

## Foliar application of chitosan and plant probiotic bacteria influencing the growth, productivity and bulb storage life of onion

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### Abstract

Onion having several health promoting phytochemical contents is an important spices in most of the countries of the world as well as vegetables in particular, and chitosan and plant probiotic bacteria are few proven natural growth enhancing agents. Here, three bio-stimulants namely Chitosan (100 and 200 ppm), *Paraburkholderia* BRRh-4 bacteria ( $1.5 \times 10^9$  CFU mL<sup>-1</sup>) and Clybio (0.1%) in singly as well as combining one another were applied at twelve different treatment combinations including control with the aim of investigating the below and above ground vegetative growth, bulb productivity and post-harvest storage performance of onion cv. "Taherpuri". The experiment was set up in Randomized Complete Block Design (RCBD) maintaining three replicates and conducted during November 2019 to June 2020 where the bio-stimulant formulations were sprayed in the foliage at 20, 40 and 60 days after transplanting (DAT). Reproductive behaviors of the onion cultivar differed significantly among the treatments and those were the resultants of statistically varied vegetative performances recorded at 30, 60 and 90 DATs. Chitosan @ 100 ppm + Clybio (0.1%) + *Paraburkholderia* BRRh-4 ( $1.5 \times 10^9$  CFU mL<sup>-1</sup>) combination exhibited statistical superiority over the other treatments in terms of plant height (53.230 cm), number of leaves (10.13/plant), leaf length (41.20 cm) and diameter (10.33 mm), number of roots (32.53/plant), root length (12.23 cm), neck and bulb diameter (11.20 mm and 4.83 cm, respectively) and bulbing ratio (0.23) at 90 DAT. While, shoot and root growth didn't vary significantly at earlier stages (at 30 DAT). Again, the highest yield (3.36 kg/plot and 10.45 t/ha) was also obtained from Chitosan @ 100 ppm + Clybio (0.1%) + *Paraburkholderia* BRRh-4 ( $1.5 \times 10^9$  CFU mL<sup>-1</sup>) treatment. The combination of chitosan and probiotic bacteria had significant influence on post-harvest storage qualities of onion where the longest shelf life/marketable life (8.17 months) with the lowest physiological weight loss and bulb rotting at 90 days after harvest was administered in Chitosan @ 100 ppm + Clybio (0.1%) + *Paraburkholderia* BRRh-4 ( $1.5 \times 10^9$  CFU mL<sup>-1</sup>) treatment. Besides the best treatment, Chitosan @ 100 ppm + Clybio (0.1%) and Chitosan @ 100 ppm + *Paraburkholderia* BRRh-4 ( $1.5 \times 10^9$  CFU mL<sup>-1</sup>) produced statistically superior results over control for onion growth and development. Therefore, chitosan and plant probiotic bacteria can be judiciously used for quality onion production in the warm and humid areas of the world.

**Keywords:** Onion (*Allium cepa* L.); shrimp shell chitosan; *Paraburkholderia* BRRh-4; clybio; root and shoot growth; bulb yield; storage quality.

**Abbreviations:** CFU\_Colony forming unit, DAH\_Days after harvest, DAT\_Days after transplanting, PLW\_Physiological loss in weight, RH\_Relative humidity, T-Max\_Maximum temperature, T-Min\_Minimum temperature

### Introduction

Onion (*Allium cepa*) is the most important member of Alliace family which is used worldwide as culinary and therapeutic spice and vegetable as well. It is an crucial ingredient in many Asian and African sauces and is primarily grown locally, with Egypt being the continent's pioneer producer (Kuete, 2017). The crop has a great economic importance and is the second most important spice crop in the world. Onion bulb is a good source of carbohydrates, proteins, vitamins (B<sub>6</sub>, C), minerals (phosphorus, calcium, magnesium, iron, manganese, sulfur), amino acids and antioxidants. In addition, a variety of secondary metabolites have been identified in onion species such as flavonoids, phenolics, phytosterols, and saponins (Ware, 2017; Marrelli et al., 2019) that are known to diminish cardiovascular ailment, diabetes and antibacterial, antiviral, anti-allergenic.

The green leaves and lowering stalks are also edible and popular. Onion is famous for its characteristics flavor and widely used to increase the taste of foods like gravies, soups, stew stuffing, fried fish and meat but can also be eaten raw or used to make pickles and chutneys (Rashid et al., 2015). In the south-Asian and African countries onion is a high-value spice crop. Households' daily kitchen chores and restaurants as well as the industries demand quality onion throughout the year. But most of the high onion consuming countries fall short of quality onion production in adequate amount. In order to increase crop productivity and improve soil fertility, the farmers indiscriminately use various inorganic fertilizers and crop boosters, which might cause several environmental and health damages (Parr et al., 1991). Also, the intensive use of inorganic fertilizers without

the addition of organic matter leads to poor soil strata, which is more sensitive to wind and rain erosion. To overcome the above problems, environmental advocates and many investigators recommended the use of beneficial soil micro-organisms and products derived from biological transformations. Chitosan, clybio and plant probiotic bacteria might be some key inclusive of such eco-friendly growth booster of crops.

Chitosan, a polycationic polymer of  $\beta$ -1,4, linked D-glucosamine chemically derived from crustaceans and soluble in organic acids is one of a range of natural compounds that have been shown to be effective in decreasing disease incidence in crops (Jitareerat et al., 2007). It is considered environmentally safe for various agricultural uses as it is easily biodegradable, biocompatible, and non-toxic to human beings (Basit et al., 2020). Chitosan and its derivatives have been reported to act as a plant growth regulator and are considered to elicit natural defense responses in plants and have been used as a natural compound to control pre- and post-harvest pathogenic diseases as well as abiotic stresses like drought, salinity, etc. (Uthairatanakij et al., 2007). However, many investigators reported that using chitosan as a foliar spray increased the vegetative growth, yield, and biochemical contents of vegetable crops (El-Miniawy et al., 2013). On the other hand, plant probiotic bacteria are naturally occurring plant-associated microorganisms that enhance the growth of the host plants including yield and may suppress diseases when applied in adequate amounts. They provide beneficial effects to host plants through the production of phytohormones, antibiotics, and lytic enzymes, fixation of atmospheric nitrogen, solubilization of soil mineral nutrients, and induction of systemic resistance in the host plants (Islam and Hossain, 2012). Clybio formulation is the mixture of three probiotics namely yeast fungus, *Bacillus natto* and *Lactobacillus*. Yeast is a natural, safe source of biofertilizer which is typically applied on soil or foliar application on crops improved growth characters and yields of vegetable crops while *Bacillus* and *Lactobacillus* can act as the soil probiotic making it a perfect medium for organic farming to enhance plant growth and protect against disease causing organisms (Ikeda et al., 2013). However, scientific information is meager on the influence of combined applications of chitosan, plant probiotic bacteria and clybio formulation on yield, yield contributing attributes and post-harvest storage life of onion. Beside yield enhancement, these growth regulators can add valuable anti-oxidants and mineral elements to the produces. Considering the above facts, the present investigation was undertaken to observe the influence of chitosan and plant probiotic bacteria on the vegetative and reproductive performance including bulb yield and physical post-harvest attributes of onion in Bangladesh condition.

## Results

### Effect on vegetative growth characters

#### Plant height

Significant variations were noticed among the treatments at 30, 60 and 90 days after transplanting (DAT) for plant height in onion and an increase in plant height was noted with the foliar application of chitosan alone or in combination with *Paraburkholderia* and clybio than control plants (Figure 1). At 30 and 60 DAT, the tallest plant (33.07 cm and 47.33 cm, respectively) was obtained from Chitosan @ 200 ppm +

*Paraburkholderia* combination ( $T_{11}$ ) and Chitosan @ 200 ppm + Clybio combination ( $T_{10}$ ) treatments, respectively having been statistically similar to that of all other treatments except  $T_1$  treatments which exhibited the shortest plant throughout the season (26.68 cm, 42.60 cm and 44.13 cm at 30, 60 and 90 DAT, respectively). While at 90 DAT the longest plant (53.20 cm) was recorded in Chitosan @ 100 ppm + Clybio + *Paraburkholderia* combination ( $T_8$ ) which was statistically succeeded by  $T_9$ ,  $T_{10}$ , and  $T_{11}$  treatments.

#### Number of leaves per plant

Number of leaves per plant varied significantly in response to various treatments at 30 and 90 DAT, whereas at 60 DAT number of leaves per plant didn't differ statistically (Table 1). At 30 DAT, maximum number of leaves per plant (3.47) was counted in Chitosan @ 200 ppm + *Paraburkholderia* combination ( $T_{11}$ ) which was closely followed by  $T_2$ ,  $T_3$  and  $T_8$  treatments. Although there was no statistical difference in the number of leaves per plant among the treatments, but numerically the highest number of leaves per plant (5.93) was recorded in Chitosan @ 200 ppm + Clybio ( $T_{10}$ ) treatment at 60 DAT. At 90 DAT, statistically maximum number of leaves per plant (10.13) was registered in Chitosan @ 100 ppm + Clybio + *Paraburkholderia* ( $T_8$ ) treatment followed by  $T_7$  and  $T_{10}$  treatments. Control plants produced minimum number of leaves per plant (3.07, 5.47 and 7.73, respectively) at all the DATs.

#### Leaf growth

Leaf length and leaf width had significant variation in response to different chitosan and probiotic treatments at different DATs (Table 1). The highest leaf length and leaf diameter was estimated 31.70 cm and 4.93 mm, respectively in plants treated with Chitosan @ 200 ppm + *Paraburkholderia* combination ( $T_{11}$ ) at 30 DAT having statistical similarity to most other treatments except  $T_1$ ,  $T_7$  and  $T_9$  for leaf length and  $T_1$  and  $T_{12}$  for leaf width. Again, at 60 and 90 DAT maximum leaf length and leaf width (41.20 cm and 46.87 cm and 6.80 mm and 10.33 mm, respectively) was resulted in the plants sprayed with Chitosan @ 100 ppm + Clybio + *Paraburkholderia* combination ( $T_8$ ). Statistically inferior growth in terms of leaf length and leaf width was noticed in untreated plants.

#### Root growth

Onion root number and root length were also significantly influenced by different levels of treatments (Table 2). Although there was no statistically significant difference in plant root number among the treatments, but numerically higher and lower number of roots (21.73 and 18.13, respectively) was counted in plants treated with Chitosan @ 100 ppm + Clybio + *Paraburkholderia* combination ( $T_8$ ) and control treatment ( $T_1$ ) at 30 DAT. Again, at 60 and 90 DAT, maximum number of roots (24.53/plant and 32.53/plant) was recorded from Chitosan @ 0 ppm + *Paraburkholderia* ( $T_3$ ) and Chitosan @ 100 ppm + Clybio + *Paraburkholderia* ( $T_8$ ) treatments, respectively. On the other hand, at 60 and 90 DAT, the lengthiest root (8.71 cm and 12.23 cm, respectively) was estimated in plants treated with Chitosan @ 100 ppm + Clybio + *Paraburkholderia* combination ( $T_8$ ) which was statistically followed by  $T_{11}$  and  $T_{12}$  treatments. Whereas, root number as well as root length at 60 and 90 DAT was noted minimum (19.07 and 25.53 and 5.87 cm and 8.75 cm, respectively) in control plants.

### **Neck diameter**

Neck diameter differed significantly in response to different treatments at 30, 60 and 90 days after transplanting of onion seedlings (Table 3). Therefore, Chitosan @ 100 ppm + Clybio + *Paraburkholderia* combination ( $T_8$ ) performed the best in terms of neck diameter at all the DATs (3.97 mm, 8.33 mm and 11.20 mm, respectively) and statistically and numerically identical neck diameter was also revealed by Chitosan @ 100 ppm + Clybio treatment ( $T_6$ ) at both 60 and 90 DAT. Furthermore, neck diameter was observed to be statistically unique at 60 and 90 DAT for all the treatments except control which exhibited minimum diameter (2.20 mm, 6.80 mm and 9.73 mm, respectively) at 30, 60 and 90 DAT.

### **Bulb diameter**

Bulb diameter was positively influenced and varied significantly due to different treatments (Table 3). Statistically maximum and numerically same bulb diameter (1.33 cm) was registered in Chitosan @ 200 ppm + Clybio ( $T_{10}$ ) and Chitosan @ 200 ppm + Clybio + *Paraburkholderia* ( $T_{12}$ ) combinations at 30 DAT which was statistically similar to all other treatments except  $T_2$  and  $T_1$  treatments. While, at 60 DAT, the highest bulb diameter (2.56 cm) was recorded from the treatment Chitosan @ 200 ppm + Clybio ( $T_{10}$ ) which was statistically identical to that of all others except  $T_1$  treatment. Again, bulb diameter at 90 DAT was noticed the highest (4.83 cm) in Chitosan @ 100 ppm + Clybio + *Paraburkholderia* combination ( $T_8$ ) which was similar to  $T_5$ ,  $T_6$ ,  $T_7$  and  $T_{10}$  treatments. But minimum bulb diameter (1.13 cm, 2.03 cm and 3.28 cm, respectively) was observed in control ( $T_1$ ) treatment at all the DATs.

### **Bulbing ratio**

At 30 DAT, significantly maximum bulbing ratio (0.30) was observed in Chitosan @ 100 ppm + Clybio + *Paraburkholderia* treatment ( $T_8$ ) followed by  $T_6$ ,  $T_{10}$ ,  $T_{11}$  and  $T_{12}$  treatments, while minimum bulbing ratio (0.20) was noted in control ( $T_1$ ) treatment (Table 3). On the other hand, the highest bulbing ratio (0.38) at 60 DAT was obtained by foliar spraying the onion plants with Chitosan @ 200 ppm ( $T_9$ ) treatment and the lowest bulbing ratio (0.28) was recorded in control ( $T_1$ ) and Chitosan @ 0 ppm + *Paraburkholderia* ( $T_3$ ). Furthermore, the lowest bulbing ratio (0.23) at 90 DAT was obtained from Chitosan @ 100 ppm + Clybio + *Paraburkholderia* combination ( $T_8$ ) which was statistically similar to all other treatments except  $T_1$  and  $T_{12}$  treatment. Thus, foliar application of different concentrations of chitosan and probiotic bacteria treatment on onion plants produced the lowest bulbing ratio at 90 DAT.

### **Effect on yield and yield contributing characters**

#### **Splitting of bulb**

Splitting of bulbs showed a significant response to different treatments (Figure 2). Maximum percentage of splitting bulb was calculated in  $T_{11}$  (11.08%) treatment followed by  $T_3$  (10.82%),  $T_7$  (11.05%),  $T_{10}$  (10.54%) which was statistically similar to the other treatments and the minimum was recorded in control  $T_1$  (5.69%) which was statistically different from other treatments.

#### **Single bulb weight and bulb yield**

Single bulb weight showed a significant response to different treatments at 30, 60, and 90 DAT (Table 4). At 30 DAT, the heaviest bulb was produced by  $T_8$  (1.96 g) treatment and the lightest bulb was produced by  $T_1$  (1.32 g) treatment. On the other hand, the highest bulb weight was observed from  $T_8$  (11.18 g) treatment at 60 DAT. Treatment  $T_{12}$  (10.70 g) produced the second-highest bulb weight and the lowest bulb weight was found in  $T_1$  (7.68 g) treatment at 60 DAT which was statistically different from all other treatments. Furthermore, the maximum individual bulb weight was recorded from  $T_8$  (42.25 g) treatment followed by  $T_6$  (40.64 g),  $T_{12}$  (39.57 g) and the minimum individual bulb weight was found from  $T_1$  (30.70 g) at 90 DAT. Following this trend, significantly maximum yield was recorded from the treatment  $T_8$  (3.36 kg/plot and 10.45 t/ha) which was statistically similar to  $T_6$  (3.08 kg/plot and 9.50 t/ha) treatment, while the minimum yield per plot was found in  $T_1$  (2.24 kg/plot and 6.89 t/ha) treatment.

### **Effect on post-harvest quality attributes**

#### **Physiological weight loss**

Significant ( $p \leq 0.05$ ) variation was observed in the percentage of weight loss according to the different treatments in various storage durations (10-90 days) (Table 5). Weight loss was noticed maximum in control ( $T_1$ ) treatment throughout the storage period of onion. On the other hand, the minimum physiological weight loss was recorded from  $T_8$  followed by  $T_5$ ,  $T_6$ ,  $T_9$ ,  $T_{12}$  treatment at 90 days of storage.

#### **Rotten bulbs (%)**

The percentage of rotten bulbs of onion at different periods of storage was significantly influenced by different treatments (Table 6). The maximum percentage of rotten bulbs (15.14 %) after 90 days of storage was noted in  $T_1$  which was statistically different from the other treatments, whereas the lowest percentage of rotten bulbs was observed in  $T_8$  (5.66 %) treatment followed by  $T_5$ ,  $T_6$ ,  $T_9$  and  $T_{12}$  treatments.

#### **Shelf life**

Shelf life of onions was greatly influenced and statistically significant ( $p \leq 0.05$ ) by different treatments (Figure 3). The longest shelf life was found in  $T_8$  (8.17 months) treatment, while the lowest shelf life was observed in  $T_1$  (3.50 months) control. However, shelf life of onions was significantly enhanced in  $T_5$  (6.83 months),  $T_6$  (6.90 months),  $T_7$  (6.14 months),  $T_{10}$  (6.50 months), and  $T_{12}$  (6.80 months) treatment, respectively.

### **Discussions**

Onion is one of the most important spices in terms of its economic value and also valuable for containing several many biologically active substances, including phenolic acids, thiosulfinates and flavonoids with proposed health-related properties including antidiabetic, antimicrobial, cardiovascular, anticancer, antioxidant effects, etc. and this important spice crop requires balanced supply of nutrient inputs, chemical as well as naturally occurring growth promoters, for successful growth, development and yield. In the present research, foliar application of chitosan @ 100 ppm when combined with both of clybio @ 0.1% and *Paraburkholderia* BRRh-4 bacteria @  $1.5 \times 10^9$  CFU mL<sup>-1</sup> or separately with each of the two at early vegetative growth phases significantly improved root and shoot growth, neck

and bulb diameter resulting in the statistically lowest bulbing ratio at 90 days after transplanting (Figure 4) compared to the control. Again, the superior vegetative growth with chitosan and probiotic bacteria ultimately improved the bulb yield and post-harvest qualities of onion with extended shelf life (Figure 5).

Shrimp shell chitosan and plant probiotic bacteria have been now-a-days used in crop production as non-chemical growth and yield promoting agents as these increase the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure and reducing the accumulation of harmful free radicals by increasing the enzymatic and other functional activities (Abdel-Mawgoud et al., 2010; Garcia-Seco et al., 2015; Yildirim et al., 2015; Emami Bistgani et al., 2017; Jiménez-Gómez et al., 2017; Hassnain et al., 2020; Chakraborty and Islam, 2022). Plant growth-promoting bacteria improve plant growth parameters by increasing the levels of auxins and cytokinins and decreasing the levels of ethylene and abscisic acid in the plants, which result in enhanced cell division and elongation of plants (Ruzzi and Aroca, 2015). Mondal et al. (2012) opined that the application of chitosan increased the key enzyme activities of nitrogen metabolism (nitrate reductase, glutamine synthetase, and protease) and improved the transportation of nitrogen (N) in the functional leaves as well as increased photosynthesis which enhanced plant growth and development. In the present investigation, chitosan, *Paraburkholderia* and clybio spray improves the vegetative growth indicating increased number of leaves, leaf length and diameter, number of roots and root length in onion over control. Alike this experiment, it is reported that foliar application of chitosan and rhizobacteria alone significantly increased plant growth and development in onion (Dilpreet et al., 2016; Kumar et al., 2017; Sutariati et al., 2019), garlic (Ahmed and Farm, 2015), potato (Shaheen et al., 2019) and pepper (Shrestha et al., 2014). Mukta et al. (2017) and Rahman et al. (2018a,b) also reported that foliar application of chitosan, *Bacillus* and *Paraburkholderia* improved root and shoot growth in treated plants compared to non-treated plants in strawberry. *Bacillus* isolates also have involvement in the modulation of plant development through the production of phytohormones, efficient in producing auxin that might stimulate root proliferation (Bashan and de-Bashan, 2005; Jiménez-Gómez et al., 2016) and nutrient uptake (Tsavkelova et al., 2006) resulting in the vegetative growth of plants as occurred here in the present experiment with onion upon application of clybio consisting of yeast fungus, *Bacillus natto* and *Lactobacillus*. The valuable components of yeast, such as cytokinins, can stimulate cell division and elongation, as well as the synthesis of proteins and nucleic acids, and can increase mineral nutrients and can potentially increase crop growth (Fawzy et al., 2012; Matter and Abou-Sreera, 2016). Lactic acid bacteria strains like *Lactobacillus* are mostly studied as probiotics organisms because they produced various antibacterial compounds, such as indole-3-acetic acid (IAA), lactic acid, hydrogen peroxide, several bacteriocins, and even antifungal compounds (Hamed et al., 2011; Axel et al., 2012; Giassi et al., 2016), which is responsible for plant growth promotion (Mohite, 2013; Shrestha et al., 2014). Reetha et al. (2014) and Tinna et al. (2020) also noted that Rhizobacteria isolates of *Pseudomonas fluorescens* and *Bacillus subtilis* significantly produced higher shoot lengths compared to control plants of onion. Enhanced bulb diameter and consequently low bulbing ratio with the use of

the rhizospheric bacteria *Azotobacter chroococcum*, *Pseudomonas fluorescens*, and *Bacillus subtilis* was reported by Čolo et al. (2014). Present investigation revealed that, foliar application of chitosan and probiotic bacteria produced the lowest bulbing ratio at harvest and that low bulbing ratios are desirable for onion growers (Abdel-Moneim et al., 2015). Splitting of bulb was also noticed in some cases which might be due to fluctuations in day and night temperature during the bulb formation (Steer, 1980) rather than the effects of treatments.

Again, the increase in yield might be due to significantly higher plant height, leaf growth and root growth with combined application of chitosan and probiotic bacteria inoculation leading to the higher synthesis of photosynthates and their better translocation to the sink, as the rate of photosynthesis is significantly correlated with the growth of onion. Čolo et al. (2014) observed that inoculation of onion with *Azotobacter chroococcum*, *Bacillus subtilis* and the mixture of the inoculants significantly increased bulb weight and yield in onion. Similarly Afify et al. (2018) examined that the microbial inoculation combination with chemical fertilizers improves the growth and productivity of plants and increases bulb weights and total bulb yield of onion. Identical observations on enhanced yield and yield contributing characters was noted in several crops through foliar application of chitosan [Sultana et al. (2017) and Parvin et al. (2019) in eggplant and tomato, Rahman et al. (2018a) and Soppelsa et al. (2019) in strawberry, Rodríguez et al. (2017) in potato, Mahmood et al. (2017) in bell pepper, Mehehub et al. (2019) in litchi], plant probiotic bacteria [Karlidag et al. (2010) and Rahman et al. (2018b) in strawberry, Yildirim et al. (2015) in cabbage, Garcia-Seco et al. (2015) in blackberry] and clybio [Akter et al. (2021) in spinach, Uddin et al. (2021) in strawberry].

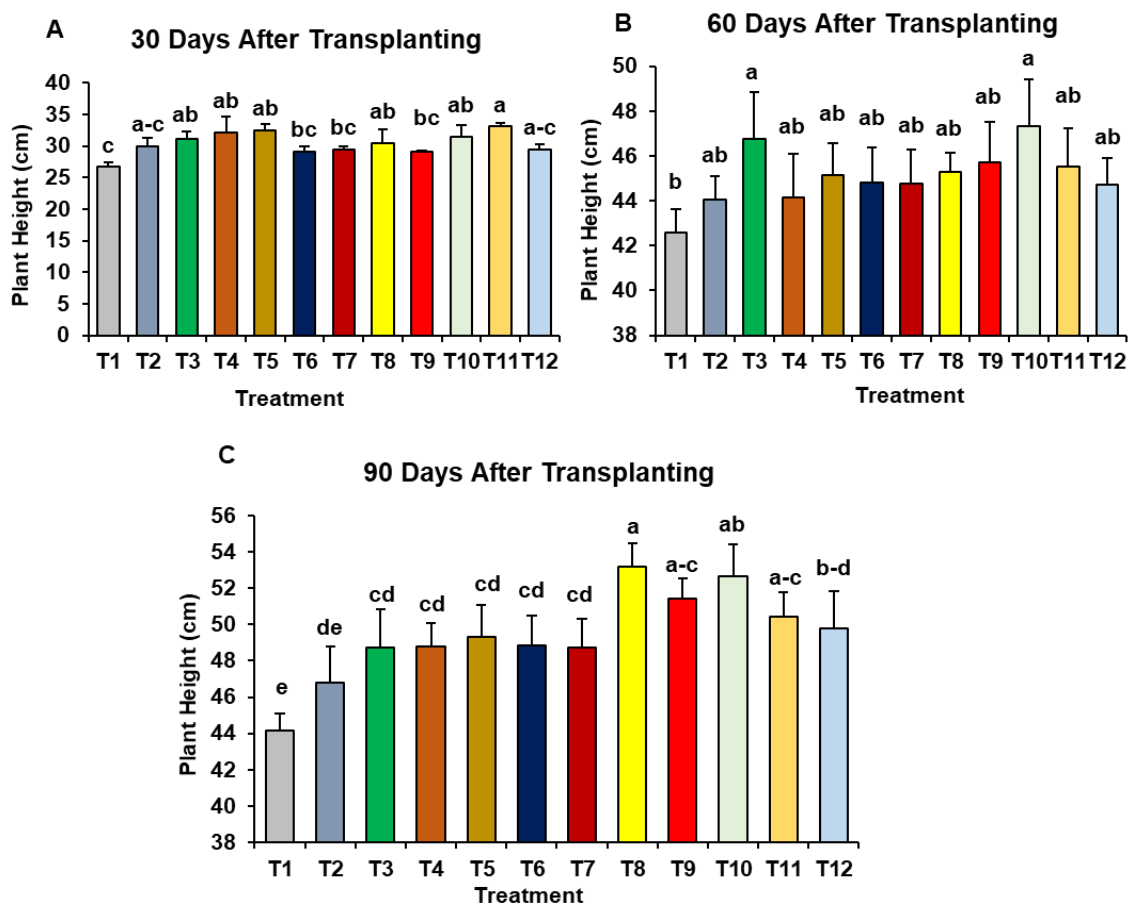
Along with higher yield, chitosan, *Paraburkholderia* and clybio combination enhanced the storability of onion through by minimizing physiological weight loss and rotten bulb percentages which might be resultant of high dry matter accumulation in onion bulb as a function of extended photosynthetic CO<sub>2</sub> fixation in leaves and its translocation to the bulb in chitosan and probiotic bacteria inoculated plants. Besides, chitosan and plant probiotic bacteria are organic growth stimulants that extend the storage through the reduction of respiration rate and water loss and prevent the colonization of pathogen, thereby improves the immune system of the onion bulb (Shehata et al., 2012; Gerjes et al., 2020). Kloepper et al. (2004) reported that *Bacillus subtilis* is a biocontrol agent because they protect plant roots from phytopathogenic fungi such as *Fusarium* and *Pythium*. Liu et al. (2016) also opined that chitosan and oligochitosan enhance ginger resistance to rhizome rot in storage. Furthermore, chitosan, as an antimicrobial agent, induces immunity of plants against many plant pathogenic bacteria and fungi and is likely to be linked with prolonging the shelf life of fruits and vegetables in storage conditions (Romanazzi et al., 2017). In the present experiment, similar phenomena might occur and thereby lower percentages of rotting bulbs were obtained from the combination of chitosan with clybio and *Paraburkholderia* BRRh-4 bacteria treatments. Finally, as a result of such inherently developed immune system and post-harvest qualities in the onion bulb significantly reduced the post-harvest loss and enhanced the shelf life up to 8 months after harvest in ambient storage conditions.

**Table 1.** Effect of chitosan and plant probiotic bacteria on number leaves per plant at different growth stages of onion

Treatment	Number of Leaves /plant			Leaf length (cm)			Leaf diameter (mm)		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T <sub>1</sub>	3.07c	5.47	7.73e	25.63b	36.40d	40.07d	4.27c	5.30c	7.87e
T <sub>2</sub>	3.40ab	5.67	8.13de	27.90ab	37.76cd	41.53cd	4.60a-c	6.20a-c	8.67d
T <sub>3</sub>	3.40ab	5.60	8.20de	28.60ab	39.27a-d	42.60b-d	4.93a	6.40a-c	8.73cd
T <sub>4</sub>	3.33a-c	5.60	8.67cd	29.93ab	38.27a-d	42.20cd	4.80ab	5.87a-c	8.80cd
T <sub>5</sub>	3.20a-c	5.53	8.53d	31.23a	38.97a-d	41.60cd	4.80ab	5.53bc	9.07b-d
T <sub>6</sub>	3.13bc	5.73	9.33b	27.40ab	38.00b-d	42.00cd	4.60a-c	6.47ab	9.60b
T <sub>7</sub>	3.33a-c	5.80	9.60ab	26.40b	39.47a-d	44.00a-c	4.47a-c	5.73a-c	9.13b-d
T <sub>8</sub>	3.40ab	5.80	10.13a	27.55ab	41.20a	46.87a	4.47a-c	6.80a	10.33a
T <sub>9</sub>	3.2a-c	5.53	9.27bc	26.77b	40.60a-c	45.73a	4.33bc	6.60ab	9.40bc
T <sub>10</sub>	3.27a-c	5.93	9.73ab	28.33ab	40.93ab	46.40a	4.53a-c	6.73a	9.20b-d
T <sub>11</sub>	3.47a	5.20	9.40b	31.70a	37.83b-d	42.20cd	4.93a	6.27a-c	9.40bc
T <sub>12</sub>	3.27a-c	5.53	9.40b	27.57ab	38.77a-d	45.33ab	4.33bc	6.33a-c	9.40bc
LSD <sub>(0.05)</sub>	0.297	ns	0.66	4.368	3.136	3.0187	0.5237	1.1374	0.6748
CV (%)	5.34	4.21	4.33	9.13	4.75	4.11	6.74	10.86	4.36

Mean values within a column followed by the same letter do not differ significantly by Fisher's protected LSD test at  $p \leq 0.05$ , ns= not significant.

[T<sub>1</sub>: Control; T<sub>2</sub>: Clybio; T<sub>3</sub>: *Paraburkholderia*; T<sub>4</sub>: Clybio + *Paraburkholderia*; T<sub>5</sub>: Chitosan @ 100 ppm; T<sub>6</sub>: Chitosan @ 100 ppm + Clybio; T<sub>7</sub>: Chitosan @ 100 ppm + *Paraburkholderia*; T<sub>8</sub>: Chitosan @ 100 ppm + Clybio + *Paraburkholderia*; T<sub>9</sub>: Chitosan @ 200 ppm; T<sub>10</sub>: Chitosan @ 200 ppm + Clybio; T<sub>11</sub>: Chitosan @ 200 ppm + *Paraburkholderia*; T<sub>12</sub>: Chitosan @ 200 ppm + Clybio + *Paraburkholderia*]



**Figure 1.** Average plant height of onion at 30 DAT (A), 60 DAT (B) and 90 DAT (C) after foliar application of chitosan and plant probiotic bacteria. Vertical bars on the top of the columns represent the standard errors of means of three replicates. Different letters indicate the statistical differences among the treatments at  $p \leq 0.05$ , as per Fisher's least significant difference test.

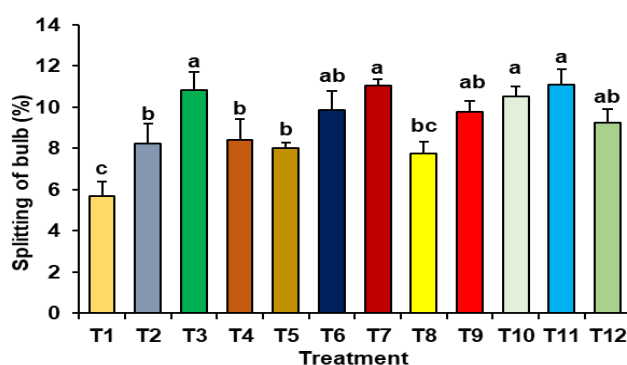
[T<sub>1</sub>: Control; T<sub>2</sub>: Clybio; T<sub>3</sub>: *Paraburkholderia*; T<sub>4</sub>: Clybio + *Paraburkholderia*; T<sub>5</sub>: Chitosan @ 100 ppm; T<sub>6</sub>: Chitosan @ 100 ppm + Clybio; T<sub>7</sub>: Chitosan @ 100 ppm + *Paraburkholderia*; T<sub>8</sub>: Chitosan @ 100 ppm + Clybio + *Paraburkholderia*; T<sub>9</sub>: Chitosan @ 200 ppm; T<sub>10</sub>: Chitosan @ 200 ppm + Clybio; T<sub>11</sub>: Chitosan @ 200 ppm + *Paraburkholderia*; T<sub>12</sub>: Chitosan @ 200 ppm + Clybio + *Paraburkholderia*]

**Table 2.** Effect of chitosan and plant probiotic bacteria on number of roots and root length at different growth stages of onion.

Treatment	Number of roots			Root length (cm)		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T <sub>1</sub>	18.13	19.07c	25.53e	3.67b	5.87e	8.75e
T <sub>2</sub>	19.13	23.60ab	29.80b-d	3.87ab	6.40d	9.61d
T <sub>3</sub>	19.60	24.53a	30.37b-d	3.97ab	6.50cd	10.05cd
T <sub>4</sub>	18.80	22.20a-c	28.93cd	3.90ab	6.60cd	9.76cd
T <sub>5</sub>	21.13	21.40a-c	30.33b-d	4.40a	6.53cd	10.37c
T <sub>6</sub>	19.00	22.07a-c	30.47a-d	3.30b	6.83b-d	10.04cd
T <sub>7</sub>	19.33	23.47ab	31.13ab	3.67b	6.90bc	10.35c
T <sub>8</sub>	21.73	21.87a-c	32.53a	3.83ab	8.71a	12.23a
T <sub>9</sub>	19.60	21.42a-c	28.67d	3.67b	6.63cd	10.29c
T <sub>10</sub>	19.93	23.93ab	30.93a-c	3.33b	6.60cd	10.37c
T <sub>11</sub>	19.53	20.93bc	31.33ab	3.53b	7.13b	11.04b
T <sub>12</sub>	20.53	22.87ab	31.60ab	3.77ab	7.30b	11.37b
LSD (0.05)	ns	3.4908	2.0909	0.7234	0.4685	0.6197
CV (%)	6.15	9.25	4.1	11.42	4.05	3.54

Mean values within a column followed by the same letter do not differ significantly by Fisher's protected LSD test at  $p \leq 0.05$ , ns= not significant.

[T<sub>1</sub>: Control; T<sub>2</sub>: Clybio; T<sub>3</sub>: *Paraburkholderia*; T<sub>4</sub>: Clybio + *Paraburkholderia*; T<sub>5</sub>: Chitosan @ 100 ppm; T<sub>6</sub>: Chitosan @ 100 ppm + Clybio; T<sub>7</sub>: Chitosan @ 100 ppm + *Paraburkholderia*; T<sub>8</sub>: Chitosan @ 100 ppm + Clybio + *Paraburkholderia*; T<sub>9</sub>: Chitosan @ 200 ppm; T<sub>10</sub>: Chitosan @ 200 ppm + Clybio; T<sub>11</sub>: Chitosan @ 200 ppm + *Paraburkholderia*; T<sub>12</sub>: Chitosan @ 200 ppm + Clybio + *Paraburkholderia*]



**Figure 2.** Influence of chitosan and plant probiotic bacteria on splitting of bulb of onion. Vertical bars on the top of the columns represent the standard errors of means of three replicates. Different letters indicate the statistical differences among the treatments at  $p \leq 0.05$ , as per Fisher's least significant difference test.

[T<sub>1</sub>: Control; T<sub>2</sub>: Clybio; T<sub>3</sub>: *Paraburkholderia*; T<sub>4</sub>: Clybio + *Paraburkholderia*; T<sub>5</sub>: Chitosan @ 100 ppm; T<sub>6</sub>: Chitosan @ 100 ppm + Clybio; T<sub>7</sub>: Chitosan @ 100 ppm + *Paraburkholderia*; T<sub>8</sub>: Chitosan @ 100 ppm + Clybio + *Paraburkholderia*; T<sub>9</sub>: Chitosan @ 200 ppm; T<sub>10</sub>: Chitosan @ 200 ppm + Clybio; T<sub>11</sub>: Chitosan @ 200 ppm + *Paraburkholderia*; T<sub>12</sub>: Chitosan @ 200 ppm + Clybio + *Paraburkholderia*]

## Materials and methods

### Site details and planting material

The field and laboratory works of experiment was carried out at the research field and laboratory of the Department of Horticulture Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur-1706, Bangladesh during November 2019 to June 2020. Soil of the experimental field was shallow red-brown terrace soil under the Salna Series (FAO, 1999) in Madhupur Tract (Agroecological Zone 28) having a pH 6.71. The soil had 1.70% organic matter, 0.115% N, 21.35 ppm soluble P and 0.24 meq./100 g soil exchangeable K. The area was characterized by heavy rainfall, excessive humidity, high temperature relatively long days from April to September and scanty rainfall, low humidity, low temperature and a short-day period from October to March. Healthy, insect-pest and pathogen free seeds of the onion cultivar "Taherpuri" were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh prior to cultivation.

### Treatment preparation and application

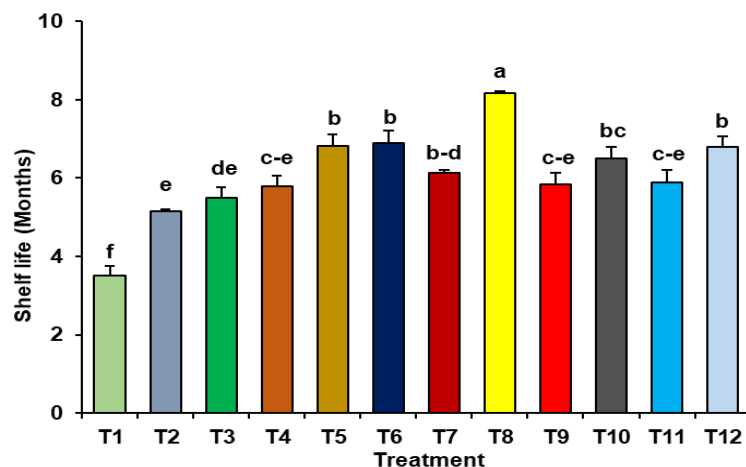
The treatment of the experiment consists of three types of naturally occurring bio-stimulants namely chitosan polymerase (95% deacetylated) in two separate concentrations (100 ppm and 200 ppm), one plant probiotic bacteria (*Paraburkholderia fungorum* BRRh-4) at  $1.5 \times 10^9$  CFU mL<sup>-1</sup> concentration and clybio (a complex formulation of three probiotics such as yeast fungus, *Bacillus natto*, and *Lactobacillus*) at 0.1% dose in alone and in combinations of any two and all the three types along with no spray. Thus, including control (T<sub>1</sub>) a total of twelve different treatment combinations were made namely T<sub>2</sub>: Clybio, T<sub>3</sub>: *Paraburkholderia*, T<sub>4</sub>: Clybio + *Paraburkholderia*, T<sub>5</sub>: Chitosan @ 100 ppm, T<sub>6</sub>: Chitosan @ 100 ppm + Clybio, T<sub>7</sub>: Chitosan @ 100 ppm + *Paraburkholderia*, T<sub>8</sub>: Chitosan @ 100 ppm +

**Table 3.** Effect of chitosan and plant probiotic bacteria on neck and bulb diameter and bulbing ratio at different growth stages of onion.

Treatment	Neck diameter (mm)			Bulb diameter (cm)			Bulbing ratio		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T <sub>1</sub>	2.20e	6.80c	9.73b	1.13c	2.03b	3.28e	0.20e	0.28b	0.29a
T <sub>2</sub>	2.77d	7.20a-c	10.07ab	1.15bc	2.38a	3.64de	0.24b-d	0.31ab	0.28ab
T <sub>3</sub>	3.00cd	7.93a-c	10.87ab	1.28a	2.52a	3.98cd	0.23c-e	0.28ab	0.28ab
T <sub>4</sub>	2.87cd	7.27a-c	10.87ab	1.27a	2.27ab	3.98cd	0.23de	0.32ab	0.28ab
T <sub>5</sub>	2.80d	6.87bc	10.40ab	1.25ab	2.43a	4.37a-c	0.22de	0.32ab	0.24b
T <sub>6</sub>	3.33bc	8.33a	11.20a	1.23ab	2.30ab	4.60ab	0.27a-c	0.36a	0.24b
T <sub>7</sub>	2.93cd	7.60a-c	10.53ab	1.23ab	2.37ab	4.50a-c	0.24cd	0.36a	0.24b
T <sub>8</sub>	3.97a	8.33a	11.20a	1.31a	2.52a	4.83a	0.30a	0.37a	0.23b
T <sub>9</sub>	3.07cd	8.20ab	11.07ab	1.29a	2.32ab	4.22bc	0.24cd	0.38a	0.27ab
T <sub>10</sub>	3.80ab	8.07a-c	10.93ab	1.33a	2.56a	4.43a-c	0.28a	0.32ab	0.25ab
T <sub>11</sub>	3.67ab	7.60a-c	10.47ab	1.32a	2.32ab	4.26bc	0.28ab	0.33ab	0.24b
T <sub>12</sub>	3.70ab	7.73a-c	10.60ab	1.33a	2.49a	4.22bc	0.28a	0.32ab	0.25ab
LSD <sub>(0.05)</sub>	0.5263	1.3956	1.3545	0.1017	0.3227	0.5533	0.0375	0.0705	0.054
CV (%)	9.79	10.76	7.5	4.76	8.02	7.89	8.86	12.67	12.39

Mean values within a column followed by the same letter do not differ significantly by Fisher's protected LSD test at  $p \leq 0.05$ , ns= not significant.

[T<sub>1</sub>: Control; T<sub>2</sub>: Clybio; T<sub>3</sub>: *Paraburkholderia*; T<sub>4</sub>: Clybio + *Paraburkholderia*; T<sub>5</sub>: Chitosan @ 100 ppm; T<sub>6</sub>: Chitosan @ 100 ppm + Clybio; T<sub>7</sub>: Chitosan @ 100 ppm + *Paraburkholderia*; T<sub>8</sub>: Chitosan @ 100 ppm + Clybio + *Paraburkholderia*; T<sub>9</sub>: Chitosan @ 200 ppm; T<sub>10</sub>: Chitosan @ 200 ppm + Clybio; T<sub>11</sub>: Chitosan @ 200 ppm + *Paraburkholderia*; T<sub>12</sub>: Chitosan @ 200 ppm + Clybio + *Paraburkholderia*]



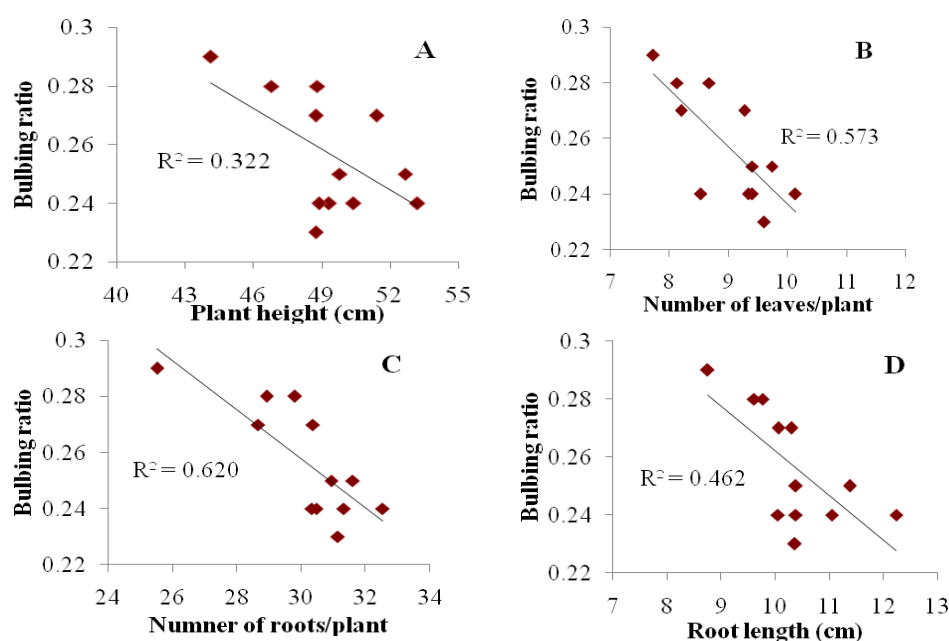
**Figure 3.** Effect of chitosan and plant probiotic bacteria on shelf life of onion in storage at room temperature ( $25 \pm 1^\circ\text{C}$ ). Vertical bars on the top of the columns represent the standard errors of means of three replicates. Different letters indicate the statistical differences among the treatments at  $p \leq 0.05$ , as per Fisher's least significant difference test. [T<sub>1</sub>: Control; T<sub>2</sub>: Clybio; T<sub>3</sub>: *Paraburkholderia*; T<sub>4</sub>: Clybio + *Paraburkholderia*; T<sub>5</sub>: Chitosan @ 100 ppm; T<sub>6</sub>: Chitosan @ 100 ppm + Clybio; T<sub>7</sub>: Chitosan @ 100 ppm + *Paraburkholderia*; T<sub>8</sub>: Chitosan @ 100 ppm + Clybio + *Paraburkholderia*; T<sub>9</sub>: Chitosan @ 200 ppm; T<sub>10</sub>: Chitosan @ 200 ppm + Clybio; T<sub>11</sub>: Chitosan @ 200 ppm + *Paraburkholderia*; T<sub>12</sub>: Chitosan @ 200 ppm + Clybio + *Paraburkholderia*]

**Table 4.** Effect of chitosan and plant probiotic bacteria on single bulb weight at different growth stages and bulb yield (kg/plot and kg/ha) of onion

Treatment	Single bulb weight (g)			Bulb yield	
	30 DAT	60 DAT	90 DAT	kg/plot	kg/ha
T <sub>1</sub>	1.32 c	7.68 d	30.70 f	2.24 d	6.89 d
T <sub>2</sub>	1.41 bc	9.73 bc	32.98 ef	2.90 bc	8.93 bc
T <sub>3</sub>	1.48 bc	9.76 bc	35.21 de	2.61 c	8.04 c
T <sub>4</sub>	1.39 bc	9.43 bc	32.82 ef	2.98 b	9.18 b
T <sub>5</sub>	1.44 bc	9.20 c	34.43 de	3.02 b	9.32 b
T <sub>6</sub>	1.45 bc	9.26 c	40.64 ab	3.08 ab	9.50 ab
T <sub>7</sub>	1.48 bc	9.45 bc	36.51 cd	2.82 bc	8.8 bc
T <sub>8</sub>	1.96 a	11.18 a	42.25 a	3.36 a	10.45 a
T <sub>9</sub>	1.4 bc	9.41bc	36.28 cd	2.93 bc	9.03 bc
T <sub>10</sub>	1.39 bc	9.60 bc	39.37 b	2.92 bc	9.04 bc
T <sub>11</sub>	1.49 bc	9.38 bc	38.11 bc	2.77 bc	8.53 bc
T <sub>12</sub>	1.55 b	10.70 ab	39.57 ab	2.83 bc	8.73 bc
LSD <sub>(0.05)</sub>	0.1773	1.3169	2.8112	0.3329	1.0606
CV	7.07	8.13	4.54	6.85	7.06

Mean values within a column followed by the same letter do not differ significantly by Fisher's protected LSD test at  $p \leq 0.05$

[T<sub>1</sub>: Control; T<sub>2</sub>: Clybio; T<sub>3</sub>: *Paraburkholderia*; T<sub>4</sub>: Clybio + *Paraburkholderia*; T<sub>5</sub>: Chitosan @ 100 ppm; T<sub>6</sub>: Chitosan @ 100 ppm + Clybio; T<sub>7</sub>: Chitosan @ 100 ppm + *Paraburkholderia*; T<sub>8</sub>: Chitosan @ 100 ppm + Clybio + *Paraburkholderia*; T<sub>9</sub>: Chitosan @ 200 ppm; T<sub>10</sub>: Chitosan @ 200 ppm + Clybio; T<sub>11</sub>: Chitosan @ 200 ppm + *Paraburkholderia*; T<sub>12</sub>: Chitosan @ 200 ppm + Clybio + *Paraburkholderia*]



**Figure 4.** Correlation graphs showing the reduced bulbing ratio in onion with relation to enhanced plant growth [plant height (A), number of leaves plant<sup>-1</sup> (B), number of roots plant<sup>-1</sup> (C) and root length (D)] recorded at 90 days after transplanting upon application of chitosan and plant probiotic bacteria

Clybio + *Paraburkholderia*, T<sub>9</sub>: Chitosan @ 200 ppm, T<sub>10</sub>: Chitosan @ 200 ppm + Clybio, T<sub>11</sub>: Chitosan @ 200 ppm + *Paraburkholderia* and T<sub>12</sub>: Chitosan @ 200 ppm + Clybio + *Paraburkholderia*. Required amount of chitosan was dissolved to 2% acetic acid and diluted in distilled water followed by heating and agitating for 30 minutes and rested to get normal temperature and pH being adjusted at 6.2 through adding 0.1N NaOH. Nutrient free *Paraburkholderia fungorum* BRRh-4 suspension at a concentration of approximately  $1.5 \times 10^9$  CFU mL<sup>-1</sup> was prepared with modifying the method of Rahman et al. (2018b) where bacterial isolates were cultured in 1000 mL nutrient broth in conical flask and agitated at 80 rpm and 27 °C for 72 hours in incubator. The broth was centrifuged at 12,000 g and the pellet was washed thrice with sterilized distilled water to remove nutrients. Clybio made up of Yeast, *Lactobacillus* and *Bacillus natto* bacteria with the power of enzyme

(indigenous microbial nutrient such as amino acid and peptide-based hormone substances) being collected from *Compass Corporation* was diluted to 0.1% concentration by adding 1 mL of clybio solute to 1000 mL of distilled water. The treatments were applied as foliar spray thrice at 20 days intervals starting from 20 days after transplanting i.e., at 20 days, 40 days and 60 days after transplanting (DAT).

#### Experimental design and layout

The experiment was laid out in randomized complete block design (RCBD) with three replications. The experiment's field was first divided into 3 blocks, each block containing 12 unit plots represented a replication where each plot received a treatment and thereby a total of 36 unit plots measuring 1.8 m × 1.8 m were prepared where the 12 treatments were randomly assigned in the 12 plots of a block. The distance between the blocks was 50 cm and between the plots was



**Table 5.** Effect of chitosan and plant probiotic bacteria on physiological weight loss (PLW) (%) of onion at 10 to 90 days after harvest.

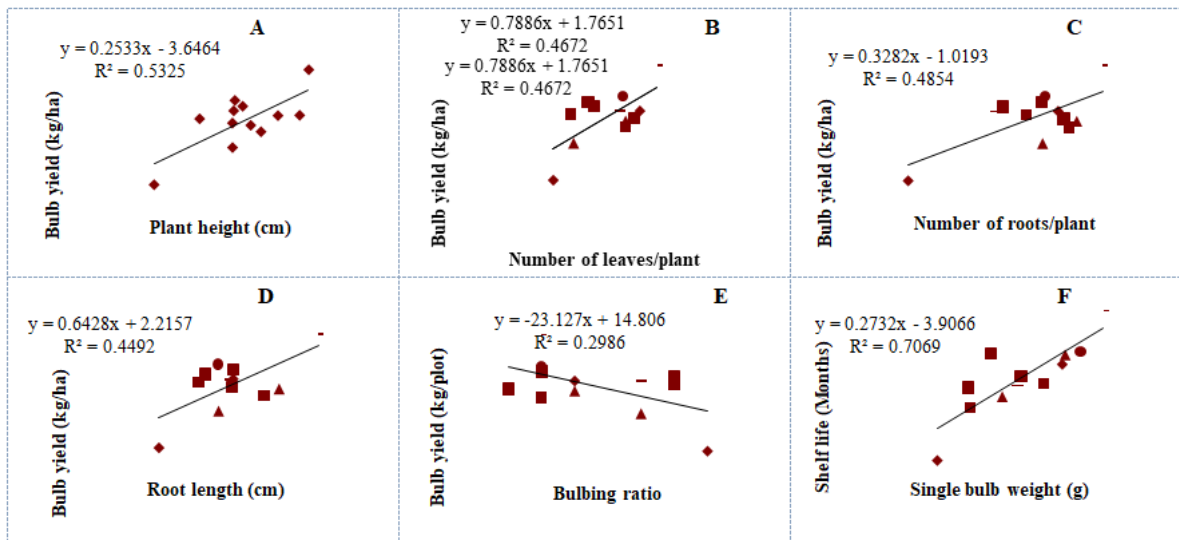
Treatment	Physiological loss in weight (%) at different days after harvest (DAH)								
	10DAH	20DAH	30DAH	40DAH	50DAH	60DAH	70DAH	80DAH	90DAH
T <sub>1</sub>	7.64 a	10.43 a	11.73 a	13.18 a	15.31 a	17.34 a	18.68 a	20.43 a	20.60 a
T <sub>2</sub>	3.43 c-f	5.44 cd	8.32 b	10.64 b	11.67 bc	12.27 bc	14.48 b	13.31 b	10.34 b
T <sub>3</sub>	4.02 b-d	5.64 b-d	8.17 b	9.53 bc	12.04 b	13.34 b	13.25 bc	12.22 bc	9.72 b
T <sub>4</sub>	4.11 bc	5.02 c-e	7.72 bc	9.22 b-d	10.49 b-d	11.55 c	12.51 c	10.41 cd	9.16 b
T <sub>5</sub>	2.49 f	3.31 f	3.88 e	5.48 g	7.15 g	9.15 de	8.48 e	6.60 fg	4.97 c
T <sub>6</sub>	2.79 ef	4.63 de	4.12 e	5.86 fg	7.42 g	9.33 de	10.65 d	9.20 de	6.28 c
T <sub>7</sub>	4.76 b	6.70 b	6.80 cd	8.63 c-e	10.06 c-f	11.84 c	12.57 c	10.91 de	9.26 b
T <sub>8</sub>	2.85 d-f	4.05 ef	4.67 e	5.73 fg	7.18 g	8.40 e	9.25 de	6.14 g	4.63 c
T <sub>9</sub>	3.74 b-e	5.70 b-d	6.39 d	7.28 ef	8.48 e-g	10.16 d	10.63 d	9.16 de	6.38 c
T <sub>10</sub>	3.90 b-e	5.27 cd	6.41 d	7.76 de	8.80 d-g	9.45 de	10.53 d	9.34 de	8.87 b
T <sub>11</sub>	3.70 b-e	5.77 bc	7.13 b-d	8.73 c-e	10.23 b-e	12.34 bc	12.68 bc	10.31 c-e	9.15 b
T <sub>12</sub>	3.54 c-f	5.06 c-e	6.22 d	7.70 de	8.31 fg	9.83 d	10.35 d	8.37 ef	6.25 c
LSD <sub>(0.05)</sub>	1.1947	1.1213	1.2713	1.6165	1.8919	1.1063	1.8375	2.0194	1.8059
CV	18.02	11.86	11.05	11.49	11.45	5.81	9.04	11.32	12.12

Mean values within a column followed by the same letter do not differ significantly by Fisher's protected LSD test at  $p \leq 0.05$ . [T<sub>1</sub>: Control; T<sub>2</sub>: Clybio; T<sub>3</sub>: *Paraburkholderia*; T<sub>4</sub>: Clybio + *Paraburkholderia*; T<sub>5</sub>: Chitosan @ 100 ppm; T<sub>6</sub>: Chitosan @ 100 ppm + Clybio; T<sub>7</sub>: Chitosan @ 100 ppm + *Paraburkholderia*; T<sub>8</sub>: Chitosan @ 100 ppm + Clybio + *Paraburkholderia*; T<sub>9</sub>: Chitosan @ 200 ppm; T<sub>10</sub>: Chitosan @ 200 ppm + Clybio; T<sub>11</sub>: Chitosan @ 200 ppm + *Paraburkholderia*; T<sub>12</sub>: Chitosan @ 200 ppm + Clybio + *Paraburkholderia*].

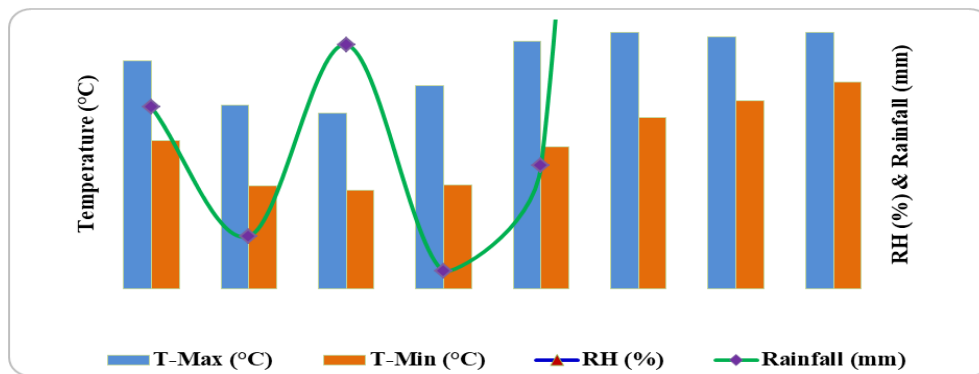
**Table 6.** Effect of chitosan and plant probiotic bacteria on rotten bulbs (%) of onion at different days after harvest (DAH).

Treatment	Rotten bulbs (%)		
	30 DAH	60 DAH	90 DAH
T <sub>1</sub>	12.21 a	14.5 a	13.04 a
T <sub>2</sub>	7.18 c-e	10.68 cd	9.31 b
T <sub>3</sub>	7.11 c-e	10.62 c-e	7.78 c
T <sub>4</sub>	7.44 c-e	11.04 c	7.15 cd
T <sub>5</sub>	6.41 e	9.66 ef	5.75 f
T <sub>6</sub>	6.71 de	9.90 d-f	5.93 f
T <sub>7</sub>	9.94 b	12.14 b	7.43 cd
T <sub>8</sub>	6.28 e	9.38 f	5.66 f
T <sub>9</sub>	7.18 c-e	10.42 c-e	5.83 f
T <sub>10</sub>	8.76 bc	11.21 bc	6.93 de
T <sub>11</sub>	6.7 de	9.90 d-f	6.23 ef
T <sub>12</sub>	8.12 cd	11.2 bc	5.87 f
LSD <sub>(0.05)</sub>	1.6613	0.9709	0.7076
CV	12.52	5.27	5.77

Mean values within a column followed by the same letter do not differ significantly by Fisher's protected LSD test at  $p \leq 0.05$ . [T<sub>1</sub>: Control; T<sub>2</sub>: Clybio; T<sub>3</sub>: *Paraburkholderia*; T<sub>4</sub>: Clybio + *Paraburkholderia*; T<sub>5</sub>: Chitosan @ 100 ppm; T<sub>6</sub>: Chitosan @ 100 ppm + Clybio; T<sub>7</sub>: Chitosan @ 100 ppm + *Paraburkholderia*; T<sub>8</sub>: Chitosan @ 100 ppm + Clybio + *Paraburkholderia*; T<sub>9</sub>: Chitosan @ 200 ppm; T<sub>10</sub>: Chitosan @ 200 ppm + Clybio; T<sub>11</sub>: Chitosan @ 200 ppm + *Paraburkholderia*; T<sub>12</sub>: Chitosan @ 200 ppm + Clybio + *Paraburkholderia*].



**Figure 5.** Correlation graphs showing the bulb yield enhancement of onion as a function of increased plant height (A), number of leaves/plant (B), number of roots/plant (C), root length (D) and reduced bulbing ratio (E) and shelf life extension with increased bulb weight (F) upon application of chitosan and plant probiotic bacteria



**Figure 6.** Average monthly maximum and minimum temperature, mean monthly relative humidity and monthly total rainfall of the growing area during November 2019 to June 2020 (Source: Department of Agricultural Engineering, BSMRAU)

30 cm with the plant spacing of 15 cm × 15 cm to facilitate different intercultural operations. As many as 144 plants were accommodated in each unit plot and a total of 5184 plants were planted in the 36 unit plots.

**Crop culturing and management**

Prior to sowing the seeds were soaked overnight in water and allowed to sprout in a piece of cloth kept under the sunshade for two days. The sprouted seeds were sown directly on a raised seedbed in the nursery on 25<sup>th</sup> November 2019. Upon germination (4-5 days after sowing), the young seedlings were exposed to dew by night and mild sunshine in the morning and evening. On 30<sup>th</sup> December 2019, 30-day old healthy, disease-free and uniform seedlings were transplanted to the main field with the spacing of 15 cm × 15 cm accommodating 144 plants in each unit plot. All intercultural operations like fertilizer management, insect-pest management, irrigation, weeding, mulching, etc were practiced as per guidelines and requirements. Before 7 days of harvest, when the plants attained maturity by showing drying up of leaves and weakening of necks, they were bent at the soil level by hands and kept as such up to harvest to fasten bulb maturity. Onion bulb was harvested by lifting with spade and hand on 25<sup>th</sup> April 2020. Care was taken so that no bulb was injured during lifting. Curing of onion was

done for three days under a shed. Tops were separated from bulbs with a knife leaving 2 cm neck at ambient temperature (25.6 ± 2.6)<sup>0</sup> C and then bulbs were stored in a well-ventilated house (25 ± 1<sup>0</sup> C) separately as per treatments.

**Measurement of growth, yield and storage qualities**

Plant vegetative growth is an important indicator for reproductive performance. At the present study with onion, vegetative parameters like plant height (cm), number of leaves per plant, leaf length (cm), leaf width (mm), neck diameter (mm), number of roots, root length (cm) and bulb diameter (cm) were measured and recorded. Bulbing ratio was also calculated through dividing the neck diameter by bulb diameter. The growth parameters were measured from randomly selected five onion plants of each treatment plot and averages were used. All the data were gathered at 30, 60 and 90 days after transplanting (DAT). Again, after harvest total number of bulbs, individual bulb weight (g), yield per plot (kg) and yield per hectare (t) were estimated by a weighing balance. Splitting of bulb (%) was recorded by the following formula-

$$\text{Splitting of bulb (\%)} = \frac{\text{Number of split bulbs} \times 100}{\text{Number of total bulbs (plants)}}$$

Physiological loss in weight (PLW) (%) and rotten bulb (%) were also recorded. The following formula were used for

calculating PLW (%) and rotten bulb (%) – PLW (%) = (Fresh or initial weight – Storage or final weight × 100/Initial weight  
Rotten bulb (%) = Number of rotten bulbs × 100/Total number of bulbs  
Shelf life of onion in storage was determined by keeping the bulbs in ambient temperature (25 ± 1 °C) in normal storage conditions at the laboratory and observing the bulb quality up to optimum marketing.

### Statistical analysis

The collected data were tabulated and analyzed statistically using MSTAT-C statistical package program. The treatment means were separated by Fisher's Least Significant Difference (LSD) test, using a p-value of ≤ 0.05 to be statistically significant (Gomez and Gomez, 1984). Correlation analysis with the treatment means were also performed for bulbing ratio, bulb yield and shelf life in relation to different vegetative growth parameters observed at 90 days after transplanting using MS Office Excel 2007.

### Conclusion

Utilization of naturally occurring chitosan biopolymer and plant probiotic bacteria has been practiced for quality enhancement of produce with multiple advantages to human health and environment. The present investigation exhibited that shrimp shell chitosan, clybio and *Paraburkholderia* in combinations enhance the vegetative and reproductive growth of onion. Among the combinations, 3-time foliar spray Chitosan (100 ppm) + Clybio (0.1%) + *Paraburkholderia* ( $1.5 \times 10^9$  CFU mL<sup>-1</sup>) significantly enhanced the growth as well as bulb production of onion at field level under semi-arid condition in Bangladesh. Furthermore, the same treatment also responded for maximum shelf life through minimizing physiological weight loss and protecting rotting of onion bulb in storage.

### Acknowledgements

The authors would like to express their heartiest gratitude to the Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh for the giving opportunity to accomplish the whole research activities; Institute of Biotechnology and Genetic Engineering, BSMRAU for offering some of the laboratory facilities and Bangladesh Agricultural Research Institute, Gazipur, Bangladesh for supplying with the planting materials.

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