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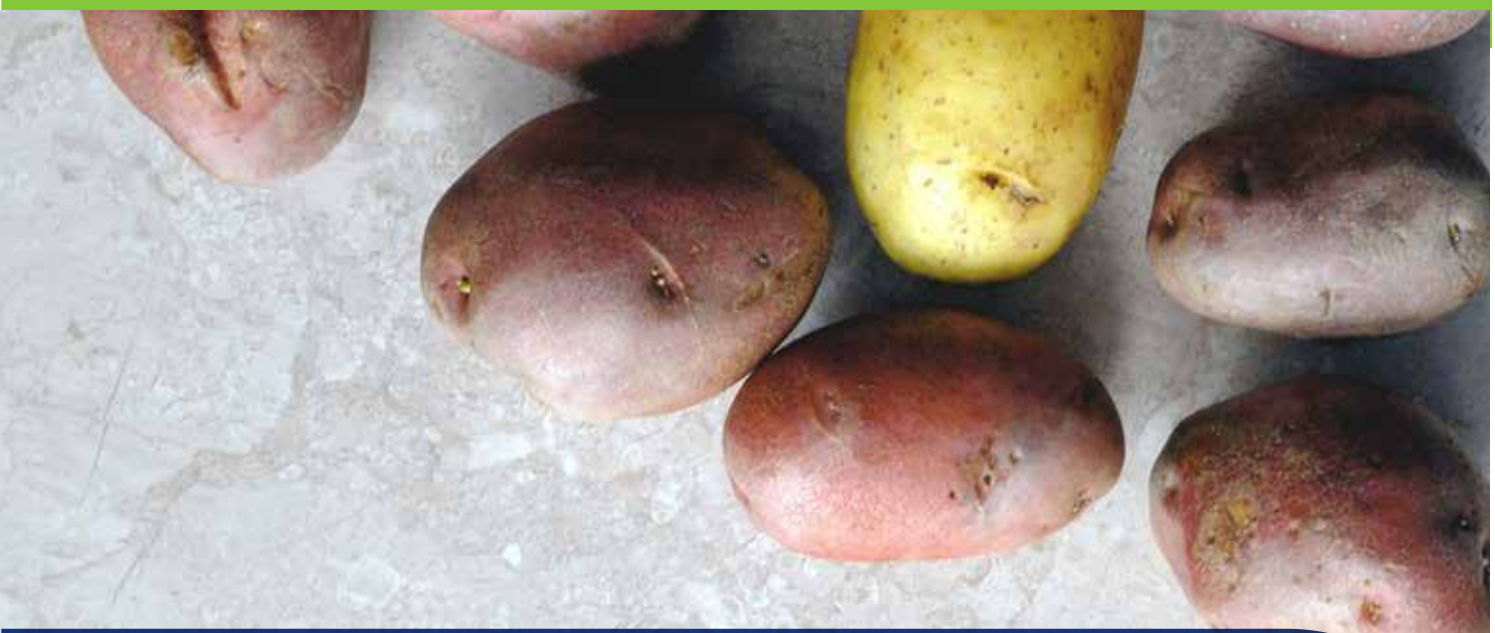
SYMBIOMA

Technology Innovations and Business Models for Valorisation of Industrial Waste Biomass in Sparsely Located Enterprises



Utilization of potato waste as a biostimulant for plant growth

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SUMMARY

In this SYMBIOMA project study we investigated if potato waste (PW) biomass could be utilized as a biostimulant/biofertilizer for plant growth. Due to that Trichoderma spp has been commercially recognized as a biofertilizer with plant growth-promoting substances, the study aimed at producing Trichoderma-enriched biostimulant/ biofertilizer for root health of the plants. The potato waste (PW) was fully utilized by Trichoderma harzianum. This process allows the solid and liquid phases to separate to be used as biostimulants on the plants through a foliar spray and the solid phase (digestate) can be utilized as a soil amendment. There was a reduction in the nitrogen and total sugar parameters, reflecting the growth of fungal biomass. The visual observation proves a predominant growth of TH on the potato dextrose agar media. This confirms the amount of TH biomass has been increased in the liquid phase of PW. The functionality of PW biostimulant was tested on Lactuca sativa during germination. It showed a significant difference in the germination rate, seed vigour index, and root elongation compared to the untreated seed of Lactuca sativa in a dose-dependent manner. Although, there are no significant changes seen in the chlorophyll pigment of Lactuca sativa after germination, which requires further screening to understand the pigment pathway at various stages of the life-cycle period. It could be suggested that the quantification of TH and other bioactive compounds responsible for the beneficial effects of PW biostimulant needs to be verified.

1 Introduction

Potato waste accounts for 12 to 20% of its total production. These wastes are derived during the processing of potatoes mainly from peels, pulp, and spoiled rejects (Osawa et al., 2018). This can be further processed to utilize the starch and remaining protein to produce valuable compounds in the circular bioeconomy. As there is an increasing interest in using beneficial microorganisms for utilizing industrial waste as an ingredient for the growth of microbes to produce secondary metabolites emerging in recent times. Fermentation is a less energy consuming technique to produce high-value compounds, the energy involved in the sterilization and pre-treatment steps of solid potato waste requires less water and electricity which consequently lower environmental impacts (Haverkort et al., 2022). This approach is a completely sustainable and economically viable method of converting waste into valorization for agriculture. The technique involved in this conversion is less carbon emission and preventing landfill which will allow the local industry to reduce the local municipal tax for waste removal.

Based on the EU Bioeconomy Strategy, the conversion of primary organic waste into useful biobased products such as food, feed, fuel, and fertilizer has been given a major priority (Iskanius, 2019). As potato waste consists of valuable nutrients that can be consumed by fungi during fermentation. Trichoderma spp has been commercially recognized as a biofertilizer with plant growth-promoting substances (Contreras-Cornejo et al., 2016). This fungus is efficient in degrading cell-wall polysaccharides which can consume potato peel and potato starch for its growth (Ben Taher et al., 2017). It has been proven that Trichoderma reesei produces very efficient endo- and exo-glucanases than Aspergillus niger (Bansal et al., 2012), thus producing cellulase through solid state fermentation (SSF) is one of the factors of fully utilizing the potato waste biomass (Miao et al., 2020). Besides, this process will allow the local potato producer to fully utilize the waste biomass to produce a huge quantity of fungal biomass as a biostimulant or biofertilizer for agriculture. This considers a circular approach to connecting the loop in the circular economy. There are various studies that proved the efficiency of Trichoderma to produce phytohormones and disease prevention against biotic and abiotic stress are attracted considerably.

2 Methods and Materials

SSF is one of the sustainable methods of producing fungal biomass with secondary metabolites (bio-active compounds or organic acids), in the circular bioeconomy. In this study, the SSF technique has been used to convert potato waste (low-value biomass) into high-value fungal biomass to improve the root health and production of Lactuca sativa.

2.1 Fermentation

The potato waste (PW) had a dry matter content of 18.3% and was autoclaved at 121°C for 30 min to immobilise other microbes before inoculation. In this study, solid-state fermentation was carried out using T. harzianum (TH) Rifai strain T-39. TH is competent against rhizosphere, abiotic stress, and stimulating plant growth which was selected to use in this study (Alias et al., 2022; Sala et al., 2022). Initially, TH of 0.2, 0.3, and 0.4 mg ((1.4 X 1010) spores per gram, supresivit brand) were dissolved in 10 mL of autoclaved MilliQ water and then added to 100g of PW in the Erlenmeyer flask. Inoculation was performed inside a laminar flow chamber and mixed thoroughly to undergo fermentation. These inoculated flasks were closed with a cotton plug and placed on an orbital shaker at 200 rpm at 25°C for 7 days to ferment. The weight of the flask was measured on daily basis throughout the fermentation period, whereas temperature, time, and period of fermentation were kept constant. After fermentation, the mixture was centrifuged at 5000 rpm for 10 min at 10°C. The supernatant liquid was collected and stored in the refrigerator for further use as a biostimulant.

2.2 Biochemical analysis

The initial PW samples and final biostimulant were measured for biochemical parameters. Moisture, ash content, and total solid% were measured gravimetrically. The physical parameters such as pH, salinity, electrical conductivity, redox, and dissolved oxygen were determined. Total glucose and protein (nitrogen) contents were analysed using the phenol- sulfuric acid method and Kjeldahl method, respectively. Chlorophyll content was estimated by acetone extraction and the absorbance was measured spectrophotometrically.

2.3 Qualitative test for TH on Potato dextrose agar (PDA)

Different concentrations of PW biostimulant were used on the PDA substrate (0.5mL, 1mL, 2mL, and 3mL PW) to screen for potential dose-response on TH growth. The petri dishes were incubated at 26°C and 60% RH under the illumination of 12 h light/12 h dark cycles, using daylight tubes 24 W/m2, 9000 lx in a climatic chamber for 6 days. Similarly, non-fermented potato wastewater (autoclaved) was incubated as a control. The substrate colonization due to the fungal growth was monitored daily by visual inspection of the culture. Pictures were taken at the end of the incubation to visually compare the presence of TH on the PDA substrates.

2.4 Seed treatment and germination

Briefly, seeds of Lactuca sativa (red lettuce) were bleached in 2% sodium hypochlorite before treatment. After bleaching, the seeds were imbibed with three different concentrations of 0.5, 1, 2, and 3% v/v of PW biostimulant in 10mL of distilled water for 16h and then plated on the petri dish. Seeds soaked with ddH2O served as a positive control and without seed treatment as a negative control. All the experimental groups (PW biostimulant treated) have 36 seeds in replicates per treatment, whereas the control groups (positive and negative) have 30 seeds each. These seeds were placed on the petri dishes (15 cm diameter) consisting of cotton wool and Whatman no.1 filter paper as two layers that were moistened with 15 mL distilled water. All these plates were incubated under a cool fluorescent light (100 µmol photons m−1 s−1) with a 16:8 h (day: night cycle) at 22 ± 2°C for 10 days. The germination was monitored throughout the culture period and the percentage of germination was observed. The percentage of normal seedlings as defined in standard germination testing was measured 10 days after imbibition.

The germination percentage (GP%) was calculated as
GP % =Number of germinated seeds / Total number of seeds × 100 Eq(1) Randomly ten healthy germinated plants were chosen to measure shoot and root length in centimetres using a scale ruler. The fresh weight of samples was recorded in grams.

Seedling vigour index (SVI) was calculated as follows:

SVI= [Mean root length (Lr)+Mean shoot length (Ls)] × Percentage of seed germination (GP) Eq(2)

Where, GP is the seed germination percentage (%); Lr is the root length (L); Ls is the shoot length (L) (Geetha et al., 2014).

Fresh leaves of 10 plants for each treatment were weighed out in 0.1–0.2 g (fresh weight, FW). The extractions were performed using 10 ml (V) of 80% acetone until the leaf turned white. The optical density was measured with UV-1800 spectrophotometer at 663nm (OD663) and 645 nm (OD645) for chlorophyll a (Chl a) and chlorophyll b (Chl b). The chlorophyll concentrations (Chl) were determined using (Lichtenthaler and Wellburn, 1983): The chlorophyll pigments were measured spectrophotometrically by the following equation.

Chlorophyll a (mg/g F.W) = (12.7 A663 -2.69 A645) • X/1000 • n Eq(3)
Chlorophyll b (mg/g F.W) = (22.9 A645 – 4.68 A663) • X/1000 • n Eq(4)
Total chlorophyll (mg/g F.W) = (20.2 A645 + 8.02 A) • X/1000 • n Eq(5)
where: A645 = absorption value at 645 nm, A663= absorption value at 663 nm, X = total volume of filtrate, n = tissue weight.

2.4 Statistical analysis

All the test samples were statistically analyzed in Microsoft Excel 2016. One-way ANOVA was used to study the significance of the groups for fermentation studies. At the end of the incubation time, germination percentage, shoot length, root lengths, and SVI were evaluated. The results were expressed as the mean root lengths ± standard deviation (SD). Ten fresh leaves were taken for chlorophyll analysis (n=10) content. The statistical difference between groups and within the treatments (different concentrations) was analyzed by Tukey HSD and the $p<0.05$ were accepted as significant.

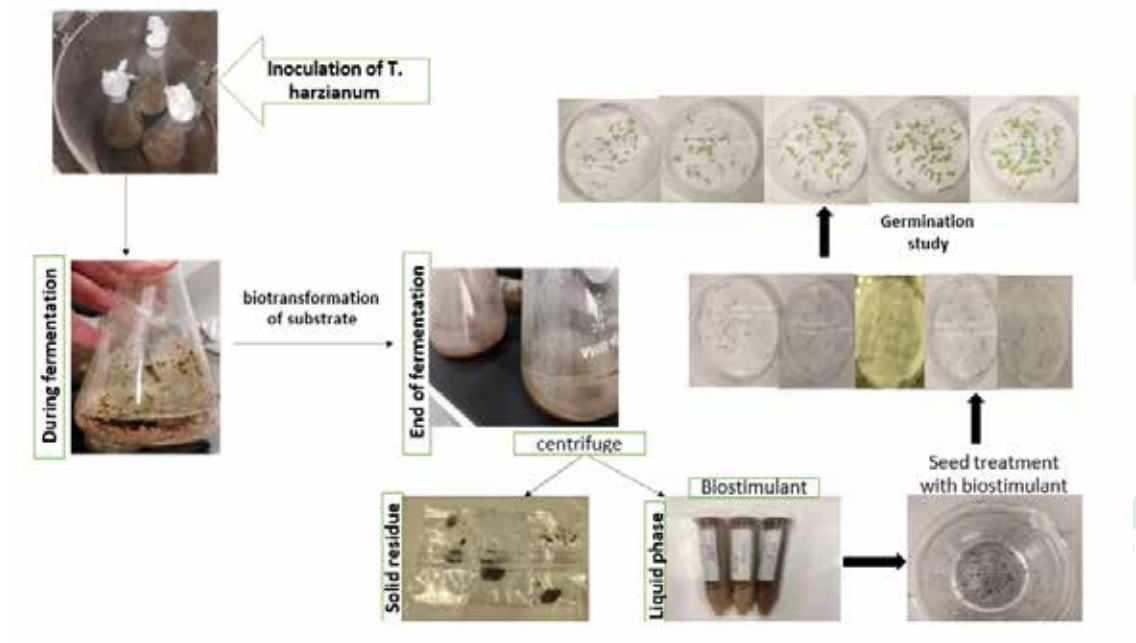

Figure 1: A schematic flow of potato waste biostimulant production and seed germination study

3 Results and Discussions

The biochemical, fungal, and functional property of PW biostimulant were studied in this work. The physical parameter such as pH, salinity, electrical conductivity, redox and dissolved oxygen were measured throughout the process. The initial and final biochemical parameters are crucial to identify the changes of fermentation.

3.1 Biochemical parameters

The fermented PW biostimulant was tested for biochemical changes. In this case, solid state fermentation found to be promising in converting the property of the substrate into useful products, which can be utilized by the fungi to produce useful secondary metabolites. The amount of sugar (12%) present in the initial biomass (non-fermented PW) allowed Trichoderma to grow. Three concentrations of T. harzianum (TH) were used to inoculate the PW. The total nitrogen content decreased in all three TH concentrations used in the fermentation process. PW contains 12% of carbohydrates (starch + simple sugar ww%) whereas after fermentation the total glucose content became 8.9 to 9% (89.7 to 90.7 mg/mL). Based on the biochemical analysis (Table 1) it is evident that most of the PW nutrients were utilized by the fungi (TH). From table 1, the biochemical parameters were not significantly different between PT1 to PT3. Electrical conductivity (EC) is directly related to potassium concentrations, and higher EC relates to the high absorption of biostimulant by plants. However, irrespective of TH spores' concentrations used in PW substrate (100 g ww), EC did not change significantly.

Nevertheless, the fermentation process enhances the separation of solid and liquid phases, PT3 had 90 mL of the liquid phase and 4.338 g (ww) of solid as a residue. In most cases, mechanical separation and high-energy processes have been used to separate the solid and phase of agro- food waste. In this method, the whole biomass was fully valorized with less energy input. This process aids the separation, as well as retention of nutrients in the liquid phase as a biostimulant, and the residual solid can be used as a soil amendment.

Table 1 Physiochemical parameters of biostimulant from potato waste

Samples	pH	Sal (ppm)	EC mS/cm	redox (mV)	DO (ppm)	Total glucose (mg/ml)	total N%	Ash (g)	liquid (mL)	TS (mg/L)	Residue DM (g)	Residue wet (g)
Non-fermented PW	4.8	3.5	6.4	231.7	-	12%*	1.3	<1%	81.7%	-	-	-
P.T.1	5.44	2.9	5.34	16.3	0.014	89.7	0.471	0.45	36	29.05	0.558	4.229
P.T.2	5.07	2.5	4.68	29.4	0.00	81.40	0.37	0.33	37	26.142	1.298	9.436
P.T.3	7.83	2.4	4.28	-266.3	0	90.7	0.578	0.267	50	14.45	0.563	4.338

All table values are mean of three replicates (n=3) of analytical trials, *total carbohydrate of wet biomass

3.2 Presence of Trichoderma in the PW biostimulant

The non-fermented PW substrate was found to be a suitable substratum for the growth of Trichoderma. As the initial pH was 4.8 was optimal for the growth of Trichoderma spp. The visual examination from Figure 2 shows that the growth of Trichoderma hazarium (TH) was predominant when a high concentration of TH spores (0.3mg/100g) was used during PW fermentation. However, at 2% and 3% concentrations the growth of TH was predominant in PT1 and PT3 not in PT2. No growth of TH at a 1% concentration of PT2 (refer Figure 2) compared to all treatment groups at a similar concentration. These differences are likely attributable to the different growth rates of the Trichoderma and the viability of active cells present in the PW biostimulant.

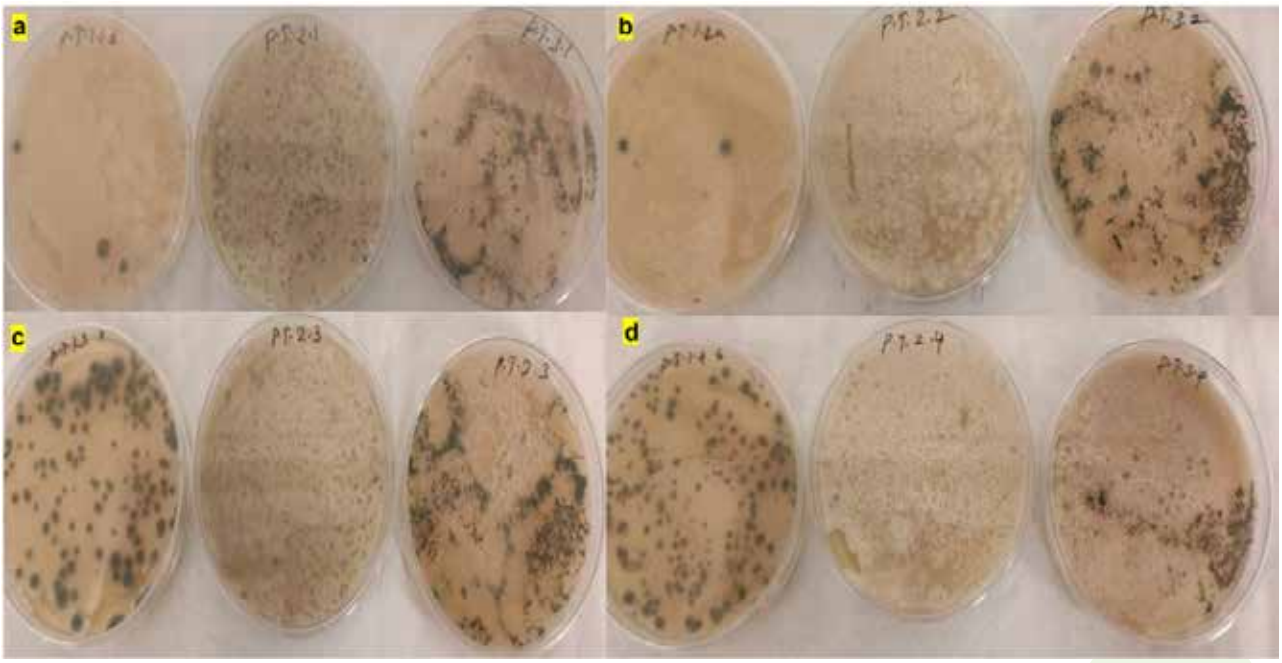

Figure 2: The growth of Trichoderma hazarium on PDA media at 0.5%, 1%, 2%, and 3% concentrations of PW biostimulant labeled as a, b, c, and d.

3.3 Seed Germination

The low concentration of Trichoderma (PT1) used in the fermentation process had a higher GP% (PT1 at 3%) than compared to all other groups. The control group seeds had low GP% i.e., 83.3% and 78% respectively, for WST and water-treated seeds (Figure 3a). This proves that low concentrations of TH spores present in the biostimulant can stimulate seed germination, which seem to be optimum to break seed dormancy. However, further repeatability of studies requires to validate GP% at various dose-response. Seed vigor index (SVI) showed a significant difference between the experimental groups and the control groups (Figure 3b); however, these differences were not significant between the treatments at three dosage levels (PT1 to PT3). This shows that PT1 and PT3 improve the SVI property of Lactuca sativa and its performance of seed lot during germination and seedling emergence. This confirms the quantity and viability of active TH cells present in the PW biostimulant.

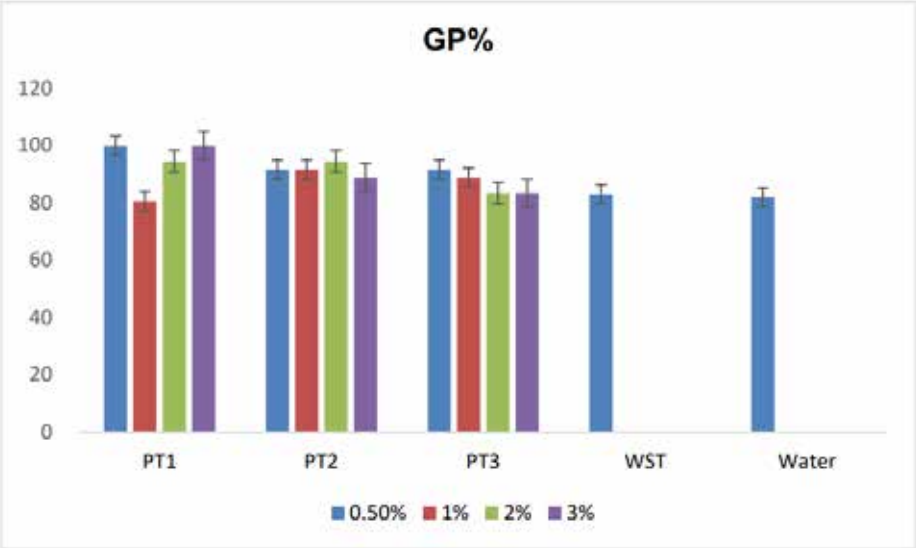

Figure 3a: Germination percentage (GP%) of Lactuca sativa seeds treated with PW biostimulant at different concentrations. PT1- low spores of TH, PT2- medium spores and PT3- high spores' concentration during fermentation. WST-without seed treatment, water- seed treated with water. All values are mean ±SD, p< 0.05

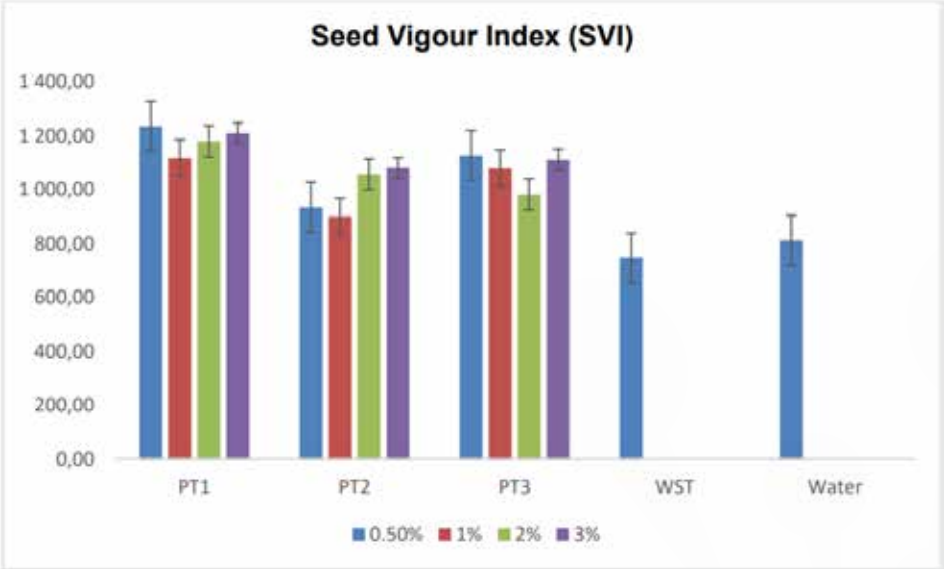

Figure 3b: Seed Vigour Index (SVI) of Lactuca sativa seeds treated with PW biostimulant at different concentrations on the 10th day of germination. PT1- low spores of TH, PT2- medium spores, and PT3- high spores' concentration during fermentation. WST-without seed treatment, water- seed treated with water. All values are mean ±SD, p< 0.05

3.4 Shoot length

The shoot length has been significantly different in PT1 at low concentration (0.5%). However, PT1 at 1%, 2%, and 3% dosages were not different from other seed treatment groups at similar concentrations (Figure 4a). As expected, PT3-treated seeds were increased in shoot length at all dosages (0.5% to 3%). Although there seems to be less difference between PT1, PT2, and PT3, the shoot elongation was significantly different ($p < 0.05$) in comparison with water- treated seeds and negative control as without seed treatment (WST). Similarly, the maximum mean weight of the fresh leaves was 0.168g for PT3 at 3% concentration (Figure 4b), although no significant difference between the treatment and between the group. Further pot experiment is required to identify the total yield of the biomass. However, control groups were 0.08g and 0.109g for WST and water. It was also noted that a few unhealthy sprouts and leaves were seen in the WST at the end of 10th day.

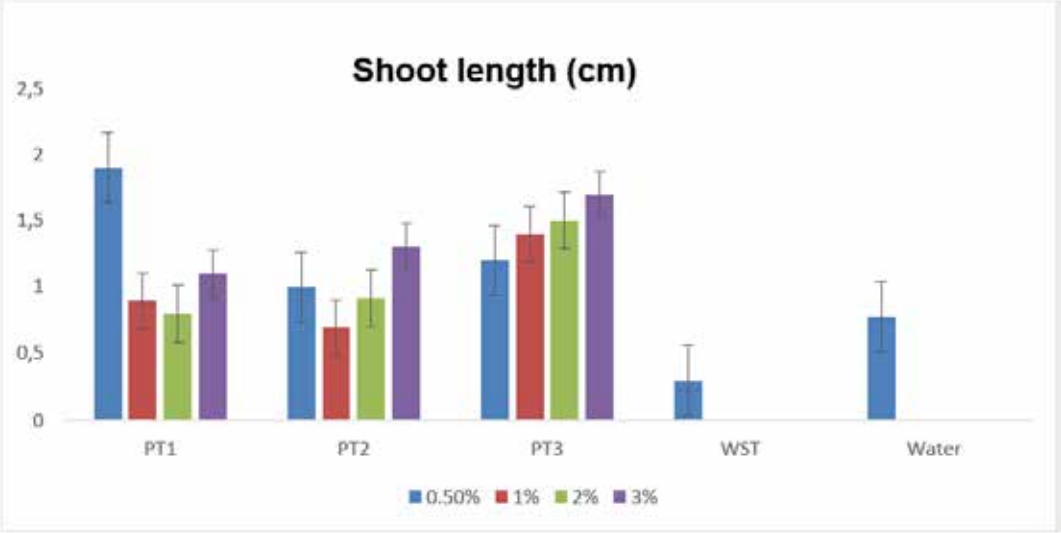

Figure 4a: Shoot length of Lactuca sativa treated with PW biostimulant at different concentrations on the 10th day of germination. PT1- low spores of TH, PT2- medium spores and PT3- high spores' concentration during fermentation. WST-without seed treatment, water- seed treated with water. All values are mean ±SD, $p < 0.05$

Figure 4a: Fresh leaves weight of Lactuca sativa treated with PW biostimulant at different concentrations on the 10th day of germination. PT1- low spores of TH, PT2- medium spores, and PT3- high spores' concentration during fermentation. WST-without seed treatment, water- seed treated with water. All values are mean ±SD, $p < 0.05$

3.5 Root Elongation

The root elongation of Lactuca sativa was evaluated after seedlings were fully grown on the 10th day of germination at different concentrations ranging from 0.5% to 3% (Figure 5a). At low and high concentrations (PT1 and PT3) root length was significantly elongated, which correlated with the presence of Trichoderma within the PW biostimulant mixture (refer Figure 2). Accordingly, the maximum mean root length of PT1 (10.46 to 11.7 cm), PT3 (10.69 to 12.1 cm), and PT2 were slightly low in growth (9.1 to 10.8) at various concentrations. While the control groups including WST and water were 6.2 cm and 8.7 cm, respectively. Similarly, from Figure 5b, it can be observed that the root weight has been improved after the PW biostimulant PT1 (0.114 to 0.139 g), PT3 (0.086 to 0.132 g), PT3 (0.108 to 0.136 g) in comparison with control it was 0.073g and 0.08g for WST and water, respectively. On the 10th day formation of lateral roots from 4 to 7 numbers (through observation) was seen in the PT1 and PT3 groups (refer Figure 6), whereas unhealthy roots and wilted roots were seen in the WST at the end of 10th day.

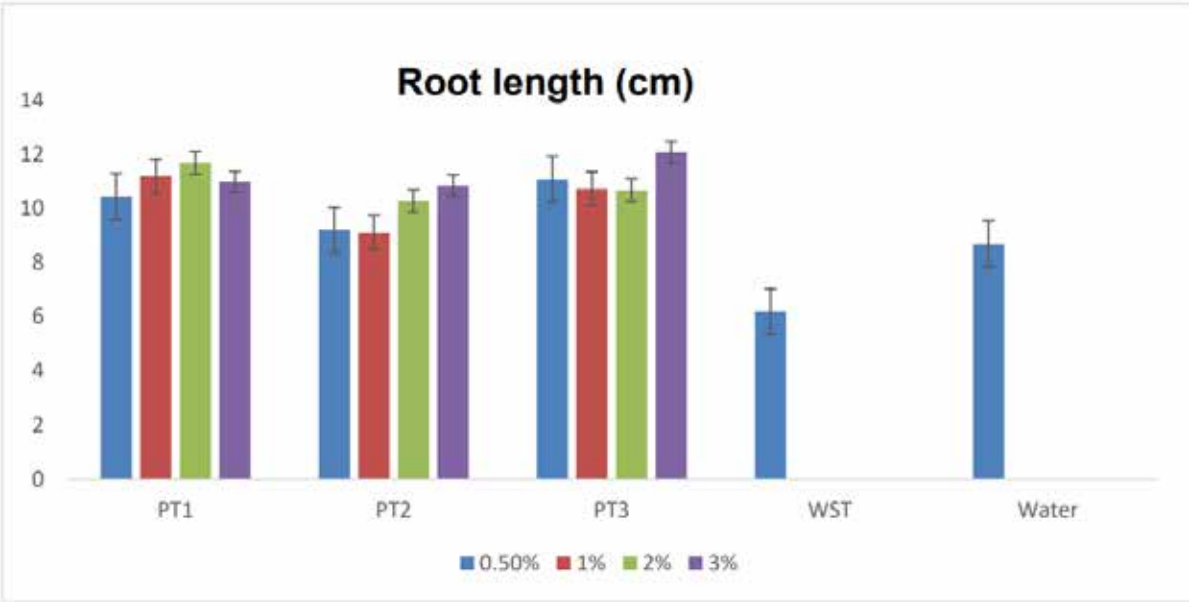

Figure 5a: Root length (cm) of Lactuca sativa treated with PW biostimulant in different concentrations on the 10th day of germination. PT1- low spores of TH, PT2- medium spores and PT3- high spores' concentration during fermentation. WST-without seed treatment, water- seed treated with water. All values are mean ±SD, p< 0.05

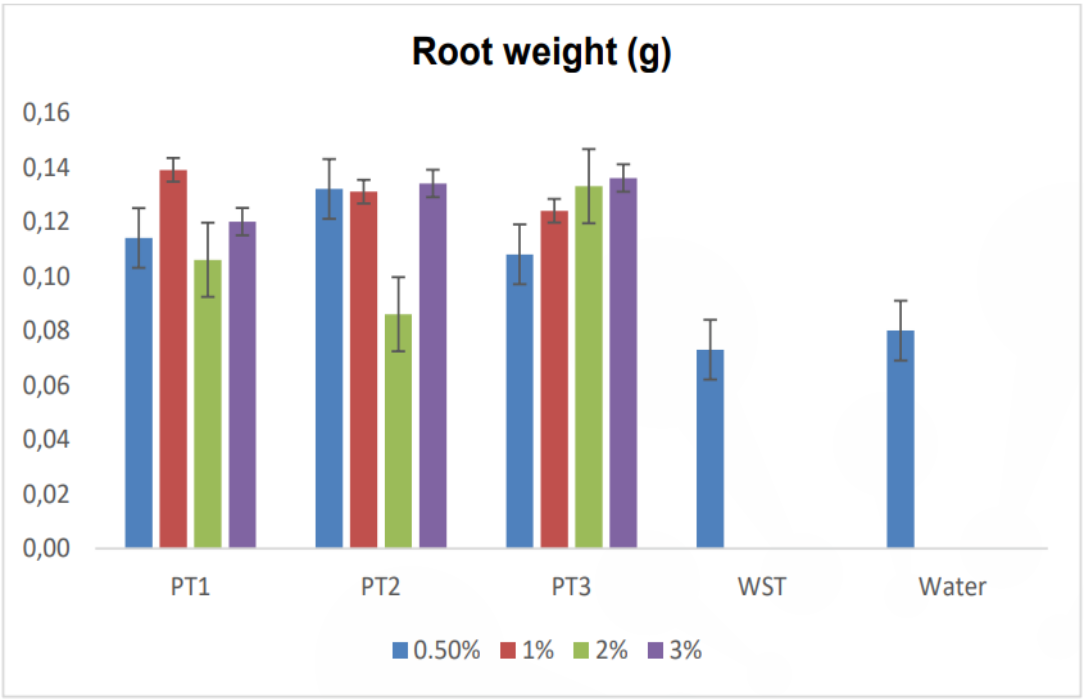

Figure 5b: Root weight (g) of Lactuca sativa seeds treated with PW biostimulant in different concentrations on the 10th day of germination. PT1- low spores of TH, PT2- medium spores, and PT3- high spores' concentration during fermentation. WST-without seed treatment, water- seed treated with water. All values are mean ±SD, p< 0.05

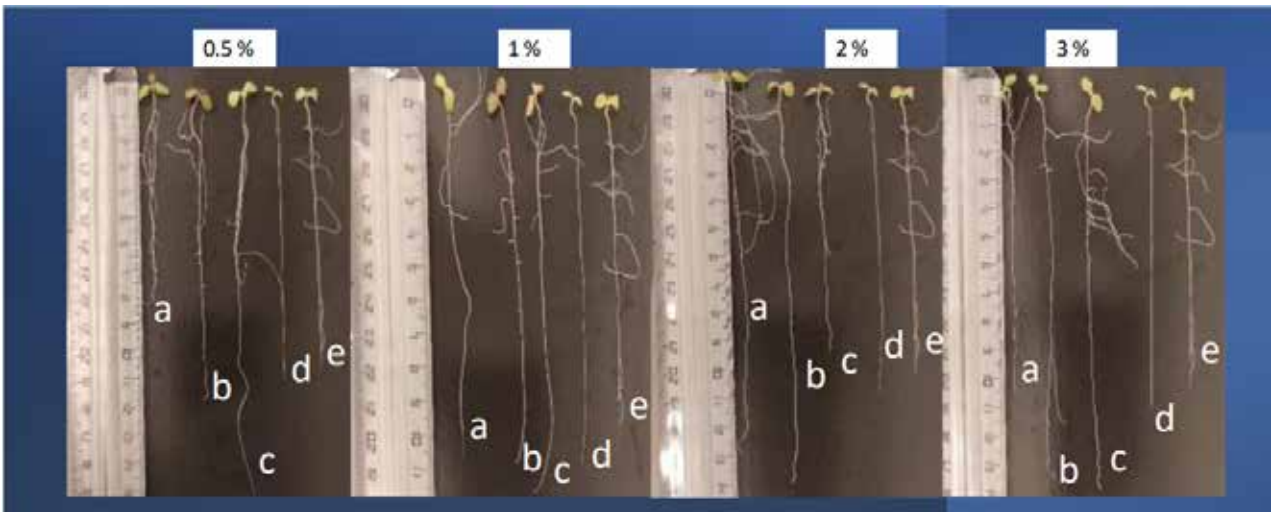

Figure 6: Effect of PW biostimulant treatment with different concentrations on root elongation of Lactuca sativa on the 10th day of germination. Picture label a-PT1, b-PT2, c-PT3, d-WST, e-Water

3.6 Chlorophyll content

The chlorophyll content of PT1 to PT3 is given in Figure 7. PW biostimulant show no significant difference in the chlorophyll content of the fresh leaves, although some variations were found between the treatments PT1 and PT3 at different concentrations. Similar variability was found between the control groups, indicating that a weak correlation between chlorophyll and instantaneous growth rate. This necessitates further study to understand the metabolic pathway of PW biostimulant on pigments. It is worth noting that PT2 (0.5% to 3%) did not show predominant differences in the GP%, shoot, and root length (Figure 3, 4 and 5), although, the chlorophyll content was higher than PT1, and control groups. This necessitates further process optimization of using TH present in the PW biostimulant at the life-cycle period of Lactuca sativa to understand the dose response on the pigment pathway.

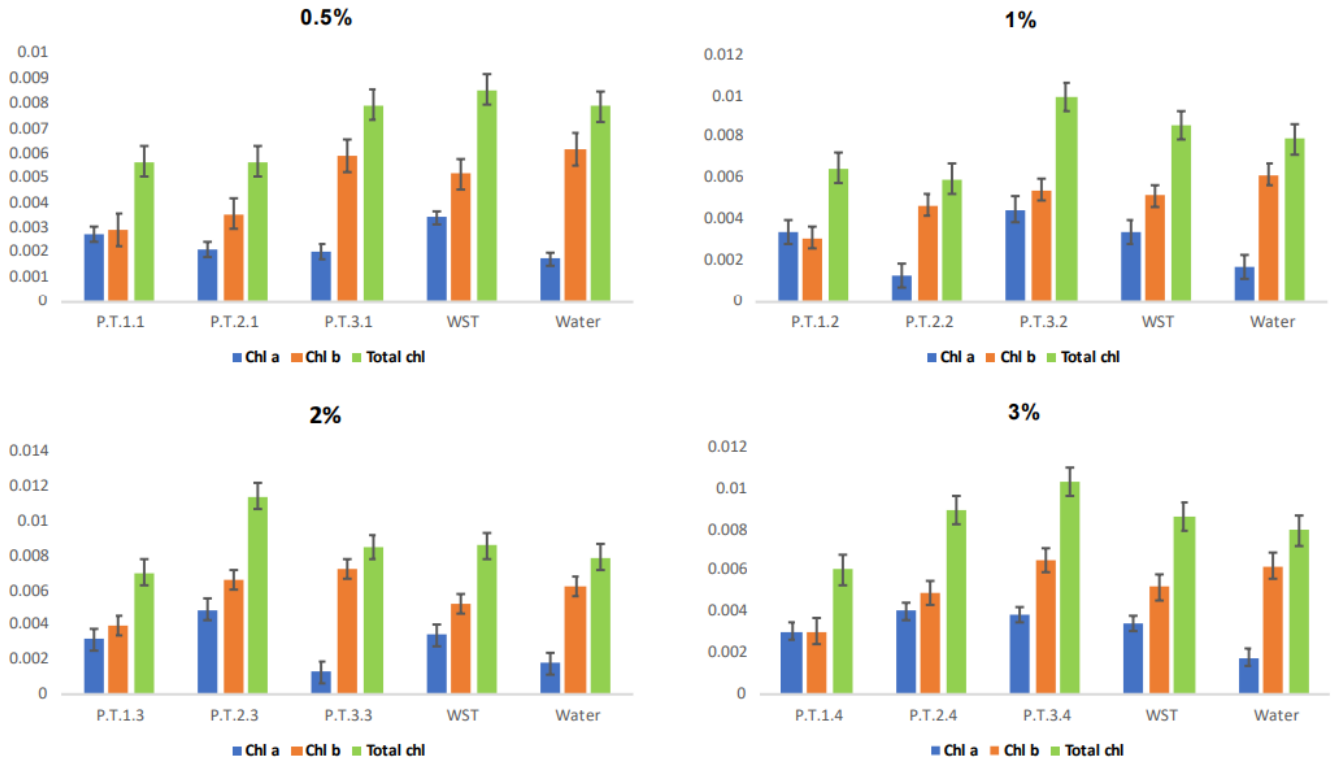

Figure 7: Effect of PW biostimulant treatment at different concentrations on chlorophyll content (mg/g) of Lactuca sativa on the 10th day of germination. All values are mean ±SD, $p < 0.05$

4 Conclusion

This screening study suggests that potato waste can be valorised as biostimulant or biofertilizer to improve root health and plant growth (Lactuca sativa). As the potato waste was used as a substrate for fungi growth, this finding opens a new pathway for using potato waste to produce a large quantity of fungal biomass (Trichoderma spp) via fermentation. Trichoderma-enriched media can be used as a foliar spray and the residual solid biomass represents a low-cost soil- amendment with a large amount of Trichoderma inoculum. There are many studies and commercial applications proven the efficacy of using Trichoderma for disease prevention in agriculture. In this screening, PW-based biostimulant improved the root elongation and seed vigour index (SVI) in a dose-dependent manner. Further studies are required to quantify the amount of Trichoderma present in PW biostimulant as well as the secondary metabolites responsible for plant growth promotion, root elongation, and protection against disease. This will allow us to scale up the process as a novel product by utilizing potato waste from the industry.

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