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ROLE OF CHITOSAN POWDER ON THE PRODUCTION OF QUALITY RICE SEEDLINGS OF BRRI dhan29

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Role of chitosan powder on the production of quality rice seedlings of BRRI dhan29 was examined in the field of Sher-e-Bangla Agricultural University, Dhaka. There were six treatments and three replications in the experiment. The treatments were as follows: T_1 = 100 g chitosan (CHT) powder/m^2, T_2 = 200 g CHT powder/m^2, T_3 = 300 g CHT powder/m^2, T_4 = 400 g CHT powder/m^2, T_5 = 500 g CHT powder/m^2, T_6 = 0 g CHT powder/m^2. A significant variation was observed in the seedlings height, biomass production, dry matter production and chemical properties of the seedbed soils due to the application of chitosan powder in the seedbed. The maximum seedlings height, fresh weight, oven dry weight was observed in the treatment T_4 and the minimum level in the treatment T_6 (control). On the other hand, the maximum level of organic carbon, organic matter and soil pH was recorded in the treatment T_5 and the minimum level in the treatment T_6 (control). Chitosan powder increased the level of organic matter in a dose dependent manner. Quality of the Boro rice seedlings were improved due to the application of chitosan powder and the seedlings strength were increased in a dose dependent manner. All the treatments were produced good quality Boro rice seedlings having more chlorophyll level and seedlings strength than the control treatments. Our results indicated that treatment T4 shows the superior results than other treatments. These results could be due some nature of soil alkalization and other some macro-micro nutritional supplementation which may be improving the strength of the seedlings. Taken together, chitosan powder could play a significant role in the quality Boro rice seedling production that might be help to increase the grain yield.

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INTRODUCTION

Boro rice is very popular in Bangladesh. Among the different groups of rice, transplanted Boro rice covers about 49.11% of total rice area and it contributes to 38.11% of the total rice production in the country (BBS, 2013). Production of Boro rice seedlings in our country is a big problem due to the cold stress and quality of the seedling is not up to the mark. Cold stress retards the seedling growth and biomass production resulting in lower the seedling strength, an indicator of quality seedlings. The population of Bangladesh is still growing by two million every year and may increase by another 30 million over the next 20 years. Thus, Bangladesh will require about 27.26 million tons of rice for the year 2020 (FAO, 2009).

Chitosan (CHT) is a natural biopolymer modified from chitin, which is the main structural component of squid pens, cell walls of some fungi and shrimp and crab shells. Chitin and chitosan are copolymers found together in nature. They are inherent to have specific properties of being environmentally friendly and easily degradable. Bangladesh is a world-leading exporter of frozen shrimps. Therefore, there are abundant raw materials for chitosan powder production. Chitosan has a wide scope of application. With high affinity and non-toxicity, it does no harm to humans and livestock (Harmed et al., 2016).

Chitosan regulates the immune system of plants and induces the excretion of resistant enzymes. It has strong effects on agriculture such as acting as the carbon source for microbes in the soil, accelerating of transformation process of organic matter into inorganic nutrients and assisting the root system of plants to absorb more nutrients from the soil (Ibrahim et al., 2015). Chitosan is absorbed to the root after being decomposed by bacteria in the soil. Application of chitosan in agriculture, even without chemical fertilizer, can increase the microbial population by large numbers, and transforms organic nutrient into inorganic nutrient, which is easily absorbed by the plant roots (Choi, 2016).The organic manures viz. sludge and spray of CHT may be used as an alternative source of N which increases efficiency of applied N (Saravanan et al., 1987). Integrated use of organic manures with the combination of inorganic fertilizers can contribute to increase N content of rice soil as well as to increase long term productivity and enhancement of ecological sustainability (Gill and Meelu, 1982).

Combined application of sludge and spray of CHT along with nitrogen fertilizer improves soil health and soil productivity but only use of nitrogenous fertilizer for a long period causes deterioration of physical condition and organic matter status and reduces crop yield. When sludge and spray of CHT are applied along with chemical fertilizers for efficient growth of crop, decline in organic carbon is arrested and the gap between potential yield and actual yield is bridged to large extent (Rabindra et al., 2005). The objective of the study is to determine the effect of chitosan powder on quality seedling production of BRRI dhan29.

MATERIALS AND METHODS

The experiment was conducted at the research field of Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from December 2015 to January 2016. The soil of the experimental plots belongs to the Agro Ecological Zone, Madhupur Tract (AEZ-28).The farm belongs to the general soil type of Deep Red Brown Terrace soils under Tejgaon soil series. Initial Soil pH was 7.2 and had organic carbon 0.63% and organic matter content is 1.09%. BRRI dhan29 were used as the test crop. There were six treatments in the experiments i.e. T_1: seedbed applied CHT powder at 100 g/m^2,T_2: seedbed applied CHT powder at 200g/m^2, T_3: seedbed applied CHT powder at 300g/m^2, T_4: seedbed applied CHT powder at 400g/m^2, T_5: seedbed applied CHT powder at 500g/m^2 and T_6: seedbed applied CHT powder at 0 g/m^2. Dry shrimp shell byproducts were collected from the Khulna region of Bangladesh. The Chitosan powder was prepared by milling the byproducts and sieving them using a sieve having 2 mm in diameter. Finally up to 2 mm size powder were used in the experiment. The prepared chitosan was used in the experiment during the final land preparation. The experiment was laid out in a Randomized Complete Block Design (factorial). Each treatment was replicated thrice. The size of a unit plot was 1 m × 1 m. Total plots in the experimental field were 18. Common procedure was followed in rising of seedlings in the nursery bed.

The nursery bed was prepared by puddling with repeated ploughing followed by laddering. Weeds were removed and irrigation was gently provided to the bed as and when needed. No fertilizer was used in the seedbed. Physical and chemical properties of soil were analyzed in the Department of Soil Science, Sher-e-Bangla Agricultural University, Dhaka-1207. The properties studied included texture, soil pH, soil organic carbon and soil organic matter. The soil was analyzed following standard methods. Soil pH was measured with the help of a glass electrode pH meter using soil suspension of 1:2.5 as described by Jackson (1962). Organic carbon in soil was determined by wet oxidation method (Walkley and Black, 1934). All the data were recorded and analyzed by using standard windows based software statistix 10 trial (https://statistix.informer.com/10.0/).

RESULTS AND DISCUSSION

Chitosan-powder-induced fresh weight of BRRI dhan29 rice seedlings

Fresh weight production of BRRI dhan29 rice seedlings were significantly increased with the chitosan powder treatments in the seedbed. The maximum fresh weight (29.14 g) production of 100 seedlings was found in the treatment T4 having 400 g CHT powder/m^2 which was significantly different from all other treatments (Fig. 1, A). The second highest fresh weight production (27.92 g) was found in the treatment T5 which was statistically identical to the treatment T3 (27.2 g) (Fig. 1, A). The production of fresh weight in the treatment T1 was 25.78 g which was statistically identical to the treatment T2 (25.5 g). The lowest fresh weight production (12.6 g) was found in the treatment T6 (control) which was significantly different from all other treatments (Fig. 1, A). These results indicate that fresh weight productions of BRRI dhan29 rice seedlings were influenced by the chitosan powder treatments and this might be due its supplementation of plant nutrients and growth regulators (REF). Chitosan promotes shoot and root growth (Tsugita *et al*., 1993; Rahman *et al.*, 2015). Application of CHT can increase the microbial population by large numbers and transforms organic nutrient into inorganic nutrient which is easily absorbed by the plant roots (Bolto *et al*., 2004). The organic manures viz. sludge and spray of CHT increases the efficiency of applied N (Saravanan *et al.*, 1987).

Chitosan-powder-influenced oven dry weight of BRRI dhan29 rice seedlings

Oven dry weight of BRRI dhan29 rice seedlings were significantly influenced due to the application of chitosan powder. The maximum oven dry weight (6.43 g) production of 100 seedlings was found in the treatment T4 having 400 g powder/m^2 which was statistically different from all other treatments (Fig. 1,B). The second highest oven dry weight (6.23 g) was found in the treatment T5 which was statistically identical to the treatment T1 (6.1 g). The production of oven dry weight in the treatment T2 was 6.07 g which was statistically identical to the treatments T3 (6.00 g) and T1. The lowest oven dry weight production (3.5 g) was found in the treatment T6 (control) which was significantly different from all other treatments (Fig. 1, B). These results indicate that oven dry weight productions of BRRI dhan29 rice seedlings were influenced by the chitosan powder applications and this might be due its nutritional support to the seedlings, improvement of growth promoting hormonal activity and could improve the biological as well as physioco-chemical properties of the seedbed soils (Tsugita*et al*., 1993; Rahmanet al*.,* 2015).

Fresh weight production of 35 days old BRRI dhan29 rice seedlings

Fresh weight production of BRRI dhan29 rice seedlings at 35 DAS were significantly increased with the chitosan powder treatments. The maximum fresh weight (44.95 g) production of 100 seedlings was found in the treatment T5 having 500 g powder/m^2 which was statistically different than all other treatments (Fig. 2, A). The second highest fresh weight production (42.2 g) was found in the treatment T4 which was statistically identical with the treatment T3 (39.8 g) (Fig 2, A). The production of fresh weight in the treatment T2 was 35.9 g which was statistically identical to the treatment T1 (31.95 g). The lowest fresh weight production (17.9 g) was found in the treatment T6 (control) which was significantly different from all other treatments (Fig. 2, A). These results indicate that fresh weight productions of BRRI dhan29 rice seedlings were influenced with the treatments and this might be due its nutritional supplementations to the soil as well as the improvement of growth promoting hormonal activity. Tsugita et al., (1993) reported that chitosan promotes shoot and root growth. CHT can increase the microbial population and transforms organic nutrient into inorganic nutrient which is easily absorbed by the plant roots (Bolto et al., 2004).

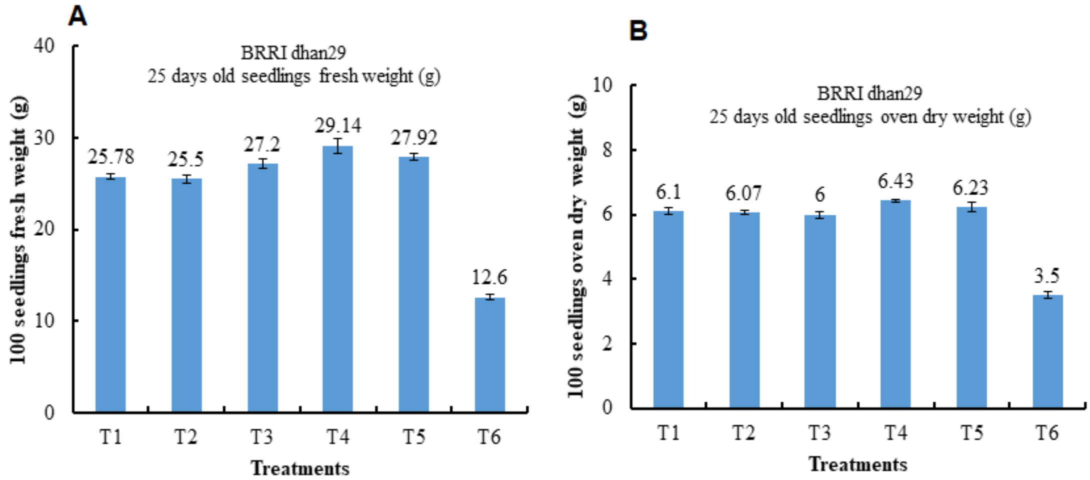

Figure 1. Effect of chitosan powder on the biomass production of 25 days old BRRI dhan29 rice seedlings; A) Chitosan powder-induced fresh weight (g) production of BRRI dhan29 rice seedlings. B) Chitosan powder-induced oven dry weight (g) production of BRRI dhan29 rice seedlings. T1=100 g powder/m^2, T2=200 g powder/m^2, T3=300 g powder/m^2, T4=400 g powder/m^2, T5=500 g powder/m^2, T6=0 g powder/m^2

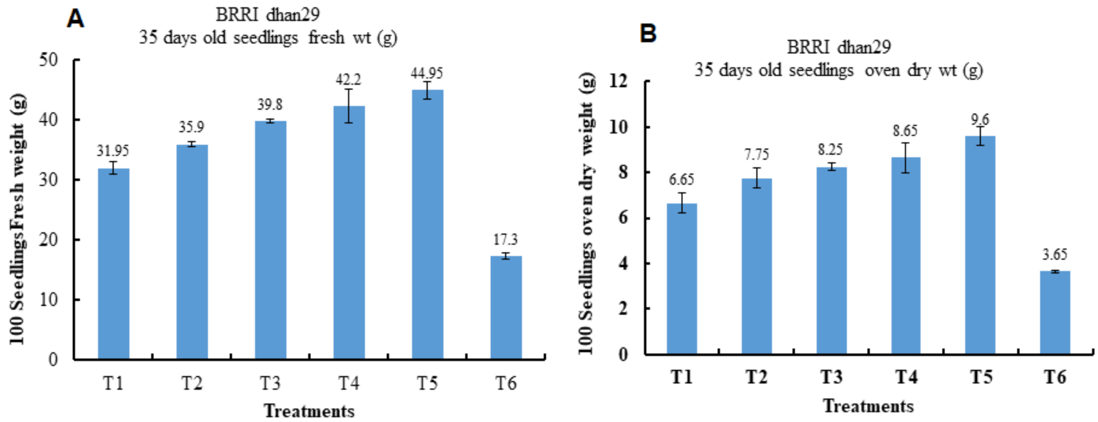

Figure 2. Effect of chitosan powder on the biomass production of 35 days old BRRI dhan29 rice seedlings; A) Chitosan powder-induced fresh weight (g) production of BRRI dhan29 rice seedlings. B) Chitosan powder-induced oven dry weight (g) production of BRRI dhan29 rice seedlings. T1=100 g powder/m^2, T2=200 g powder/m^2, T3=300 g powder/m^2, T4=400 g powder/m^2, T5=500 g powder/m^2, T6=0 g powder/m^2

Oven dry weight production of 35 days old BRRI dhan29 rice seedlings

Oven dry weight production of 35 days old BRRI dhan29 rice seedlings were significantly increased due to the chitosan powder application in the seedbed. The maximum oven dry weight (9.6 g) of 100 seedlings was found in the treatment T5 having 500 g powder/m^2 which was statistically different from all other treatments (Fig 2, B). The second highest oven dry weight production (8.65 g) was found in the treatment T4 which was statistically identical to the treatment T3 (8.25 g). The production of oven dry weight in the treatment T2 was (7.75 g) which was statistically identical to the treatments T1 (6.65 g).

The lowest oven dry weight production (3.65 g) was found in the treatment T6 (control) which was significantly lower from all other treatments (Fig. 2, B). These results indicate that oven dry weight productions of BRRI dhan29 rice seedlings were significantly influenced by the chitosan powder applications. These results might be due the macro-micro nutritional support, alkalization effect of the soil environment as well as physioco-chemical properties of the seedbed soils.

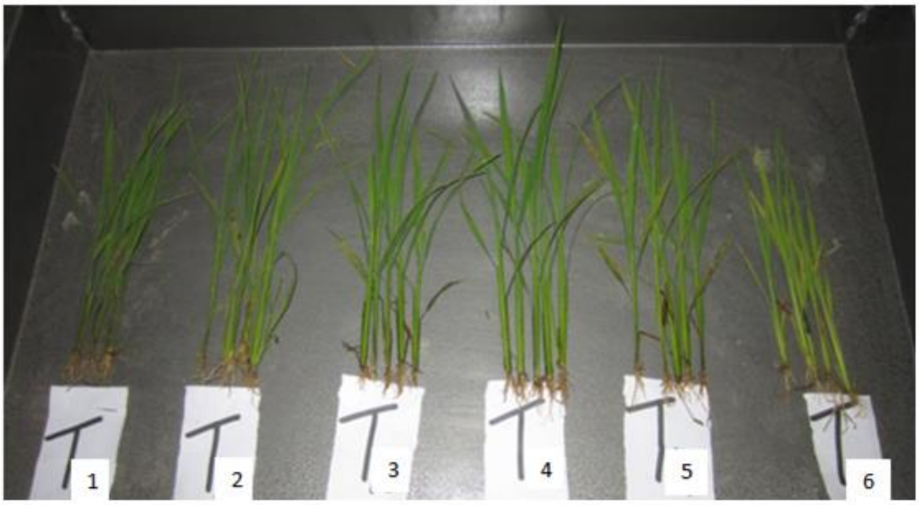

Figure 3. Typical picture of the 35 days old BRRI dhan29 rice seedlings produced with the treatments of chitosan powder in the seedbed. T_1 = 100 g powder/m^2, T_2 = 200 g powder/m^2, T_3 = 300 g powder/m^2, T_4 = 400 g powder/m^2, T_5 = 500 g powder/m^2, T_6 = 0 g powder/m^2

Table 1. Effects of chitosan powder on seedlings height (cm) and seedling strength (mg/cm) of BRRI dhan29 at 35 DAS

Treatments	Seedling height (cm)	Oven dry wt (mg/seedling)	Seedling strength (mg/cm)
T_1: seedbed applied CHT powder @ 100 g/m^2	15.35c	255.60d	16.66c
T_2: seedbed applied CHT powder @ 200g/m^2	16.18b	287.20c	17.70c
T_3: seedbed applied CHT powder @ 300g/m^2	16.37b	318.40b	19.47b
T_4: seedbed applied CHT powder @ 400g/m^2	17.13a	337.60ab	19.72b
T_5: seedbed applied CHT powder @ 500g/m^2	16.51ab	359.60a	21.78a
T_6: seedbed applied CHT powder @ 0g/m^2	12.83d	138.40e	10.80d
LSD (0.05)	0.65	22.09	1.60
CV (%)	2.27	3.27	4.96
Level of significance	*	**	**

Values in a column are significantly different at $p \leq 0.05$ applying LSD.
** = Significant at 1% level of probability, *= Significant at 5% level of probability

Seedling height (cm)

Seedling height was found to be statistically significant in all of the treatments used in the experiment. The maximum seedling height (17.13 cm) was obtained in the T_4 treatment having CHT powder @ 400 g/m^2 which was statistically identical to the treatment T5. The second highest seedlings height (16.51 cm) was found in the treatment T5 and it was statically identical with the treatments T2, T3 (Table 1). The seedlings height (15.35 cm) was found in the treatment T1 that was also significantly different than the control treatment T6. The minimum seedling height (12.83 cm) was observed in the control treatment T_6 having no CHT powder in the seedbed (Table 1). All the treatments produced higher seedlings height than the control treatment. According to the seedling height the treatment combinations were were ranked in the order of T4> T_5>T_3>T_2>T_1>T_6. This result indicated that chitosan could be functional against cold stress through salicylic acid priming (Issak, 2013). Our results suggested that Boro rice seedlings production were improved by using the chitosan powder in the seedbed. These results were supported by Boonlertnirun *et al.* (2008) who found that application of chitosan stimulate the seedling height significantly. Chitosan functions against drought stress through the reduction of stomatal aperture (Issak et al*.,* 2013).

Single Seedling oven dry wt. (mg)

Single seedling oven dry weight was found to be statistically significant in all of the treatments used in the experiment. The maximum single seedling oven dried weight (359.60 mg) was obtained in the T_5 treatment having CHT powder @ 500g/m^2 and minimum seedling weight (138.40 mg) was obtained in the T_6 control treatment having CHT powder @ 0 g/m^2 (Table 1). All the treatments except T1 increased the seedlings oven dry weight and the increments were more than double. According to the seedling oven dry weight production the treatments were ranked in the order of T_5> T_4 >T_3>T_1>T_2>T_6. These results were supported by Boonlertnirun et al*.* (2008) who found that application of chitosan stimulate the seedling dry matter weight significantly.

CHT-powder-induced seedling strength (mg/cm) of BRRI dhan29

Seedling strength is a strong indicator for measuring good quality seedlings. Seedling strength of BRRI dhan29 was found to be statistically significant. The maximum seedling strength (21.79 mg/cm) was obtained in the T_5 treatment having CHT powder @ 500 g/m^2. The second height seedlings strength (19.72) was found in the treatment T4 and it was statistically identical with the treatment T3 (19.47) (Table 1). In the treatment T2, seedling strength was found (17.70) which were statistically identical with the treatmentsT1 (16.66).The minimum seedling strength (10.80 mg/cm) was obtained in the T_6 treatment (control) (Table 1). All the treatments showed stronger seedling strength than control treatment. According to the seedling strength the treatments may be ranked in the order of T_5> T_4 >T_3>T_2>T_1>T_6. The seedlings strength were near about double in the treatments T3, T4, T5 indicating a very good quality rice seedling that might be increase the growth and yield of Boro rice. These results were supported by Boonlertnirun et al*.* (2008) who found that application of chitosan stimulate the seedling strength significantly.

Organic carbon status of the post transplanted rice seedbed soils

Application of chitosan powder in the seedbed soil tends to increment of organic carbon content. The organic carbon content was increased with increasing the level of chitosan powder in the seedbed soils. Maximum organic carbon content (1.18 %) was found in the treatment T5 having applications of 500 g/m^2 of chitosan in the seedbed which was statistically identical to the treatments T4 (1.08%) and T3 (0.97%) (Table 2). However, minimum carbon content (0.62%) was found in the treatment T6 which was statistically identical to the treatment T2 (Table 2). The organic carbon content was increased in a dose dependent manner, it might be due to the use of chitosan containing high amount of organic carbon level. This result suggested that chitosan powder application may increase the level of organic matter in soils. Rahman et al*.* (2015) reported that soil application of the chitosan powder increase the level of soil organic carbon.

Table 2. Organic carbon and organic matter status of the post transplanted rice seedbed soils influenced by the chitosan powder

Treatments	Post transplanted Rice seedbed soil	
	% OC	%OM
T_1: CHT powder @ 100 g/m^2	0.77e	1.32f
T_2: CHT powder @ 200g/m^2	0.87d	1.49d
T_3: CHT powder @ 300g/m^2	0.97c	1.67c
T_4: CHT powder @ 400g/m^2	1.08b	1.87b
T_5: CHT powder @ 500g/m^2	1.18a	2.04a
T_6: CHT powder @ 0g/m^2	0.62f	1.06f
CV	3.69	3.70
LSD	0.095	0.165
Level of significance	**	*

Values in a column are significantly different at $p \leq 0.05$ applying LSD.
** = Significant at 1% level of probability, *= Significant at 5% level of probability

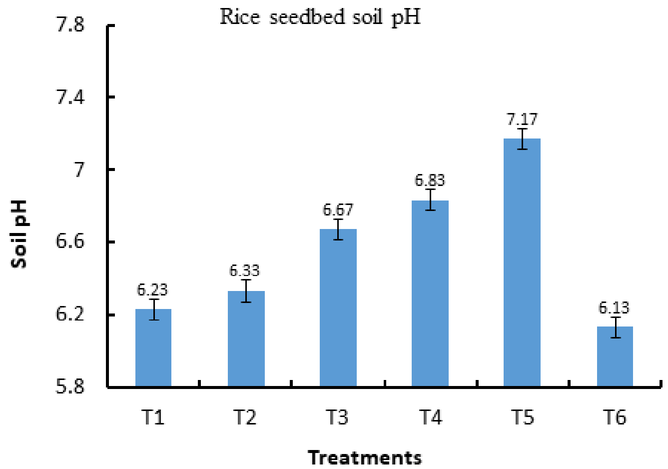

Figure 4. Chitosan-powder-induced alkalization of the post transplanted rice seedbed soils of BRRI dhan29. Mean was calculated from three replicates for each treatment

Chitosan-powder-induced alkalization of the post transplanted rice seedbed soil

A significant variation was found in the rice seedbed soil alkalization at 35 days after sowing due to the different treatment combinations (Fig. 4). The alkalization value of the rice seedbed soil was ranged from 6.13 to 7.17 (Fig. 4). The highest alkalization value (7.17) was recorded in T_5 treatment having 500 g/m^2 chitosan. On the other hand, seedbed soil alkalization of the control treatment T6 was 6.13 having no chitosan in the seedbed soils. The alkalization of the control seedbed soil T_6 was the lowest; it might be due to the non-application of the chitosan in the seedbed soils. Application of chitosan might be neutralized the seedbed soils, which might be improved the seedbed soil environment due to the increment of soil alkalization levels. Many causes could be involved in the improvement of seedbed soil environment due to the application of the chitosan in the seedbed soils. Nutrient supplementation will be increased due to the increment of alkalization levels which will improve the biological and physico-chemical properties of soil.

Analytical composition of the CHT powder

Analytical results revealed that a number of essential (macro and micro elements) were supplied due to the application of the modified CHT in the rice seedbed soils. With the alkaline behavior of the materials increased the pH level of the seedbed soils. Many factors could be involved in the supper growth, development and yield increment of the rice seedlings. The above mentioned nutritional supplementation and some other growth promoting hormone could be involved in the mechanisms.

Table 4. Composition of the CHT which was used in the research work

Name of the nutrients	Nutrient content
Nitrogen (N)	4.06 %
Phosphorus (P)	.643 %
Potassium (K)	0.28 %
Sulphur (S)	0.092 %
Calcium (Ca)	2.43 %
Magnesium (Mg)	0.36 %
Zinc (Zn)	92.03 ppm
Boron(B)	152 ppm
Organic Carbon (OC)	7.52%
Organic Matter (OM)	12.96%
pH of the CHT powder	8.73

CONCLUSION

The research was conducted to improve our understanding in BRRI dhan29 rice seedlings responses to CHT powder application. Our results indicated beneficial effects of CHT powder application in Boro rice seedlings production. The results also indicate that seedbed applied method of chitosan would be highly effective to produce good quality Boro rice seedlings that could play influential role to increase grain yield of Boro rice .The overall results of the present study demonstrated that rice may be grown successfully for obtaining maximum yield with the application of CHT powder in the seedbed applied method.

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