds. After 20 days the plants were carefully uprooted by hand, cleaned, and analysed for root length, shoot length, number of leaves and leaf area.

- **Chlorophyll estimation**

0.1gm of leaf from control and treated plants were cut into fine pieces and grounded in mortar and pestle along with 80% acetone and was centrifuged at 5000 rpm for 5 mins. The supernatant was transferred to test-tube and the final volume was made to 10 ml. Test tubes were wrapped with black paper to prevent photooxidation of the pigment, absorbance was measured at 663nm and 645 nm UV-Visible spectrophotometer 117 (Arnon, 1949). Experiment was performed in three sets. Chlorophyll content (mg/g) was calculated using the formula (Gogoi and Basumatary, 2018).

$$Chlorophyll\ a\ (mg/g) = \frac{\{12.69(A_{663}) - 2.69(A_{645})\} X\ V}{1000\ X\ W}$$

$$Chlorophyll\ b\ (mg/g) = \frac{\{22.9(A_{645}) - 4.68(A_{663})\} X\ V}{1000\ X\ W}$$

$$Total\ chlorophyll\ (mg/g) = \frac{\{20.2(AA_{645}) - 8.02(A_{663})\} X\ V}{1000\ X\ W}$$

- **Protein estimation**

500mg of plant tissue was homogenized with potassium phosphate buffer (pH 7.0, 50mM) containing 0.1M EDTA and polyvinylpyrolidone (1%). The homogenate was filtered through cheese cloth and extract was homogenozed at 15,000 rpm for 15 minutes at 4°C. the supernatant was used for the estimation of protein by the method of Lowry, *et al.*, (1951) against bovine serum albumin as standard. Triplicates were maintained for the experiment.

STATISTICAL ANALYSES

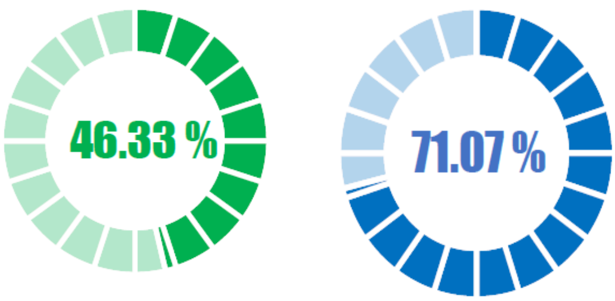
The data obtained was subjected to independent sample t Test, α= 0.05 using SPSS 16.0.

RESULT AND DISCUSSION

Both the supply and demand of goods have increased in the modern world due to the population explosion. The population's needs are challenging for the agricultural and horticulture sectors to satisfy. Farmers frequently employ fertilisers, which are harmful to human health, to increase crop yield. Gelatin, a biostimulant that may be readily produced from byproducts of the fish industry, can be used to promote the growth of plants and crops.

The extracted gelatin (Figure 2.) was brownish yellow in colour having fishy odour. Texture of extracted gelatin was rough and flaky. The percentage yield of gelatin from Ghol scale waste was found to be 35.52 ± 0.69 %. The percentage yield is affected by the method of pretreatment and extraction. The percentage yield was found to be more than gelatin extracted from skin of tiger tooth croaker which ranged from 3.55-7.46% (Koli, *et al.*, 2012) but less than croaker skin gelatin which was 75.6 ± 1.4 % (Silva, *et al.*, 2014).

Figure 2: Gelatin extracted from Ghol fish (*Protonibea diacanthus*) scales



CT (Control) **GAT (Ghol scale Gelatin treatment)**

Figure 3. Percentage of Seed Germination

The percentage germination of mung seeds was found to be more in case of Gelatin (GAT) treated seeds as compared to the control (CT). In control group only 46.33 % of seeds germinated while 71.07 % germination was achieved in gelatin treated seed. Similar findings of effect of biostimulant (plant and animal origin) have been reported in beans (Carvalho, *et al.*, 2013), parsley, celery, leek (Yildirim, *et al.*, 2000). Overall crop yield can be affected by germination percentage of seeds thus by use of gelatin biostimulants the germination percentage can be improved.

The application of ghol scale gelatin improved all the plant growth indices under study. Significant increase in root length, shoot length, number of leaves and leaf area was found. 32% increase in root length, 9% increase in shoot length and leaf area and 31% increase in number of leaves was observed. Wilson *et al.*, (2018) reported increase in total leaf area and dry weight of plant parts above the ground of few plants like cucumber, pepper, tomato, corn, broccoli, arugula as a result of application of gelatin capsules in vicinity of seeds. Grabowska, et al., (2012) has also reported that using aminoplant as biostimulant spray has led to increased root length and diameter in the test plants as compared to control. According to Kavipriya, *et al.*, 2016 seaweed extracts have positive effect on mung seed germination and growth. Similarly plant growth enhancement in broccoli by soy protein coating was observed by Amirkhani, *et al.*, 2016.

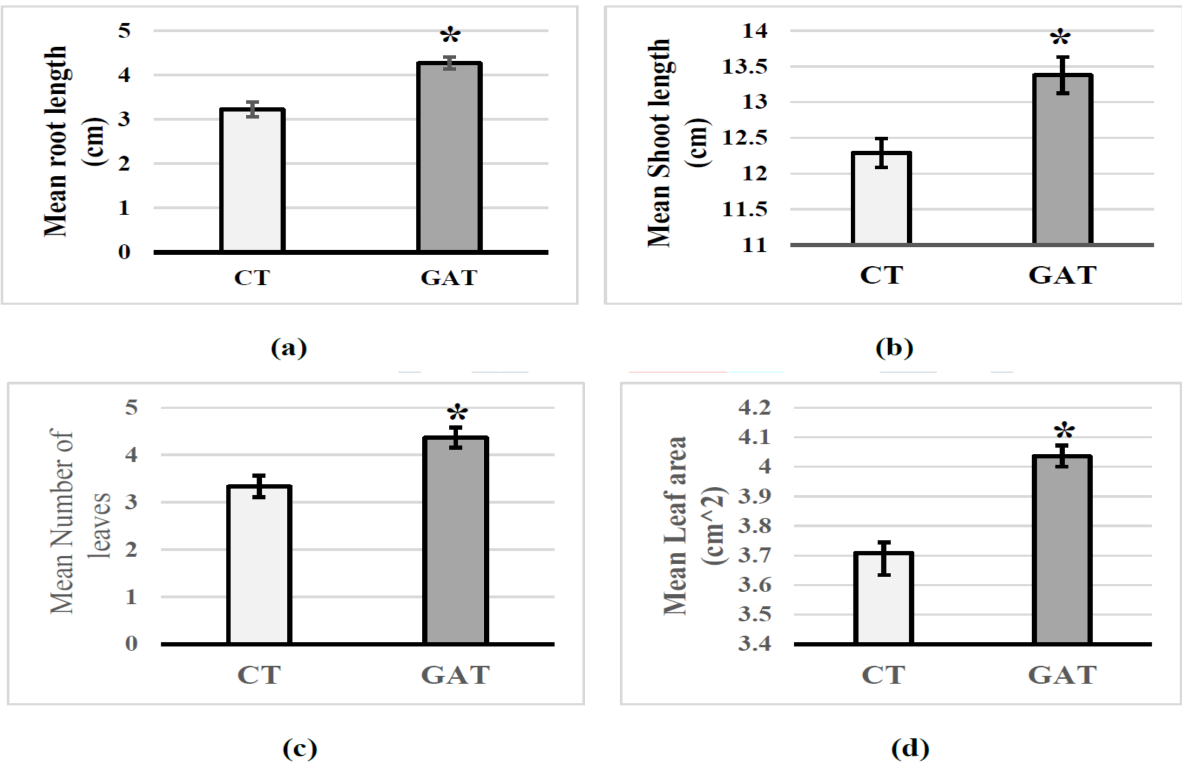


Figure 4. Effect of Gelatin treatment on Mung plant growth parameters.
Each figure displays the comparison between control (CT) and gelatin treated seed (GAT). Bars represent standard error of mean. ***** indicates significant difference. (a) Mean root length (cm), (b) Mean shoot length (cm), (c) Mean number of leaves, (d) Mean total leaf area (cm^2)

A considerable increase in chlorophyll content in leaves of *vigna radiata* were observed in gelatin treated plant in comparison to control. The chlorophyll a content increased from 0.25 to 0.56 mg/g, chlorophyll b from 0.29 to 0.97 mg/g leading to increase in total chlorophyll from 0.54 to 1.53 mg/g. Xu and Mou (2017) also reported increase in the amount of chlorophyll a, b and total chlorophyll in lettuce plant by using protein hydrolysate obtained from fish as a biostimulant. Seaweed extract, *moringa oleifera* extract, and amino acid foliar spray have enhanced chlorophyll pigments in potato, *Eruca vesicaria* and lettuce plant (Arafa, *et al.*, 2011, Abdalla, 2013, Naroozlo *et al.*, 2019). Increase in total chlorophyll may lead to enhanced yield as studied in wheat, barley and oats (Sid'Ko, *et al.*, 2017).

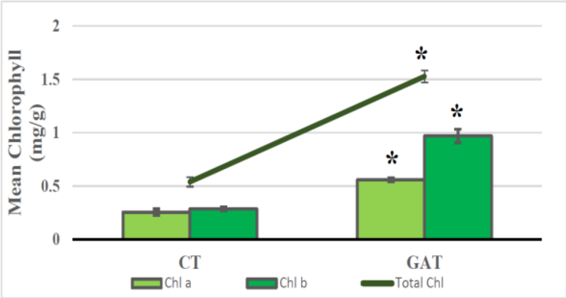


Figure 5. Effect of Gelatin treatment on photosynthetic pigments. (* indicates significant difference)

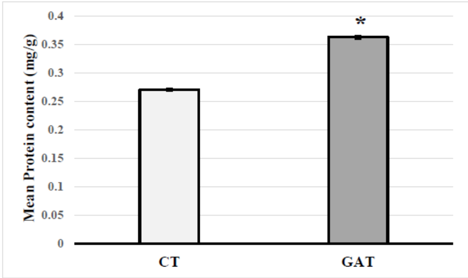


Figure 6. Effect of Gelatin treatment on plant protein content. (* indicates significant difference)

Figure 6. depicts the significant increase in protein content of the gelatin treated mung plant in comparison to the untreated control. In plants treated with GAT, a 34% increase in protein content was noted. The higher protein content in the treated plant may be due to the gelatin's stimulation of plant growth. Puglisi et al. (2020) showed that treatment of the lettuce seedling with S. quadricauda extract resulted in a 38% increase in total protein. Increased protein content of the roots and leaves of maize seedlings were observed after application of humic material derived from leonardites (Conselvan *et al.*, 2017).

CONCLUSION:

Biostimulants are chemicals derived from living organisms that help overall growth of plants and leads to better yield. The current study's findings imply that employing gelatin from ghol fish scales as a biostimulant source can enhance plant growth and result in the valorisation of scale waste. Proline and hydroxyproline, two amino acids crucial for plant growth and stress tolerance, are abundant in gelatin. The current study supports the usage of scale gelatin made from fish waste as a biostimulant. After being given gelatin treatment, the seedlings' growth metrics improved. The measured metrics (% of seed germination, root length, shoot length, number of leaves, leaf area, chlorophyll, and protein content) indicated a substantial improvement in the treated seeds when compared to the untreated control. Comparing the treated and untreated green gram, it was found that the treated plant had higher levels of protein and chlorophyll, which shows that a biostimulant made from fish waste might be utilised to boost productivity's quantity and quality.

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