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ASSESSMENT OF PLANT BIOSTIMULANTING EFFICIENCY OF SAGO BAGASSE HYDROLYSATE (SBH) FOR IMPROVED PLANT GROWTH

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ABSTRACT

In our study, we aimed to produce a plant biostimulant using sago bagasse, an abundant by- product of sago industry. One step enzymatic hydrolysis was carried out to produce sago bagasse hydrolysate (SBH) by amylase. The concentration of sugar in the SBH was much higher as compared to the native SB. Further, plant bio stimulating efficiency of SBH was investigated through germination study using six different field crops. Among the various concentrations of SBH, significant improvements in the shoot and root length were observed at 1.5% of SBH treated seedlings compared to the control. Similarly, all the tested field crops at SBH 1.5% registered increased biomass, IAA and lipid accumulation compared to the other treatments. Biostimulating efficiency of SBH might be brought by the presence of various sugars in the SBH. Hence, based on these findings it can be concluding that, SBH has the potential to enhance plant growth of various agricultural crops.

KEYWORDS: Sago bagasse hydrolysate (SBH), Plant growth promotion, Biostimulant, Biomass, Lipid accumulation.

1. INTRODUCTION

In the recent years, the application of excessive chemical fertilizers posing non convivial effects to environment by accumulating of nitrate and ammonia in soil and water (Banerjee et al., 2011). These inconveniences gained negative impact among the farmers and utilization of organic products have been increased in the past few years. Particularly, plant biostimulants are expected to reach 12% over $2,200 million by 2018 (Anonymous, 2013). Biostimulants enhancing plant growth at low concentration by improving plant nutrition and metabolism (Schiavon et al., 2008; Khan et al., 2009). Commercial biostimulants are available in different formulations and they are generally classified into three types based on their original matrix: humic substances, seaweed extract, and amino acid containing products (Kauffman et al., 2007). Also, biostimulants are widely formulated from various industrial by products (Ugolini et al., 2015; Illera-Vives et al., 2015; Kim et al., 2010). In fact, utilization of these industrial by products have a possible opportunity to improve the extensive production of organic fertilizers thereby its increase waste management strategy and minimizing environmental pollution (Lomascolo et al., 2012). Only protein rich industrial by products has been valorized for biostimulant characteristics. However, these successful biostimulants are not economically feasible because of the substrate expenditures and cost effective production process. For these reasons, research in the selection of suitable substrate has been increasing in the recent years. Starch abundant industrial by products such as sugar cane bagasse, wheat bran and other provenance are not explored for biostimulant characteristics. However, nutrients representing in the starch are abundant organic substance in the form of carbohydrates. Starch industrial by products are rich in organic carbon, they suggest an opportunity for the development of new carbon-rich organic amendments that could serve the dual purpose of improving plant growth and beneficial to environment. In plants, as like amino acids, carbohydrates also playing a significant role in the plant growth and metabolic process (Smeekens, 2000; Rolland et al., 2002). Based on the fact above stated, SB was selected as an interesting substrate in the production of biostimulants using enzymatic hydrolysis.

2. MATERIALS AND METHOD

2.1 Sample collection

Sago Bagasse (SB) was collected from Sago Serve industry, Salem, Tamil Nadu. The collected SB was washed with distilled water for about three to four times till all the unwanted debris were removed. The samples were then dried under sunlight for five hours and finely powdered using a mixture grinder. The powdered sample was stored in an airtight container and kept at room temperature for further experiments.

2.2 Enzymatic hydrolysis of sago bagasse

SBH was prepared according to the method of Valachova and Horvathova (2007). Briefly, 1g of sago starch was dissolved in 100ml of distilled water and the pH was adjusted to 7.0 with 0.1N NaOH or HCl. The sago dispersion was sterilized for 30 min. Then, it was cooled and 200U of amylase was added. Enzymatic digestion was performed at 37°C under gentle agitation. The hydrolysis was performed for 4 h and the activity of the enzyme was stopped by heating the dispersion at 80°C for 20 min.

2.3 Reducing sugar estimation

After the enzymatic hydrolysis, total carbohydrate content of SB and SBH was quantified using 3,5-dinitrosalicylic acid (DNS) As described by Miller (1959). Aliquots of SB and SBH was mixed with the 4ml of DNS reagent and kept under the boiling water-bath for 5mins. To this, 1ml of Rochelle salt solution was added and absorbance was recorded at 540nm in the UV spectrophotometer. Standard curve was blotted using known concentration of glucose.

2.4 Biostimulating efficiency of SBH

SBH solution, 0.5%, 0.1%, and 1.5% (w/v) were selected for seed germination study using six different field crops (*Saseme indicum*, *Zea mays*, *vigna mungo*, *Oryza sativa*, *Setaria italic* and *Trigonella* foeunm graecum). Germination experiment was conducted on plastic germination box (10cm×8cm), and experiments were replicated three times. About 15 seeds were placed on the thin layer of cotton and filter paper wetted with 15 ml of each formulation. Seeds were then allowed to germinate at 37°C for 7 days. Following germination, seedling morphology (seedling shoot and root length) and biomass was recorded compared to the control.

2.5 Biomass

Fresh and dry weight of the seedlings were analyzed after seven days of treatment. The Fresh weight (g) of individual treatments were measured immediately after the seedling harvest. Dry weight was measured by drying the samples under hot air oven for 6 h at 70°C.

2.6 IAA Estimation

For IAA estimation, seedlings were extracted with 7.5ml of 50% aqueous acetone with continuous agitation and then filtered. The extracts were acidified to pH of 2.5 with 5 N H_2SO4 and extracted 3 times with an equal volume of peroxide free ether. Ether was evaporated and redissolved in 1 ml of 95% ethanol, 4 ml of Salkowski reagent and incubated for 20 minutes. Absorbance was measured using 480 nm under UV spectrophotometer.

2.7 Lipid accumulation

Seven-day-old seedlings were stained with 0.1% (w/v) Nile Red in acetone for 10min at room temperature. After a brief rinsing with distilled water, seedlings were placed in UV transilluminator at 312 nm for lipid accumulation.

3. RESULT AND DISCUSSIONS

Detrimental effect of chemical fertilizers is a serious threat to the environment due to the various chemical reactions such as hydrolysis, photolysis and decomposition. Considering these issues, innovative application in the agricultural system is highly needed, that regard as a reasonable and effective approach in sustainable agriculture. Therefore, recent efforts have been channelized towards the production of low cost, eco friendly biostimulants because, that has some important properties, including acceleration targeted metabolic pathway, slow release of essential nutrients and other multifunctional qualities (Vasir and Labhasetwar 2007; Agasti et al. 2010; Nair et al. 2010). Accordingly, in our study SB was selected as an interesting starting material to obtain SBH abundant with various sugars. As expected, in reducing sugar assay, the higher sugar content was obtained for SBH (237µg/ml), which was 69.57% higher as compared to the native SB (Fig.1). This result was consistent with a previous study (Kong, 2018).

Further, plant growth promoting ability of formulated SBH was assessed by seed germination study. Application of SBH increased seed germination traits for all the crops and highest shoot and root length were recorded in the 1.5% of SBH, followed by SBH 1 and 0.5% treated seedlings. The SBH treated with *Seteria italic* shown highest shoot length as compare to the other SBH treated field crops. Root length and biomass also increased linearly based on the SBH concentration (Fig.2 & Table.1). In the previous study, amino acid, seaweeds and humic acid derived biostimulants enhance the seed germination traits; which has been attributed the presence various active ingredients like amino acids, plant hormones and humic substances (Parrado et al., 2008). C abundant chitosan also reported for the improved seed germination of maize seedling (Guan et al., 2009). Besides, Sugar such as glucose and sucrose are known to influence seedling development (Borisjuk, et al., 2002). Based on the previous reports, it could be suggest that, SBH abundant with various sugars might improved seedling growth by the acceleration of glycolytic pathway.

In seed germination experiment, we found that higher concentration of SBH (SBH 1.5%) has shown improved seedling growth as compared SBH 0.5 and 1%. Further, to evaluate the effect of SBH on plant hormones, IAA estimation was performed using Salkowsky's reagent. SBH increased the highest IAA content at SBH 1.5% compared with the other formulations (Table.1). There are few *in vitro* studies investigated the connection of sugar on the stimulation of plant hormones. In *Arabidopsis thaliana*, exogenous sugar supply triggers an accumulation of auxin and increases the auxin flux in the hypocotyls (Lilley, et al., 2012; Sairanen et al., 2012). Moreover, sucrose affect the level of auxin in plants (Ljung et al., 2015). Based on the available data, it can be assumed that, SBH probably regulated the seedling growth via the acceleration of plant hormones. This interpretation was supported by the observation of higher IAA content in the SBH treated seedlings than the control. Hence, it suggested that, SBH sugars may have an impact on the activation of auxins and other conjugation pathways.

Moreover, the influence of SBH on lipid accumulation was evaluated by the nile red staining. As compared to the control, lipid content also increased linearly from 0.5 to 1.5% of SBH treatments (Fig.3). A significant increase in the lipid accumulation pattern suggested that, the higher dosage of SBH (1.5%) increased that higher lipid accumulation and this result was attributed to the greater availability of sugar. In plants, sugar acts as a substrates for the synthesis of storage lipids; and the relationship between the stimulation and accumulation of lipid in the SBH treatment may be due to the increased carbon flux through glycolysis. The characteristic response of SBH on plant growth, IAA, lipid accumulation provides evidence that, SBH has the biostimulating efficiency for various agricultural crops.

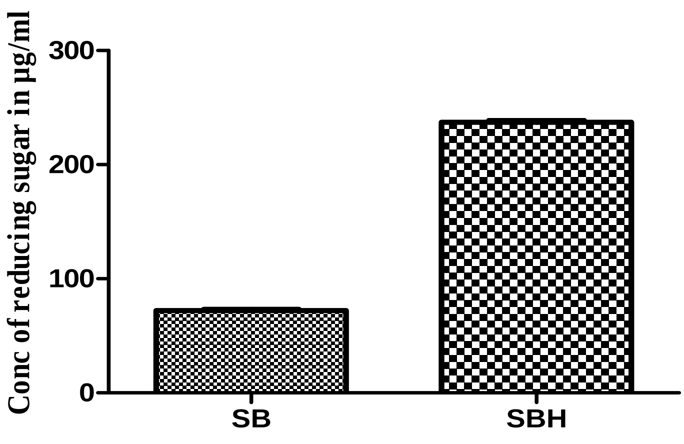

Fig. 1: Estimation of reducing sugar in SB and SBH.

Fig. 2: Biostimulant efficiency of SBH on seed germination.

(a) Control; (b) SBH 0.5%; (c) SBH 1%; (c) SBH 1.5%. There were 3 replicated of 10 seeds/ treatment.

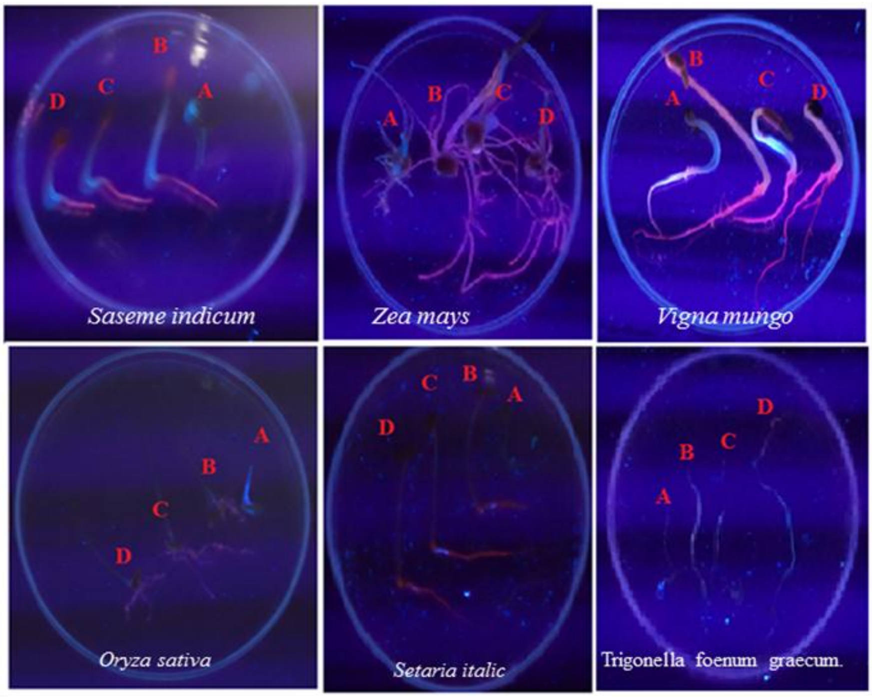

Fig. 3: Effect of SBH on lipid accumulation.

(a) Control; (b) SBH 0.5%; (c) SBH 1%; (c) SBH 1.5%. There were 3 replicated of 10 seeds/ treatment.

Table 1: Effect of SBH on plant morphological traits, biomass and IAA content.

Field crops	Shoot length (Cm)	Root length (Cm)	Fresh weight (g)	Dry weight (g)	IAA
Oryza sativa Control	4.05± 0.05	0.45±0.05	3.03±0.03	1.51±0.01	19.54±0.03
0.5% SBH	4.40±0.10	0.75±0.05	3.74±0.05	1.85±0.01	20.67±0.02
1% SBH	5.10± 0.10	0.80±0.10	4.53±0.01	2.26±0.01	21.07±0.06
1.5% SBH	5.35± 0.15	1.30±0.10	5.23±0.02	2.63±0.01	21.35±0.05
Sesame indicum Control	2.15±0.15	1.25±0.25	4.48±0.01	2.26±0.01	17.64±0.01
0.5% SBH	4.35±0.15	2.15±0.15	8.33±0.01	4.15±0.01	22.89±0.015
1% SBH	5.15±0.15	3.50±0.20	8.43±0.03	4.21±0.01	24.77±0.015
1.5% SBH	5.60±0.20	4.10±0.10	8.98±0.01	4.50±0.01	27.35±0.005
Zea mays Control	1.40±0.10	1.700±0.050	8.640±0.01	4.33±0.01	26.47±0.01
0.5% SBH	1.80±0.10	1.850±0.15	11.02±0.01	5.51±0.01	28.97±0.015
1% SBH	1.80±0.10	2.250±0.15	11.89±0.01	5.96±0.01	30.66±0.015
1.5% SBH	2.05±0.15	2.800±0.10	12.06±0.01	6.31±0.01	32.14±0.02
Vigna mungo Control	3.10±0.10	3.15±0.15	6.83±0.03	3.41±0.01	23.03±0.03
0.5% SBH	4.60±0.10	5.75±0.05	7.18±0.01	3.61±0.015	25.88±0.02
1% SBH	4.85±0.05	6.10±0.10	8.33±0.01	4.18±0.010	27.65±0.01
1.5% SBH	5.15±0.15	6.65±0.15	8.49±0.01	4.23±0.015	32.31±0.015
Seteria italic Control	2.50±0.10	1.80±0.10	1.21±0.01	0.61±0.015	14.66±0.015
0.5% SBH	3.80±0.10	4.05±0.15	2.41±0.01	1.21±0.015	18.04±0.02
1% SBH	6.05±0.15	5.20±0.10	2.71±0.01	1.36±0.010	18.97±0.02
1.5% SBH	6.25±o.05	5.75±0.05	3.16±0.01	1.58±0.010	20.06±0.005
Triconella fornum graecum Control	4.15±0.15	0.40±0.10	1.03±0.03	0.52±0.020	7.560±0.020
0.5% SBH	5.25±0.25	0.65±0.15	1.82±0.02	0.91±0.015	9.435±0.015
1% SBH	5.40±0.10	0.65±0.15	2.11±0.01	1.070±0.02	11.43±0.015
1.5% SBH	6.40±0.10	0.80±0.10	2.21±0.01	1.115±0.01	13.65±0.02

Data was reported as the mean of three measures ± SE.

4. CONCLUSIONS

The eco-friendly nature of plant biostimulants function as the key player in the sustainable agriculture by improving soil plant growth and stress tolerance. The successful plant biostimulant should meet various innovative strategies related to the function of the active ingredients and their proper application to the field of agriculture. In our study, valorization of SB promoted plant growth and biomass in a dose dependent fashion. Moreover, SBH positively regulated the plant growth, IAA acceleration and lipid accumulation in the tested field crops. Hence, we conclude that, SBH could act as plant biostimulant for improved crop productivity. Nevertheless, further studies on the evaluation of SBH in plant physiology and molecular characteristics may open various platforms for the sustainable farming systems.

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