EFFECT OF CHITOSAN RAW MATERIAL POWDER AND SEEDLING AGE ON THE PERFORMANCE OF BORO RICE (BRRI dhan88)

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*This is to certify that thesis entitled, **"EFFECT OF CHITOSAN RAW MATERIAL POWDER AND SEEDLING AGE ON THE PERFORMANCE OF BORO RICE (BRRI dhan88)"** submitted to the Department of Soil science, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in SOIL SCIENCE**, embodies the result of a piece of bona-fide research work carried out by **ISRAT JAHAN KEYA**, Registration No. **19-10227** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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Dedicated T0

My

Beloved Parents

And

Respected Teachers

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The Author

EFFECT OF CHITOSAN RAW MATERIAL POWDER AND SEEDLING AGE ON THE PERFORMANCE OF BORO RICE (BRRI dhan88)

ABSTRACT

A pot experiment was conducted under the net house of Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh, during November, 2019 to April, 2020 to investigate the effect of chitosan raw material powder and seedling age on the performance of BRRI dhan88. The experiment was consisted of two factors and following Randomized Complete Block Design (RCBD) with four replications. Factor A: Level of chitosan raw material powder viz; C_0 = 0%, C_1 = 0.1%, C_2 = 0.2%, C_3 = 0.3%, C_4 = 0.4% and C_5 = 0.5% and Factor B: Different seedling age viz; S_1 = 25 days old seedling, S_2 = 30 days old seedling and S_3 = 35 days old seedling. Experimental results revealed that among different levels of chitosan raw material powder, seedling treated with C_4 (0.4 %) treatment and S_3 (35 days old seedling) perform well and recorded the maximum average seedling height (20.45 cm), fresh weight per 100 seedling (44.6 g), oven dry weight per 100 seedling (17.2 g), seedling strength (8.41 mgcm^{-1}) , effective tillers hill^{-1} (22.75), minimum days for first flowering (74.5), minimum days for 50 % flowering (77.2), minimum days for 100 % flowering (79.25), maximum grain yield pot^{-1} (68.91g), maximum straw yield pot^{-1} (85.5 g), maximum biological yield pot^{-1} (153.45 g). Chitosan raw material powder also have some positive residual effect on pH, total nitrogen, organic carbon and organic matter. Chitosan raw material powder strongly improved the seedling characters at low temperature conditions, yield and yield attributing characters of BRRI dhan88. Thus, for cultivation of BRRI dhan88, it is suggested that optimum level (0.4%) of chitosan raw material powder could be applied in the seedbed soil along with transplanting young (35 days old) seedling perform well for obtaining higher grain yield.

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LIST OF ABBREVIATIONS

FULL FORM	ABBREVIATIONS
Agro-Ecological Zone	AEZ
Analysis of Variance	ANOVA
And others	et al.
Bangladesh Agricultural Research Institute	BARI
Bangladesh Bureau of Statistics	BBS
Bangladesh Rice Research Institute	BRRI
Centimeter	cm
Days After Transplanting	DAT
Degree Celsius	°C
Duncan's Multiple Range Test	DMRT
Emulsifiable concentrate	EC
Food and Agriculture Organization	FAO
Gram	g
Harvest Index	H.I.
Least Significant Difference	LSD
Mean sea level	MSL
Milimeter	mm
Milligram	mg
Muriate of Potash	MoP
Nanometer	nm
Non-significant	NS.
Number	No.
Parts Per Million	ppm
Percentage	%
Percentage of Coefficient of Variance	CV%
Randomized Complete Block Design	RCBD
Serial	Sl.
Soil Resource Development Institute	SRDI
Triple super phosphate	TSP
United States Department of Agriculture	USDA
Weight	Wt.

CHAPTER I

CHAPTER I

INTRODUCTION

Globally rice (*Oryza sativa*) is the third major cereal grain (Rahman M. *et al.*, 2021). It is a food staple for more than 3.5 billion people around the world, particularly in Asia, Latin America, and parts of Africa (National Geographic Society, 2022). In the 2021/2022 crop year, about 509.87 million metric tons of rice was consumed worldwide, up from 437.18 million metric tons in the 2008/2009 crop year (M. shahbandeh, 2022). It is the predominant dietary energy source for 17 countries in Asia and the Pacific, 9 countries in North and South America and 8 countries in Africa. Rice provides 20% of the world's dietary energy supply, while wheat supplies 19% and maize (corn) 5% (FAO, 2011). Broad capability in different ecosystems and less cultivation risk, several farmers preferred rice cultivation instead of other crops. World population is increasing and it is assumed that 14,886 million tons (MT) of foods need to be produced in 2050 to meet up the food demand (Mohammad Fakhrul Islam S. *et al.*, 2020). Worldwide 513.02 MT rice is produced where China produces 29% of the total, followed by India (25.1%), Bangladesh (7.0%), Indonesia (6.1%), Vietnam (5.3%), and Thailand (3.8%) (USDA, 2022).

Rice is also the staple food in Bangladesh and accounting for approximately 78 percent of the country's total net cropped areas cultivation. The country achieves an autarky to meet up the rice demand for its 169.04 million peoples from 11.55 million hectares of cultivated gross area (Nasim M. *et al.*, 2021). In Bangladesh, food security is equivalent to rice security (Kabir M.S. *et al.*, 2020). Annual per capita rice consumption is still the highest in the world (Salam *et al.*, 2019). Rice is cultivated in three seasons namely Aus, Aman and Boro throughout the year. Since independence, rice production has been increased three-fold from approximately 11 MT in 1971–72 to about 36.6 MT in 2019–20 (BBS, 2020). This revolution has transformed the country from so called "Bottomless Basket" to a "Full of Food Basket". After a long period, rice production in Bangladesh has risen significantly after 1990-1991, especially during two periods: 1996-1997 and 2000-01, as well as from 2009-10 to 2013-14. Improved loan distribution policies (credit deposits directly to farmers' 10 Taka bank accounts), well organized fertilizer supplies, availability of high-quality seeds by the public and commercial sectors, and technical interventions (e.g., genetic improvements of varieties for favorable and unfavorable ecosystems) make it possible to make Bangladesh as one of the largest contributors of rice in the world (Rabbi S. *et al.*, 2020). Bangladesh recently placed the third position worldwide in rice production, behind China and India, with a production volume of 3.6 crore tones (Rahman M. *et al.*, 2021).

"Boro" is the dry season irrigated rice crop planted from December to early February and harvested between April and June. It is known for high productivity in deep water areas. Water management in Boro is more systematic as it is an irrigated crop. Consequently, this crop responds well to higher doses of fertilizers resulting in higher production. Being a winter season crop, it is spared from insect-pest infestation. Moreover, lower winter temperature during the early crop growth period facilitates the accumulation of photosynthates, thereby increasing carbon: nitrogen ratio. Temperature rises during the ripening period, further facilitating the process. Variations in these parameters cause variation in yields across the Boro growing areas. The Boro rice cultivars have to be of short duration having physiological and plant type parameters to shorten the vegetative growth phase and more efficient dry matter accumulation. These would mean cold tolerance, lower loss of water due to transpiration, shade efficiency, less tillering and more effective tillers. In the wake of the COVID-19 pandemic in March 2020, policy concern for food security escalated globally, sensing the impending disruption in production, value chain and trade (Mobarok *et al.*, 2021). On-farm activities, postharvest processing and manufacturing, trade and distribution, retail market, and regulatory processes were affected by COVID-19. A dream for 'zero hunger' by 2030 face gigantic hurdle due to the pandemic situation. With an unexpected global deadlock, a big concern for a steady rice supply has frustrated the rice farmers, consumers, and policymakers (Kathiresan *et al.*, 2020). Two consecutive bumper harvests of Boro rice (dry season rice), which is the central pillar of rice security in the country, have pacified the rice security concern nationally and internationally. The highest ever Boro rice production of 20.8 million tonnes in 2021 is a big step towards a resilient rice system in Bangladesh (MOA, 2021). It is 6% higher than in 2020 (USDA, 2021). When the first lock-down in March 2020 was imposed countrywide, Boro rice was at the flowering stage. A deep uncertainty spread over the successful harvest of the main rice. However, farmers in Bangladesh presented a good harvest braving the pandemic, lock-down, and disaster (DAE, 2020).

BRRI dhan88 is a short-term variety of rice in Boro season. Its heredity line is BRRI dhan29-SC3-28-16-10-8-HR1 (Com). The lineage was invented through somaclonal variation and conventional method selection and later the final heredity line selection was done in row method from the sheaf at the BRRI Regional Office, Comilla. BRRI dhan88 was released by the National Seed Board for the Boro season in 2018 as it was satisfactory in various regional offices of BRRI and in different parts of the country (cultivation area of BRRI dhan28). The amylase content of rice is 26.3% and protein is 9.8%. The weights of 100 ripened rice are 22.1 grams. The average yield of the newly released BRRI dhan88 is 7 tonnes per hectare which is 0.5 tonnes higher than BRRI dhan28. Pest and disease infection in BRRI dhan88 is comparatively less than the other conventional varieties. The life span of the variety is 140-143 days which is 3-4 days earlier than that of BRRI dhan28, so it can be cultivated in haor area. This variety is shorter than BRRI dhan28 and more prone to shedding. Since this variety is capable of resist tilting and paddy does not fall from the sheaf, it can be harvested with the help of machine (BRRI, 2018).

Chitosan is a derivative of chitin and is considered the second most common polymer in the world after cellulose (Pandey *et al.*, 2018). Chitosan, as well as chitin, are classified as polysaccharides containing randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl glucosamine units. Chitosan is mainly obtained from leftovers obtained from sea food processing such as crab and shrimp shells, as well as fish scales. Furthermore, the potential to receive it from waste fungal mycelium is also indicated (Kumari *et al.*, 2017). Chitosan is a natural biopolymer which stimulates growth and increases yield of plants as well as induces the immune system of plants (Sultana *et al.*, 2019). They are inherent to have specific properties of being environmentally friendly and easily degradable (Boonlertnirun *et al.*, 2008). Moreover, chitosan not only activates the cells, but also improves its disease and insect resistant ability at field and storage (Sultana *et al.*, 2019). 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 (Bolto *et al.*, 2004). Moreover, plants treated with chitosan may be less prone to environmental stress such as drought, salinity and temperature (Pongprayoon *et al.*, 2013).

Objectives:

Considering the above facts, the present experiment was undertaken with the followingobjectives:

1. To examine effect of chitosan raw material powder and seedling age on the performance of BRRI dhan88

CHAPTER II

CHAPTER II

REVIEW OF LITERATURE

The agriculture sector is the building block of an economy with more than 60% of the world population depending on it for livelihood. Among the many crops, rice is the most important income source. It is the staple food for more than half of the world population. In spite of its huge demand, rice production has been dwindling due to various constraints. Chitosan is an excellent choice for agricultural applications owing to its non-toxic, biodegradable nature. Extensive studies of the regulatory effects of chitosan on various crops have been carried out worldwide by different workers. Since review of literature forms a bridge between the past and present research works related to problem, which helps an investigator to draw a satisfactory conclusion, an effort was thus made to present some research works related to the present study in this section. This chapter includes the available information regarding the effect of chitosan on rice.

Divya K. *et al.* (2022) conducted an experiment to study the effect of Chitosan nanoparticles (ChNP) as a growth promoter in improving the yield and biological activity of rice. 1 $mgml^{-1}$ of ChNP was applied as a seed, soil, foliar and combination treatments and the growth and yield parameters were measured to understand the best mode of application. The combination treatment of seed, soil and the foliar application was found to be most efficient. The cellular uptake of ChNP was also studied to deduce the mechanism of action. The soil toxicity of ChNP was studied prior to application and was found to be non-toxic.

Elshayb O.M. *et al.* (2022) observed the effects of urea–chitosan nanohybrid as a slow released source of nitrogen fertilizer on rice. The effects of fertilization applications, namely: CU: control treatment; U_1: application of a full recommended dose of classical urea (165 kg N ha^{-1}); U_2: adding recommended dose of classical urea by 80% + exogenous urea–chitosan nanohybrid 250 mg NL^{-1}; U_3: adding recommended dose of classical urea by 80% + exogenous urea–chitosan nanohybrid 500 mg NL^{-1}; U_4: adding recommended dose of classical urea by 60% + exogenous urea–chitosan nanohybrid 250 mg NL^{-1}; U_5: adding recommended dose of classical urea by 60% +

exogenous urea–chitosan nanohybrid 500 mg NL^{-1}; U_6: adding recommended dose of classical urea by 40% + exogenous urea–chitosan nanohybrid 250 mg NL^{-1}; and U_7: adding recommended dose of classical urea by 40% + exogenous urea–chitosan nanohybrid 500 mg NL^{-1} on growth indicators, yield-related components, grain productivity, and N uptake status of rice plants were investigated during two successive seasons. As a result, significant achievements concerning growth, yield and yield-related traits were obtained when rice plants were fertilized with exogenous urea–chitosan nanohybrid (i.e., 500 mg NL^{-1}) + 60% classical urea without a significant decline in the studied traits compared to the full recommended dose of classical urea. Accordingly, this investigation revealed that chitosan nanohybrid at 500 mg NL^{-1} as a compensatory alternative can be used in saving 40% of classical urea requirement.

Jia-Yi Y. *et al.* (2022) carried out an experiment on chitosan-based silicon nanoparticles (Chsi-NPs) that primarily consists of C (57.9%), O (31.3%), N (5.6%), and Si (3.5%) and are 10–180 nm in size. The effect on the foliage applied on rice planted on soil contaminated with 104 $mgkg^{-1}$ arsenic (As); low (3 mgL^{-1}) and high (15 mgL^{-1}) doses of the foliar Chsi-NPs are administered during the rice grain filling stage. The results showed that the higher dose foliar Chsi-NPs treatment reduced the As concentration in the grain by 61.2% but increased As concentration in the leaves by 47.1% compared to the control treatment. The foliar spraying of the Chsi-NPs inhibited As transport to the grain by facilitating the attachment of As to the cell wall, with higher doses of the foliar Chsi-NPs treatment increased by 8.7%. The foliar spraying of Chsi-NPs increased the malondialdehyde levels by 18.4%, the catalase activity by 49.0% and the glutathione activity by 99.0%. These results indicated that the foliar Chsi-NPs application was effective for alleviating As toxicity and accumulation in rice.

Riseh R.S. *et al.* (2022) observed that Chitosan is a candidate polymer for the encapsulation of probiotic bacteria for agricultural purposes. The attractive features of chitosan include its biocompatibility with other materials, easy digestion and dissolution, non-toxicity, high adsorption power, and potential biodegradation in nature, as well as its wide availability and cost-effectiveness. Chitosan has therefore become very important in a number of industries. In agriculture, the use of this polymer has effectively solved the main problems of bacterial encapsulation, including degradability, survival, and long-term performance.

Ahmed T. *et al.* (2021) reported that the green synthesis of chitosan-magnesium (CS-Mg) nanocomposite and its antimicrobial activity against two rice pathogens namely *Acidovorax oryzae* and *Rhizoctonia solani* for the first time. The green MgO nanoparticles synthesized by using a native *Bacillus sp.* strain RNT_3, were used to fabricate CS-Mg nanocomposite utilizing one-pot synthesis method. The synthesis of CS-Mg nanocomposite was further confirmed by using UV–vis spectroscopy, whereas, FTIR and XRD analysis showed the capping of CS-Mg nanocomposites by different functional groups together with their crystalline structure, respectively. Besides, SEM and TEM images revealed the spherical shape along with the particles size ranging from 29 to 60 nm. Moreover, EDS analysis confirmed the elemental purity of nanocomposite. The CS-Mg nanocomposite showed remarkable antimicrobial activity against *A. oryzae* and *R. solani* and significantly inhibited the growth as compared to non-treated control. The ultra-structure studies showed damaged structure of cell wall and internal cellular organelles after treatment with 100 μgmL^{-1} CS-Mg nanocomposite. The results of this study indicated that CS-Mg nanocomposite-based antimicrobial agents could be considered as promising nanopesticides against phytopathogens in plant disease management.

Kociecka J. *et al.* (2021) carried out an experiment on cereals using chitosan, its derivatives, and nanoparticles. Research into the use of chitosan in agriculture is growing in popularity. Since 2000, 188 original scientific articles indexed in Web of Science, Scopus, and Google Scholar databases have been published on this topic. These have focused mainly on wheat (34.3%), maize (26.3%), and rice (24.2%). It was shown that research on other cereals such as millets and sorghum is scarce and should be expanded to better understand the impact of chitosan use. This review demonstrates that this chitosan is highly effective against the most dangerous diseases and pathogens for cereals. Furthermore, it also contributes to improving yield and chlorophyll content, as well as some plant growth parameters. Additionally, it induces excellent resistance to drought, salt, and low temperature stress and reduces their negative impact on cereals. However, further studies are needed to demonstrate the full field efficacy of chitosan.

Faqir Y. *et al.* (2021) reviewed on chitosan in modern agriculture production and concluded that plant growth-promoting properties of chitosan as a growth regulator, pest/disease resistance, signaling regulation, effect on nuclear deformation, and apoptosis. Chitosan can improve the plant defense mechanism by stimulating photochemistry and enzymes related to photosynthesis. Furthermore, electrophysiological modification induced by chitosan can practically enable it to be utilized as an herbicide. Chitosan has an excellent role in improving soil fertility and plant growth as well as plant growth promoters. It is concluded; chitosan can play a key role in modern agriculture production and could be a valuable source promoting agricultural ecosystem sustainability. Future suggestions will be based on current achievements and also notable gaps. In addition, chitosan has a huge contribution to reducing fertilizers pollution, managing agricultural pests and pathogens in modern day agriculture.

Moolphuerk N. *et al.* (2021) was investigated the ability of chitosan to promote rice growth, physiological traits, and photosynthetic performance in rice seedlings under drought stress. Rice seedlings (*Oryza sativa* L. cv. KDML105) were treated with 100 mgl^{-1} low and high molecular weight (MW) chitosan via a combination of seed priming and foliar spray. The seedlings were subjected to drought stress by withholding water for 4 days, which resulted in the U-shaped (scale 5) leaf rolling. The results showed that drought significantly decreased shoot and root growth. Chitosan application, particularly with high MW chitosan, improved shoots and root growth under drought stress. Chitosan treatment also alleviated the effects of drought stress by elevating relative water content, as well as reducing electrolyte leakage and malondialdehyde content. Antioxidant enzyme activities, including guaiacol peroxidase (GPX) and ascorbate peroxidase (APX), were increased in response to chitosan. Additionally, treating the plant with chitosan improved photosynthetic efficiency as evidenced by increased CO_2 response, the maximum rate of Rubisco carboxylase activity (Vc_{max}), and photosynthetic rate. We conclude that the exogenous application of chitosan aids the plant in coping with the severity of drought stress.

While both low and high MW chitosan's were effective at alleviating the effect of drought on rice seedlings, high MW chitosan might have a slight advantage with respect to increased effective duration.

Stanely-Raja V. *et al.* (2021) reported that reduced pathogen resistance and management of the left-over rice stubble are among the most important challenges faced in rice cultivation. A novel and eco-friendly strategy to synthesize 'Fungal Chitosan' (FC) from *Aspergillus niger* using rice straw could serve as a sustainable treatment approach to improve both disease resistance and yields, while also effectively managing the rice stubble waste. The FC treatment promoted germination as well as growth parameters in rice varieties, TN_1 (high yielding-susceptible) and PTB_{33} (low yielding-resistant) better than a commercial chitosan (PC). Treatments of exogenously applied FC to plants produced direct toxicity to *Xoo*, and reduced the BLB disease index by 39.9% in TN_1. The capability of FC to trigger a cascade of defense pathways was evident from the measurable changes in the kinetics of defense enzymes, peroxidase (POD) and polyphenol oxidase (PPO). FC treatment increased levels of POD in TN_1 by 59.4%, which was 35.3% greater than that of untreated PTB_{33}. Therefore, the study demonstrated the effectiveness of FC treatments for use in agriculture as a potential bio stimulant as well as protective agent against bacterial leaf blight, BLB, of rice (*Oryza sativa*) that could be produced from stubble waste and improve rice stubble management strategies.

Abdullah Y. *et al.* (2020) investigated the antibacterial activity of biosynthesized chitosan nanoparticles (CSNPs) and zinc oxide nanoparticles (ZnONPs) against rice pathogen *Xanthomonas oryzae pv. oryzae* (Xoo). The formation of CSNPs and ZnONPs in the reaction mixture was confirmed by using UV-vis spectroscopy at 300–550 nm. Moreover, CSNPs and ZnONPs with strong antibacterial activity against *Xoo* were further characterized by scanning and transmission electron microscopy, Fourier-transform infrared spectroscopy, and X-ray diffraction. Compared with the corresponding chitosan and ZnO alone, CSNPs and ZnONPs showed greater inhibition in the growth of Xoo, which may be mainly attributed to the reduction in biofilm formation and swimming, cell membrane damage, reactive oxygen species production, and apoptosis of bacterial cells.

Ahmed *et al*. (2020) conducted a field experiment to examine effect of chitosan-raw-materials of on yield maximization of BRRI dhan49. The experiment was laid out in a Randomized Complete Block Design (RCBD) having four treatments with five replications. The chitosan-raw-materials were applied in different doses and methods (Seedbed and main field applied methods). The treatment combinations were as follows: T_1: Seedbed applied @ 0 gm^{-2} + Main field applied @ 0 tha^{-1} (Control); T_2: Seedbed applied @ 0 gm^{-2} + Main field applied @ 0.5 tha^{-1}; T_3: Seedbed applied @ 250 gm^{-2} + Main field applied @ 0 tha^{-1}; T_4: Seedbed applied @ 250 gm^{-2} + Main field applied @ 0.5 tha^{-1}. Seedlings height, dry matter production and seedlings strength were significantly increased with the application of chitosan raw materials in the seedbed. Seedlings, treated with chitosan raw materials, had a profound effect on grain yield and yield traits of T. Aman rice (BRRI dhan49). The yield traits like effective tillershills^{-1}, 1000 grain weight and filled grainpanicle^{-1} were increased due to the effect of chitosan-raw-materials on rice seedlings. Maximum grain yield (6.64 tha^{-1}) was found in the treatment T_4 which was statistically identical with the grain yield (6.51 tha^{-1}) of treatment T_3. Minimum grain yield (5.28 tha^{-1}) was found in the control treatment T_1 which was statistically identical with the grain yield of treatment T_2. Moreover, it was also observed that primary tillers production became earlier, number of effective tillers became higher, flowering and maturity time became earlier, resulting more yield.

Chakraborty M. *et al.* (2020) reported that chitosan (CHT) emerged as a promising agent used as a plant growth promoter and also as an antimicrobial agent. It induces plant growth by influencing plant physiological processes like nutrient uptake, cell division, cell elongation, enzymatic activation and synthesis of protein that can eventually lead to increased yield. It also acts as a catalyst to inhibit the growth of plant pathogens, and alter plant defense responses by triggering multiple useful metabolic pathways.

Divya K. *et al.* (2020) recommended that chitosan nanoparticles (ChNP) as a cost-effective alternative for chemical fungicides with potential biocontrol efficacy. ChNP was found to suppress 90% disease in detached leaf assay and 75% under greenhouse conditions. The enzyme specific activity of all the defense enzymes was significantly higher than the chemical control. The peroxidase, phenylalanine ammonia-lyase and chitinase enzymes were found to be the most active defense enzymes with 0.19,7.28 and 118.16 U/min/ml/mg protein compared to 0.01, 4.99 and 62.22 U/min/ml/mg protein for control. ChNP, a non-toxic biodegradable biopolymer, can be an effective biocontrol agent against rice sheath blight pathogen (ShB) caused by *Rhizoctonia solani*. It is a potent plant immunity booster that can be used as a suitable alternative to commercially available chemical fungicides.

Sathiyabama M. *et al.* (2020) conducted an experiment to prepare chitosan guar nanoparticle (CGNP) with high antimicrobial activity to use as a bio protectant against rice phytopathogens. Nanoparticles were prepared using sodium tripolyphosphate by the ionic gelation method. The physic chemical properties of nanoparticles were characterized through DLS, FTIR, TEM, SEM, AFM and XRD. The application of CGNP to rice seeds stimulated seed germination and seedling growth. CGNP showed growth inhibition towards rice pathogens *P. grisea* and *X. oryzae* under in-vitro condition. Excised rice leaves treated with CGNP and challenged with *P. grisea* showed no blast disease symptom whereas control leaves showed very high blast disease symptom.

Xie X. *et al.* (2020) explored the effects of 0.5 mgL^{-1} COS on plant growth promotion in rice seedlings by measuring root and stem length, investigating biochemical factors in whole plants via proteomic analysis, and confirming upregulated and downregulated genes by real-time quantitative PCR. Pathway enrichment results showed that COS promoted root and stem growth, and stimulated metabolic (biosynthetic and catabolic processes) and photosynthesis in rice plants during the seedling stage. Expression levels of genes related to chlorophyll *a*-b binding, RNA binding, catabolic processes and calcium ion binding were upregulated following COS treatment. Furthermore, comparative analysis indicated that numerous proteins involved in the biosynthesis, metabolic (catabolic) processes and photosynthesis pathways were upregulated. The findings indicate that COS may upregulate calcium ion binding, photosynthesis, RNA binding and catabolism proteins associated with plant growth during the rice seedling stage.

Hidangmayum A. *et al.* (2019) concluded that chitosan enhances the physiological response and mitigates the adverse effect of abiotic stresses through stress transduction pathway via secondary messenger(s). Chitosan treatment stimulates photosynthetic rate, stomatal closure through ABA synthesis; enhances antioxidant enzymes via nitric oxide and hydrogen peroxide signaling pathways, and induces production of organic acids, sugars, amino acids and other metabolites which are required for the osmotic adjustment, stress signaling, and energy metabolism under stresses. It is also known to form complexes with heavy metals and used as tool for phytoremediation and bioremediation of soil. Besides, this is used as anti-transparent compound through foliar application in many plants thus reducing water use and ensures protection from other negative effects.

Malerba M. *et al.* (2018) reported that among the search for biological methods to avoid the application of chemical products in agriculture has led to investigating the use of biopolymers-based materials, the best results were obtained from those based on the biopolymer chitosan (CHT). CHT, available in large quantities from the deacetylation of chitin, has multiple advantages: it is safe, inexpensive and can be easily associated with other compounds to achieve better performance. In this review, we have summarized the latest researches of the application of CHT on plant productivity, plant protection against the attack of pathogens and extension of the commercial life of detached fruits.

Issak, M. and Sultana, A. (2017) carried out an experiment to observed the role of chitosan powder on the production of quality rice seedlings of BRRI dhan29. There were six treatments and three replications in the experiment. The treatments were as follows: T_1 = 100 g chitosan (CHT) powderm^{-2}, T_2 = 200 g CHT powderm^{-2}, T_3 = 300 g CHT powderm^{-2}, T_4 = 400 g CHT powderm^{-2}, T_5 = 500 g CHT powderm^{-2}, T_6 = 0 g CHT powderm^{-2}. A significant variation was observed in the seedling's 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.

Hassan O. *et al.* (2017) concluded that Chitosan and oligo chitosan are the well-known biocontrol agents because of their nontoxic, biodegradable and biocompatible properties. Chitosan is considered the most abundant natural polymer with dual effect: Firstly, it controls pathogenic microorganisms by preventing their growth and sporulation, reducing spore viability and germination and by disrupting their cell membrane. Secondly, it induces of different defense responses in host plants by inducing and/or inhibiting different biochemical activities during the plant pathogen interaction. Chitosan has been assayed for controlling numerous pre and post-harvest diseases of many crops. Chitosan also has the positive effect of enriching biodiversity in the rhizosphere. The meteorological effect on chitosan is little evaluated. For achieving the goal of sustainable agriculture, chitosan will become a popular plant protectant.

Phothi R. *et al.* (2017) conduct research aimed to study the effects of chitosan on physiology, photosynthesis and biomass of rice cultivar RD47 under elevated ozone. Rice samples were grown at indoor climate-controlled chambers, allowing the inlet air to pass through charcoal filters. For combined effects of chitosan and ozone, rice was soaked and sprayed with chitosan 0.05% (W/V) under elevated ozone concentration at 40 ppb (Chi+EO_{340}) and 70 ppb (Chi+EO_{370}). Control groups (CF) with no additional ozone were also studied. Samples were analyzed weekly for tiller number per plants, leaf area, leaf chlorophyll, photosynthesis, shoot biomass, root biomass and total biomass. The results obviously showed that ozone at the concentration of both 40 and 70 ppb caused negative effects on rice physiology, photosynthesis and biomass. The 70-ppb concentration, particularly, caused severe damage. Whilst soaking and spraying with chitosan could significantly reduce the harmful effects of ozone compared with the control group. For the samples soaked and sprayed with chitosan under elevated ozone for 21 days, Chi+EO_{340} and Chi+EO_{370} significantly performed more photosynthesis and contained more leaf chlorophyll than EO_{340} and EO_{370}, respectively ($p = 0.05$). In addition, chitosan could reduce the ozone negative effects and increased higher physiology and photosynthesis rate. However, there was no significant difference in biomass compared with the control group. Even through, ozone has been gradually increasing which made plants at risk, chitosan treatment could significantly ameliorate the effect of ozone and serve as a plant growth promoter with no harmful to human being.

Theerakarunwong C.D. *et al.* (2016) carried out research aims to study the effect of chitosan on 10 rice cultivars. Number of leafs, tiller number per plant, stem height, leaf area, photosynthesis rate and dry weight were analyzed. Seeding was immersed by chitosan concentration level at 0.01, 0.05 and 0.10% w/v and transplanted in the tray for 2 weeks and further grew in 8-inch pot contained soil and sprayed with all those chitosan solutions compared to the control group. The results showed the increasing of all studied factors. The concentration of chitosan at 0.05% w/v would obviously cause more effect than chitosan concentration at 0.01 and 0.10% w/v. Especially to the photosynthetic activity of RD_{41}, RD_{49} and RD_{47}, the activity increased up to 41.93, 37.80and 34.05%, respectively compared to the control group. Also increasing of number of leafs, tiller number per plant, leaf area, total dry weight and stem height. Overall, the application of chitosan to the growth of rice was significantly enhanced which affected to the photochemical reaction of rice especially to RD_{41}, RD_{47}, RD_{49} and PL_2.

Chamnanmanoontham N. *et al.* (2015) investigated the growth promoting responses induced by chitosan at the physiological and molecular level in rice (*Oryza sativa* L.) seedlings. The combination of the degree of deacetylation (DD), molecular weight and concentration of chitosan had differing effects on the rice seedling growth. For the best enhancement, oligomeric chitosan with an 80% DD applied at 40 mgL^{-1} significantly enhanced the vegetative growth, in terms of the leaf and root fresh weights and dry weights of rice seedlings compared to the control. At the proteomics level, of the 352 rice leaf proteins that could be resolved using the Multi Experiment Viewer software, 105 showed a significantly different expression level in rice leaves treated with chitosan compared to the control. Co-expression network analysis revealed nine of these proteins had significant co-expression with other genes from the three main biochemical network systems of photosynthesis, carbohydrate metabolism and cell redox homeostasis. More than 90% of the genes positively co-expressed with these nine chitosan-responsive proteins were localized in chloroplasts, suggesting that chitosan enhanced the plant growth of rice seedlings via multiple and complex networks between the nucleus and chloroplast.

Kananont *et al.* (2015) conducted an experiment with Fermented chitin waste (FCW) with three levels of 0.25, 0.50 or 1.00% (w/w) FCW in comparison with chemical fertilizer application. The application of FCW resulted in an increased photosynthetic pigment concentration and enhanced photosynthesis rate, leading to a significantly higher tiller number, shoot biomass and grain yield. At 30 d after transplantation (DAT), the rice plants grown with 0.5% (w/w) FCW-supplemented soil showed the highest level of new leaf photosynthetic pigments and photosynthesis rate, but they were not significantly different from the plants grown in soil supplemented with 1.0% (w/w) FCW. However, at 60 DAT, the plants grown under 1.0% (w/w) FCW had a significantly higher photosynthesis rate than plants grown in 0.5% (w/w) FCW supplemented soil. The addition of 0.5 or 1.0% (w/w) FCW increased the grain yield 2.7-fold and 4.3-fold, respectively, compared to that with chemical fertilizer application. The addition of FCW significantly increased the soil pH and organic matter, nitrogen, phosphorus and potassium contents.

Katiyar D. *et al.* (2015) concluded that chitosan enhanced the efficacy of plants to reduce the deleterious effect of unfavorable conditions as well as on plant growth. Chitosan affects various physiological responses like plant immunity, defense mechanisms involving various enzymes such as, phenylalanine ammonium lyase, polyphenol oxidase, tyrosine ammonia lyase and antioxidant enzymes viz., activities superoxide dismutase, catalase and peroxide against adverse conditions. Recent studies have shown that chitosan induces mechanisms in plants against various biotic (fungi, bacteria, and insects) and abiotic (salinity, drought, heavy metal and cold) stresses and helps in formation of barriers that enhances plant's productivity. This paper takes a closer look at the physiological responses of chitosan molecule.

CHAPTER III

CHAPTER III
MATERIALS AND METHODS

The experiment was conducted at Sher-e-Bangla Agricultural University, Dhaka to investigate the effect of chitosan raw material powder and seedling age on growth, yield contributing characters and yield of Boro rice cv. BRRI dhan88. Materials used and methodologies followed in the present investigation have been described in this chapter.

3.1 Experimental Period

The experiment was conducted during the period from November, 2019 to April, 2020 in Boro season.

3.2 Description of Experimental Site

3.2.1 Geographical location

The experiment was conducted in the Agronomy field of Sher-e-Bangla Agricultural University (SAU). The experimental site is geographically situated at 23°77′ N latitude and 90°33′ E longitude at an altitude of 8.6 meter above sea level (Anon et al., 2004).

3.2.2 Agro-ecological zone

The experimental site belongs to the Agro-ecological zone (AEZ) of “The Modhupur Tract”, AEZ-28 (Anon et al., 1988a). This was a region of complex relief and soils developed over the Modhupur clay, where floodplain sediments buried the dissected edges of the Modhupur Tract leaving small hillocks of red soils as ‘islands’ surrounded by floodplain (Anon et al.,1988b). For better understanding about the experimental site has been shown in the Map of AEZ of Bangladesh in Appendix-I.

3.2.3 Soil

The soil of the experimental field belongs to the General soil type, Shallow Red Brown Terrace Soils under Tejgaon soil series. Soil pH ranges from 5.4–5.8 (Anon et al., 1989). The land was above flood level and sufficient sunshine was available during the experimental period. Soil samples from 0–15 cm depths were collected from the Sher-e-Bangla Agricultural University (SAU) farm field. The soil analyses were done at Soil Resource and Development Institute (SRDI), Dhaka. The morphological and physicochemical properties of the soil are presented in below table:

Table 1: Morphological characteristics of the experimental area

MORPHOLOGICAL FEATURES	CHARACTERISTICS
Location	Sher-e-Bangla Agricultural University soil research field, Dhaka
AEZ	AEZ-28, ModhupurTract
General soil type	Shallow Red Brown Terrace Soil
Land type	Highland
Soil series	Tejgaon
Topography	Fairly leveled

Table 2: The initial physical and chemical characteristics of soil used in this experiment

PHYSICAL CHARACTERISTICS	
Constituents	Percent (%)
Sand	26
Silt	45
Clay	29
Textural class	Silty clay
Soil characteristics	Value
pH	5.8
Organic carbon (%)	0.5
Organic matter (%)	0.87
Total nitrogen (%)	0.04
Available P (ppm)	20.54
Exchangeable K (mg/100 g soil)	0.10

3.2.4 Climate & weather

The climate of the experimental site was subtropical, characterized by the winter season from November to February and the pre-monsoon period or hot season from

March to April and the monsoon period from May to October (Edris et al., 1979). Meteorological data related to the temperature, relative humidity and rainfall during the experiment period was collected from Bangladesh Meteorological Department (Climate division), Sher-e-Bangla Nagar, Dhaka and has been presented in Appendix-II.

3.3 Experimental Materials

BRRI dhan88 and different level of chitosan raw material powder were used as experimental materials for this experiment. The important characteristics of these are mentioned below:

3.3.1 BRRI dhan88

Rice (*Oryza sativa*) variety BRRI dhan88 was used as planting material. BRRI developed this variety and released in 2018. It is the most popular & high yielding Boro variety suitable for planted at 15th Dec-30th December. This variety attains a height of 100 cm. The life cycle of this variety is 140-143 days. Grain yield is around 7 tha^{-1} and 1000 grain weight is 20-22 g. The seeds of this variety were collected from Bangladesh Rice Research Institute (BRRI), Gazipur. Seeds contain 76% carbohydrate, 26.3% amylose and 9.8% protein.

3.3.2 Chitosan Raw Materials

The composition of chitosan raw material powder is given below:

Table 3: Compositions of the chitosan raw material powder which was used in the research work

NAME OF THE NUTRIENTS	NUTRIENT CONTENT
Nitrogen (N)	7.03%
Phosphorus (P)	0.643%
Potassium (K)	0.28%
Sulphur (S)	0.092%
Calcium (Ca)	2.43%
Magnesium (Mg)	0.36%
Zinc (Zn)	92.03 ppm

NAME OF THE NUTRIENTS	NUTRIENT CONTENT
Boron (B)	152 ppm
Organic Carbon (OC)	7.52%
Organic Matter (OM)	12.96%

3.4 Seed Collection & Sprouting

BRRI dhan88 was collected from BRRI (Bangladesh Rice Research Institute), Joydebpur, Gazipur. Healthy and disease-free seeds were selected, following standard technique. Seeds were immersed in water in a bucket for 24 hrs. These were then taken out of water and kept in gunny bags. The seeds started sprouting after 48 hrs which were suitable for sowing in 72 hrs.

3.5 Experimental Treatments

There were two factors in the experiment namely chitosan raw material powder level and different age of seedling as mentioned below:

Factor A: Level of chitosan raw material powder (w/w) (6) viz*;*

C_0= 0%

C_1= 0.1%

C_2= 0.2%

C_3= 0.3%

C_4= 0.4%

C_5= 0.5%

Factor B: Seedling age (3) viz;

S_1= 25 days old seedlings, S_2= 30 days old seedlings and S_3=35 days old seedlings.

3.6 Sowing Time

Generally, farmers prepared the seedbed and sow their seed in the seedbed during the month of October to November and transplant seedling in the main filed at the month of November to December. In this experiment sowing was done at 29th November, 2019. 25, 30 & 35 days seedlings were taken to observe the yield variations between optimum time and the chitosan raw material powder treated seedling.

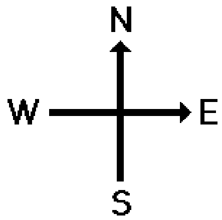
3.7 Seed Pot Preparation and Application of Chitosan Raw Material Powder

2-inch plastic pots were used for raising seedling. Field moist soil was collected from Sher-e-Bangla Agricultural University farm and then mixed with different levels of chitosan raw material powder according to the treatment. Then the pot was filled with 1 kg chitosan raw material treated soil. After that 100 seeds were sown in the pot for raising seedlings.

3.8 Experimental Design

The experiment was laid out in Randomized Complete Block Design (RCBD) with four replications. Total 72 unit pots were made for the experiment with 18 treatment combinations (6 chitosan level × 3 seedling age). Each pot will be of required size. The layout of the experiment is given below:

R_1	R_2	R_3	R_4
C_0S_1	C_5S_3	C_5S_3	C_4S_1
C_0S_2	C_5S_2	C_5S_2	
C_0S_3	C_5S_1	C_5S_1	C_4S_3
C_1S_1	C_4S_3	C_4S_3	C_5S_1
C_1S_2	C_4S_2	C_4S_2	C_5S_2
C_1S_3	C_4S_1	C_4S_1	C_5S_3
C_2S_1	C_3S_3	C_3S_3	C_0S_1
C_2S_2	C_3S_2	C_3S_2	C_0S_2
C_2S_3	C_3S_1	C_3S_1	C_0S_3
C_3S_1	C_0S_1	C_2S_3	C_1S_1
C_3S_2	C_0S_2	C_2S_2	C_1S_2
C_3S_3	C_0S_3	C_2S_1	C_1S_3
C_4S_1	C_1S_1	C_1S_3	C_3S_1
C_4S_2	C_1S_2	C_1S_2	C_3S_2
C_4S_3	C_1S_3	C_1S_1	C_3S_3
C_5S_1	C_2S_1	C_0S_1	C_2S_1
C_5S_2	C_2S_2	C_0S_2	C_2S_2
C_5S_3	C_2S_3	C_0S_3	C_2S_3



LEGEND

Chitosan level viz;

C_0= 0% Chitosan,

C_1= 0.1% Chitosan,

C_2= 0.2% Chitosan,

C_3= 0.3%Chitosan,

C_4= 0.4% Chitosan

And

C_5= 0.5% Chitosan

Different age of seedlings viz;

S_1= 25 days old seedlings,

S_2= 30 days old seedlings

And

S_3= 35 days old seedlings

Figure 1: Layout of the experiment

3.9 Detail of Experimental Preparation

3.9.1 Selection and preparation of the pot

Earthen pots of having 15 cm diameter, 15 cm height with a hole at the center of the bottom were used. Silt soil was used in the experiment. The upper edge diameter of the pots was 30 cm (r= 15 cm). While filling with soil, the upper two cm of the pot was kept vacant so that irrigation can be provided using a hose pipe. As such the diameter of the upper soil surface was 15 cm and the area of the upper soil surface was (πr^2 = 3.14x 0.15x 0.15=0.07 m^2). The preparation of the pot was done in 22^{nd} December, 2019.

3.9.2 Fertilizer management

The following doses of fertilizer were applied for cultivation of T. Boro rice (FRG, 2018).

Table 4: Doses of fertilizers used for the cultivation

Fertilizers	Quantity ($kgha^{-1}$)	Fertilizer given pot^{-1} (g)
Urea	300	2.1
TSP	100	0.7
MoP	120	0.84
Gypsum	60	0.42

Plant Macronutrients (*viz*. nitrogen, phosphorus, potash, sulfur) for rice were given through urea, triple super phosphate, muriate of potash, and gypsum, respectively. All of the fertilizers except urea were applied as basal dose at the time of filling pot with soil. Urea (300 $kgha^{-1}$) was applied in equal three splits. The first dose of urea was applied at 12 days after transplanting (DAT). The second dose of urea was added as top dressing at 27 days after transplanting and third dose was applied at 42 days after transplanting recommended by BRRI.

3.9.3 Seedling transplanting in the pot

The seedling of rice was transplanted to the pot according to the treatment. One seedling was transplanted in each pot.

3.10 Intercultural Operations

3.10.1 Application of irrigation water

Irrigation water was added to each pot according to the critical stage. It was given by using water pipe.

3.10.2 Weeding

The crop was infested with some common weeds, which were controlled by uprooting and removed them three times from the pot during the period of experiment. Weeding was done after 20, 30 and 45 days after transplanting.

3.10.3 Plant protection measures

The crop was attacked by yellow rice stem borer (*Scirpopagain certulas*) at the panicle initiation stage which was successfully controlled with Sumithion @ 1.5 Lha^{-1}. Yet to keep the crop growth in normal, Basudin was applied at tillering stage @17 $kgha^{-1}$ while Diazinon 60 EC @ 850 $mlha^{-1}$ was applied to control rice bug and leaf hopper. Application of insecticide was applied at 16th January, 2020. Crop was protected from birds during the grain filling period by using net and covering the experimental site.

3.11 General Observations of the Experimental Field

Regular observations were made to see the growth and visual different of the crops due to application of different treatment were applied in the experimental pot. In general, the plant looked nice with normal green plants. Incidence of stem borer, green leaf hopper, leaf roller was observed during tillering stage and there were also some rice bugs were present in the experimental pot. But any bacterial and fungal disease was not observed. The flowering was not uniform.

3.12 Crop Sampling and Data Collection

Pot from each replication were randomly selected and marked with sample card. Different data were recorded from selected plants at various growth stages. The rice plant was harvested depending upon the maturity of grains and harvesting was done manually from each plot. Maturity of crop was determined when 80–90% of the grains become golden yellow in color. Harvesting date was 18th April, 2020 and 21th April, 2020. Harvesting was done in the morning to avoid shattering. Prior to harvesting, randomly selected plant from each replication pot were separately harvested for recording yield attributes and other data. The harvested plants were tied into bundles and carried to the threshing floor of the Soil Field Laboratory. Threshing was done by hand. The grains were cleaned and sun dried to moisture content of 12%. Straw was also sun dried properly. Finally grain and straw yields pot^{-1} were recorded.

3.13 Harvesting and Threshing

Harvesting was done when 90% of the crops became brown in color. The matured crop was cut and collected manually. The harvested crops were threshed, cleaned and processed. Grain yield and straw yield was recorded plot wise and moisture of straw was calculated on oven dry basis.

3.14 Field Operation

The different field operations performed during the course of present investigation is given below in chronological order in list form.

Table 5: List of schedules of field operations done during the course of experimentation

Operations	Working Dates
Collection of field moist soil	7th December, 2019
Different level of chitosan raw material powder was mixed with field moist soil	8th December, 2019
Filling the pot with chitosan raw material powder mixed soil	8th December, 2019
Seed sowing	9th December, 2019
Collection and preparation of the main pot	22nd December, 2019
Application of fertilizers (1/3rdUrea, TSP, MoP, Gypsum)	22nd, 27th December, 2019 & 1st January 2020
Intercultural Operations	**Working Dates**
1st Weeding	14th January, 2020
2nd Weeding	24th January, 2020

Intercultural Operations	Working Dates
3rd Weeding	8th February, 2020
1st split application of urea	5th January,2020
2nd split application of urea	20th January,2020
3rd split application of urea	5th February, 2020
Insecticide application	16th January, 2020
Harvesting and threshing	18th & 21st April, 2020

3.15 Data Collection

The data were recorded on the following parameters

I. Average seedling height (cm)
II. Fresh weight per 100 seedlings (g)
III. Oven dry weight per 100 seedlings (g)
IV. Seedling strength ($mgcm^{-1}$)
V. Number of tillers pot^{-1} at different DAT
VI. Number of effective tillers pot^{-1}
VII. Days to first flowering
VIII. Days to 50% flowering
IX. Days to 100% flowering
X. Unfilled grain weight (g)
XI. Grain yield pot^{-1} (g)
XII. Straw yield pot^{-1} (g)
XIII. Biological yield pot^{-1} (g)
XIV. Harvest index (%)

3.16 Procedure of Data Collection

I. Average seedling height (cm)

The heights of 30 seedlings during transplanting were measured with a meter scale from the ground level to tip of seedlings and the mean heights were expressed in cm.

II. Fresh weight per 100 seedlings (g)

Fresh weight of 100 seedlings were collected during transplanting time from each treatment and then weighed by using a digital electric balance and the mean weight were expressed in gram.

III. Oven dry weight per 100 seedlings (g)

Different treated 100 seedlings were collected from seedbed and then sun dried. The sun-dried seedling again dried in oven and weighted by using a digital electric balance & their mean was expressed in gram.

IV. Seedling strength (mgcm^{-1})

Seedling strength was measured by using the following formula:

$$\text{Seedling strength} = \frac{\text{Oven dry weight per seedling}}{\text{Average seedling height}}\ \text{mgcm}^{-1}$$

V. Number of tillers pot^{-1}

Number of tillers pot^{-1} were counted at 10 days' interval up to 60 DAT from pre-selected hills and finally averaged as their number pot^{-1}. Only those tillers having three or more leaves were considered for counting.

VI. Number of effective tillers pot^{-1}

The total number of effective tillers pot^{-1} was counted as the number of panicles bearing tillers per hill. Data on effective tiller per pot were recorded at harvesting time and average value was recorded.

VII. Days to first flowering

The date of flower blooming was recorded from the number of days of 1st the date of flower blooming after transplanting.

VIII. Days to 50% flowering

After completion of 50% flowering, data was recorded from the number of days of 50% flowering after transplanting.

IX. Days to 100% flowering

After completion of 100% flowering, data was recorded from the number of days of 100% flowering after transplanting.

X. Unfilled grains yield pot^{-1} (g)

Unfilled grain weight was measuring by using a digital electric balance and the mean weight were expressed in gram.

XI. Grain yield pot^{-1} (g)

Grain yield from each pot were taken expressed as g on about 12% moisture basis. Grain moisture content was measured by using a digital moisture tester.

XII. Straw yield pot^{-1} (g)

Straw obtained from each pot were sun dried and weighed carefully and finally converted to g.

XIII. Biological yield pot^{-1} (g)

The summation of grain yield and above ground straw yield was the biological yield. Biological yield pot^{-1} (g) = (Grain yield pot^{-1}+ straw yield pot^{-1}) g.

XIV. Harvest index (%)

Harvest index was calculated on dry weight basis with the help of following formula:

$$\text{Harvest index (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

Here, Biological yield= Grain yield+ straw yield

3.17 Chemical Analysis of Seed Pot Soil after Seedling Transplantation

Soil pH

Soil pH was measured with the help of a Glass electrode pH meter using soil and water at the ratio of 1:2.5 as described by Jackson (1962).

Organic C

Organic carbon in soil was determined by Walkley and Black (1934) Wet Oxidation Method. The underlying principle is to oxidize the organic carbon with an excess of 1N $K_2Cr_2O_7$ in presence of conc. H_2SO_4 and to titrate the residual $K_2Cr_2O_7$ solution with 1N $FeSO_4$ solution. To obtain the organic matter content, the amount of organic carbon was multiplied by the Van Bemmelen factor, 1.73. The result was expressed as percentage.

Total nitrogen

Total N content of soil were determined followed by the Micro Kjeldahl method. One gram of oven dry ground soil sample was taken into micro jeldahl flask to which 1.1 gm catalyst mixture (K_2SO_4:$CuSO_4$:$5H_2O$ Se in the ratio of 100:10:1), and 6 ml H_2SO_4 were added. The flasks were swirled and heated 200 ^{0}C and added 3 ml H_2O_2 and then heating at 360 ^{0}C was continued until the digest was clear and colorless. After cooling, the content was taken into 100 ml volumetric flask and the volume was made up to the mark with distilled water. A reagent blank was prepared in a similar manner. These digests were used for nitrogen determination. Then 20 ml digest solution was transferred into the distillation flask, then 10 ml of H_3BO_3 indicator solution was taken into a 250 ml conical flask which is marked to indicate a volume of 50 ml and placed the flask under the condenser outlet of the distillation apparatus so that the delivery end dipped in the acid. Add sufficient amount of 10 N-NaOH solutions in the container connecting with distillation apparatus. Water runs through the condenser of distillation apparatus was checked. Operating switch of the distillation apparatus collected the distillate. The conical flask was removed by washing the delivery outlet of the distillation apparatus with distilled water. Finally, the distillates were titrated with standard 0.01 N H_2SO_4 until the color changes from green to pink. The amount of N was calculated using the following formula: % N = (T-B) × N × 0.014 × 100/S. Where, T = Sample titration (ml) value of standard H_2SO_4, B = Blank titration (ml) value of standard H_2SO_4, N = Strength of H_2SO_4 and S = Sample weight in gram.

Organic Matter

Total organic matter content of soil were determined followed by the Walkley-Black wet oxidation method. The method measures easily oxidizable carbon using sulfuric acid and potassium dichromate. The Walkley-Black method works very well on soils with low organic matter (<2.0%); however, the method is less suitable on soils with very high organic matter (>8.0%) as the dichromate reagent is consumed and may not oxidize all organic carbon. Soils with high chloride may interfere with the Walkley-Black method.

3.18 Data Analysis Technique

The collected data were compiled and analyzed statistically using the analysis of variance (ANOVA) technique with the help of a computer package program name STATISTIX 10. The significance of the difference among the treatment means was estimated by the Duncan's Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez, 1984).

CHAPTER IV

CHAPTER IV

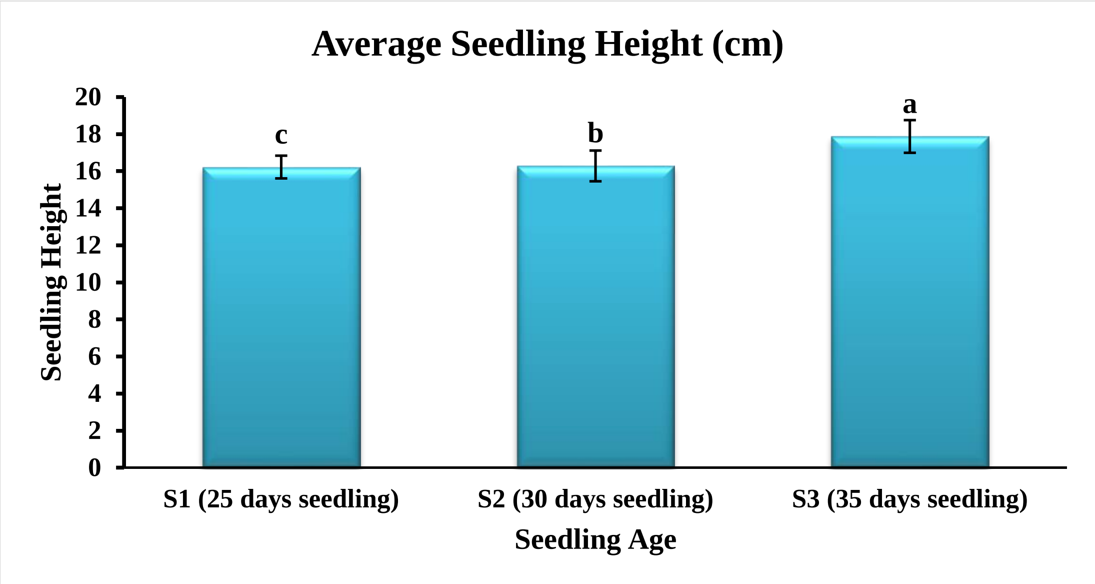
RESULTS AND DISCUSSION

This chapter comprises of the presentation and discussion of the results obtained due to application of chitosan raw material powder in seedbed soil of boro rice (cv. BRRI dhan88). The results of the present investigation have been presented, discussed and compared as far as possible with the results of the researchers.

4.1 Average seedling height (cm)

4.1.1 Effect of chitosan raw material powder level on average seedling height of BRRI dhan88

Seedling height was significantly influenced by different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%**)** (Figure 2). Highest seedling height was found from C_3 (18.33 cm) and lowest seedling height was found from C_0 (14.42 cm). Seedling height at transplanting, the treatments may be ranked in the order of C_3> C_2> C_4> C_1> C_5> C_0. Katiyar D. *et al.* (2015) concluded that chitosan enhanced the efficacy of plants to reduce the deleterious effect of unfavorable conditions as well as on plant growth. The increased seedling height through the application of chitosan was also reported by many other scientists (Issak, M. and Sultana, A. 2017).

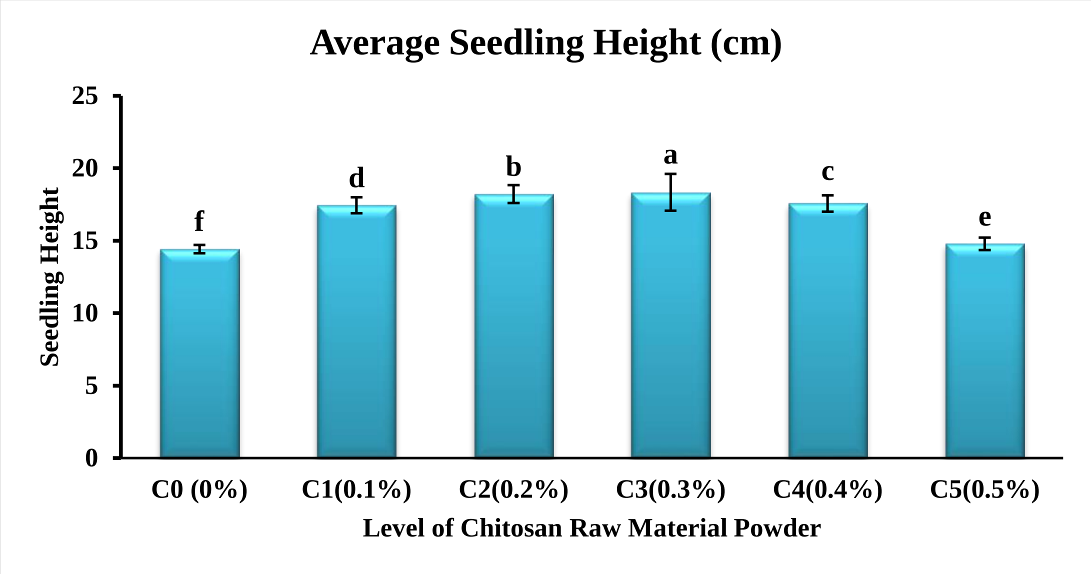


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 2: Effect of chitosan raw material powder level on average seedling height of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.1.2 Effect of seedling age on average seedling height of BRRI dhan88

Seedling height was significantly influenced by different seedling age S_1 (25 days old seedling), S_2 (30 days old seedling) and S_3 (35 days old seedling) (Figure 3). Highest seedling height was found from S_3 (17.87 cm) and lowest seedling height was found from S_1 (16.22 cm). Seedling height at transplanting, seedling age may be ranked in the order of $S_3 > S_2 > S_1$.

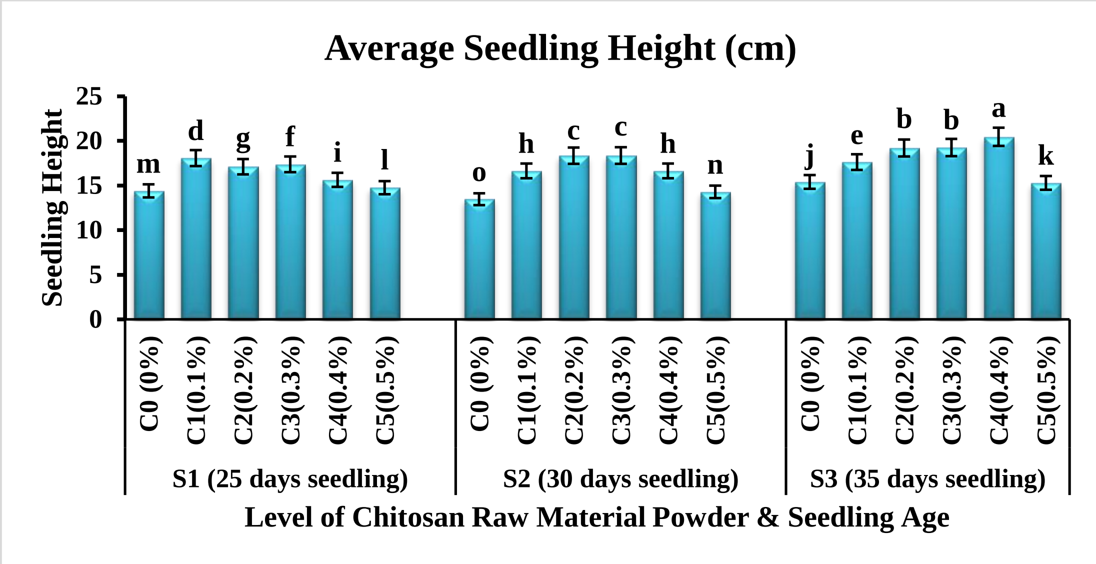


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 3: Effect of seedling age on average seedling height of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.1.3 Combined effect of chitosan raw material powder level & seedling age on average seedling height of BRRI dhan88

Seedling treated with different levels of chitosan raw material powder along with different ages of seedling significantly effect on average seedling height of BRRI dhan88 (Figure 4). Experimental result showed that, the maximum average seedling height (20.45 cm) was obtained in S_3C_4 treatment combination whereas the minimum seedling height (13.47 cm) was obtained in S_2C_0 treatment combination.



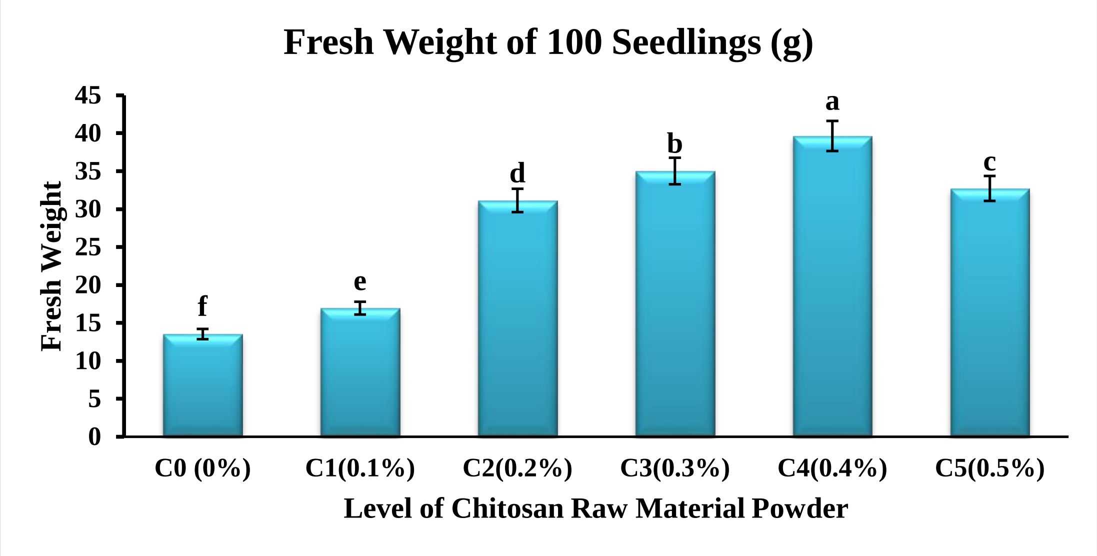
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 4: Combined effect of chitosan raw material powder level & seedling age on average seedling height of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.2 Fresh weight of 100 seedlings (g)

4.2.1 Effect of chitosan raw material powder level on fresh weight of 100 seedlings of BRRI dhan88

Application of different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%**)** had significantly effect on fresh weight seedling^{-1} (Figure 5). Highest fresh weight was found from C_4 (39.64 g) and lowest fresh weight was found from C_0 (13.54 g). In fresh weight of seedlings, the treatments may be ranked in the order of C_4> C_3> C_5> C_2> C_1> C_0. Issak, M. and Sultana, A. (2017) carried out an experiment to observed the role of chitosan powder on the production of quality rice seedlings of BRRI dhan29. The maximum fresh weight was observed in the treatment T_4 (400 g CHT powder/m^2) and the minimum level in the treatment T_6 (0 g CHT powder/m^2). Divya K. *et al.* (2022); Ahmed *et al*. (2020); Malerba M. *et al.* (2018) found the similar result that fresh weight of chitosan treated seedlings were significantly higher than control.

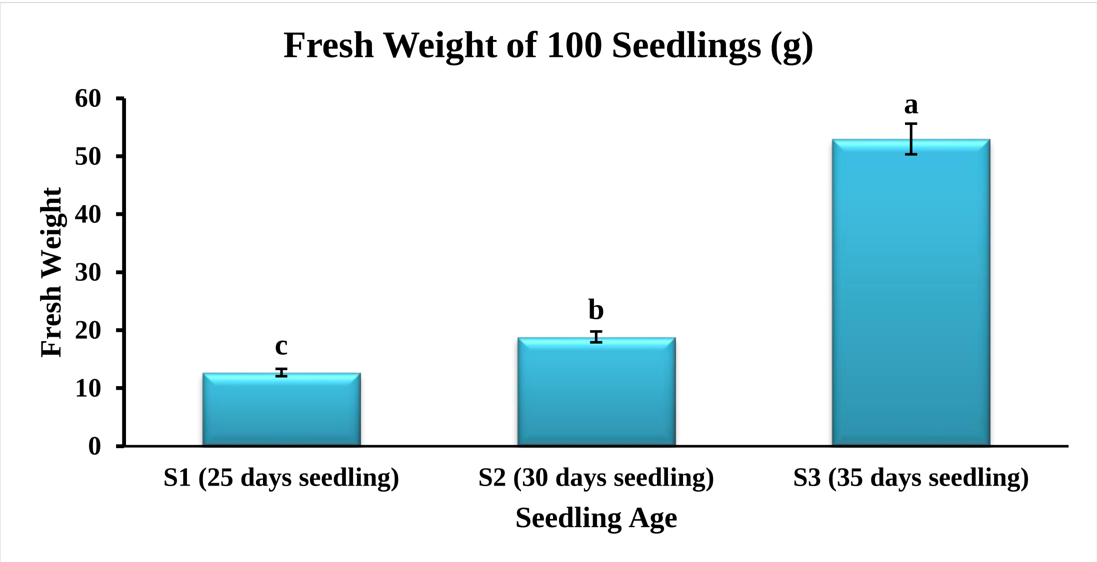


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 5: Effect of chitosan raw material powder level on fresh weight of 100 seedlings of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.2.2 Effect of seedling age on fresh weight of 100 seedlings of BRRI dhan88

Seedling age (S_1= 25 days old seedling, S_2= 30 days old seedling and S_3= 35 days old seedling) had significantly effect on fresh weight seedling^{-1} (Figure 6). Highest fresh weight was found from S_3 (52.96 g) and lowest fresh weight was found from S_1 (12.71 g). In fresh weight of seedlings, seedling age may be ranked in the order of S_3> S_2> S_1.

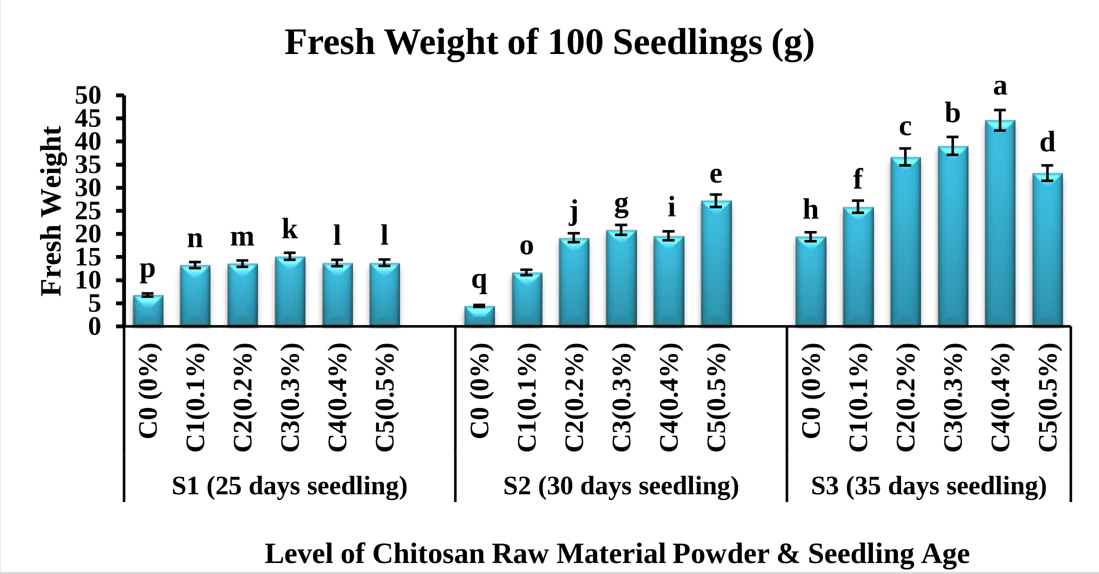


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 6: Effect of seedling age on fresh weight of 100 seedlings of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.2.3 Combined effect of chitosan raw material powder level & seedling age on fresh weight of 100 seedlings of BRRI dhan88

Seedling treated with different levels of chitosan raw material powder along with different ages of seedling significantly effect on fresh weight seedling^{-1} of BRRI dhan88 (Figure 7). Experimental result showed that, the maximum fresh weight seedling^{-1} (85.6 g) was obtained in S_3C_4 treatment combination whereas the minimum fresh weight seedling^{-1} (4.43 g) was obtained in S_2C_0 treatment combination.



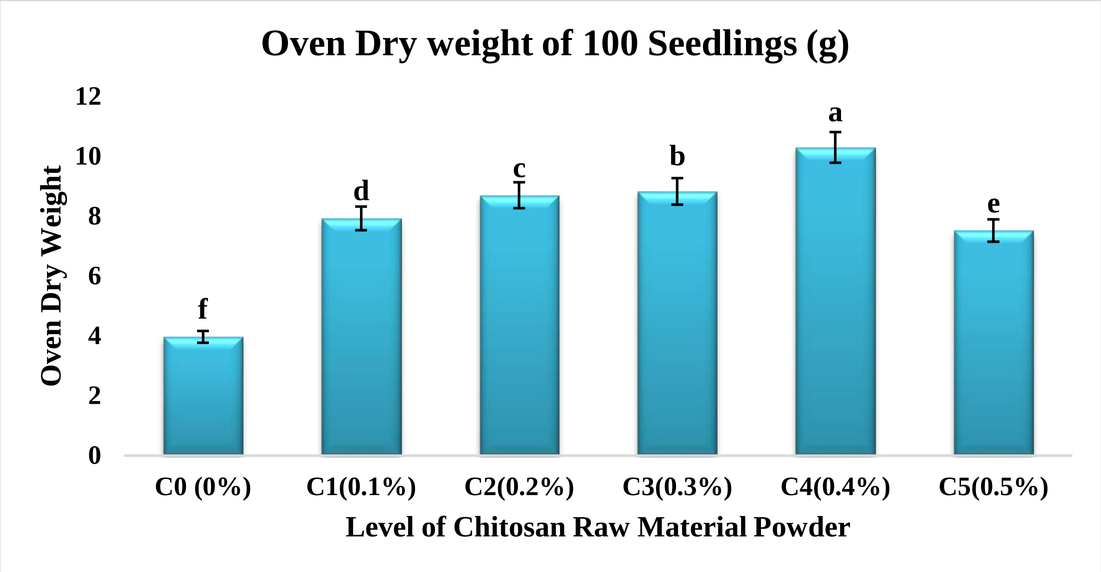
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 7: Combined effect of chitosan raw material powder level & seedling age on fresh weight of 100 seedlings of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.3 Oven dry weight per 100 seedlings (g)

4.3.1 Effect of chitosan raw material powder level on oven dry weight of 100 seedlings of BRRI dhan88

Oven dry weight was significantly influenced by different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%) (Figure 8). Highest oven dry weight was found from C_4 (10.3 g) and lowest oven dry weight was found from C_0 (3.97 g). In oven dry weight of seedlings, the treatments may be ranked in the order of C_4> C_3> C_2> C_1> C_5> C_0. Issak, M. and Sultana, A. (2017) showed that the maximum oven dry weight was obtained in the treatment T_4 (400 g CHT powder/m^2) and the minimum level in the treatment T_6 (0 g CHT powder/m^2). The increased oven dry weight of seedlings through the application of chitosan was also observed by many other scientists (Divya K. *et al.* 2022; Ahmed *et al*. 2020; Malerba M. *et al.* 2018).

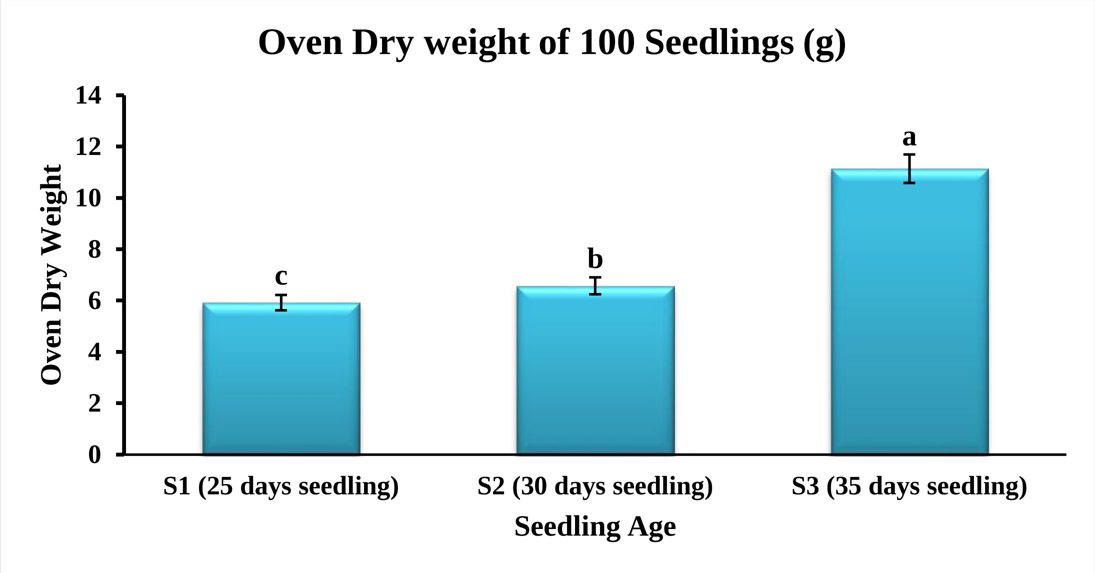


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 8: Effect of chitosan raw material powder level on oven dry weight of 100 seedlings of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.3.2 Effect of seedling age on oven dry weight of 100 seedlings of BRRI dhan88

oven dry weight was significantly influenced by different seedling age S_1 (25 days old seedling), S_2 (30 days old seedling) and S_3 (35 days old seedling) (Figure 9). Highest oven dry weight was found from S_3 (11.14 gm) and lowest oven dry weight was found from S_1 (5.92 gm). oven dry weight at transplanting, seedling age may be ranked in the order of $S_3 > S_2 > S_1$.

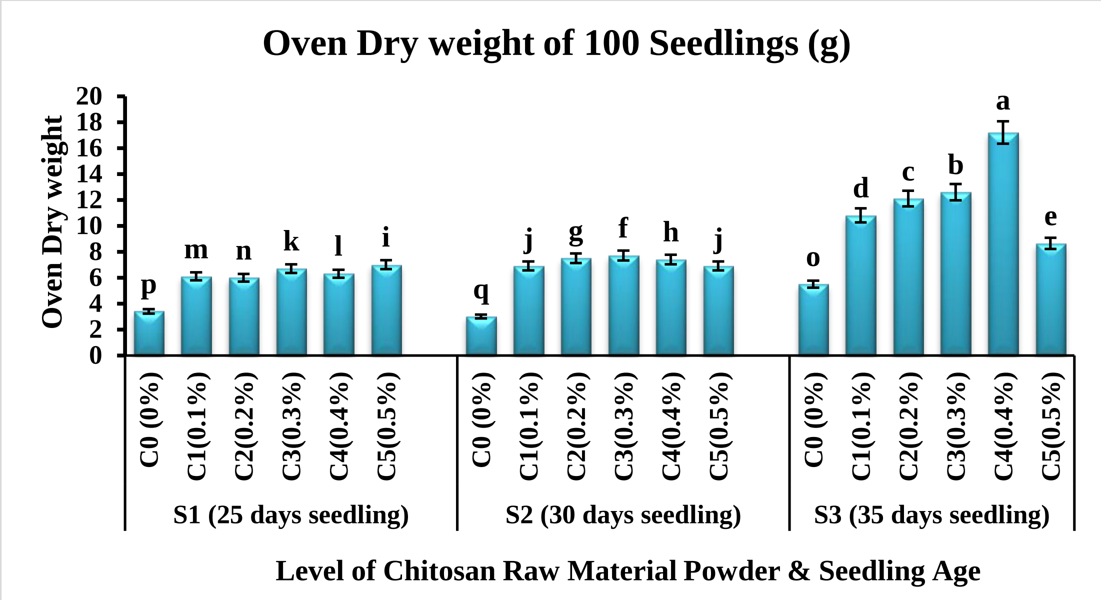


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 9: Effect of seedling age on oven dry weight of 100 seedlings of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.3.3 Combined effect of chitosan raw material powder level & seedling age on oven dry weight of 100 seedlings of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on oven dry weight seedling^{-1} of BRRI dhan88 (Figure 10). Experimental result showed that, the maximum oven dry weight seedling^{-1} (17.2 g) was obtained in S_3C_4 treatment combination whereas the minimum oven dry weight seedling^{-1} (3 g) was obtained in S_2C_0 treatment combination.



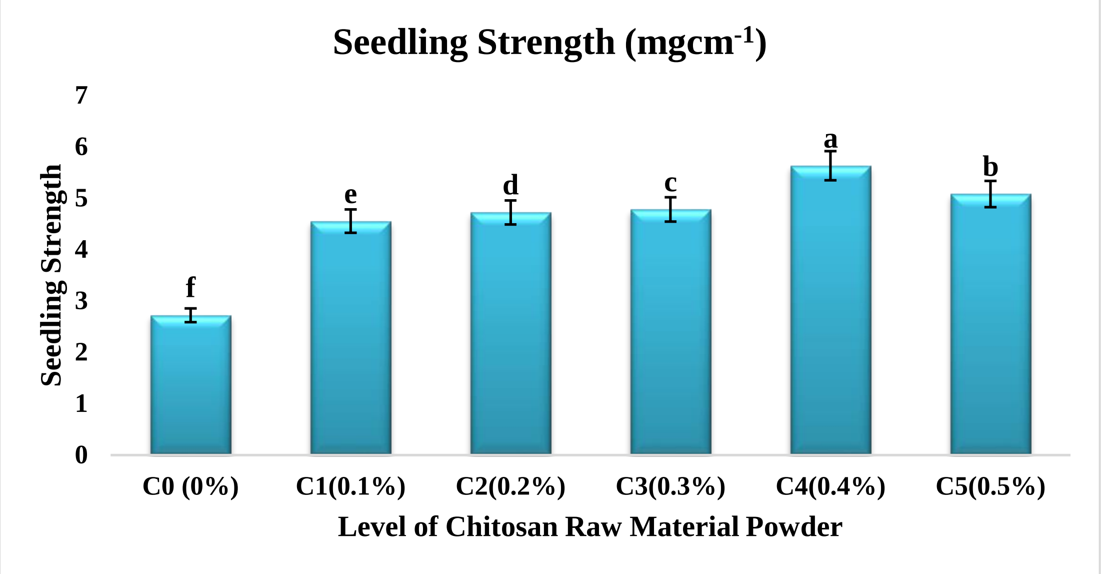
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 10: Combined effect of chitosan raw material powder level & seedling age on oven dry weight of 100 seedlings of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.4 Seedling strength ($mgcm^{-1}$)

4.4.1 Effect of chitosan raw material powder level on seedling strength of BRRI dhan88

Application of different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%**)** had significantly effect on seedling strength seedling^{-1} (Figure 11). Highest seedling strength was found from C_4 (5.63 $mgcm^{-1}$) and lowest seedling strength was found from C_0 (2.72 $mgcm^{-1}$). For seedling strength, the treatments may be ranked in the order of C_4> C_5> C_3> C_2> C_1> C_0. Malerba M. *et al.* (2018) revealed that chitosan has an excellent role in improving seedling strength. Divya K. *et al.* (2022); Ahmed *et al*. (2020) found the similar result that seedling strength of chitosan treated seedlings were significantly higher than control.

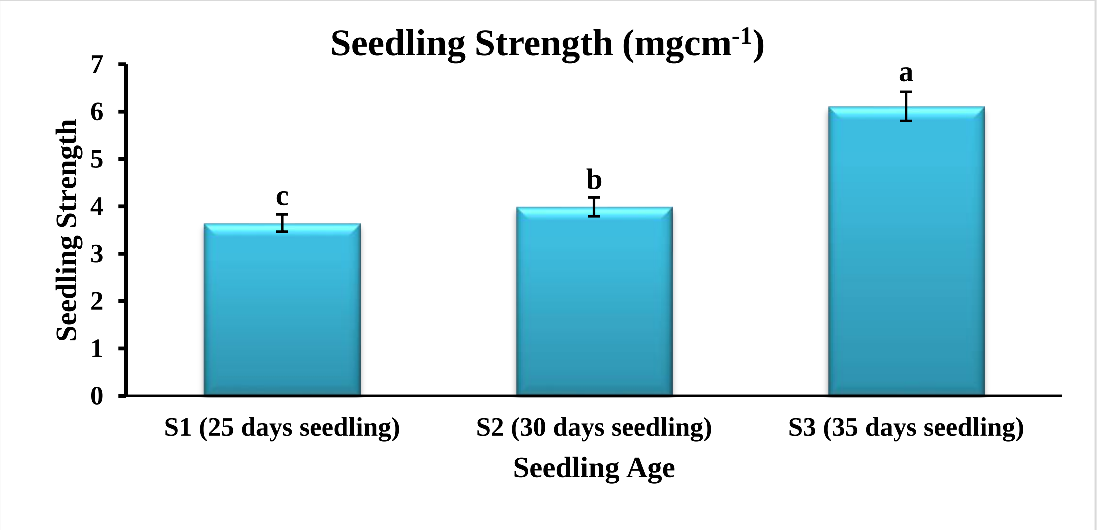


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 11: Effect of chitosan raw material powder level on seedling strength of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.4.2 Effect of seedling age on seedling strength of BRRI dhan88

Seedling age (S_1= 25 days old seedling, S_2= 30 days old seedling and S_3= 35 days old seedling) had significantly effect on seedling strength seedling^{-1} (Figure 12). Highest seedling strength was found from S_3 (6.11 mgcm^{-1}) and lowest seedling strength was found from S_1 (3.65 mgcm^{-1}). For seedling strength, seedling age may be ranked in the order of $S_3 > S_2 > S_1$.

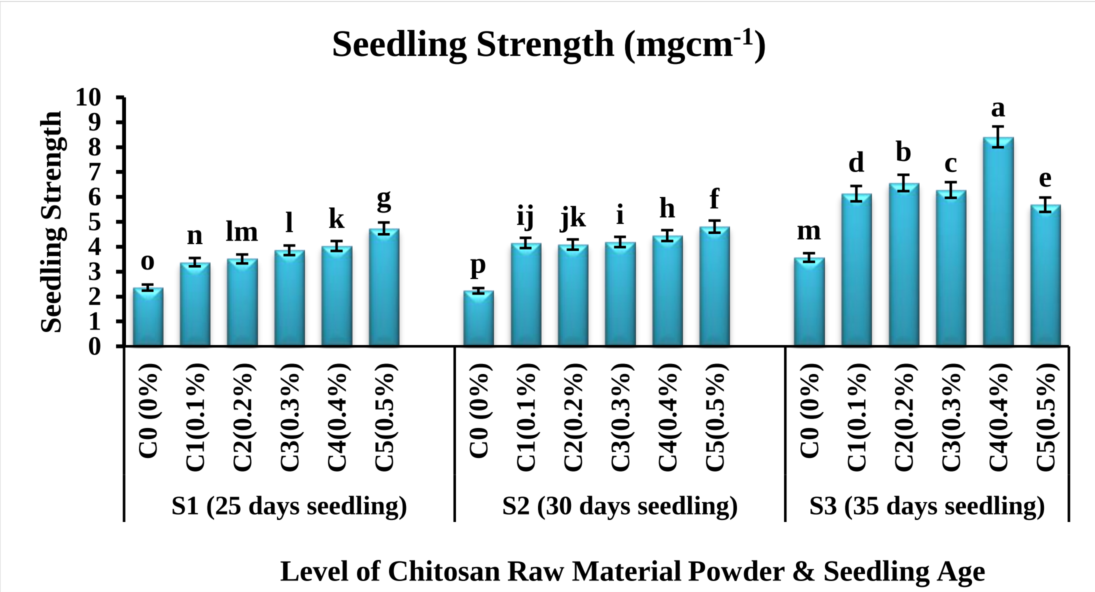


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 12: Effect of seedling age on seedling strength of BRRI dhan88. Bars with different letters are significantly different at p ≤ 0.05 applying DMRT

4.4.3 Combined effect of chitosan raw material powder level & seedling age on seedling strength of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on seedling strength of BRRI dhan88 (Figure 13). Experimental result showed that, the maximum seedling strength (8.41 $mgcm^{-1}$) was obtained in S_3C_4 treatment combination whereas the minimum seedling strength (2.23 $mgcm^{-1}$) was obtained in S_2C_0 treatment combination.



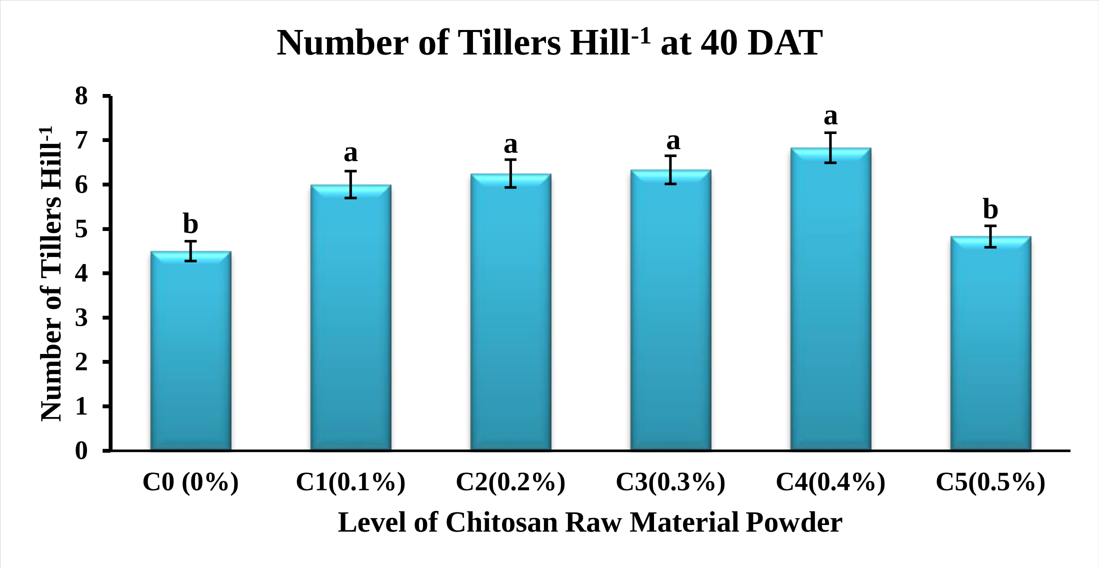
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 13: Combined effect of chitosan raw material powder level & seedling age on seedling strength of BRRI dhan88. Bars with different letters are significantly different at p ≤ 0.05 applying DMRT

4.5 Number of tillers $hill^{-1}$ at 40 DAT

4.5.1 Effect of chitosan raw material powder level on number of tillers $hill^{-1}$ at 40 DAT of BRRI dhan88

Tiller number was significantly influenced by different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%**)** (Figure 14). Highest tiller number was found from C_4 (6.83) which was statistically identical with C_3 (6.33), C_2 (6.25) and C_1 (6). On the other hand, lowest tiller number was found from C_0 (4.5) which was identical with C_5 (4.83). Tiller number at 40 DAT, the treatments may be ranked in the order of $C_4 > C_3 > C_2 > C_1 > C_5 > C_0$. Faqir Y. *et al.* (2021) revealed that chitosan has an excellent role in improving tiller number of plants. The increased tiller number through the application of chitosan was also reported by many other scientists (Divya K. *et al.* 2022; Ahmed *et al*. 2020; Issak, M. and Sultana, A. 2017).

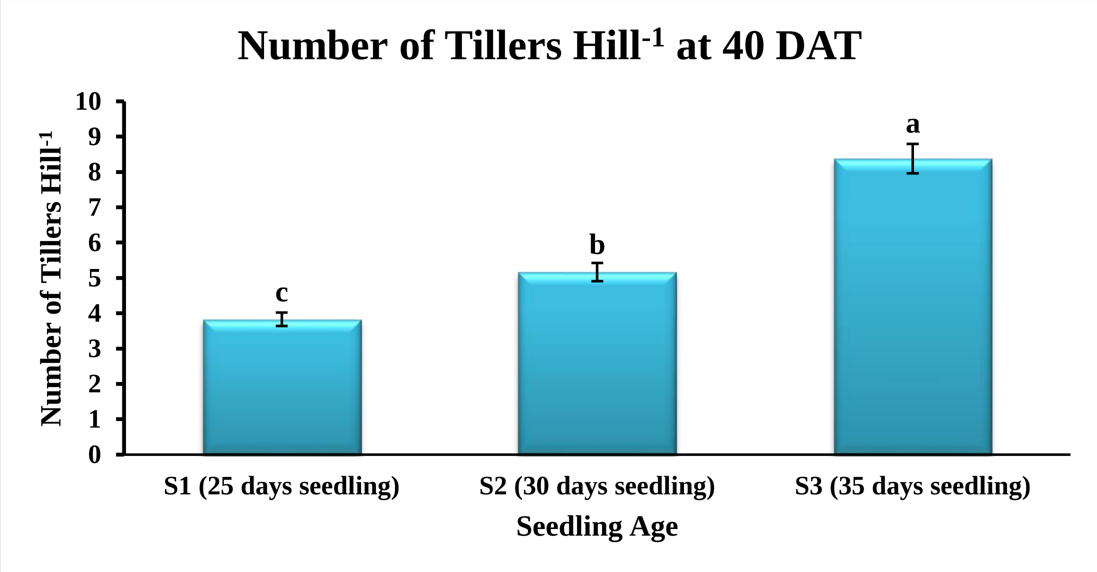


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 14: Effect of chitosan raw material powder level on number of tillers $hill^{-1}$ at 40 DAT of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.5.2 Effect of seedling age on number of tillers $hill^{-1}$ at 40 DAT of BRRI dhan88

Tiller number was significantly influenced by different seedling age S_1 (25 days old seedling), S_2 (30 days old seedling) and S_3 (35 days old seedling) (Figure 15). Highest tiller number was found from S_3 (8.38). On the other hand, lowest tiller number was found from S_1 (3.83). Tiller number at 40 DAT, seedling age may be ranked in the order of $S_3 > S_2 > S_1$.

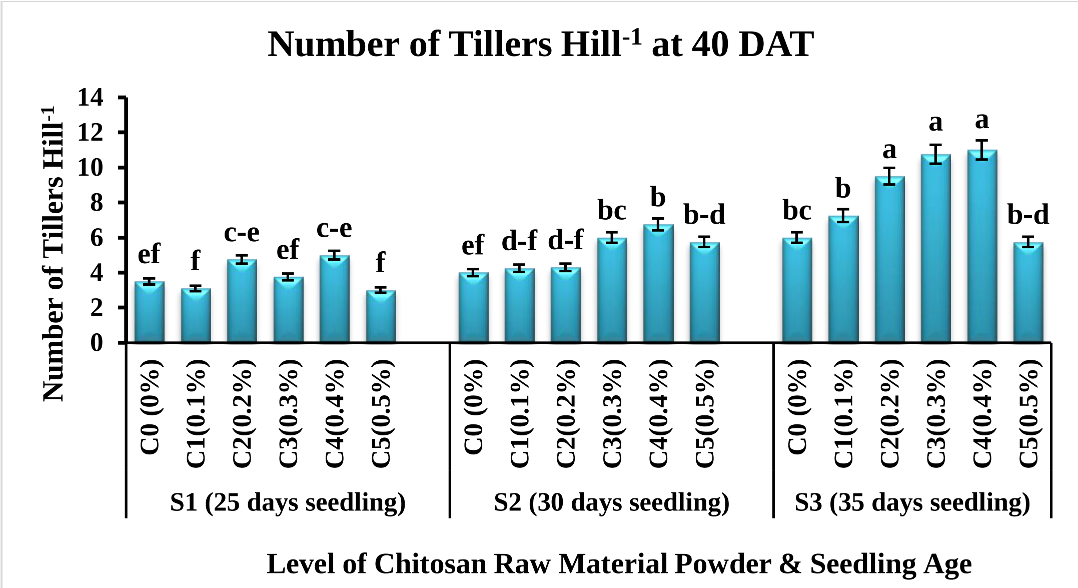


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 15: Effect of seedling age on number of tillers hill^{-1} at 40 DAT of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.5.3 Combined effect of chitosan raw material powder level & seedling age on number of tillers hill^{-1} at 40 DAT of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on number of tillers hill^{-1} at 40 DAT of BRRI dhan88 (Figure 16). Experiment result revealed that, the maximum tiller number hill^{-1} (11) was obtained in S_3C_4 treatment combination which was statistically identical with S_3C_3 (10.75) and S_3C_2 (9.5). Whereas the minimum tiller number hill^{-1} (3) was obtained in S_1C_5 treatment combination which was statistically identical with S_1C_1 (3.1), S_1C_0 (3.5), S_1C_3 (3.75), S_2C_0 (4), S_2C_1 (4.25) and S_2C_2 (4.3).



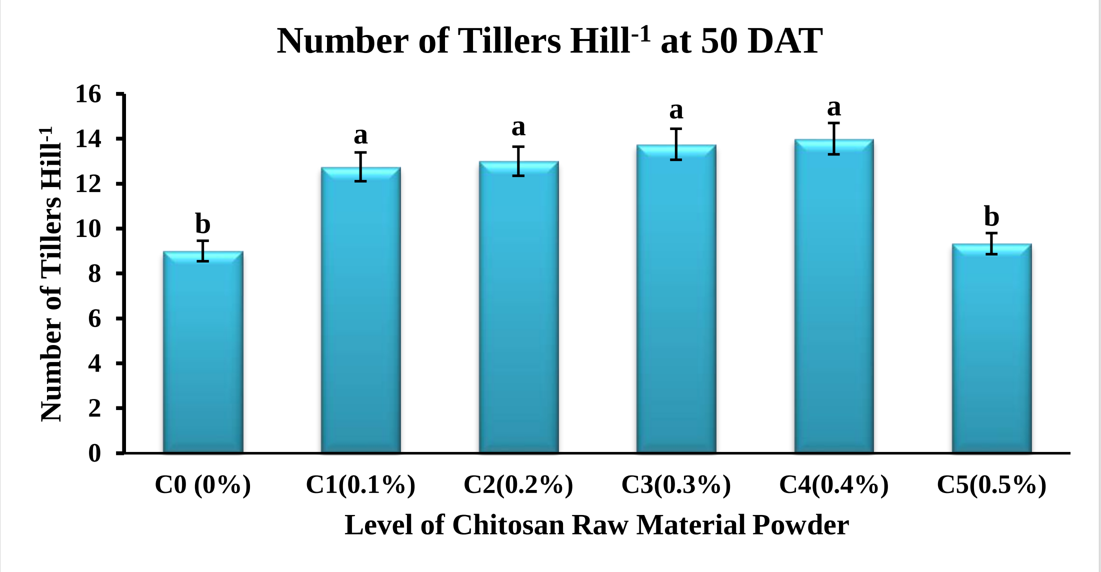
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 16: Combined effect of chitosan raw material powder level & seedling age on number of tillers hill^{-1} at 40 DAT of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.6 Number of tillers hill^{-1} at 50 DAT

4.6.1 Effect of chitosan raw material powder level on number of tillers hill^{-1} at 50 DAT of BRRI dhan88

Application of different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%**)** had significantly effect on tiller number (Figure 17). Highest tiller number was found from C_4 (14) which was statistically identical with C_3 (13.75), C_2 (13) and C_1 (12.75). On the other hand, lowest tiller number was found from C_0 (9) which was statistically identical with C_5 (9.33). In tiller number at 50 DAT, the treatments may be ranked in the order of C_4> C_3> C_2> C_1> C_5> C_0. Issak, M. and Sultana, A. (2017) carried out an experiment to observed the role of chitosan powder on the production of quality rice seedlings of BRRI dhan29. The maximum tiller number was observed in the treatment T_4 (400 g CHT powder/m^2) and the minimum level in the treatment T_6 (0 g CHT powder/m^2). Divya K. *et al.* (2022); Ahmed *et al*. (2020); Malerba M. *et al.* (2018) found the similar result that tiller number of chitosan treated plants were significantly higher than control.

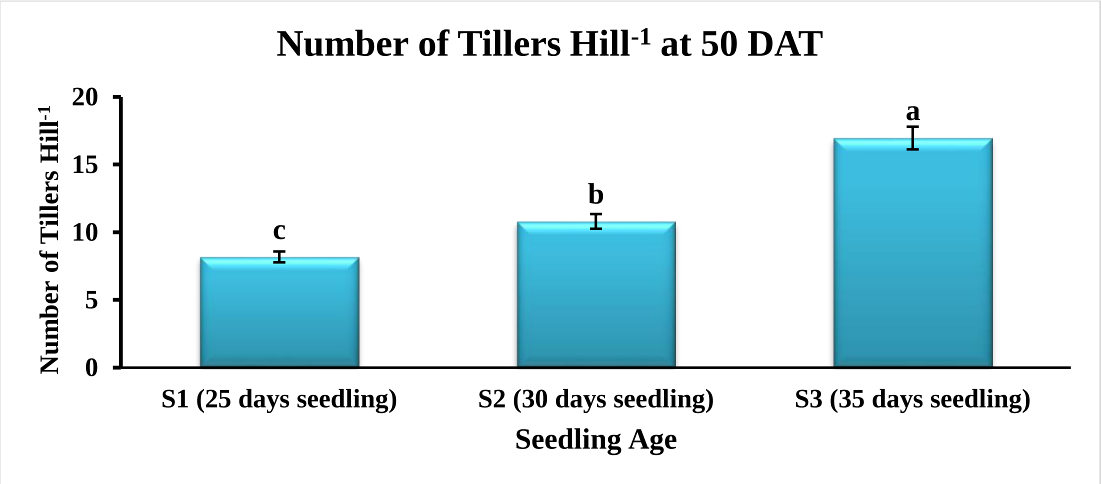


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 17: Effect of chitosan raw material powder level on number of tillers hill^{-1} at 50 DAT of BRRI dhan88. Bars with different letters are significantly different at p ≤ 0.05 applying DMRT

4.6.2 Effect of seedling age on number of tillers hill^{-1} at 50 DAT of BRRI dhan88

Seedling age (S_1= 25 days old seedling, S_2= 30 days old seedling and S_3= 35 days old seedling) had significantly effect on tiller number (Figure 18). Highest tiller number was found from S_3 (16.96). On the other hand, lowest tiller number was found from S_1 (8.17). In tiller number at 50 DAT, seedling age may be ranked in the order of S_3> S_2> S_1.

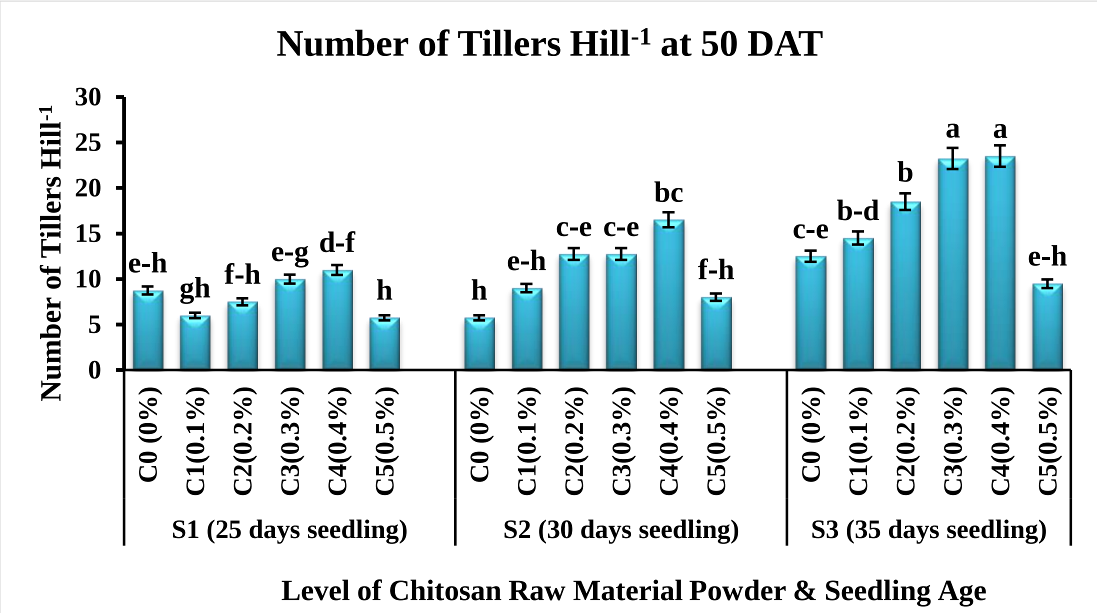


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 18: Effect of seedling age on number of tillers hill^{-1} at 50 DAT of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.6.3 Combined effect of chitosan raw material powder level & seedling age on number of tillers hill^{-1} at 50 DAT of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on number of tillers hill^{-1} at 50 DAT of BRRI dhan88 (Figure 19). Experiment result revealed that, the maximum tiller number hill^{-1} (23.5) was obtained in S_3C_4 treatment combination which was statistically identical with S_3C_3 (23.25). Whereas the minimum tiller number hill^{-1} (5.75) was obtained in S_1C_5 treatment combination which was statistically identical with S_2C_0 (5.75), S_1C_1 (6), S_1C_2 (7.5), S_2C_5 (8), S_1C_0 (8.75), S_2C_1 (9) and S_3C_5 (9.5).



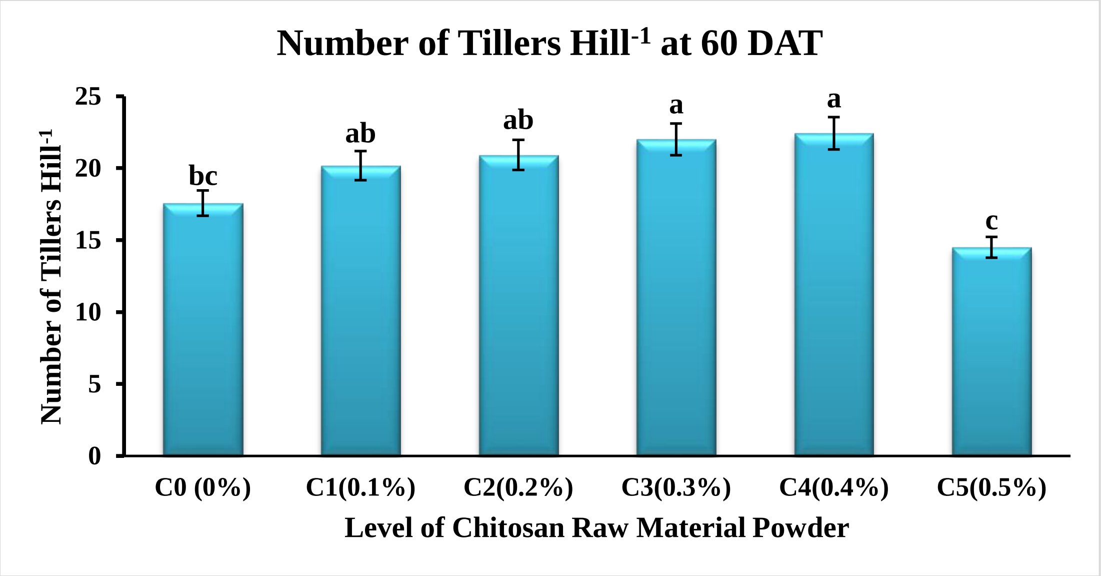
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 19: Combined effect of chitosan raw material powder level & seedling age on number of tillers hill^{-1} at 50 DAT of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.7 Number of tillers hill^{-1} at 60 DAT

4.7.1 Effect of chitosan raw material powder level on number of tillers hill^{-1} at 60 DAT of BRRI dhan88

Tiller number was significantly influenced by different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%) (Figure 20). Highest tiller number was found from C_4 (22.42) was statistically identical with C_3 (22), C_2 (20.92) and C_1 (20.17). On the other hand, lowest tiller number was found from C_5 (14.5) was statistically identical with C_0 (17.58). In tiller number at 60 DAT, the treatments may be ranked in the order of C_4> C_3> C_2> C_1> C_0> C_5. Issak, M. and Sultana, A. (2017) showed that the maximum tiller number was obtained in the treatment T_4 (400 g CHT powder/m^2) and the minimum level in the treatment T_6 (0 g CHT powder/m^2). The increased tiller number of plants through the application of Chitosan was also observed by many other scientists (Divya K. *et al.* 2022; Ahmed *et al*. 2020; Malerba M. *et al.* 2018).

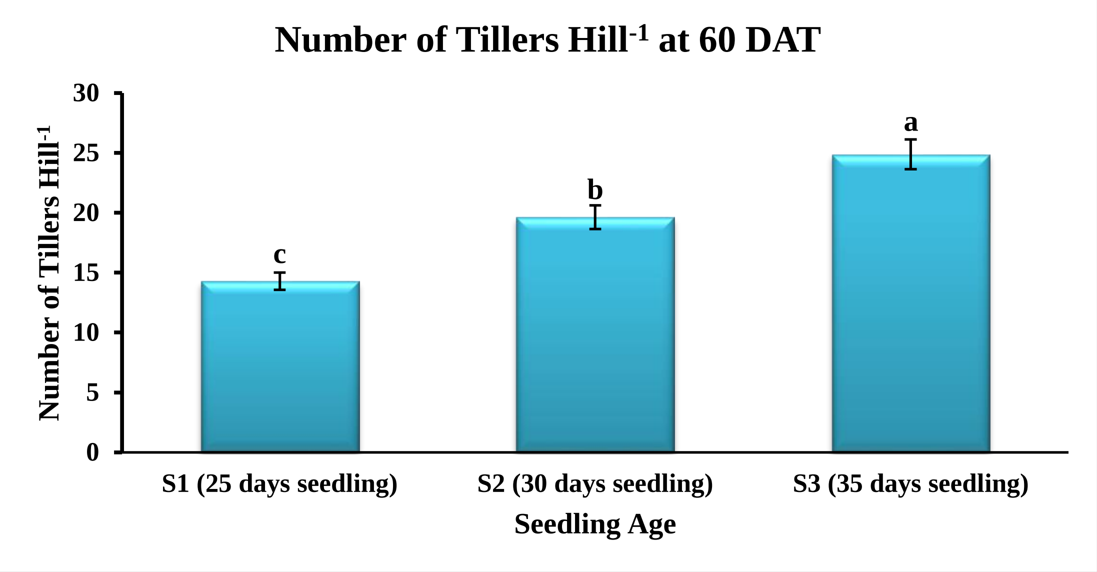


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 20: Effect of chitosan raw material powder level on number of tillers hill^{-1} at 60 DAT of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.7.2 Effect of seedling age on number of tillers hill^{-1} at 60 DAT of BRRI dhan88

Tiller number was significantly influenced by different seedling age S_1 (25 days old seedling), S_2 (30 days old seedling) and S_3 (35 days old seedling) (Figure 21). Highest tiller number was found from S_3 (24.88). On the other hand, lowest tiller number was found from S_1 (14.29). In tiller number at 60 DAT, seedling age may be ranked in the order of $S_3 > S_2 > S_1$.

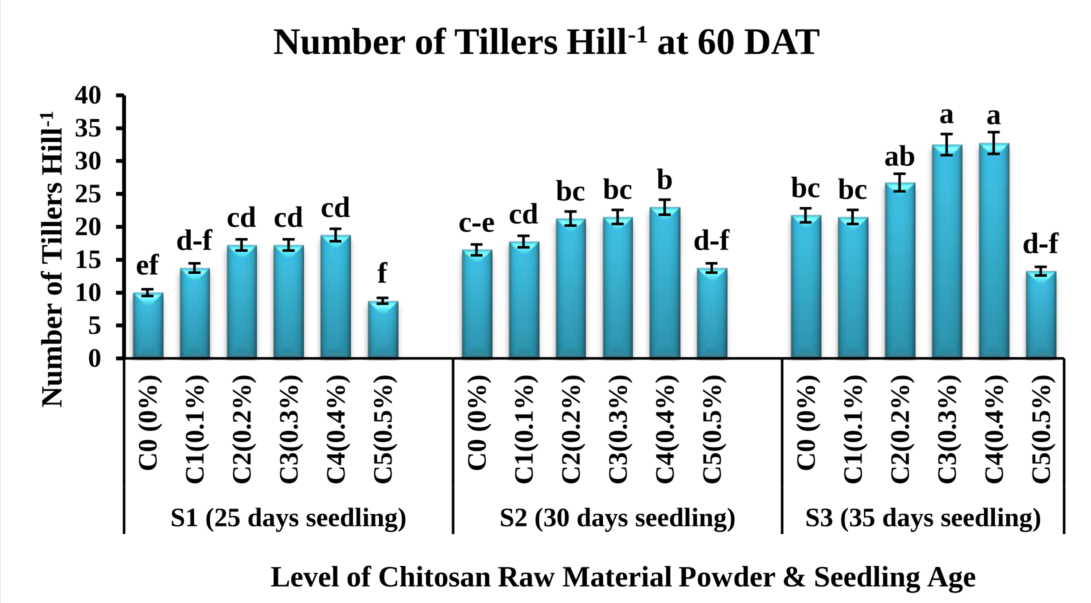


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 21: Effect of seedling age on number of tillers hill^{-1} at 60 DAT of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.7.3 Combined effect of chitosan raw material powder level & seedling age on number of tillers hill^{-1} at 60 DAT of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on number of tillers hill^{-1} at 60 DAT of BRRI dhan88 (Figure 22). Experiment result revealed that, the maximum tiller number hill^{-1} (32.75) was obtained in S_3C_4 treatment combination which was statistically identical with S_3C_3 (32.5) and S_3C_2 (26.75). Whereas the minimum tiller number hill^{-1} (8.75) was obtained in S_1C_5 treatment combination which was statistically identical with S_1C_0 (10), S_3C_5 (13.25), S_2C_5 (13.75) and S_1C_1 (13.75).



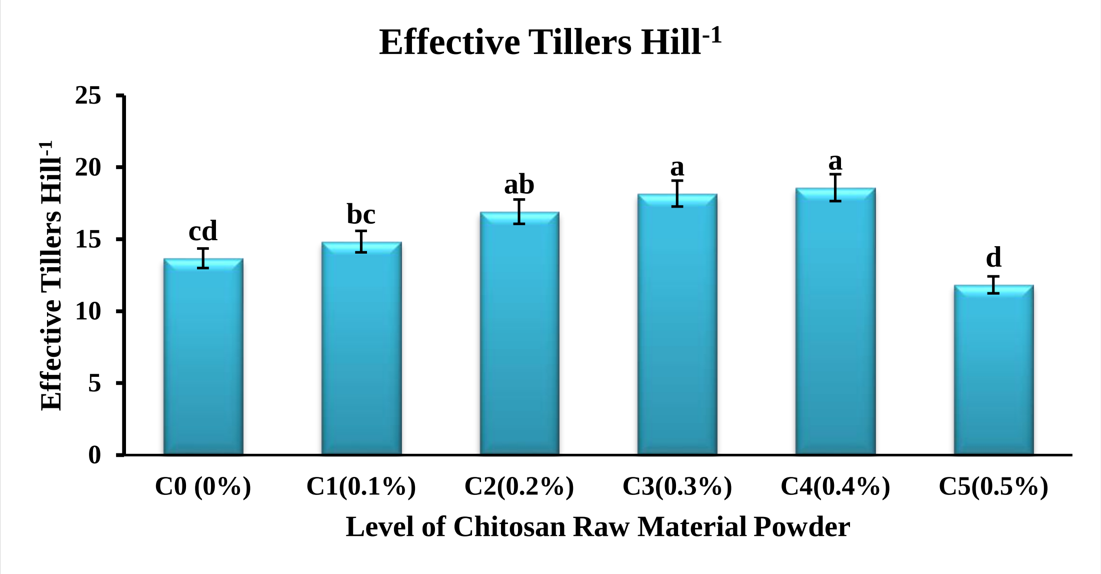
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 22: Combined effect of chitosan raw material powder level & seedling age on number of tillers hill^{-1} at 60 DAT of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.8 Number of Effective tillers hill^{-1}

4.8.1 Effect of chitosan raw material powder level on number of effective tiller hill^{-1} of BRRI dhan88

Application of different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%**)** had significantly effect on effective tiller (Figure 23). Highest effective tiller was found from C_4 (18.58) which was statistically identical with C_3 (18.17) and C_2 (16.92). On the other hand, lowest effective tiller was found from C_5 (11.83) which was statistically identical with C_0 (13.67). For effective tiller hill^{-1}, the treatments may be ranked in the order of C_4> C_3> C_2> C_1> C_0> C_5. Malerba M. *et al.* (2018) revealed that chitosan has an excellent role in improving effective tiller. Divya K. *et al.* (2022); Ahmed *et al*. (2020) found the similar result that effective tiller of chitosan treated plants were significantly higher than control.

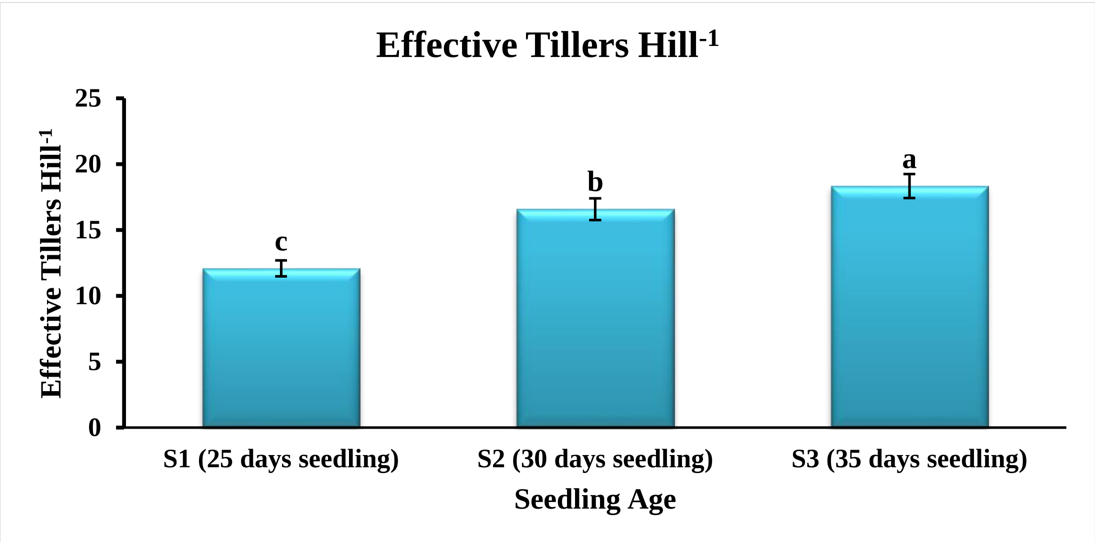


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 23: Effect of chitosan raw material powder level on number of effective tiller hill^{-1} of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.8.2 Effect of seedling age on number of effective tiller hill^{-1} of BRRI dhan88

Seedling age (S_1= 25 days old seedling, S_2= 30 days old seedling and S_3= 35 days old seedling) had significantly effect on effective tiller (Figure 24). Highest effective tiller was found from S_3 (18.33). On the other hand, lowest effective tiller was found from S_1 (12.08). For effective tiller hill^{-1}, seedling age may be ranked in the order of S_3> S_2> S_1.

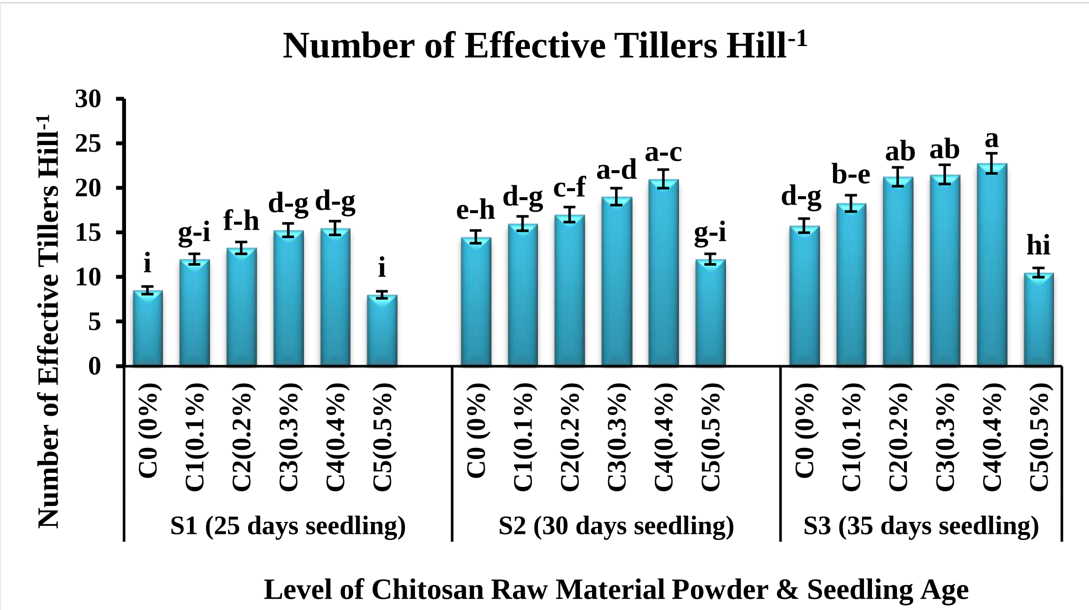


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 24: Effect of seedling age on number of effective tiller hill^{-1} of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.8.3 Combined effect of chitosan raw material powder level & seedling age on number of effective tiller hill^{-1} of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on effective tillers hill^{-1} of BRRI dhan88 (Figure 25). Experimental result revealed that, the maximum effective tillers hill^{-1} (22.75) was obtained in S_3C_4 treatment combination which was statistically identical with S_3C_3 (21.5), S_3C_2 (21.25), S_2C_4 (21) and S_2C_3 (19). Whereas the minimum tiller number hill^{-1} (8) was obtained in S_1C_5 treatment combination which was statistically identical with S_1C_0 (8.5), S_3C_5 (10.5), S_1C_1 (12) and S_2C_5 (12).



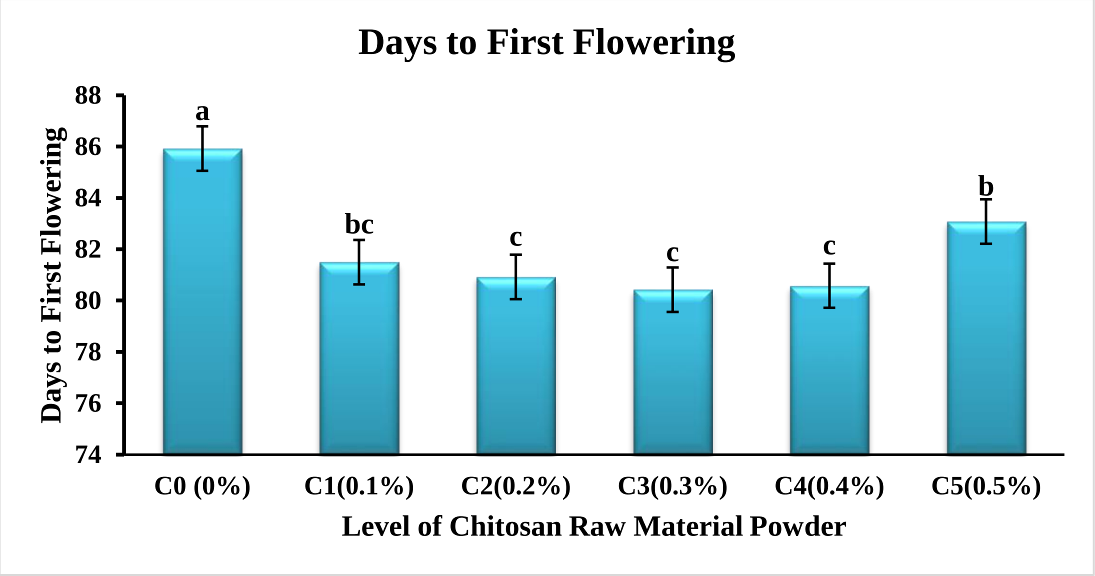
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 25: Combined effect of chitosan raw material powder level & seedling age on number of effective tiller hill^{-1} of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.9 Days to first flowering

4.9.1 Effect of chitosan raw material powder level on days to first flowering of BRRI dhan88

Days to first flowering was significantly influenced by different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%) (Figure 26). Highest flowering time were found from C_0 (85.92) and lowest flowering time was found from C_3 (80.42) which was statistically similar with C_4 (80.58), C_2 (80.92) and C_1 (81.5). In days to first flowering, the treatments may be ranked in the order of C_0> C_5> C_1> C_2> C_4> C_3. Faqir Y. *et al.* (2021) revealed that chitosan has an excellent role on flowering time. The flowering time through the application of Chitosan was also reported by many other scientists (Divya K. *et al.* 2022; Ahmed *et al*. 2020; Issak, M. and Sultana, A. 2017).

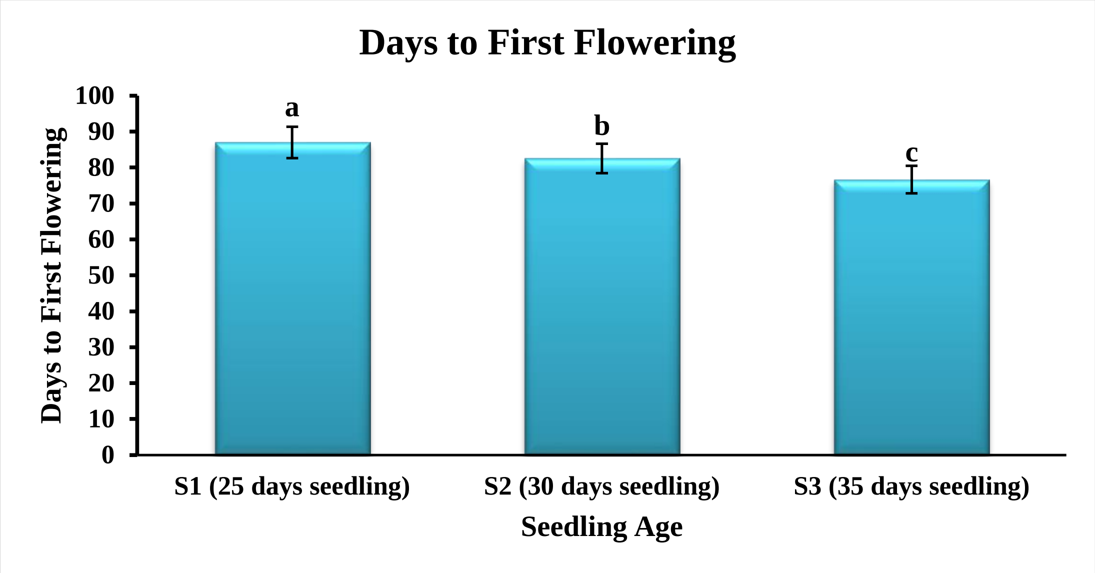


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 26: Effect of chitosan raw material powder level on days to first flowering of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.9.2 Effect of chitosan seedling age on days to first flowering of BRRI dhan88

Days to first flowering was significantly influenced by different seedling age S_1 (25 days old seedling), S_2 (30 days old seedling) and S_3 (35 days old seedling) (Figure 27). Highest flowering time were found from S_1 (87) and lowest flowering time was found from S_3 (76.67). In days to first flowering, seedling age may be ranked in the order of $S_1 > S_2 > S_3$.

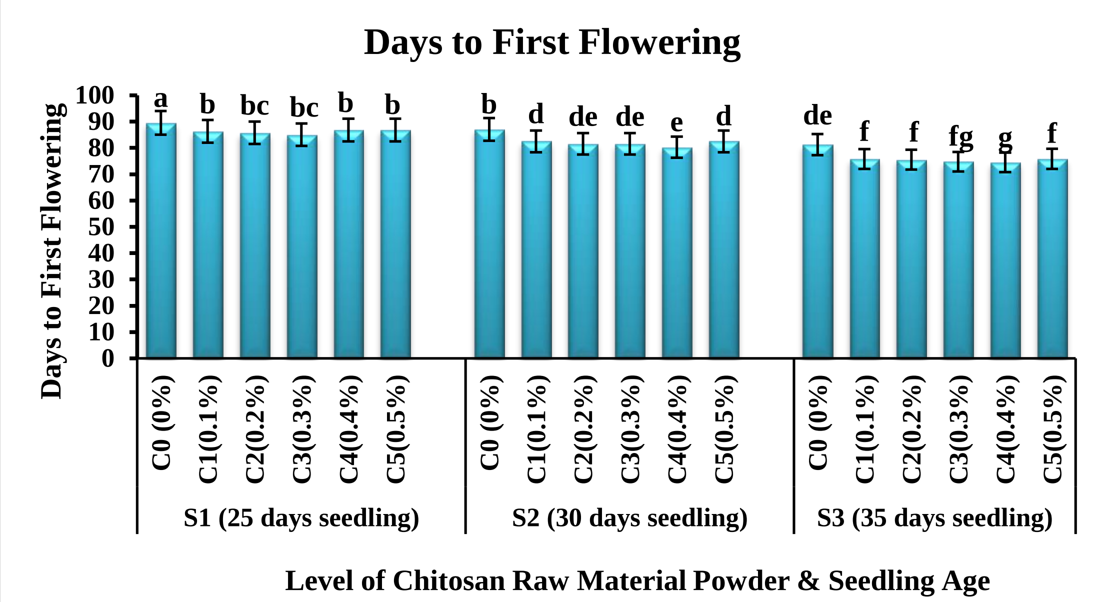


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 27: Effect of seedling age on days to first flowering of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.9.3 Combined effect of chitosan raw material powder level & seedling age on days to first flowering of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on days to first flowering of BRRI dhan88 (Figure 28). Experimental result revealed that, the maximum days for first flowering (89.5) was obtained in S_1C_0 treatment combination. Whereas the minimum days for first flowering (74.5) was obtained in S_3C_4 treatment combination which was statistically similar with S_3C_3 (74.75) treatment combination.



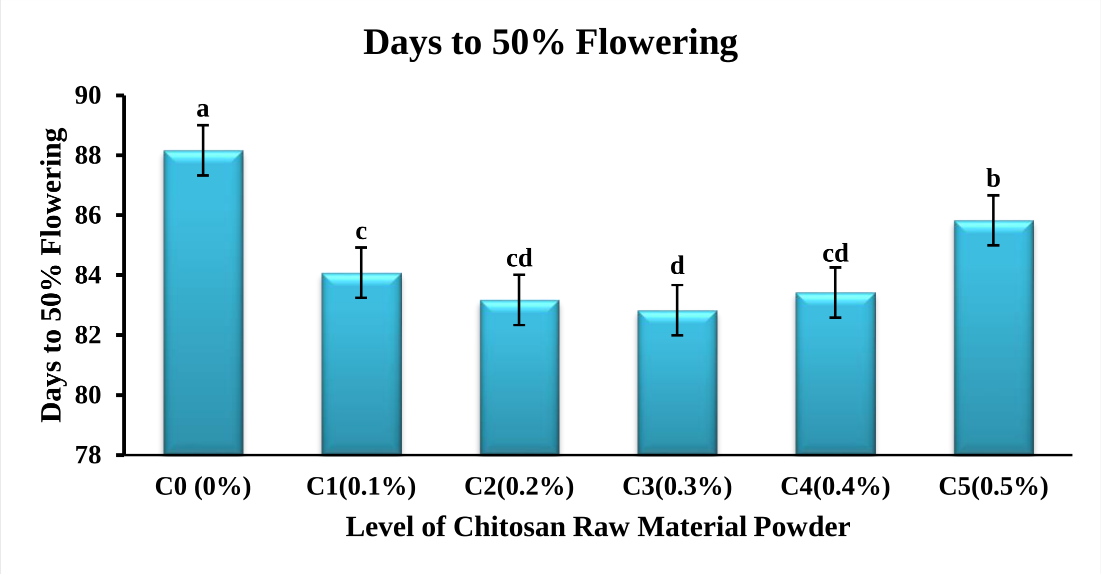
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 28: Combined effect of chitosan raw material powder level & seedling age on days to first flowering of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.10 Days to 50% flowering

4.10.1 Effect of chitosan raw material powder level on days to 50% flowering of BRRI dhan88

Application of different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%**)** had significantly effect on days to 50% flowering (Figure 29). Highest flowering time was found from C_0 (88.17) and lowest flowering time was found from C_3 (82.83) which was statistically similar with C_2 (83.17) and C_4 (83.42). In days to 50% flowering, the treatments may be ranked in the order of C_0> C_5> C_1> C_4> C_2> C_3. Issak, M. and Sultana, A. (2017) carried out an experiment to observed the role of chitosan powder on the flowering time of BRRI dhan29. Divya K. *et al.* (2022); Ahmed *et al*. (2020); Malerba M. *et al.* (2018) found that flowering time of chitosan treated plants were significantly varies.

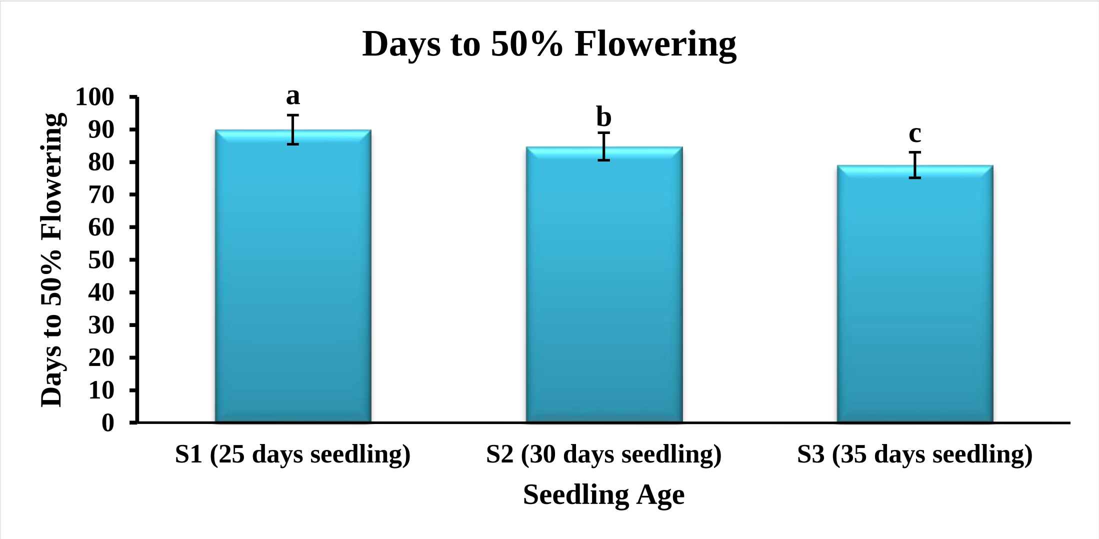


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 29: Effect of chitosan raw material powder level on days to 50% flowering of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.10.2 Effect of seedling age on days to 50% flowering of BRRI dhan88

Seedling age (S_1= 25 days old seedling, S_2= 30 days old seedling and S_3= 35 days old seedling) had significantly effect on days to 50% flowering (Figure 30). Highest flowering time was found from S_1 (89.92) and lowest flowering time was found from S_3 (79.08). In days to 50% flowering, seedling age may be ranked in the order of S_1> S_2> S_3.

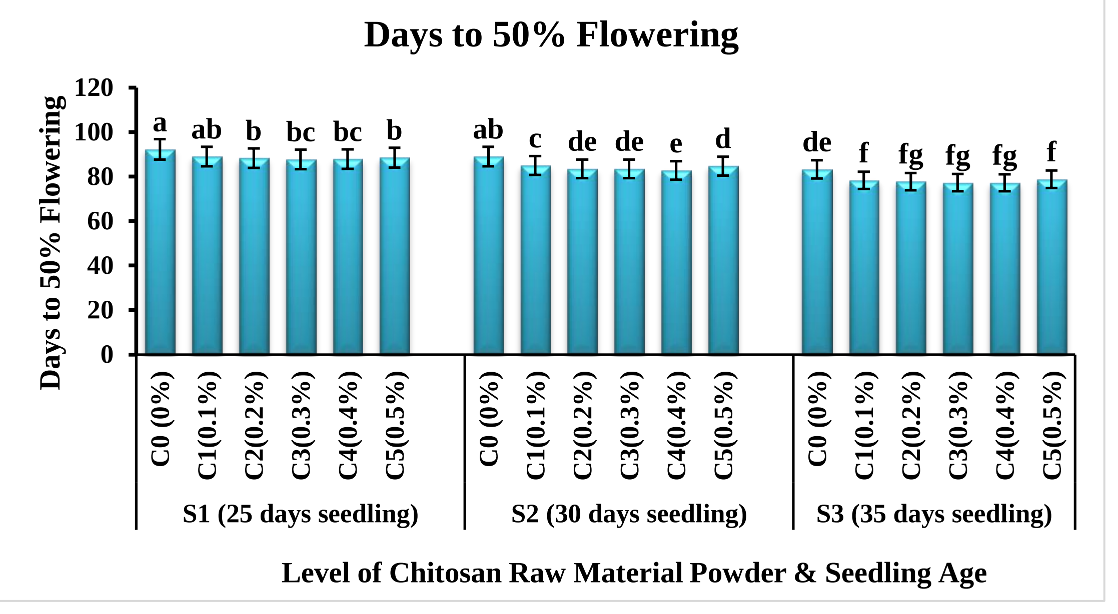


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 30: Effect of seedling age on days to 50% flowering of BRRI dhan88. Bars with different letters are significantly different at p ≤ 0.05 applying DMRT

4.10.3 Combined effect of chitosan raw material powder level & seedling age on days to 50% flowering of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on days to 50% flowering of BRRI dhan88 (Figure 31). Experimental result revealed that, the maximum days for 50% flowering (92.25) was obtained in S_1C_0 treatment combination which was statistically similar with S_1C_1 (89) and S_2C_0 (89) treatment combination. Whereas the minimum days for 50% flowering (77.2) was obtained in S_3C_4 treatment combination which was statistically similar with S_3C_2 (77.75) and S_3C_1 (78.25) treatment combination.



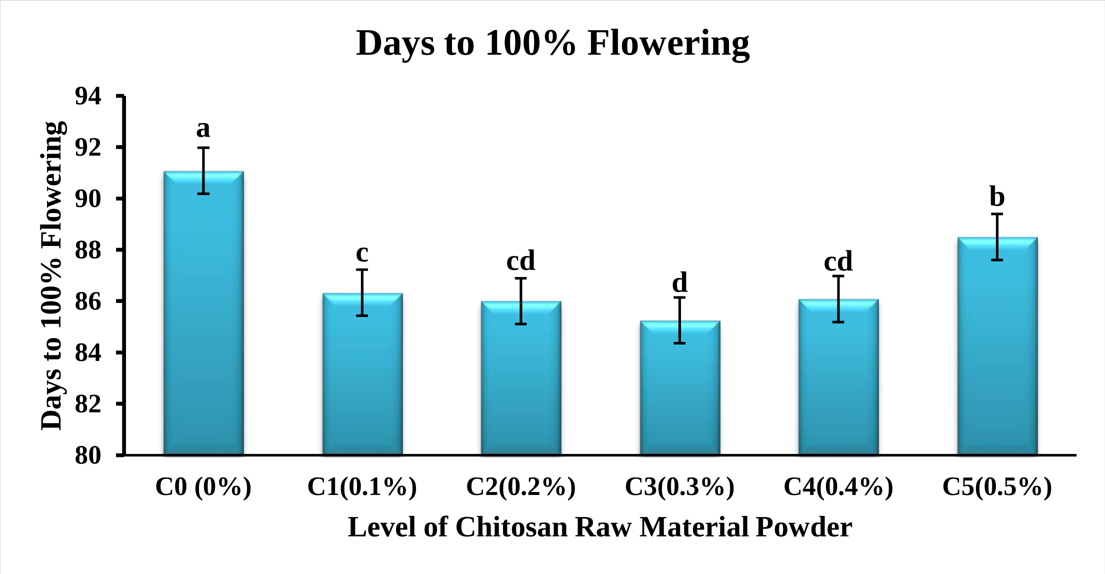
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 31: Combined effect of chitosan raw material powder level & seedling age on days to 50% flowering of BRRI dhan88. Bars with different letters are significantly different at p ≤ 0.05 applying DMRT

4.11 Days to 100% flowering

4.11.1 Effect of chitosan raw material powder level on days to 100% flowering of BRRI dhan88

Days to 100% flowering was significantly influenced by different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%) (Figure 32). Highest flowering time was found from C_0 (91.08) and lowest flowering time was found from C_3 (85.25) which was statistically similar with C_2 (86) and C_4 (86.08). In days to 100% flowering, the treatments may be ranked in the order of $C_0 > C_5 > C_1 > C_4 > C_2 > C_3$. Issak, M. and Sultana, A. (2017) showed that the role of chitosan powder on the flowering time of BRRI dhan29. The flowering time of plants through the application of Chitosan was also observed by many other scientists (Divya K. *et al.* 2022; Ahmed *et al*. 2020; Malerba M. *et al.* 2018).

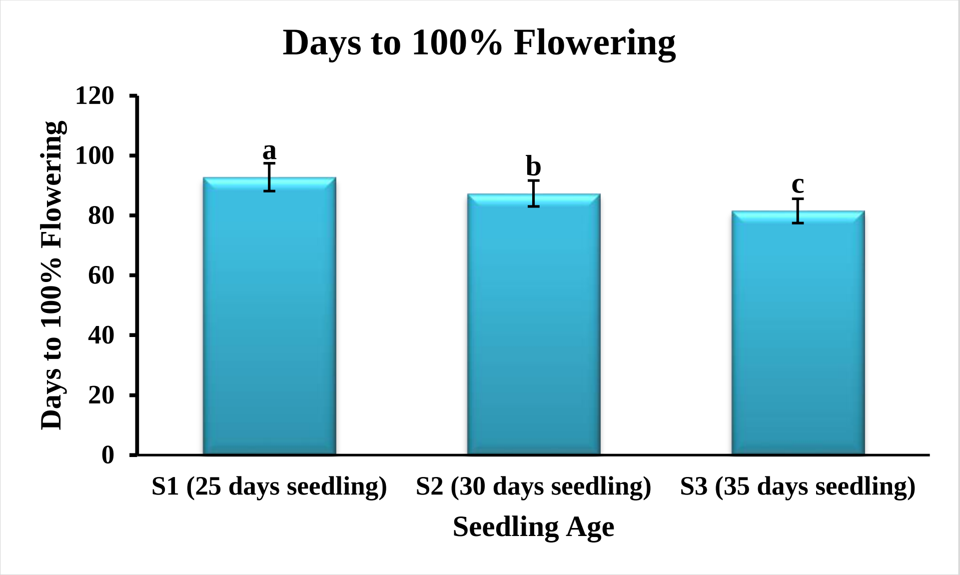


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 32: Effect of chitosan raw material powder level on days to 100% flowering of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.11.2 Effect of seedling age on days to 100% flowering of BRRI dhan88

Days to 100% flowering was significantly influenced by different seedling age S_1 (25 days old seedling), S_2 (30 days old seedling) and S_3 (35 days old seedling) (Figure 33). Highest flowering time was found from S_1 (92.75) and lowest flowering time was found from S_3 (81.54). In days to 100% flowering, seedling age may be ranked in the order of $S_1 > S_2 > S_3$.

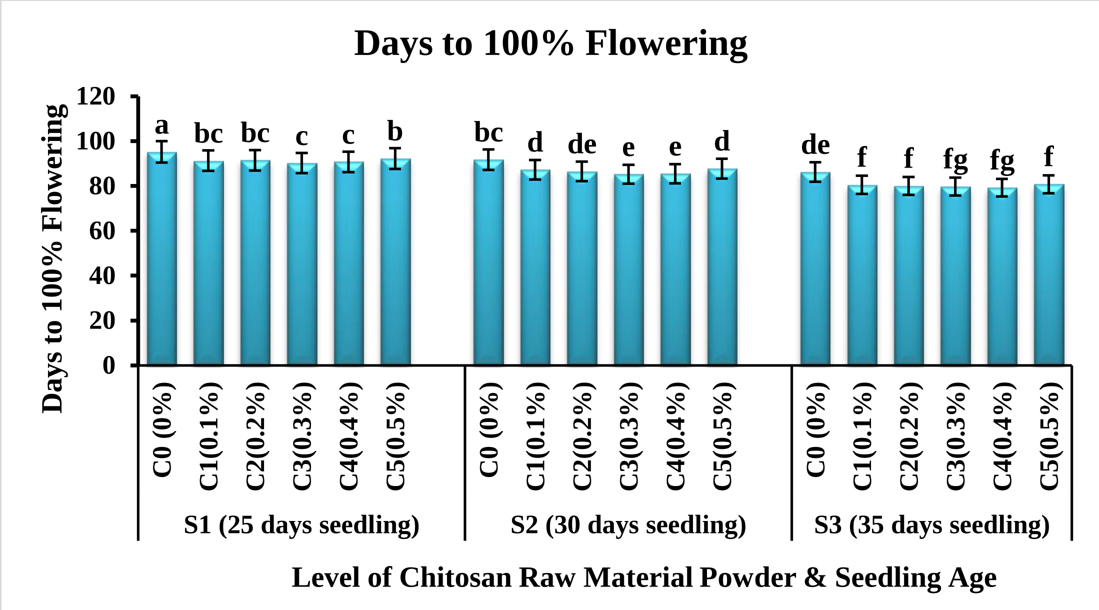


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 33: Effect of seedling age on days to 100% flowering of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.11.3 Combined effect of chitosan raw material powder level & seedling age on days to 100% flowering of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on days to 100% flowering of BRRI dhan88 (Figure 34). Experimental result revealed that, the maximum days for 100% flowering (95.25) was obtained in S_1C_0 treatment combination. Whereas the minimum days for 100% flowering (79.25) was obtained in S_3C_4 treatment combination which was statistically similar with S_3C_3 (79.75), S_3C_2 (80), S_3C_1 (80.5) and S_3C_5 (80.75) treatment combination.



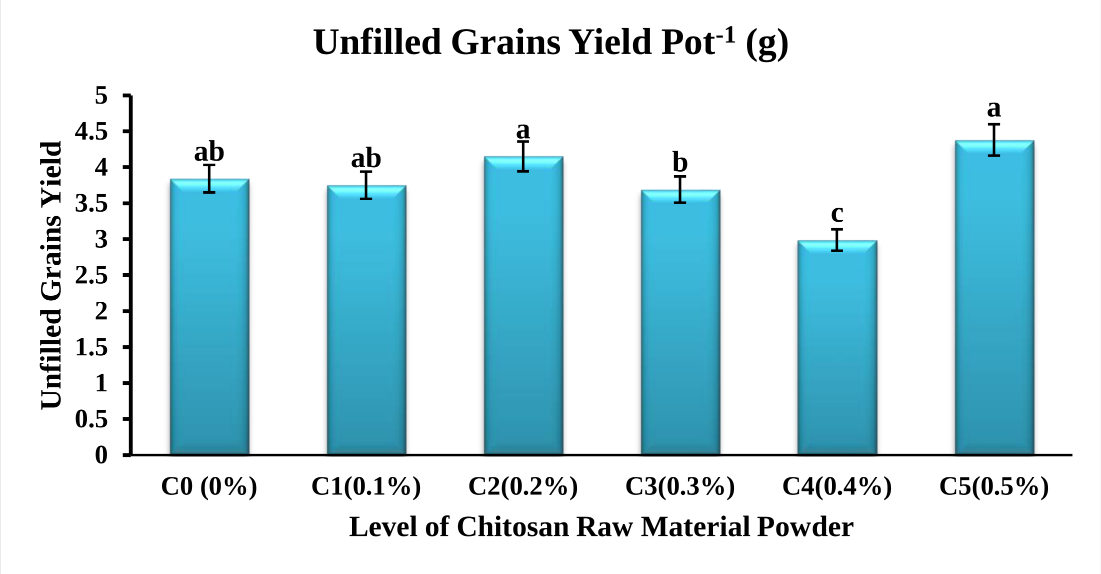
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 34: Combined effect of chitosan raw material powder level & seedling age on days to 100% flowering of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.12 Unfilled grains yield pot^{-1} (g)

4.12.1 Effect of chitosan raw material powder level on unfilled grains yield of BRRI dhan88

Application of different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%) had effect on unfilled grains yield pot^{-1} (g) (Figure 35). Highest unfilled grains yield was found from C_5 (4.38 g) which was statistically similar with C_2 (4.15 g), C_0 (3.84 g) and C_1 (3.75 g). On the other hand, lowest unfilled grains yield was found from C_4 (2.99 g). For unfilled grains yield pot^{-1}, the treatments may be ranked in the order of $C_5 > C_2 > C_0 > C_1 > C_3 > C_4$. Malerba M. *et al.* (2018) revealed that chitosan has an excellent role on unfilled grains yield. Divya K. *et al.* (2022); Ahmed *et al.* (2020) found that unfilled grains yield of chitosan treated plants were varies.

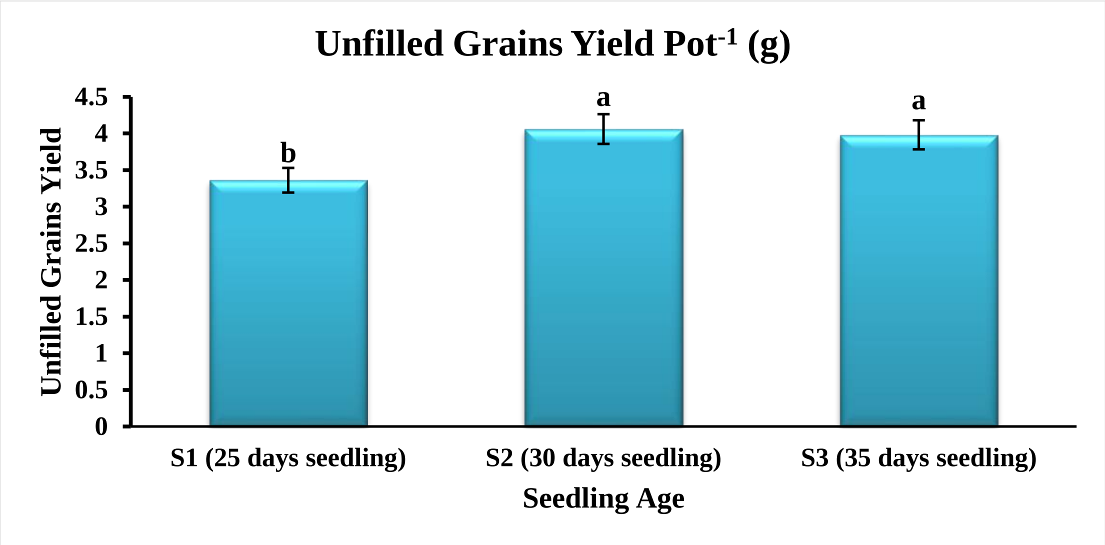


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 35: Effect of chitosan raw material powder level on unfilled grains yield of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.12.2 Effect of seedling age on unfilled grains yield of BRRI dhan88

Seedling age (S_1= 25 days old seedling, S_2= 30 days old seedling and S_3= 35 days old seedling) had effect on unfilled grains yield pot^{-1} (g) (Figure 36). Highest unfilled grains yield was found from S_2 (4.06 g) and lowest unfilled grains yield was found from S_1 (3.36 g) was statistically similar with S_3 (3.98 g). For unfilled grains yield, seedling age may be ranked in the order of S_2> S_3> S_1.

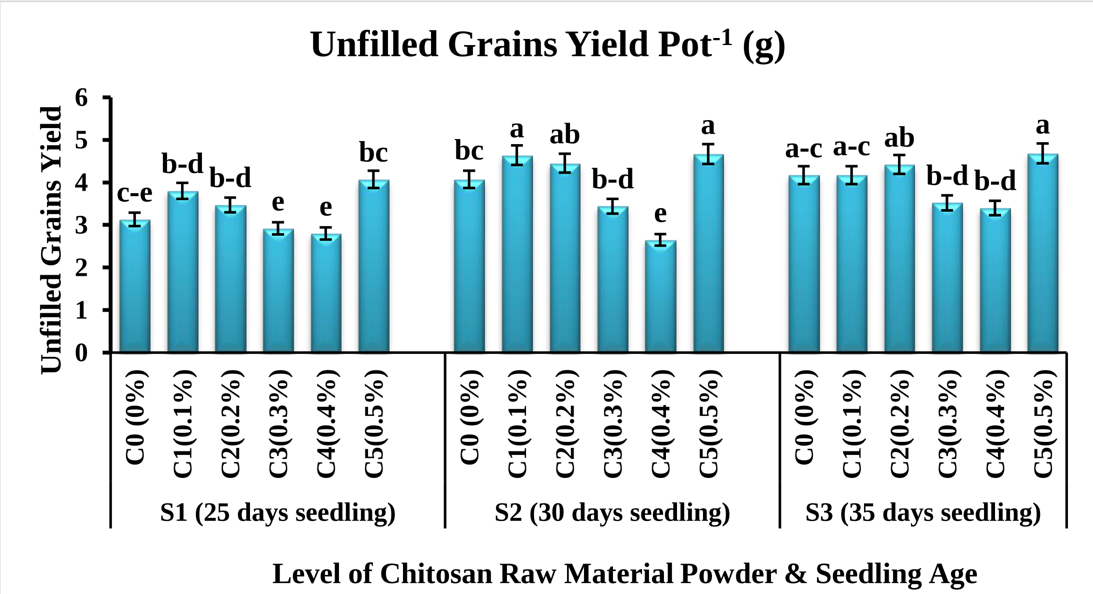


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 36: Effect of seedling age on unfilled grains yield of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.12.3 Combined effect of chitosan raw material powder level & seedling age on unfilled grains yield of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on unfilled grains yield pot^{-1} of BRRI dhan88 (Figure 37). Experimental result revealed that, the maximum unfilled grains yield pot^{-1} (4.68 g) was obtained in S_3C_5 treatment combination which was statistically similar with S_2C_5 (4.67 g), S_2C_1 (4.64 g), S_2C_2 (4.45 g), S_3C_2 (4.42 g), S_3C_1 (4.17 g) and S_3C_0 (4.17 g) treatment combination. Whereas the minimum unfilled grains yield pot^{-1} (2.65 g) was obtained in S_2C_4 treatment combination which was statistically similar with S_1C_4 (2.8 g), S_1C_3 (2.92 g) and S_1C_0 (3.13 g) treatment combination.



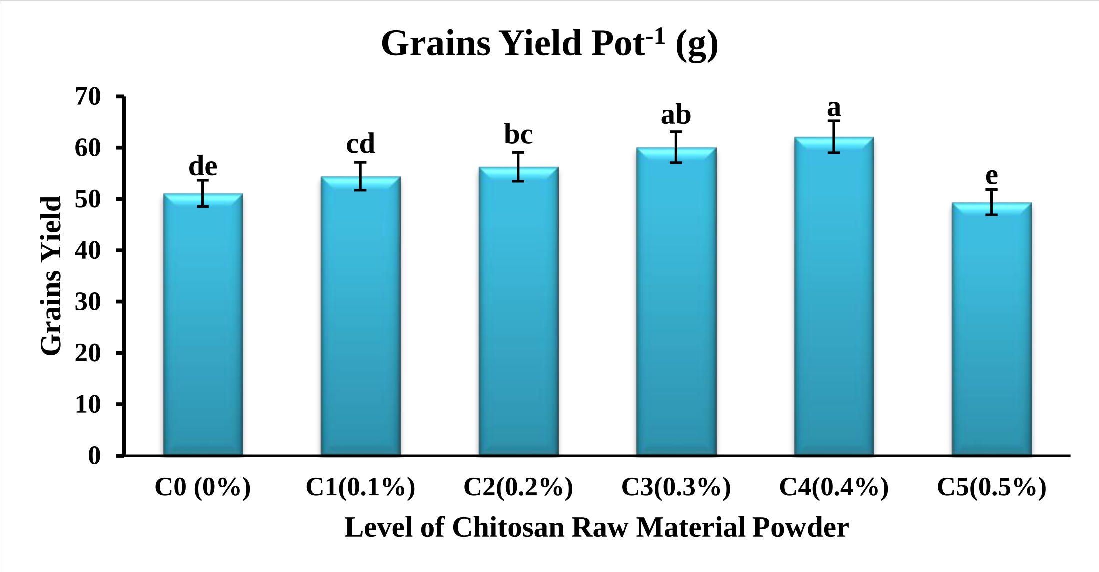
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 37: Combined effect of chitosan raw material powder level & seedling age on unfilled grains yield pot^{-1}of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.13 Grains yield pot^{-1} (g)

4.13.1 Effect of chitosan raw material powder level on grains yield of BRRI dhan88

Grains yield pot^{-1} was significantly influenced by different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%) (Figure 38). Highest grains yield pot^{-1} was found from C_4 (62.15 g) which was statistically identical with C_3 (60.11 g). On the other hand, lowest grains yield pot^{-1} was found from C_5 (49.39 g) which was statistically identical with C_0 (51.11 g). For grains yield pot^{-1}, the treatments may be ranked in the order of C_4> C_3> C_2> C_1> C_0> C_5. Faqir Y. *et al.* (2021) revealed that chitosan has an excellent role in improving grains yield. The increased grains yield through the application of Chitosan was also reported by many other scientists (Divya K. *et al.* 2022; Ahmed *et al*. 2020; Issak, M. and Sultana, A. 2017).



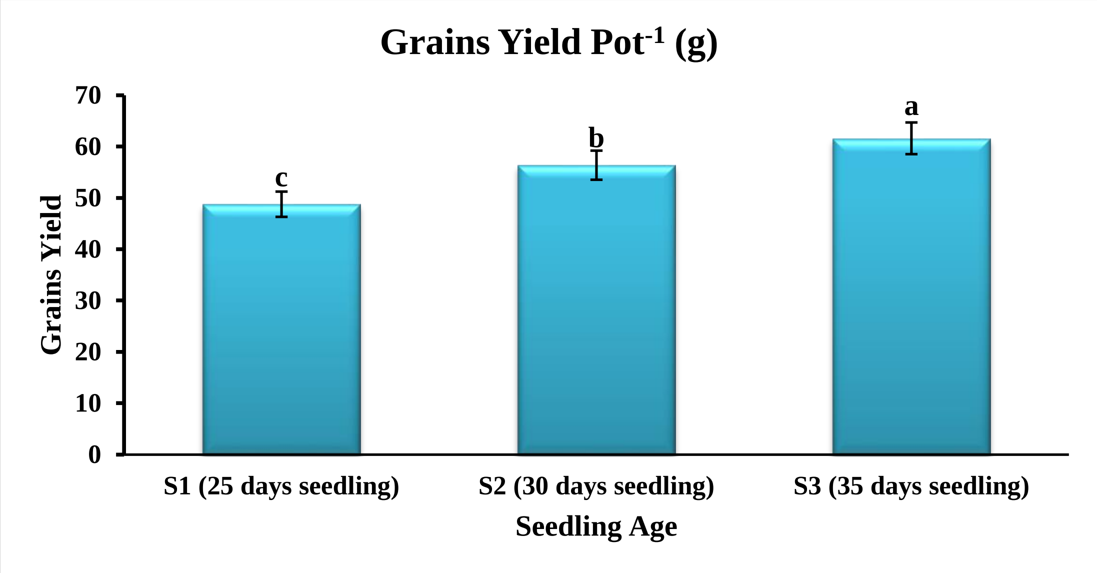
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 38: Effect of chitosan raw material powder level on grains yield of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.13.2 Effect of seedling age on grains yield of BRRI dhan88

Grains yield pot^{-1} was significantly influenced by different seedling age S_1 (25 days old seedling), S_2 (30 days old seedling) and S_3 (35 days old seedling) (Figure 39).

Highest grains yield pot^{-1} was found from S_3 (61.6 g) and lowest grains yield pot^{-1} was found from S_1 (48.76 g). For grains yield pot^{-1}, seedling age may be ranked in the order of $S_3 > S_2 > S_1$.

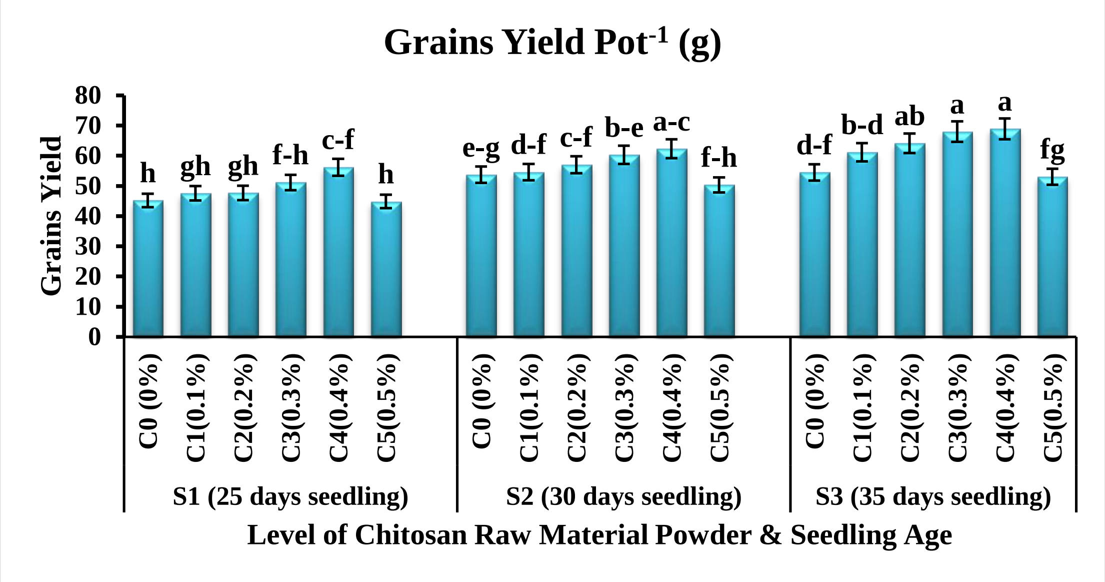


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 39: Effect of seedling age on grains yield of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.13.3 Combined effect of chitosan raw material powder level & seedling age on grains yield of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on grains yield pot^{-1} of BRRI dhan88 (Figure 40). Experimental result revealed that, the maximum grains yield pot^{-1} (68.91 g) was obtained in S_3C_4 treatment combination which was statistically similar with S_3C_3 (67.95 g), S_3C_2 (64.12 g) and S_2C_4 (62.31 g) treatment combination. Whereas the minimum grains yield pot^{-1} (44.82 g) was obtained in S_1C_5 treatment combination which was statistically similar with S_1C_0 (45.19 g), S_1C_1 (47.54 g), S_1C_2 (47.63 g), S_1C_3 (51.15 g) and S_2C_5 (50.31 g) treatment combination.



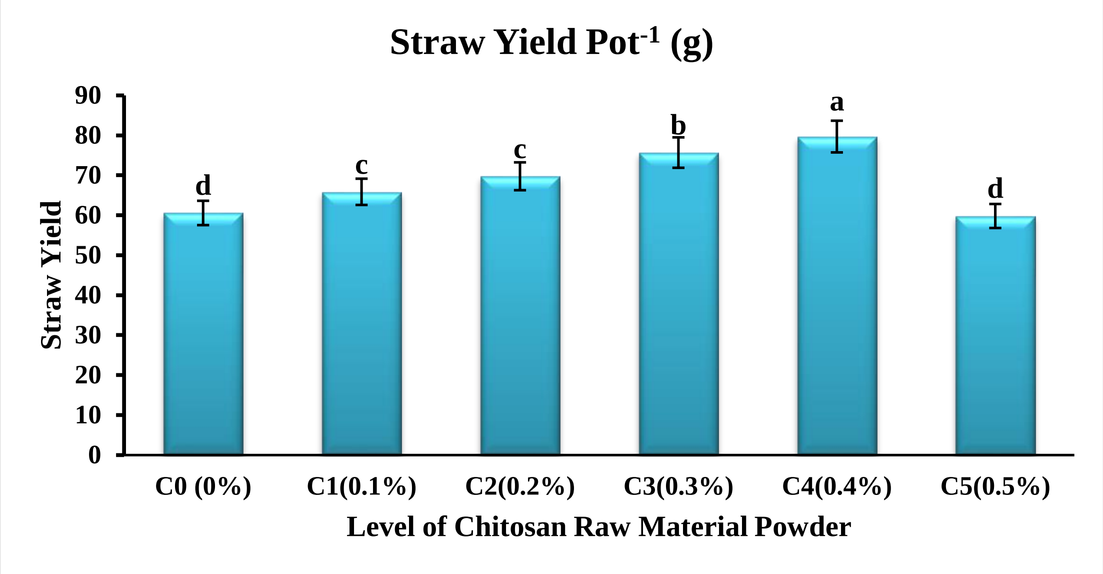
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 40: Combined effect of chitosan raw material powder level & seedling age on grains yield of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.14 Straw yield pot^{-1} (g)

4.14.1 Effect of chitosan raw material powder level on straw yield of BRRI dhan88

Application of different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%) had significantly effect on straw yield pot^{-1} (Figure 41). Highest straw yield pot^{-1} was found from C_4 (79.67 g) and lowest straw yield pot^{-1} was found from C_5 (59.83 g) was statistically identical with C_0 (60.58 g). In straw yield pot^{-1} of seedlings, the treatments may be ranked in the order of C_4> C_3> C_2> C_1> C_0> C_5. Issak, M. and Sultana, A. (2017) carried out an experiment to observed the role of chitosan powder on the straw yield of BRRI dhan29. Divya K. *et al.* (2022); Ahmed *et al*. (2020); Malerba M. *et al.* (2018) found that straw yield of chitosan treated seedlings were significantly varies.

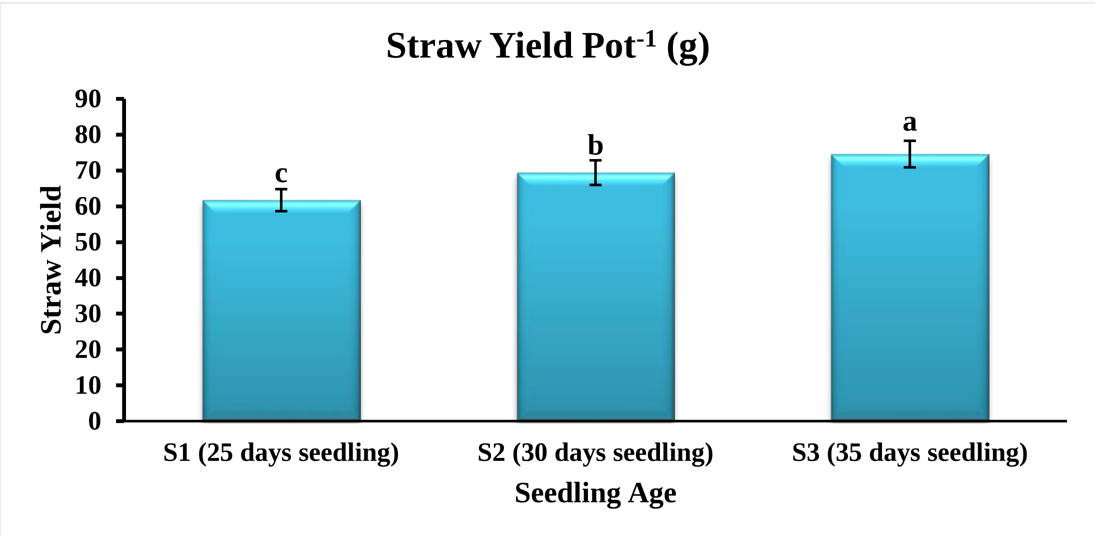


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 41: Effect of chitosan raw material powder level on straw yield of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.14.2 Effect of seedling age on straw yield of BRRI dhan88

Seedling age (S_1= 25 days old seedling, S_2= 30 days old seedling and S_3= 35 days old seedling) had significantly effect on straw yield pot^{-1} (Figure 42). Highest straw yield pot^{-1} was found from S_3 (74.58 g) and lowest straw yield pot^{-1} was found from S_1 (61.71 g). In straw yield pot^{-1} of seedlings, straw yield pot^{-1} may be ranked in the order of S_3> S_2> S_1.

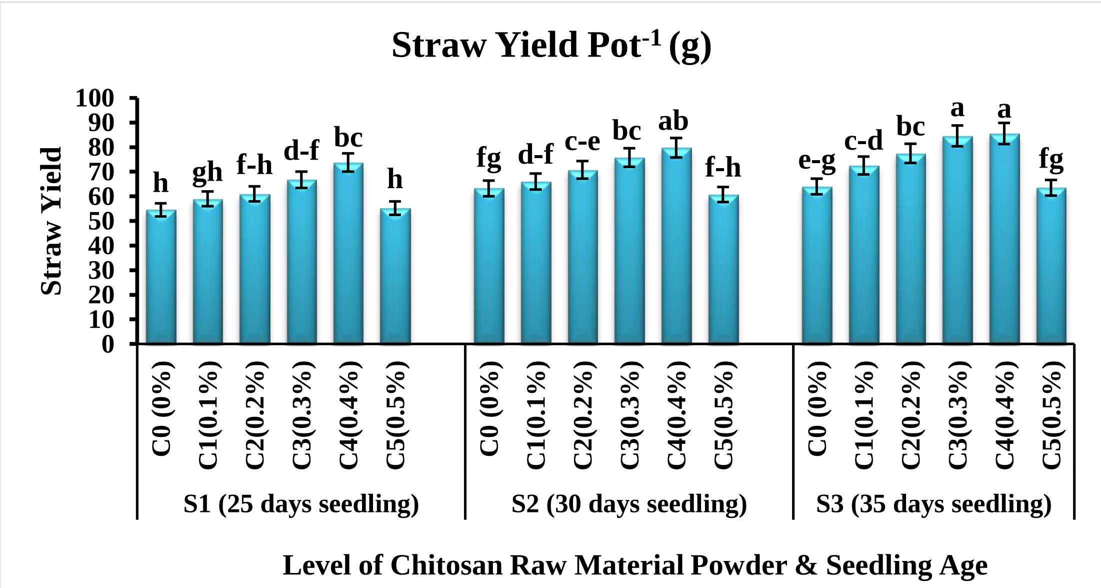


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 42: Effect of seedling age on straw yield of BRRI dhan88. Bars with different letters are significantly different at p ≤ 0.05 applying DMRT

4.14.3 Combined effect of chitosan raw material powder level & seedling age on straw yield of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on straw yield pot^{-1} of BRRI dhan88 (Figure 43). Experimental result revealed that, the maximum straw yield pot^{-1} (85.5 g) was obtained in S_3C_4 treatment combination which was statistically similar with S_3C_3 (84.5 g) and S_2C_4 (79.75 g) treatment combination. Whereas the minimum straw yield pot^{-1} (54.5 g) was obtained in S_1C_0 treatment combination which was statistically similar with S_1C_5 (55.25 g), S_1C_1 (59 g), S_2C_5 (60.75 g) and S_1C_2 (61 g) treatment combination.



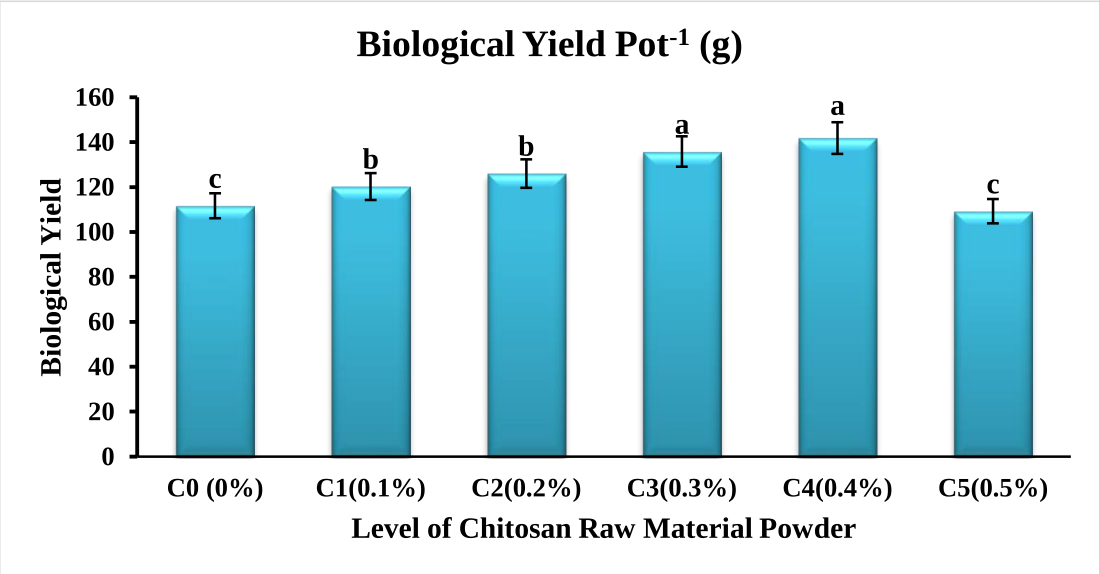
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 43: Combined effect of chitosan raw material powder level & seedling age on straw yield of BRRI dhan88. Bars with different letters are significantly different at p ≤ 0.05 applying DMRT

4.15 Biological yield pot^{-1} (g)

4.15.1 Effect of chitosan raw material powder level on biological yield of BRRI dhan88

Biological yield pot^{-1} was significantly influenced by different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%) (Figurer 44). Highest biological yield pot^{-1} was found from C_4 (141.82 g) which was statistically identical with C_3 (135.78 g). On the other hand, lowest biological yield pot^{-1} was found from C_5 (109.23 g) was statistically identical with C_0 (111.7 g). In biological yield pot^{-1}, the treatments may be ranked in the order of C_4> C_3> C_2> C_1> C_0> C_5. Issak, M. and Sultana, A. (2017) showed that the maximum biological yield was obtained in the treatment T_4 (400 g CHT powder/m^2) and the minimum level in the treatment T_6 (0 g CHT powder/m^2). The increased biological yield through the application of Chitosan was also observed by many other scientists (Divya K. *et al.* 2022; Ahmed *et al*. 2020; Malerba M. *et al.* 2018).

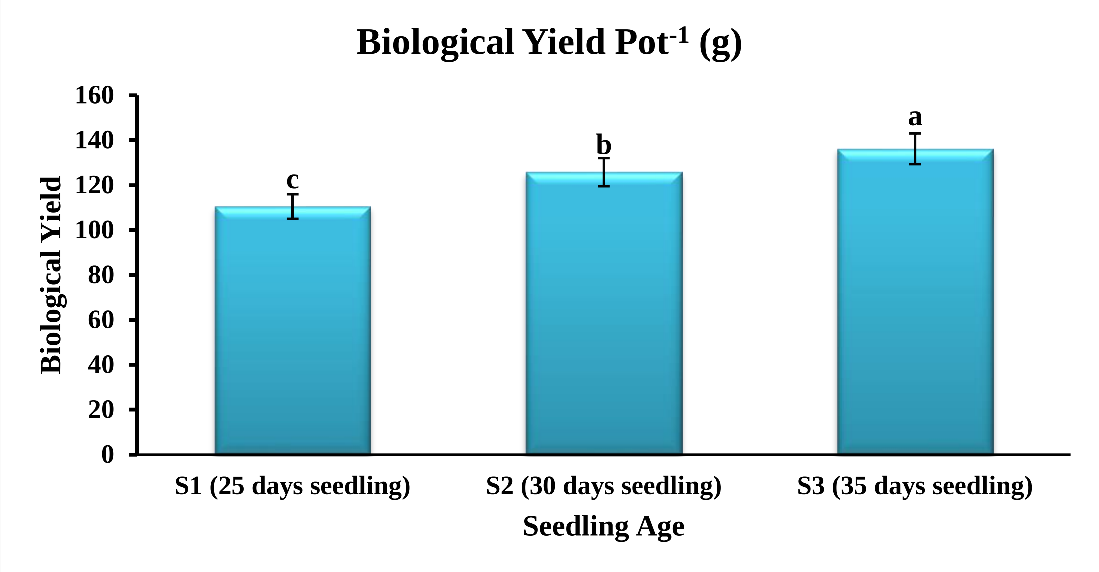


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 44: Effect of chitosan raw material powder level on biological yield of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.15.2 Effect of seedling age on biological yield of BRRI dhan88

Biological yield pot^{-1} was significantly influenced by different seedling age S_1 (25 days old seedling), S_2 (30 days old seedling) and S_3 (35 days old seedling) (Figurer 45). Highest biological yield pot^{-1} was found from S_3 (136.19 g). and lowest biological yield pot^{-1} was found from S_1 (110.46 g). In biological yield pot^{-1}, seedling age may be ranked in the order of $S_3 > S_2 > S_1$.

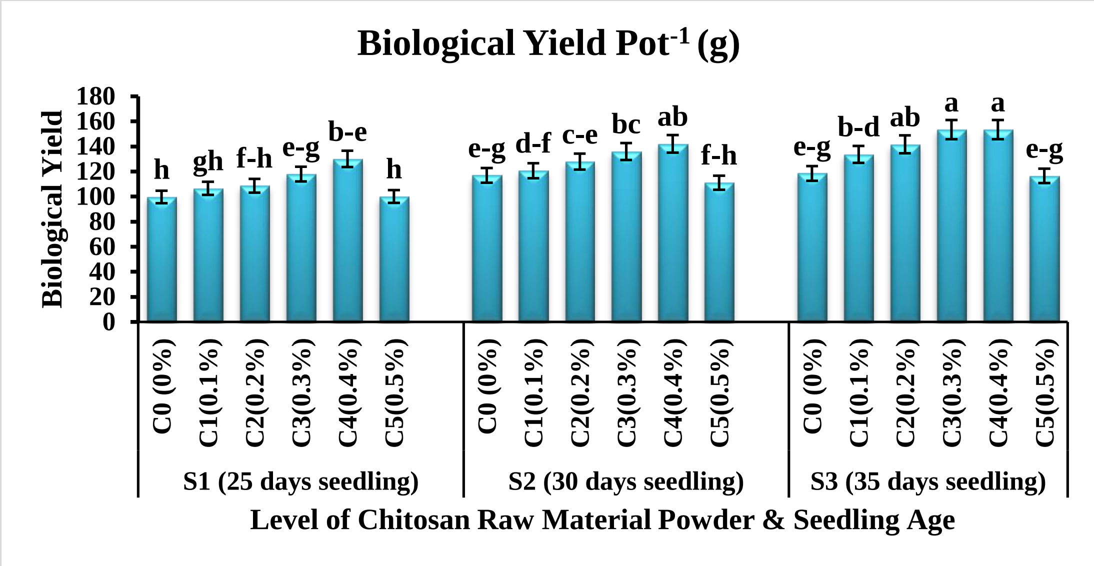


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 45: Effect of seedling age on biological yield of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.15.3 Combined effect of chitosan raw material powder level & seedling age on biological yield of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on biological yield pot^{-1} of BRRI dhan88 (Figure 46). Experimental result revealed that, the maximum biological yield pot^{-1} (153.45 g) was obtained in S_3C_4 treatment combination which was statistically similar with S_3C_3 (153.41 g), S_3C_2 (141.62 g) and S_2C_4 (142.06 g) treatment combination. Whereas the minimum biological yield pot^{-1} (99.69 g) was obtained in S_1C_0 treatment combination which was statistically similar with S_1C_5 (100.07 g), S_1C_1 (106.54 g), S_1C_2 (108.63 g) and S_2C_5 (111.06 g) treatment combination.



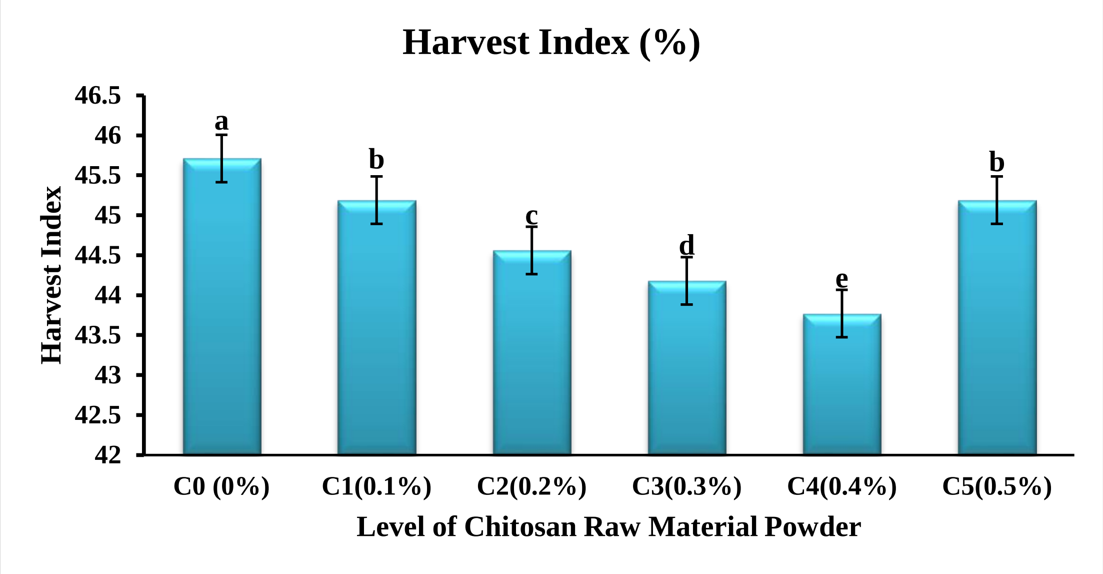
Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 46: Combined effect of chitosan raw material powder level & seedling age on biological yield of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.16 Harvest index (%)

4.16.1 Effect of chitosan raw material powder level on harvest index of BRRI dhan88

Application of different levels of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%) had significantly effect on harvest index (Figure 47). Highest harvest index was found from C_0 (45.71%) and lowest harvest index was found from C_4 (43.77%). For harvest index, the treatments may be ranked in the order of C_0> C_1> C_5> C_2> C_3> C_4. Malerba M. *et al.* (2018) revealed that chitosan has an excellent role in improving harvest index. Divya K. *et al.* (2022); Ahmed *et al*. (2020) found the similar result that harvest index of chitosan treated plants were significantly varies.

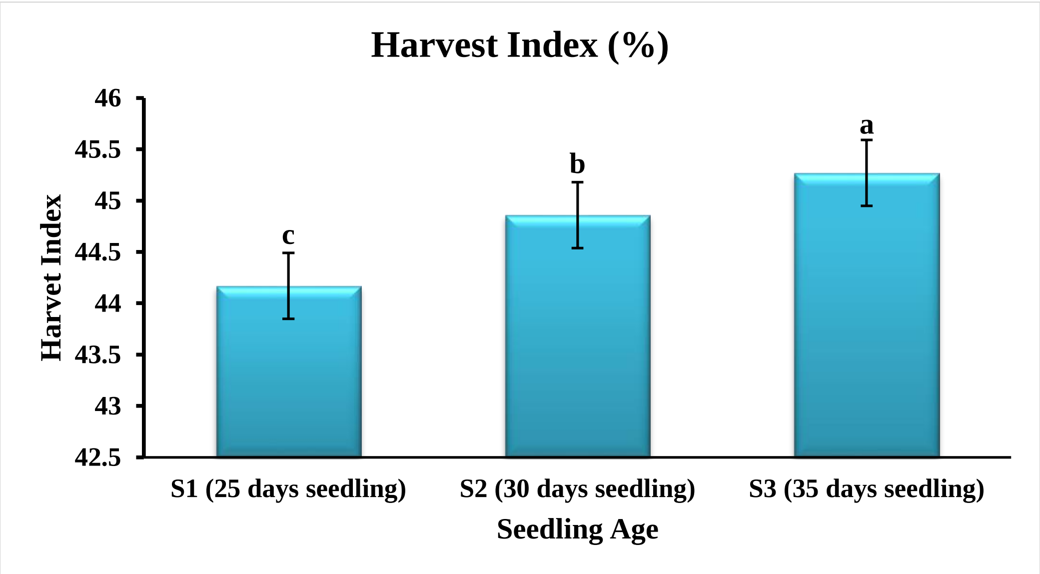


Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder.

Figure 47: Effect of chitosan raw material powder level on harvest index of BRRI dhan88. Bars with different letters are significantly different at $p \leq 0.05$ applying DMRT

4.16.2 Effect of seedling age on harvest index of BRRI dhan88

Seedling age (S_1= 25 days old seedling, S_2= 30 days old seedling and S_3= 35 days old seedling) had significantly effect on harvest index (Figure 48). Highest harvest index was found from S_3 (45.27%) and lowest harvest index was found from S_1 (44.17%). For harvest index, seedling age may be ranked in the order of S_3> S_2> S_1.

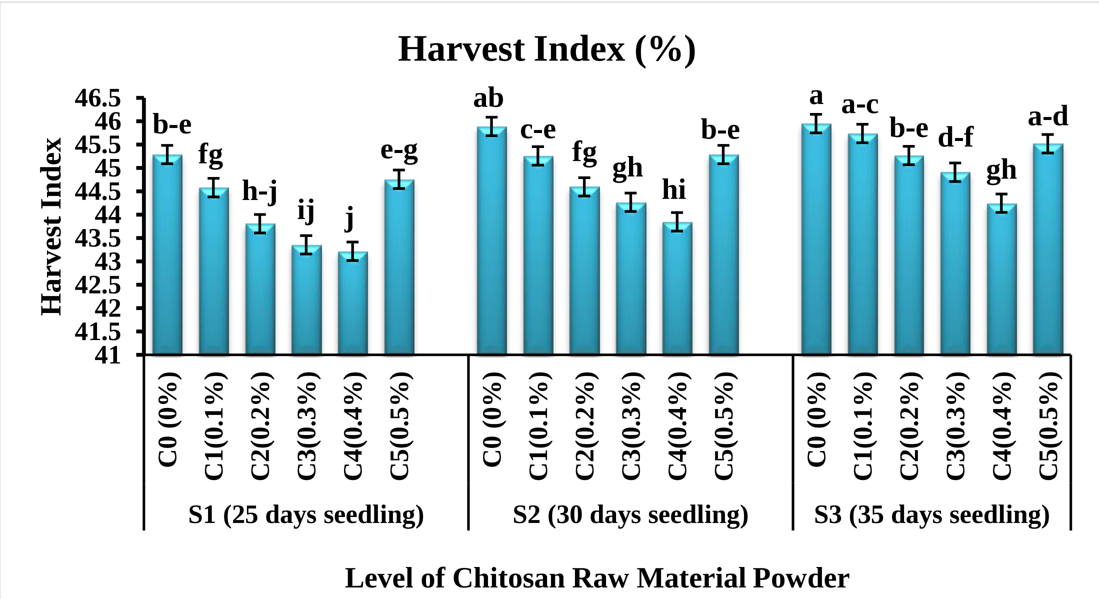


Here, S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 48: Effect of seedling age on harvest index of BRRI dhan88. Bars with different letters are significantly different at p ≤ 0.05 applying DMRT

4.16.3 Combined effect of chitosan raw material powder level & seedling age on harvest index of BRRI dhan88

Seedling treated with different levels of chitosan along with different ages of seedling significantly effect on harvest index of BRRI dhan88 (Figure 49). Experimental result revealed that, the maximum harvest index (45.95%) was obtained in S_3C_0 treatment combination which was statistically similar with S_2C_0 (45.89%), S_3C_1 (45.74%) and S_3C_5 (45.52%) treatment combination. Whereas the minimum harvest index (43.22%) was obtained in S_1C_4 treatment combination which was statistically similar with S_1C_3 (43.36%) and S_1C_2 (43.81%) treatment combination.



Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder. S_1= 25 days old seedling, S_2= 30 days old seedling, S_3= 35 days old seedling.

Figure 49: Combined effect of chitosan raw material powder level & seedling age on harvest index of BRRI dhan88. Bars with different letters are significantly different at p ≤ 0.05 applying DMRT

4.17 Chemical Properties of seedbed soils after transplant

4.17.1 Soil pH

Application of different level of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%**)** influenced seedbed soil pH from initial level (5.8) (Table 10). Among different treatments, C_5 treatment increasing seedbed soil pH (6.6) comparable control treatment due to reason that chitosan raw material powder has higher pH which influenced the soil pH in the seedbed soil.

4.17.2 Total Nitrogen

Application of different level of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%**)** influenced total nitrogen percentage from initial level (0.04%) (Table 10). Among different treatments, C_4 treatment recorded the highest total nitrogen (0.14%) comparable control treatment due to reason that application of chitosan raw material powder increasing the nutrient supplying capacity to the soil result in increasing total nitrogen percentage in the seedbed soil.

4.17.3 Organic Carbon

Chitosan raw material powder contents 7.52 % organic carbon. Application of different level of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%) influenced organic carbon percentages comparable to control treatment. The maximum organic carbon (0.77%) was recorded in C_5 treatment comparable to control treatment. Application of chitosan raw material powder increases the organic carbon present in the seedbed soil. But higher amount of organic carbon creates toxicity and compactness of soil in the root zone of the plant. As a result, plant cannot uptake essential nutrients result in poor growth and development.

4.17.4 Organic Matter

Chitosan raw material powder contents 12.96 % organic matter Application of different level of chitosan raw material powder (C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5%) influenced organic carbon percentages and organic matter comparable to control treatment. The maximum organic matter (1.32%) was recorded in C_5 treatment comparable to control treatment. Application of chitosan raw material powder increases organic matter content in the seedbed soil. But higher amount of organic matter creates toxicity and compactness of soil in the root zone of the plant. As a result, plant cannot uptake essential nutrients result in poor growth and development.

Table 6: Effect of chitosan raw material powder on pH, total nitrogen, organic carbon and organic matter of seedbed soil after seedling transplant

Treatment combinations	pH		Total Nitrogen (%)		Organic Carbon (%)		Organic Matter (%)	
	Initial	**After**	**Initial**	**After**	**Initial**	**After**	**Initial**	**After**
C_0	5.8	5.9d	0.04	0.05e	0.5	0.6d	0.87	0.88e
C_1	5.8	6.1c	0.04	0.06e	0.5	0.7c	0.87	1.03d
C_2	5.8	6c	0.04	0.1c	0.5	0.69c	0.87	1.01d
C_3	5.8	6.4b	0.04	0.08d	0.5	0.72b	0.87	1.09c
C_4	5.8	6.4b	0.04	0.14a	0.5	0.75a	0.87	1.24b
C_5	5.8	6.6a	0.04	0.12b	0.5	0.77a	0.87	1.32a
LSD (0.05)	0	0.18	0	0.003	0	0.02	0	0.03
CV (%)	0	1.97	0	2.34	0	2.62	0	2.16

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability. Here, C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% chitosan raw material powder

CHAPTER V

CHAPTER V

SUMMARY AND CONCLUSION

An experiment was carried out at Sher-e-Bangla Agricultural University, Dhaka during the period from November, 2019 to April, 2020. The study was aimed to determine the effect of chitosan raw material powder and seedling age on growth, yield contributing characters and yield of boro rice cv. BRRI dhan88. The experiment was consisted of two factors and followed Randomized Complete Block Design (RCBD) with four replications. Factor A: chitosan raw material powder level viz; C_0= 0%, C_1= 0.1%, C_2= 0.2%, C_3= 0.3%, C_4= 0.4% and C_5= 0.5% of chitosan raw material powder and Factor B: Different ages of seedling viz; S_1= 25 days old seedling, S_2= 30 days old seedling and S_3= 35 days old seedling. Chitosan was applied and mixed in the soil before transplanting of rice. Chitosan effect data were recorded on average seedling height (cm), fresh weight of 100 seedlings (g), oven dry weight of 100 seedlings (g), seedling strength ($mgcm^{-1}$), number of total tillers $hill^{-1}$ at 40, 50 & 60 DAT, number of effective tillers $hill^{-1}$, days to first flowering, days to 50% flowering, days to 100% flowering, unfilled grains yield pot^{-1} (g), grains yield pot^{-1} (g), straw yield pot^{-1} (g), biological yield pot^{-1} (g), harvest index (%) & nutrient content in soil. Application of chitosan had a profound influence on morphological, reproductive, yield attributes and grain yield of rice. Chitosan also improves and increase pH, total nitrogen, organic carbon and organic matter of seedbed soil. Among the treatment combinations, C_4 (0.4%) & S_3 (35 days old seedlings) perform best among the significantly varied parameters compare to other combinations. But in case of non-significant parameters the treatment may differ.

In case of different chitosan raw material powder level & seedling age, experimental result showed that the maximum average seedling height (20.45 cm), fresh weight of 100 seedlings (44.6 g), oven dry weight of 100 seedlings (17.2 g), seedling strength (8.41 $mgcm^{-1}$), tiller number pot^{-1} (11) at 40 DAT, tiller number pot^{-1} (23.5) at 50 DAT, tiller number pot^{-1} (32.75) at 60 DAT and effective tillers pot^{-1} (22.75) were obtained in S_3C_4 treatment combination. The maximum days for first flowering (89.5), for 50% flowering (92.25) and for 100% flowering (95.25) were obtained in

S_1C_0 treatment combination. The maximum unfilled grains weight pot^{-1} (4.68 g) was obtained in S_3C_5 treatment combination. The maximum grains weight pot^{-1} (68.91 g), straw weight pot^{-1} (85.5 g), biological yield pot^{-1} (153.45 g) were obtained in S_3C_4 and harvest index (45.95%) was obtained in S_3C_0 treatment combination. The maximum pH (6.6), organic carbon (0.77%) and organic matter (1.32%) of seedbed soil after seedling transplant were recorded in C_5 treatment. The highest total nitrogen (0.14%) in seedbed soil after seedling transplant was recorded in C_4 treatment.

Whereas the minimum average seedling height (13.47 cm), fresh weight of 100 seedlings (4.43 g), oven dry weight of 100 seedlings (3 g) and seedling strength (2.23 mgcm^{-1}) were obtained in S_2C_0 treatment combination. The minimum tiller number pot^{-1} (3) at 40 DAT, tiller number pot^{-1} (5.75) at 50 DAT, tiller number pot^{-1} (8.75) at 60 DAT and effective tillers pot^{-1} (8) were obtained in S_1C_5 treatment combination. The minimum days for first flowering (74.5), for 50% flowering (77.2) and for 100% flowering (79.25) were obtained in S_3C_4 treatment combination. The minimum unfilled grains weight pot^{-1} (2.65 g) was obtained in S_2C_4 treatment combination. The minimum grains weight pot^{-1} (44.82 g) was obtained in S_1C_5 and straw weight pot^{-1} (54.5 g), biological yield pot^{-1} (99.69 g) were obtained in S_1C_0 treatment combination. The minimum harvest index (43.22%) was obtained in S_1C_4 treatment combination. The lowest pH (5.9), total nitrogen (0.05%), organic carbon (0.6%) and organic matter (0.88%) of seedbed soil after seedling transplant were recorded in C_0 treatment. Based on the experimental results, it might be concluded that:

I. Chitosan raw material powder & seedling age influenced morphological characters, yield attributes and grain yield of rice cv. BRRI dhan88 over control.

II. All the treatments (C_1, C_2, C_3, C_4, C_5) using chitosan raw material powder showed better performance than control (C_0) and the performance of the treatment C_4 was the superior than all the treatments. S_3 showed better performance than S_2 and S_1.

III. The chitosan raw material powder improved chemical properties of soil for sustainable agriculture.

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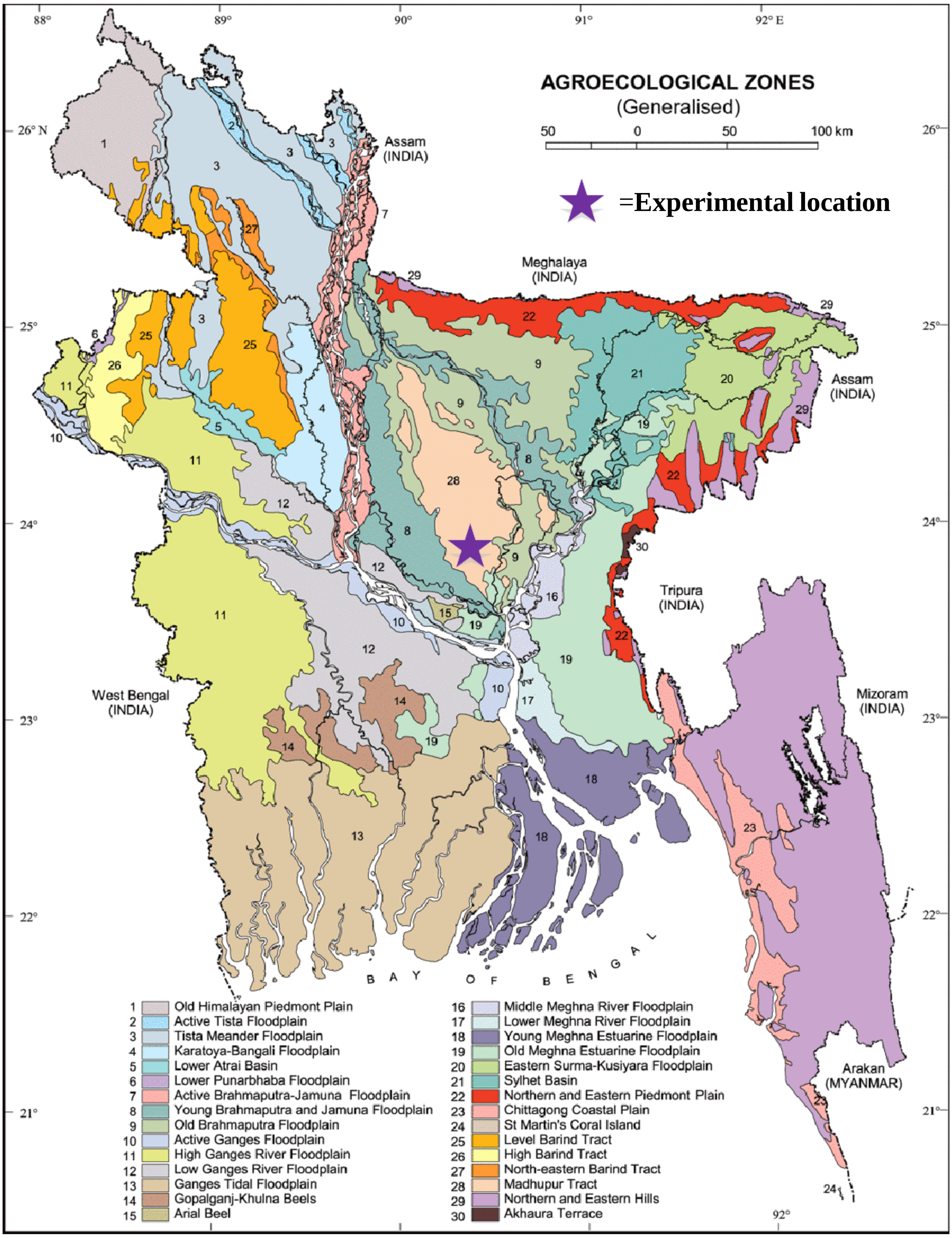
APPENDICES

Appendix I: Map showing the experimental location under study



Appendix II: Monthly meteorological information during the period from November, 2019 to April, 2020

Year	Month	Air Temperature (^{0}C)		Relative Humidity (%)	Total Rainfall (mm)
		Maximum	Minimum		
2019	November	29.6	19.8	53	00
	December	28.8	19.1	47	00
2020	January	25.5	13.1	41	00
	February	25.9	14	34	7.7
	March	31.7	20.2	60	73
	April	32.7	23.8	74	168

Source: Metrological Centre, Agargaon, Dhaka (Climate Division)

Appendix III: Analysis of variance (mean square) of average seedling height, fresh weight, oven dry weight and seedling strength of BRRI dhan88

Source	df	Average seedling height (cm)	Fresh weight (g)	Oven dry weight (g)	Seedling strength (mg/cm)
Replication	2	0.029	0.021	0.021	0.02
Seedling Age (S)	2	15.679*	8466.01*	145.948*	31.979*
Chitosan (C)	5	27.152*	986.56*	41.194*	8.77*
C×S	10	2.993*	511.659*	10.0004*	1.419*
Error	34	0.0005	$2.96E^{-04}$	$2.95E^{-04}$	0.0003
Total	53				

* = Significant at 5% level of probability

Appendix IV: Analysis of variance (mean square) of number of tillers hill^{-1} at different DAT and number of effective tillers hill^{-1} of BRRI dhan88

Source	df	Number of tillers hill^{-1}			Effective tillers hill^{-1}
		40 DAT	50 DAT	60 DAT	
Replication	3	1.903	18.426	21.384	4.889
Seedling Age (S)	2	130.792*	488.847*	672.056*	249.5*
Chitosan (C)	5	10.125*	59.356*	109.981*	85.7*
C×S	10	10.092*	76.597*	136.406*	41.1*
Error	51	1.236	8.044	21.041	8.879
Total	71				

* = Significant at 5% level of probability

Appendix V: Analysis of variance (mean square) of days to first flowering, days to 50 % flowering and days to 100 % flowering of BRRI dhan88

Source	df	Days to first flowering	Days to 50 % flowering	Days to 100 % flowering
Replication	3	0.606	0.537	1.31
Seedling Age (S)	2	644.681*	704.667*	754.042*
Chitosan (C)	5	53.814*	50.6*	57.625*
C×S	10	3.147	3.817*	3.442*
Error	51	2.254	1.87	1.32
Total	71			

* = Significant at 5% level of probability

Appendix VI: Analysis of variance (mean square) of unfilled grains yield pot-1, grains yield pot-1, straw yield pot-1, biological yield pot-1 and harvest index of BRRI dhan88

Source	df	Unfilled grains yield pot^{-1} (g)	Grain yield pot^{-1} (g)	Straw yield pot^{-1} (g)	Biological yield pot^{-1} (g)	Harvest index (%)
Replication	3	0.54749	30.47	28.26	117.4	0.335
Seedling Age (S)	2	3.505*	1001.4*	1006.68*	4016.15*	7.46*
Chitosan (C)	5	2.729*	296.99*	773.99*	2025.12*	6.286*
C×S	10	0.925	17.35	17.25	69.15	0.134
Error	51	0.686	22.55	22.93	90.86	0.183
Total	71					

* = Significant at 5% level of probability

Appendix VII: Analysis of variance (mean square) of pH, total nitrogen, organic carbon and organic matter of seedbed soil after seedling transplant

Source	df	pH	Total Nitrogen (%)	Organic Carbon (%)	Organic Matter (%)
Treatment	5	0.44*	$5.72E^{-03}$*	0.042*	0.098*
Error	18	0.015	$3.3E^{-06}$	0.0003	0.00055
Total	23				

* = Significant at 1% level of probability