

**DIRECT AND RESIDUAL EFFECT OF ORGANIC MATERIAL
(SHRIMP SHELL POWDER) ON RADISH-SPINACH CROPPING
SYSTEM**

RUKAIA TABASSUM



DEPARTMENT OF SOIL SCIENCE

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

DHAKA-1207

JUNE, 2022

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SYSTEM**

By

RUKAIA TABASSUM

Reg. No.: 15-06421

E-mail: rukaia6421@gmail.com

Mobile: 01521201700

*A Thesis Submitted to
The Department of Soil Science,
Sher-e-Bangla Agricultural University, Dhaka-1207
In partial fulfillment of the requirements
For the degree of*

MASTER OF SCIENCE (M.S.)

IN

SOIL SCIENCE

SEMESTER: JANUARY-JUNE, 2022

Approved by:

.....
Prof. Dr. Mohammad Issak
Supervisor
Department of Soil Science
Sher-e-Bangla Agricultural University
Dhaka-1207

.....
Prof. Dr. Md. Asaduzzaman Khan
Co-supervisor
Department of Soil Science
Sher-e-Bangla Agricultural University
Dhaka-1207

.....
Prof. Dr. Mohammad Saiful Islam Bhuiyan
Chairman
Department of soil science
Examination committee
Sher-e-Bangla Agricultural University
Dhaka-1207



**DEPARTMENT OF SOIL SCIENCE
SHER-E-BANGLA AGRICULTURAL UNIVERSITY,
DHAKA-1207**



Prof. Dr. Mohammad Issak
Department of soil science
Mobile :01716-238645
Email:
mdissaksau07@yahoo.com

CERTIFICATE

*This is to certify that thesis entitled, “**DIRECT AND RESIDUAL EFFECT OF ORGANIC MATERIAL (SHRIMP SHELL POWDER) ON RADISH - SPINACH CROPPING SYSTEM**” 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 bonafide research work carried out by **RUKAIA TABASSUM**, Registration No.15-06421 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*


I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Date: JUNE, 2022

Place: Dhaka, Bangladesh

.....

Prof. Dr. Mohammad Issak
Supervisor
Department of Soil Science
Sher-e-Bangla Agricultural University



**Dedicated To
My Beloved Parents
And
Respected Teachers**

ACKNOWLEDGEMENTS

All praises to the almighty ALLAH who enables me to complete the thesis for the degree of degree of Master of Science (M.S.) in Soil Science.

I feel much pleasure to express my gratitude, sincere appreciation and heartfelt indebtedness to my reverend research supervisor, **Prof. Dr. Mohammad Issak**, Department of Soil Science, Sher-e-Bangla Agricultural University for his scholastic guidance, support, encouragement, valuable suggestions and constructive criticism throughout the research work and guidance in preparation of manuscript of this thesis.

I also express my gratefulness to respected co-supervisor, **Prof. Dr. Md. Asaduzzaman khan**, Department of Soil Science, Sher-e-Bangla Agricultural University for his helpful suggestion and valuable advice during the preparation of this manuscript.

I would like to express my sincere gratitude to my respectable teacher **Prof. Dr. Mohammad Saiful Islam Bhuiyan**, chairman, Department of Soil Science, Sher-e-Bangla Agricultural University for his valuable advice and providing necessary facilities to conduct the research work.

I would like to express my deepest respect and thanks to all my honorable teachers of the Department of Soil Science, Sher-e-Bangla Agricultural University for their valuable suggestions, instructions, cordial help and encouragement.

I also express my sincere appreciation and gratitude to Ministry of Science and Technology, The People's Republic of Bangladesh for awarding me National Science and Technology (NST) fellowship.

I am extremely thankful to all MS student of January-June/2020 semester, all officers and staffs of the Department of Soil Science, Sher-e-Bangla Agricultural University for their kind support and sincere cooperation during my study.

Finally, I express my deepest sense of gratitude to my beloved parents, sisters, brothers, other family members, relatives, well-wishers and friends for their inspiration, help and encouragement throughout the study.

JUNE, 2022
SAU, DHAKA

The Author

ABSTRACT

Two consecutive pot experiments were conducted under the net house of Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh, during November 2020 to January 2021, to investigate direct and residual the effect of organic material (shrimp shell powder) on yield performance of radish- spinach cropping system. BARI mulashak-1 was used as first crop and BARI palonshak-1 was used as second crop. After harvesting first crop (radish), the second crop (spinach) was sown without using any plant nutrients. The experiment was consisted of single factor with eight (8) treatments & four (4) replications following Completely Randomized Block Design (RCBD). The treatments were, T₁ : 0% shrimp shell powder, T₂: 0.1% shrimp shell powder, T₃: 0.2% shrimp shell powder, T₄: 0.3% shrimp shell powder , T₅: 0.4% shrimp shell powder , T₆: 0.5% shrimp shell powder, T₇: 0.75% shrimp shell powder and T₈: 1% shrimp shell powder. Result revealed that significant variation was observed in germination (%), plant height (cm pot⁻¹), fresh weight (g pot⁻¹), oven dry weight (g pot⁻¹) pot of radish and spinach. In case of first crop (radish), the yield and yield contributing characters increased significantly and the treatment T₄ and T₅ show similar and better performance than others but in the treatment T₇ and T₈, negative response was observed as germination (%) has dropped suddenly. In case of second crop (spinach), the yield and yield contributing characters positively responded with the increased rate of organic material (shrimp shell powder) and highest plant height (34.28cm pot⁻¹), highest average fresh weight (308g pot⁻¹) and average oven dry weight (20.5g pot⁻¹) was observed in treatment T₈ compared to control treatment T₁. Significant variation over the control was also observed on soil nutrient content like soil OC (%) , total N (%), available phosphorus (ppm) & available sulphur (ppm) content. The result showed that application of shrimp shell powder improves soil pH in both post- harvest soil of first crop and post-harvest soil of residual crop. So, overall result indicated that application of organic material (shrimp shell powder) in soil has significant impact on growth and yield of first crop (radish) and second crop (spinach) and on chemical properties of soil.

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LISTS OF ABBREVIATIONS

FULL FORM	ABBREVIATIONS
Agro-Ecological Zone	AEZ
Analysis of Variance	ANOVA
And others	<i>et al.</i>
Bangladesh Agricultural Research Institute	BARI
Bangladesh Bureau of Statistics	BBS
Bangladesh Rice Research Institute	BRRI
Centimeter	Cm
Chitosan	CHT
Days After Sowing	DAS
Degree Celsius	°C
Duncan's Multiple Range Test	DMRT
Food and Agriculture Organization	FAO
Gram	G
Hectre	Ha
Least Significant Difference	LSD
Number	No.
Nitrogen	N
Organic carbon	OC
Organic matter	OM
Parts Per Million	Ppm
Phosphorus	P
Percentage	%
Coefficient of Variance	CV%
Randomized Complete Block Design	RCBD
Residual	Res.
Shrimp shell powder	SS powder
Serial	Sl.
Soil Resource Development Institute	SRDI
Sulphur	S
United States Department of Agriculture	USDA
Weight	Wt.



CHAPTER I

INTRODUCTION

Sustainable, safe and nutritious food productions are the major challenges for global food security which meet dietary needs and food preferences for an active and healthy life. According to the Bangladesh Bureau of Statistics (2018), approximately 12,000 farmers in Bangladesh produce organic crops on around 7,000 hectares of land. At present, consumers are becoming conscious and critical about the quality of food products for a healthy life (Mottalib *et al.*, 2018). In Bangladesh, every year, a noticeable number of people are affected by agrochemicals and suffering from diseases (Shammi *et al.*, 2020).

Vegetables is one of the most important source of minerals and nutrients. At present, the average consumption of vegetables is 75 g/day whereas the recommended per capita vegetable consumption is 250 g/day according to Food and Agriculture Organization (FAO, 2017). Bangladesh is now the third largest producer of vegetables in the world (BBS, 2020). Bangladesh is now considered as one of the fastest growing vegetable producers in the world as the country managed to make the total production almost doubled in the last decade and the annual demand for vegetables in Bangladesh is about 13.25 million tons whereas the production is only 3.73 million tons (Rahman *et al.*, 2020). Through organic vegetables production both diversification and commercialization of crops can be enhanced in our country. In organic production, organic manure is supplied by organic matter.

The global demand of organic vegetables is augmenting day by day. The demand for organic produce is increasing with increasing awareness (Bhatta *et al.*, 2008). In 2020, Asia was accounted for more than three-fourths of the world vegetables production. Organic farming is the key component for sustainable agriculture. For safe environment, soil and human health, a need arises to adopt organic farming. Organic vegetables production increases the quality of the produce which increases the income of the farmer.

Radish (*Raphanus sativus*) is a common and popular crop in Bangladesh and is a good source of vitamin A. It is a winter season-short duration vegetables used

as root vegetables. It can be consumed fresh as in salad or cooking any dish. Radish is rich in antioxidant and minerals like calcium and potassium. Radish leaf is rich in vitamin A, B and C. In Bangladesh, annual production of radish is 3.22 lakh tones in around 66582.8 acre of land (BBS, 2021). Nitrogen application upholds the overall growth, yield and quality of radish (Brintha and Seran, 2009). Spinach (*Spinacia oleracea L.*) is a common vegetable crop grown everywhere in Bangladesh. It is an annual plant belongs to the family Chenopodiaceae. In Bangladesh, annual production of radish is 61.8 lakh tones in around 24,509 acre of land (BBS 2021). It is a highly desirable leafy vegetables and has high nutritional value and it is also called a life protective food (Tewani *et al.*, 2016). It contains about Ca (215mg), K (611mg), Mg (55mg) along with vitamin C, A and K (USDA, 2016).

Application of plant growth regulator (PGR) seems to be one of the important practices in view of convenience, cost and labor efficiency. Recently, there has been global realization of the important role of PGRs in agriculture for better growth and yield of crops. Chitosan, a new plant growth regulator like GA3 that may have many uses to improve the growth, yield and yield attributes of plant. Chitosan are structural components in the cuticle of crustacean, insects, mollusk and in the cell wall of fungi and plant pathogens (Boonlertnirum and boonroung, 2008).

A new organic substance, shrimp shell powder can be used for improvement of soil fertility. When the degree of deacetylation of chitin reaches about 50% (depending on the origin of the polymer), it becomes soluble in aqueous acidic media and is called chitosan (Rinaudo, 2006). Chitosan has wide scope of application with high affinity and no toxicity and it has no harm to humans and livestock (Hamed *et al.*, 2016). Good quality vegetables has great demand and has enormous benefits for the human health due to their color, good flavor and nutrient contents (vitamins, minerals and amino acids) (Xing *et al.*, 2016). While Bangladeshi vegetables are exported to more than 38 countries, the top ten buyers are concentrated in two regions: Europe (the United Kingdom and Italy) and the Middle East (EPB, 2016). Chitosan is a natural biopolymer modified from chitin, which is the main structural component of squid pens, fungi shell wall and shrimp and crab shells. Chitosan is a derivative of chitin and is considered the second

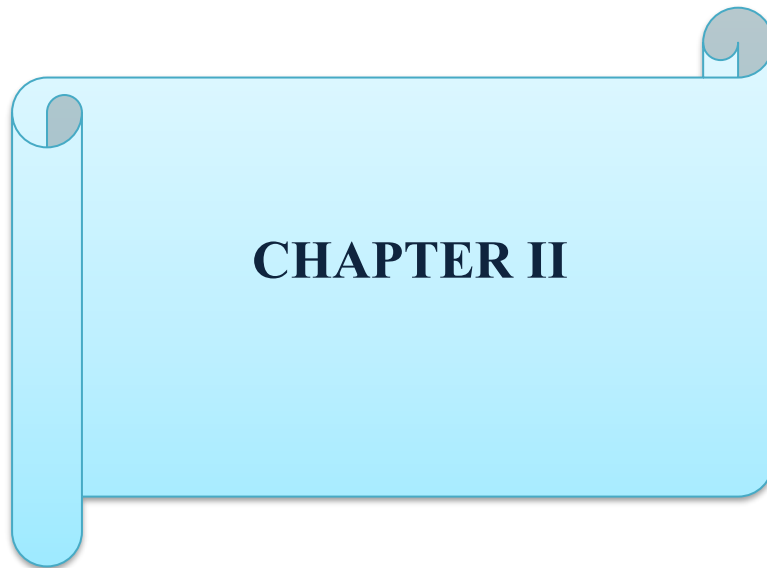
most common polymer in the world after cellulose (Pandey *et al.*, 2018). Chitosan treatment stimulates photosynthesis process, stomatal closure by synthesis of ABA; increases antioxidant enzymes via nitric oxide and hydrogen peroxide signaling pathways and production of organic acids, sugars, amino acids and other metabolites are enhanced which are required for the osmotic adjustment, stress signaling, and energy metabolism under stresses (Hidangmayum *et al.*, 2019). Chitosan is used as natural seed treatment and plant growth enhancer and plant treated with chitosan showed significantly greater number of branches/plant than untreated control. In agriculture, chitosan is used primarily as a natural seed treatment and plant growth enhancer and as an ecologically friendly bio-pesticide substance that boosts the innate ability of plants to defend themselves against fungal infections (Linden *et al.*, 2000). Bacteria decompose chitosan in the soil which is then absorbed in the plant roots. Application of chitosan in agriculture, even not using chemical fertilizer, can increase the microbial population by large numbers, and transforms organic nutrient into inorganic nutrient, which is easily absorbed by the plant roots (Choi, 2016). It plays a significant role in the quality rice seedling production that help to increase the grain yield (Issak *et al.*, 2017). Chitosan acts as a bio-fungicide, bio-bactericide, and bio-virucide, which stimulates defense mechanism of plants against the pathogen, thus the immune system of fruits and vegetables plants is enhanced (Kaya *et al.*, 2017). It also enhanced the nutrient uptake efficiency and mimic cadmium stress in radish plant (farouk, 2011). Seed soaked in chitosan solution before planting tends to stimulate plant height and also increase the plant height with increasing the concentration of oligochitosan (Sultana, 2015). Chitosan controls the immune system of plant by secretion of resisting enzymes. Moreover, plants treated with chitosan may be less prone to environmental stress such as drought, salinity and temperature (Pongprayoon *et al.*, 2013).

In agriculture, chitosan is used as a fertilizer (Lemondé *et al.*, 2011), phytosanitary products and also used to trigger plant defense mechanisms (Le devedec, 2008). It plays an important role in the stimulation of plant growth and in mobilization of soil nutrients (Le devedec, 2008). Chitosan is highly soluble in dilute organic acid such as acetic acid, lactic acid etc making it easy and useful in applications in agriculture and in aqueous solutions at various concentrations.

Bangladesh is one of the largest exporters and producers of shrimp along with the production 1200 tones chitosan in every year to make an annual export of Chitosan worth \$1.2 billion, which is 3.34% of the country's present export earnings (BBS, 2020). By 2022, overall global supply of Chitosan will be only 70,000 tons against a demand for 155,500 tons. Therefore, we have abundant raw materials for shrimp shell powder production. It has strong effects on agriculture such as acting as the carbon source for microbes in the soil, accelerating of transformation process of organic matter into inorganic nutrients and assisting the rootsystem of plants to absorb more nutrients from the soil (Ibrahim *et al.*, 2015). Chemical properties of rice growing soils improve due to the residual effect of the rawmaterial of chitosan powder. (Sultana *et al.*, 2020).

Limited information is available on the effect of organic growth promoting substance like shrimp shell powder on the growth and yield of leafy vegetables in Bangladesh. Therefore, Two consecutive pot experiments were conducted to find out yield performance of (BARI palonshak-1 and BARI mulashak-1) and to investigate the effect on soil as influenced by the organic material (Shrimp shell powder) under the Agro Ecological Zone (AEZ-28) Madhupur Tract. By considering the above described reasons, the proposed research work was undertaken to achieve the following objectives:

Objective: 1. To observe the effect of organic material (shrimp shell powder) on radish-spinach cropping system



CHAPTER II

CHAPTER II

REVIEW OF LITERATURE

Growth and development of radish and spinach are greatly influenced by the environmental factors, variety and cultural practices. Among these factors organic materials play notable role regarding the growth and development of experimental crops. An endeavor was made in this section to collect and study the consequential information available in the country and abroad regarding organic vegetables farming using organic material (Shrimp shell powder) to acquire knowledge which is helpful in executing the present research work and subsequently writing up the results and discussion. Most of reports showed that there is positive effect of application of shrimp shell powder on crops and vegetables. Some of the related reports are reviewed below:

Saravanan *et al.* (1987) reported that the organic manures viz. sludge and spray of chitosan increases the efficiency of applied N and he used the treatments as combined application of bio-organic and chemical fertilizers and observed the combined effects on physical properties, nitrogen transformation and yield of rice in submerged soils.

Oka and Pivonia (2003) demonstrated that many chitinolytic organisms establish beneficial symbiotic interactions with plants (mycorrhiza and *Rhizobium* spp.), favoring vegetal absorption of certain nutrients and especially nitrogen fixation. For example, amendments of chitin together with fertilizers as urea have been used to improve soil microbiota, to control pathogenic organisms and to strengthen plant nutrition, all these showing better results than the controls in tomato, carnation and grazing.

Ouyang and langlai (2003) carried out an experiment and revealed that seeds of non-heading Chinese cabbage dressed with chitosan at the rate 0.4-0.6 mg g⁻¹ seed and spraying with 20-40 mg L⁻¹ in the leaf increased fresh weight.

Synowiecki and Nadia (2003) revealed that chitosan or chitin derivatives enhanced plant resistance and boosts up plant immunity and so plant do not easily affected by germs and cures the disease by itself. It increases the yield.

Botlo *et al.* (2004) found in his experiment on Ion exchange for the removal of naturalorganic matter that chitosan can increase the microbial population and transforms organic nutrient into inorganic nutrient which can be uptaken easily by plants.

Lee *et al.* (2005) investigate the effects of chitosan on the productivity and nutritional quality of soybean (*Glycine max L.*) sprouts. Soybean seeds were soaked in solutions containing 1,000 ppm chitosan of low (<10 kDa), medium (50 to 100 kDa), and high (>1,000 kDa) molecular weight. Within 1 day of treatment, a significant increase in respiration, 5%, was observed in the sprouts treated with high molecular weight chitosan. The growth- improving effects of chitosan were proportional to the molecular weight of the chitosan molecule used in the experiment. Surprisingly, chitosan application did not result in any significant reduction in vitamin C content or postharvest chlorophyll formation, traits that determine the nutritional and marketing values of soybean sprouts. Results revealed that soaking soybean seeds in a solution of chitosan, especially of high molecular weight, may effectively enhance the productivity of soybean sprouts without adverse effects on the nutritional and postharvest characteristics.

Hu *et al.* (2006) revealed that with increasing of chitosan acid solution, the water stable aggregate and permeability coefficient of soil increased, however, bulk destiny,CEC and pH of the soil were reduced, the soil EC decreased firstly and then raised. The changes of physical and chemical properties appeared stable when the dose of chitosan exceeded 0.45% (chitosan mass concentration relative to dry soil) on the soil.

Martinez *et al.* (2007) showed in his experiment that the best response was obtained when tomato seeds were treated with 1 mg/L chitosan during four hours, as this concentration stimulated significantly plant dry weight, although the other indicators were not modified.

Boonlertnirun *et al.* (2008) carried out an experiment to observe the effect of chitosan application in rice production and found that, chitosan is an actual biopolymer which stimulates growth and increases yield of rice as well as induces the immune system of rice plants and chitosan increases seedling dry matter, tiller number and strength significantly.

Asghari-Zakaria *et al.* (2009) investigated in their experiment on effect of soluble chitosan on plantlets that at 750 and 1000 mg L⁻¹ of chitosan application the culture medium failed to solidify. Application of 500 mg L⁻¹ of soluble chitosan increased the shoot fresh weight, but its lower concentrations did not significantly affect this trait ($P < 0.05$). The 5 and 15 mg L⁻¹ of soluble chitosan led to a significant increase in root fresh and dry weight whereas, higher concentrations, especially 500 mg L⁻¹, significantly decreased root fresh weight of plantlets. Application of 500 mg L⁻¹ chitosan resulted in improved acclimatization of plantlets in the greenhouse as expressed by significant ($P < 0.05$) increase in mini tuber number and yield, compared to the control.

El-Tantawy *et al.* (2009) noticed in this experiment on tomato plants that chitosan spraying significantly increased all vegetative parameters (plant height plant⁻¹ and number of both branches and leaves plant⁻¹), fresh and dry weight of different plant organs.

Guan *et al.* (2009) carried out an experiment on maize plant and showed that application of oligochitosan enhances maize seedlings growth and 60 mg L⁻¹ chitosan spray has positive role on root, shoot and leaves of several crop plants and in low temperature chitosan stimulates the growth of maize plant compared to control. It was also found that different chitosan concentration significantly induce the seed germination in maize.

Abdel-Mawgoud *et al.* (2010) conducted a pot experiment and they reported that application of chitosan at 2 mg L⁻¹ increased the yield of strawberry plant and number and weight of fruit increased.

Sultana (2010) from BAEC, Bangladesh reported that chitosan plays a significant role on growth and productivity of Maize (*Zea mays* L.) Plants on which the oligochitosan was applied for its potential use as plant growth promoter. The

application of oligochitosan (molecular weight 7,000 Da) as foliar spraying @ 25, 50 and 75 mg L⁻¹ was done on maize. The findings revealed that the application of oligochitosan at the concentration of 75 mg L⁻¹ plays a significant role in terms of weight of cob and weight of seeds per Maize and ultimately maize yield.

Ziani *et al.* (2010) found that chitosan treated seeds grew successfully (with longer, and better developed radicles and greener hypocotyles) than untreated seeds. The incorporation of nutrients (nitrogen) from chitosan could potentially be connected to the observed growth of plants.

Dzung *et al.* (2011) reported that as a result of spraying seedlings with chitosan solutions of the molecular weight of 2kDa, the content of chlorophylls and carotenoids in leaves increased by 15.36% comparison to the control.

Chookhongkha *et al.* (2012) observed that the seedlings grown in the soil mixed with 1.0% (w/w) high MW of chitosan presented the greatest growth rate and chlorophyll content, and a higher number of dark green leaves followed by medium and low MW of chitosan at 30 days after transplant (DAT) of rice plants. A comparison of chitosan concentrations at 0.5 and 1.0% (w/w) on the growth and seed productivity was done in the green house. Results revealed that, the greatest seed yield indicated by fruit fresh weight plant-1, fruit numbers plant-1, seed numbers fruit-1, and seed weight plant-1 was observed in the plants grown in the soil treated with 1.0% high MW of chitosan.

Mondal *et al.*, (2012) investigated the effect of different concentrations of chitosan viz., 0, 50, 75, 100 and 125 ppm on okra. The result revealed that, morphological parameters like plant height, leaf number of okra had significantly increased by spraying of chitosan @ 100 or 125 ppm at 25, 40 and 55 DAS of okra.

Nguyen and Tran (2013) performed an experiment to know the effect of chitosan solution application for rice production in Vietnam and reported that the chitosan produced from shrimp shells using dilute acetic acid proved effective in controlling plants infection by microbial agents leading to higher yields. The study showed that the yields of rice significantly increased (~31%) after applying

chitosan solution. So, applying chitosan increased rice production and reduced cost of significantly.

Nguyen Van *et al.* (2013) found that application of chitosan can increase the chlorophyll content which in turn enhanced the nutrient uptake of plant. The authors demonstrated that after spraying the seedlings with chitosan of the molecular weight of 600 kD a three times, an increase of the content of nitrate, phosphorus and potassium in leaves by respectively 9.8–27.4%, 17.3–30.4% and 30–45% was observed. It was also proved that seedlings sprayed with a 10–50 ppm chitosan solution were characterized by increased intensity of net photosynthesis.

Hameed *et al.* (2014) found that chitosan is able to mitigate the injuries of salt stress and increase salt tolerance of wheat seeds and seedlings growth. All the chitosan priming treatments efficiently improved germination rate and shoot and root length. However, tested chitosan priming treatments had beneficial effects on seedgermination and seedling vigor. Moreover, chitosan is able to mitigate the injuries of salt stress and increase salt tolerance of wheat seeds and seedlings.

Farooq and Nawaz, (2014); Khaleel *et al.* (1981), and Matsumoto *et al.* (1999) showed that FCW application to the soil also led to an increased organic matter in the soil. OM improves the physical, chemical and biological properties of soil, as well as giving a better soil aggregation, available water content and enhanced cation exchange capacity, leading to improved soil fertility. The results found that @ 1% FCW the organic matter differ significantly from 0.5% FCW, 0.25% FCW and the rest of the treatment. Organic matter level ranges from 1.82 to 2.35 among the treatment. @ 1% FCW the highest organic matter level was obtained (2.35) and lowest organic matter level was obtained (1.82) with CF = soil supplemented with chemical fertilizer.

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 productivity. This paper takes a closer look at the physiological responses of chitosan molecule.

Munshi (2015) stated that organic carbon content in the postharvest soil was affected by different treatments of modified chitosan and ranged from 0.63% to 0.97%. Maximum organic carbon content (0.97%) was found in T₄ treatment (4.0 t ha⁻¹ modified chitosan) and minimum organic carbon content (0.63%) was found in T₅ treatment (without modified chitosan).

Rahman (2015) reported after his experiment that higher organic matter content was found in the soils having treated with modified chitosan than control where no application of modified chitosan. The result also suggests that modified chitosan application would be helpful to improve the sustainable soil health and maximum organic carbon content (1.14 %) was found in the treatment T₅ having applications of 200 g of modified chitosan in the seedbed and minimum carbon content (0.94%) was found in the treatment T₁ (control).

Sathiyabama *et al.* (2015) found that yield of tomato plants increased with chitosan treatment and the synthesized Cu- chitosan nanoparticles exhibited antibacterial activity against gram negative and gram positive bacteria.

Agbodjato *et al.* (2016) reported that Chitosan has a positive effect on rhizobacteria growth, where Chitosan possesses a symbiotic relation with growth promoting rhizobacteria, thus triggered germination rate and improving plant nutrient uptake

Wang *et al.* (2016) found that chitosan can accelerate the speed of germination of wheat seed and improve the benefits for seedling growth under low temperature stress. Moreover, chitosan is able to mitigate the injuries of salt stress and increases salt tolerance of wheat seeds and seedlings.

Islam (2016) reported that soils with chitosan treatment (500g m⁻²) have higher organic matter content (2.04%) than control (no chitosan) and application of chitosan in the seedbed soil tends to increment of organic carbon content.

Rabbi *et al.* (2016) carried out experiment on mungbean plant using of different concentrations of chitosan viz., 0 (control), 25, 50, 75 and 100 ppm as treatments at 30 and 40 DAS. Results showed that foliar application of chitosan @ 50 ppm enhanced morphological characters such as plant height, number of branches, number of leaves and leaf area plant⁻¹ significantly.

Hassan *et al.* (2017) concluded that Chitosan and oligo chitosan are the well-known bio control 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. For achieving the goal of sustainable agriculture, chitosan will become a popular plant protectant.

Issak and Sultana (2017) observed in their experiment of role of chitosan on the production of quality rice seedlings that chitosan has the role in quality of rice seedlings of BBRI dhan29 and fresh weight of the produce. Out of six treatments T₄ (400g chitosan powder m⁻²) showed superior results and minimum observation was found in the treatment T₆ (control).

Duan *et al.* (2017) revealed that chitosan had a positive effect on seed germination and can accelerate the contents of Chlorophyll, Chlorophyll a, Chlorophyll b, total Chlorophyll content and soluble protein of Barley. It can accelerate the speed of germination of seed and improve the benefits for seedling growth under low temperature stress.

Sultana *et al.* (2017) investigated the effect of oligo-chitosan (O. chitosan) on growth, yield attributes and economic yield of tomato and egg-plant under Bangladesh conditions. Three levels of oligo-chitosan concentration viz. 0 (control), 60 and 100 ppm were used. Oligo chitosan was sprayed five times after sowing. The results indicated that plant height and number of flowers plant-1 increased with increasing concentration of chitosan spray till 100 ppm. Foliar spray with 60 and 100 ppm O-chitosan were effective in increasing total yield plot G1 of tomato (41.67 and 38.30 kg, respectively) than control (22.79 kg). The acidity and protein content in tomato has been significantly ($p < 0.05$) decreased from plant treated with 60 ppm chitosan whereas, 100 ppm chitosan application significantly ($p < 0.05$) increased protein content in eggplant. The powerful antioxidant (phenolic content) component was found to be increased ($p < 0.05$) significantly with chitosan application in eggplant but decreased only with lower dose in tomato. It was concluded that the growth and functional components of tomato and eggplant enhanced when the foliar application of oligo-chitosan was done at early growth stage.

Rahman *et al.* (2018) conducted a field experiment on strawberry plant. Foliar applications of chitosan on strawberry significantly increased plant growth and fruit yield (up to 42% higher) compared to untreated control. Increased fruit yield was attributed to higher plant growth, individual fruit weight and total fruit weight/plant due to the chitosan application. Surprisingly, the fruit from plants sprayed with chitosan also had significantly higher contents (up to 2.6-fold) of carotenoids, anthocyanins, flavonoids and phenolics compared to untreated control. Total antioxidant activities in fruit of chitosan treated plants were also significantly higher (ca. 2-fold) ($p < 0.05$) than untreated control.

Esyanti *et al.* (2019) found in their experiment that chitosan was able to promote growth of chilli plant and increase the resistance of *Phytophthora* infection and application of chitosan increases chlorophyll production and number of leaves/plant.

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.

Ahmed *et al.* (2020) found that the application of chitosan raw material in theseedbed have positive influence on the height of seedling in seedbed.

Chakraborty *et al.* (2020) reported that chitosan 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.

Sultana *et al.* (2020) found in a field experiment on BRR1 dhan29 that there is residual effect of chitosan on soil. The total nitrogen content, soil pH, organic carbon and organic matter status in the post-harvest-soils were increased due to the residual effect of the powder in rice growing soils. The maximum value of the pH (7.01), organic carbon content (0.72%) and organic matter content (1.24%) in the post- harvest soils were found in the treatment T₄ and lowest values were observed in the control treatment (T₅).

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.

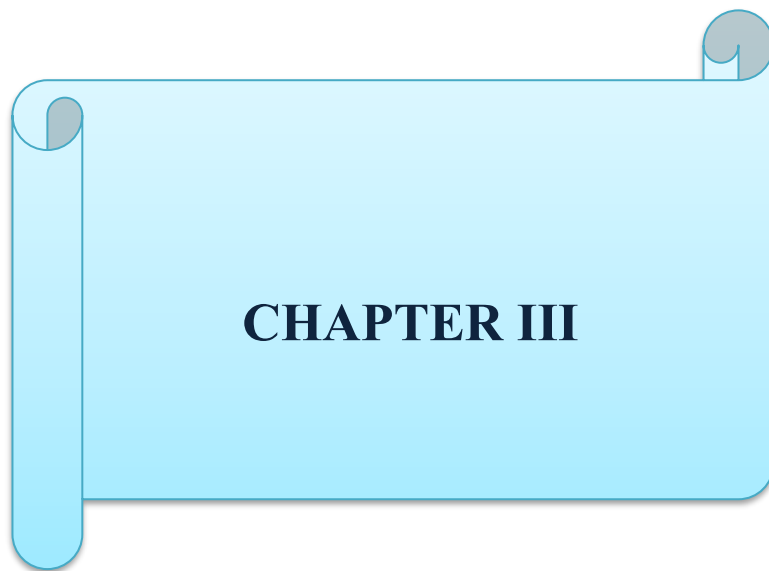
Riseh R.S. *et al.* (2022) observed that Chitosan is a candidate polymer for the encapsulation of probiotic bacteria for agricultural purposes. The attractive features of chitosan include its biocompatibility with other materials, easy digestion and dissolution, non-toxicity, high adsorption power, and 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.

Ahmad *et al.* (2022) observed the highest length of fruits was achieved at 75 ppm and the highest average diameter at 100 ppm chitosan concentration. The impact of chitosan on the total number of fruits and the total weight showed a positive result. Chitosan concentrations of 75–100 ppm showed the best performance in disease control. Foliar applications of chitosan showed increased vegetative growth, better cucumber fruit quality and plant disease control compared to control plants (0 ppm).

Divya *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 mg/ml of chitosan nanoparticles 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 chitosan nanoparticles was also studied to deduce the mechanism of action. The soil toxicity of chitosan nanoparticles 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; U₁ : application of a full recommended dose of classical urea (165 kg N ha⁻¹); U₂ : adding recommended dose of classical urea by 80% + exogenous urea–chitosan nanohybrid 250 mg N L⁻¹; U₃ : adding recommended dose of classical urea by 80% + exogenous urea–chitosan nanohybrid 500 mg N L⁻¹; U₄ : adding recommended dose of classical urea by 60% + exogenous urea–chitosan 26 nanohybrid 250 mg N L⁻¹; U₅ : adding recommended dose of classical urea by 60% + exogenous urea–chitosan nanohybrid 500 mg N L⁻¹; U₆ : adding recommended dose of classical urea by 40% + exogenous urea–chitosan nanohybrid 250 mg N L⁻¹; and U₇ : adding recommended dose of classical urea by 40% + exogenous urea–chitosan nanohybrid 500 mg N L⁻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 N L⁻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 N L⁻¹ as a compensatory alternative can be used in saving 40% of classical urea requirement.

Kazimi and Saxena (2023) found that 100 ppm level of foliar spraying chitosan have effect on average plant height, medium number of leaves, leaves area, chlorophyll content and fruit weight of tomato whereas 4ml L⁻¹ improved the mineral situation in cucumber.



CHAPTER III

CHAPTER III

MATERIALS AND METHOD

The chapter contains material and method of two consecutive experiments carried out both in same place. The pot experiments were conducted at the net house of sher-e-bangla Agricultural University, Dhaka-1207 to investigate the direct and residual effect of organic material (Shrimp shell powder) on BARI mulashak-1 and BARI palonshak-1 (at germination, different days after sowing and on yield and on soil characteristics). Materials used and methodologies followed in the present investigation have been described in this chapter.

3.1 Experimental period

The two pot experiments were conducted during the period from November 2020 to February 2021 in boro season.

3.2 Description of experimental site

3.2.1 Geographical location

The experiment was conducted in the the net house of sher-e-bangla Agricultural University, Dhaka-1207. 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., 2004).

3.2.2 Agro-ecological region:

The experimental site belongs to the Agro-ecological zone (AEZ) of “The Madhupur Tract”, AEZ-28 (Anon., 1988 a). This was a region of complex relief and soils developed over the Madhupur clay, where floodplain sediments buried the dissected edges of the Madhupur Tract leaving small hillocks of red soils as ‘islands’ surrounded by floodplain (Anon., 1988 b). 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 having Soil pH ranges from 5.4–5.8 (Anon., 1989). The land was above flood level and sufficient sunshine was available during the experimental period. The morphological and physicochemical properties of the soil are presented in below table.

Table 1: Morphological characteristics of the experimental site

Morphological features	Characteristics
Location	Sher-e-Bangla Agricultural University Farm, Dhaka.
AEZ	Madhupur Tract
General Soil Type	Red Brown Terrace Soil
Land Type	Medium high land
Soil series	Tejgaon
Topography	Fairly leveled

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

Characteristics	Value	Name of the methods
% sand	25	Marshals textural triangular method
% Silt	62	Marshals textural triangular method
% Clay	37	Marshals textural triangular method
Textural Class	Silty clay loam	Marshals textural triangular method
pH	5.9	Glass electrode pH meter method
Total N (%)	0.04	Kjeldahl method
Available P (ppm)	25.0	Olsen method
Available S (ppm)	40.0	Barium chloride extraction method
OC (%)	0.6	Wet oxidation method
OM (%)	1.03	Wet oxidation method

3.2.4 Weather and Climate

The climate of the experimental site was subtropical, characterized by the winter season from November to February (Rabi season). Meteorological data related to temperature, relative humidity and rainfall during the experiment period was collected from Bangladesh Meteorological Department (Climate Divison), Sher-e-Bangla Nagar, Dhaka and has been presented in Appendix-II

3.3 Experimental Details

3.3.1 Experimental materials

In this study, seeds of radish (first crop) and spinach (second crop) were used which was collected Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Dhaka. BARI mulashak-1 was the first crop of the experiment and BARI palonshak-1 was the second crop of the experiment. The seeds were soaked overnight and then sowing was done using 20 seeds/pot.

3.3.2 Collection and preparation of organic material (Shrimp shell powder)

The organic material (Shrimp shell by-product) was collected from the Khulna region of Bangladesh and sieving the powder using 30 mesh sieves to prepare the usable shrimp shell powder following a new traditional method. Dried shrimp shells were used as the raw material. Analytical results of the shrimp shell powder revealed that number of essential micro and macro elements were available in the powder. The powder is alkaline in nature and good source of organic nitrogen.

Table 3: Chemical composition of the organic material (shrimp shell powder) which was used in research.

Name of the nutrients	Nutrient content
pH	8.7
Organic Carbon (OC)	17.00%
Organic Matter (OM)	29.31%
Total Nitrogen (N)	12.61%
Phosphorus (P)	0.67%
Potassium (K)	0.14%
Sulphur (S)	0.10%
Calcium (Ca)	0.0094%
Magnesium (Mg)	0.00029%
Zinc (Zn)	0.012%
Boron(B)	26.21ppm

3.3.3 Experimental treatment

The single factor experiment was compared with 8 (eight) concentrations of organic material (shrimp shell powder) treatments viz.

T₁ = 0% organic material (shrimp shell powder)

T₂ = 0.1% organic material (shrimp shell powder)

T₃ = 0.2% organic material (shrimp shell powder)

T₄ = 0.3% organic material (shrimp shell powder)

T₅ = 0.4% organic material (shrimp shell powder)

T₆ = 0.5% organic material (shrimp shell powder)

T₇ = 0.75% organic material (shrimp shell powder)

T₈ = 1% organic material (shrimp shell powder)

3.3.4 Experimental design and layout

The experiment was conducted in the Rabi season. The experiment was laid out in Randomized Complete Block Design (RCBD) with single factor consist of eight treatments. Each treatment was replicated by four. Total 32 pots were prepared for the experiment. It was done in the net house of the SAU farm. The used pot size in the experiment was 14 inch. The layout of the experiment is given below in figure 1:

T1	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R1)</div> <div style="text-align: center;">(R2)</div> </div> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R3)</div> <div style="text-align: center;">(R4)</div> </div>
T2	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R1)</div> <div style="text-align: center;">(R2)</div> </div> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R3)</div> <div style="text-align: center;">(R4)</div> </div>
T3	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R1)</div> <div style="text-align: center;">(R2)</div> </div> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R3)</div> <div style="text-align: center;">(R4)</div> </div>
T4	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R1)</div> <div style="text-align: center;">(R2)</div> </div> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R3)</div> <div style="text-align: center;">(R4)</div> </div>
T5	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R1)</div> <div style="text-align: center;">(R2)</div> </div> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R3)</div> <div style="text-align: center;">(R4)</div> </div>
T6	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R1)</div> <div style="text-align: center;">(R2)</div> </div> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R3)</div> <div style="text-align: center;">(R4)</div> </div>
T7	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R1)</div> <div style="text-align: center;">(R2)</div> </div> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R3)</div> <div style="text-align: center;">(R4)</div> </div>
T8	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R1)</div> <div style="text-align: center;">(R2)</div> </div> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">(R3)</div> <div style="text-align: center;">(R4)</div> </div>

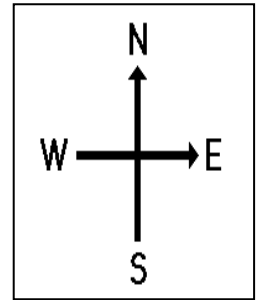


Figure 1: Layout of the experiment

3.4 Preparation of Organic Material for the pot

Well decomposed cowdung was used which was collected from Sher-e-Bangla Agricultural University (SAU) farm. And ratio of cowdung and soil was 3:1. Then raw shrimp shell powder was mixed properly with it. No synthetic fertilizer was used.

3.5 Growing of experimental crops (Radish and Spinach)

3.5.1 Pot preparation

Field moist soil was collected from Sher-e-Bangla Agricultural University farm then mixed with different level of shrimp shell powder according with treatment requirements. Plant propagules, inert materials, visible insects and pests were removed from this experimental soil. While filling with soil, the upper one inch of the pot was kept vacant so that irrigation can be provided using a hose pipe.

3.5.2 Sowing of seeds

Water soaked seeds were used for sowing. 20 seeds pot⁻¹ were sown by hand. On November 2, 2020 BARI mulashak-1 (First crop) seeds were sown on the prepared pot soil. On 12 December, 2020, BARI palonshak-1 (second crop) seeds were sown on the same pot soil after harvesting of radish (first crop).

3.5.3 Germination of seeds

Number of germinated seeds (both first crop and second crop) were counted in each pot then percent germination was calculated.

3.5.4 Intercultural Operation

3.5.4.1 Fertilizer management

Cowdung was used as common dose in every pot. No chemical fertilizer was used in this experiment. Only organic manure prepared from shrimp shell powder was used as per treatment during pot preparation.

3.5.4.2 Weeding

Hand weeding was done when required. No chemical herbicides was used in the experimental pots. And weed infestation is very low in case of radish (first crop) and spinach (second crop).

3.5.4.3 Irrigation

Irrigation was given during every morning and afternoon or when required by sprayer.

3.5.4.4 Harvesting

Harvesting of radish (first crop) was done at 10 December, 2020 (after 40 DAS).

Harvesting of spinach (second crop) was done at 21 January, 2021 (after 40 DAS).

3.6 Data collection

The data were recorded at various characterization which is given below-

3.6.1 Germination percentage

The number of germinated seed of all experimental pots was recorded after 12 days of sowing. Then the number was expressed as percentage.

3.6.2 Average plant height (cm) of radish (First crop)

Plant height (cm) of radish was measured with a meter scale from the ground level to tip of the main branch at 10 DAS, 30 DAS and 40 DAS (during harvesting). The mean heights were expressed in cm.

3.6.3 Average plant height (cm) of spinach (Second crop)

Plant height (cm) of spinach was measured with a meter scale from the ground level to tip of the main branch at 40 DAS (during harvesting). The mean heights were expressed in cm.

3.6.4 Average fresh weight (g pot⁻¹) of radish (First crop)

After harvesting from each pot, the weight of whole radish plants was recorded by using a digital electric balance. The mean weights were expressed in gram.

3.6.5 Average fresh weight (g pot⁻¹) of spinach (Second crop)

After harvesting from each pot, the weight of whole spinach plants was recorded by using a digital electric balance. The mean weights were expressed in gram.

3.6.6 Average Oven dry weight (g pot⁻¹) of radish (First crop)

After harvesting, radish plants of each pot were dried in the sun. Then, sun dried radish plants were dried in oven in the laboratory and then oven dried weight was recorded by using a digital electric balance. The mean oven dried weights were expressed in gram.

3.6.7 Average oven dry weight (g pot⁻¹) of spinach (Second crop)

After harvesting, spinach plants of each pot were dried in the sun. Then, sun dried spinach plants were dried in oven in the laboratory and then oven dried weight

was recorded by using a digital electric balance. The mean oven dried weights were expressed in gram.

3.7 Analysis of Soil samples

Composite soil samples were collected from pots of each treatment. Total 16 samples were collected. Among those, eight (8) were residual soil samples. Chemical properties of soil samples were analyzed in the laboratory of Department of Soil Science of Sher-e- Bangla Agricultural University, Sher-e- Bangla Nagar, Dhaka-1207.

The soil was analyzed following standard methods:

3.7.1 Particle size analysis

Particle size analysis of soil was done by Hydrometer Method and then textural class was determined by plotting the values of % sand, % silt and % clay to the “Marshal’s Textural Triangular Coordinate” according to the USDA system.

3.7.2 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).

3.7.3 Organic C (%)

Organic carbon in soil was determined by wet oxidation method of Walkley and Black (1934). 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. 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. Dilute the mass in the flask with about 150-200 mL of distilled water by mixing conc. H_3PO_4 and 2 mL of diphenylamine indicator. The organic carbon was calculated by the following formula:

$$\% \text{ Organic carbon} = (B-T) \times N \times 0.003 \times 1.3 \times 100/W$$

Where, B= Blank, T = Treatment, N = Normality, W = weight of soil, 1.724 = Van Bemmelen factor.

To obtain the organic matter content, the amount of organic carbon was multiplied by the Van Bemmelen factor, 1.73. The result was expressed in percentage. Therefore, % Organic matter = % Organic carbon x 1.724

3.7.4 Total Nitrogen (%)

Total N content of soil were determined by the Micro Kjeldahl method. One gram of oven dry ground soil sample was taken into micro kjeldahl flask to which 1.1 gm catalyst mixture (K_2SO_4 : $CuSO_4 \cdot 5H_2O$: Se in the ratio of 100:10:1), and 6 ml H_2SO_4 were added. The flasks were swirled and heated $2000^\circ C$ and added 3 ml H_2O_2 and then heating at $3600^\circ C$ 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 10N-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:

$$\% \text{ of total N in soil} = (T-B) \times N \times 0.014 \times D \times 100/W.$$

Where, T = Treatment, B = Blank, N = Normality, D = Dillution factor, W = Weight of soil.

3.7.5 Available phosphorus determination

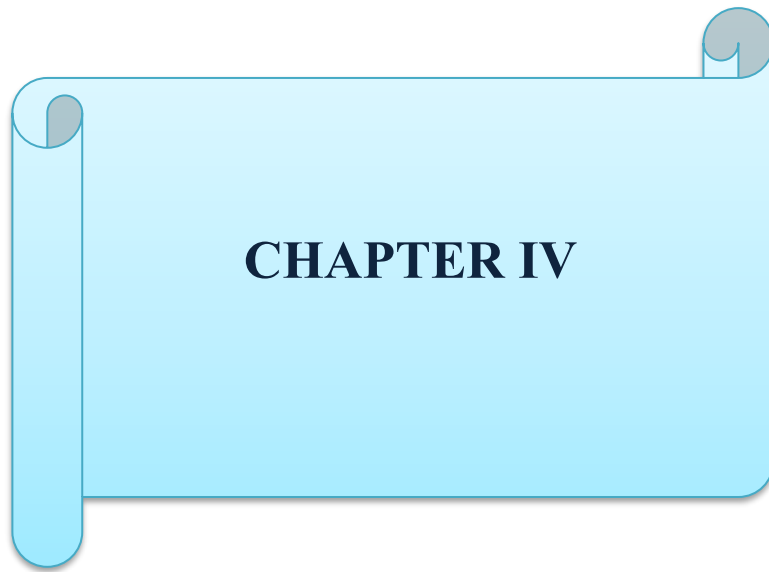
Available P was extracted from the soil with 0.5 M $NaHCO_3$ solutions, pH 8.5 (Olsen *et al.*, 1954). Phosphorus in the extract was then determined by developing blue color with reduction of phosphomolybdate complex and the color intensity were measured calorimetrically at 660 nm wavelength and readings were calibrated with the standard P curve.

3.7.6 Available sulphur determination

Available sulphur in soil was determined by calcium chloride extraction method. Available S was extracted from the soil with CaCl_2 solution. The available S as the sulphate form may be determined by the turbidity of suspended barium sulphate and hence this is known as turbidimetric method. The turbidity of suspended BaSO_4 is produced by treating the soil extract with $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ crystals. The intensity of the turbidity was then measured by a spectrophotometer at 420nm wavelengths. The procedure by which barium sulphate is precipitated must be carefully controlled as the properties of suspension are influenced by the velocity of reaction. The BaCl_2 was added to the sulphate solution in the solid state as crystals of definite size (20-26 mesh) and not as solution. Readings of the turbidity were calibrated with the standard S curve.

3.8 Statistical analysis

The collected data of plant and soil were analyzed statistically following the analysis of variance (ANOVA) technique and the mean differences were adjudged by LSD test using the statistical computer software named statistix 10.



CHAPTER IV

CHAPTER IV

RESULTS AND DISCUSSION

Results obtained from the present study have been presented and discussed in this chapter with a view to studying the direct and residual effect of organic material (shrimp shell powder) on radish-spinach cropping system. The results have been discussed and possible interpretations are given under the following headings.

4.1 Germination %

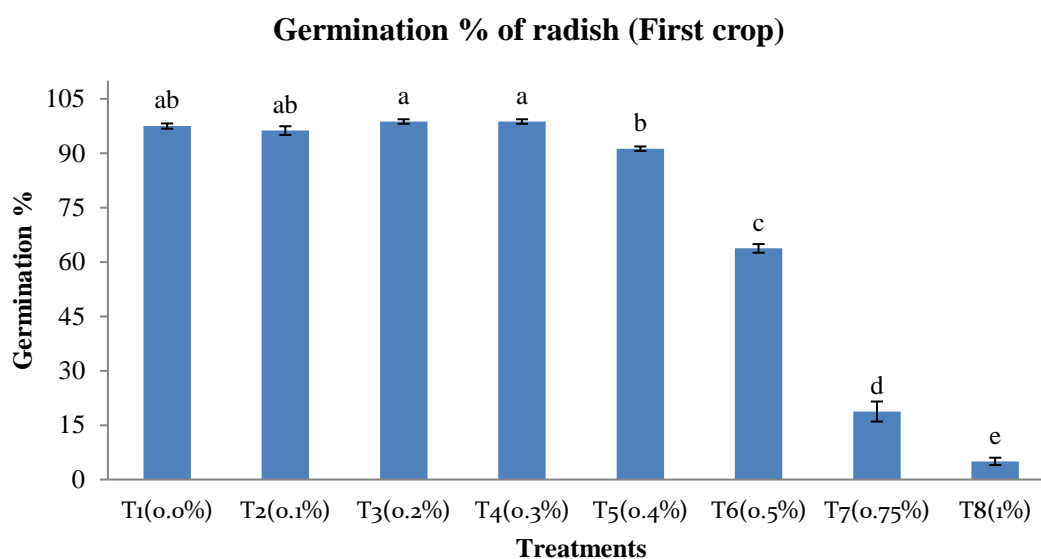
4.1.1 Effect of organic material (Shrimp shell powder) on germination % of radish (first crop)

In this experiment, different levels of organic material (shrimp shell powder) significantly influenced germination percentage of radish (Figure-2A). Experimental result revealed that the highest germination percentage of radish (98.8%) was observed in treatment T₄ (0.3% shrimp shell powder) which was statistically similar with T₃ (98.75%), T₂ (96.3%) and T₁ (97.5%) treatment. Sudden fall of germination percentage was observed in treatment T₇ (0.75% shrimp shell powder) and T₈ (1% shrimp shell powder). While the lowest germination percentage of radish (5%) was observed in treatment T₈ (1% shrimp shell powder). The general significant reduction in the seed germination was induced by the highest dose of the shrimp shell powder could probably be attributed to an inhibition of water uptake which was necessary for seed germination or may be for any hormonal reason. However, an excessive dose of the shrimp shell powder is likely to inhibit the enzyme released required for germination. As a result, at higher concentration radish seeds could not germinate and could not produce healthy seedlings and the mean germination percentage decreased gradually from treatment T₅, T₆, T₇ and T₈ respectively.

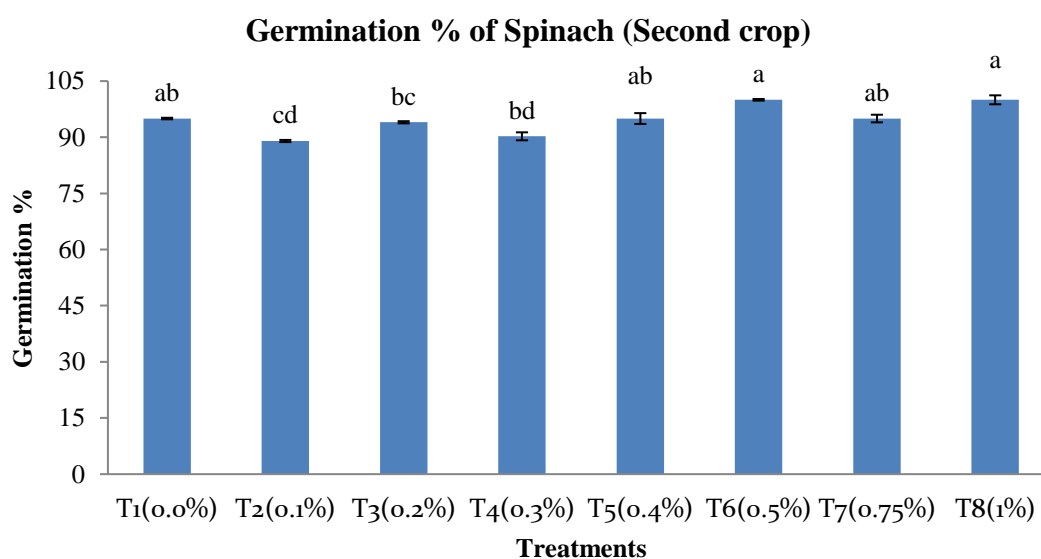
4.1.2 Residual effect of organic material (Shrimp shell powder) on germination % of spinach (second crop)

The germination percentage of second crop, spinach was significantly influenced by the residual effect of various levels of organic material (shrimp shell powder) application (Figure-2B). According to the experimental findings, the highest germination percentage of spinach (100%) was observed in treatment T₆ (0.5% shrimp shell powder) and T₈ (1% shrimp shell powder), which was statistically similar with treatment T₁ (95%), T₅ (95%) and T₇ (95%). While the lowest germination percentage of spinach was observed in treatment T₂ (89%) which was statistically similar with T₄ (90.25%). The similar data was found by Duan *et al.*, (2017) who revealed that chitosan had a positive effect on seed germination and accelerate the speed of germination of seeds. Organic materials application improves plants' ability to grow with less water, can accelerate growth and germination and improve the quality of vegetable production. Wang *et al.*, (2016) found the similar findings that chitosan can speed up the germination of wheat seeds and improve seedling growth.

(A)



(B)



Here, T₁: 0%= 0 gm (SS powder) in 9 kg soil, T₂: 0.1%= 9 gm (SS powder) in 9 kg soil, T₃: 0.2%=18 gm (SS powder) in 9 kg soil, T₄: 0.3%=27 gm (SS powder) in 9 kg soil, T₅: 0.4%=36 gm (SS powder) in 9 kg soil, T₆: 0.5%= 45 gm (SS powder) in 9 kg soil, T₇:0.75%= 67.5 gm (SS powder) in 9 kg soil and T₈: 1%= 90 gm (SS powder) in 9 kg soil.

Figure 2: A) Effect of different levels of organic material (Shrimp shell powder) on germination percentage of radish (first crop), B) Residual effect of different levels of organic material (Shrimp shell powder) on germination percentage of Spinach (Second crop)

4.2 Plant height (cm)

4.2.1 Effect of different levels of organic material (Shrimp shell powder) on plant height of Radish (First crop) at 10 DAS

Plant height is an essential character of the vegetative stage of the crop plant and indirectly impacts on yield. Application of different doses of organic material (shrimp shell powder) significantly influenced on average plant height (cm) of radish at 10 DAS (Figure-3A). Experimental result revealed that the highest average plant height (14.74 cm) was observed in treatment T₂ (0.1% shrimp shell powder) which was statistically similar with T₁ (14.41 cm) and T₃ (14.2 cm). The significant increase in plant height observed with certain levels of organic materials application could be attributed to an increase in the availability of cytokinin to shoots which in turn plays a role in cell elongation via cell division or cell elongation. Whereas, the lowest average plant height (1.38 cm) was observed in treatment T₈ (1% shrimp shell powder) which was statistically similar with treatment T₇ (2.59 cm). As a result of lowest germination percentage at high concentration of shrimp shell powder, the average plant height (cm) decreased because of low number of healthy seedlings.

4.2.2 Effect of different levels of organic material (shrimp shell powder) on average plant height (cm) of radish (1st crop) at 30 DAS

At 30 DAS, different doses of organic material (shrimp shell powder) application had shown significant effect on average plant height (cm) (Figure-3B). The highest average plant height (27.4 cm) was observed in the T₅ (0.4% shrimp shell powder) which was statistically similar to the T₂ (23.46cm), T₃ (25.75 cm), T₄ (26.98 cm) and T₆ (25.7 cm) treatments. The lowest plant height (8.94 cm) was found in T₈ (1% shrimp shell powder) treatment at 30 DAS. John *et al.*, (1997) and Dzung *et al.*, (2011) found similar findings that chitosan can promote cell division of root cells by activating plant hormones like auxin and cytokinin which later enhance nutrient

uptake of plant. T₈ treatment (1% shrimp shell powder) showed lowest plant height because of low seed germination percentage at higher concentration of shrimp shell powder and production of weak seedlings. Similar findings were seen by Nahar *et al.*, (2012) that chitosan could produce positive results at a very low concentration. Vasudevan *et al.*, (2002) reported that the use of chitosan formulation could accelerate the length of root and shoot growth of plant.

4.2.3 Effect of different levels of organic material (shrimp shell powder) on average plant height (cm) of radish (1st crop) at 40 DAS during harvesting

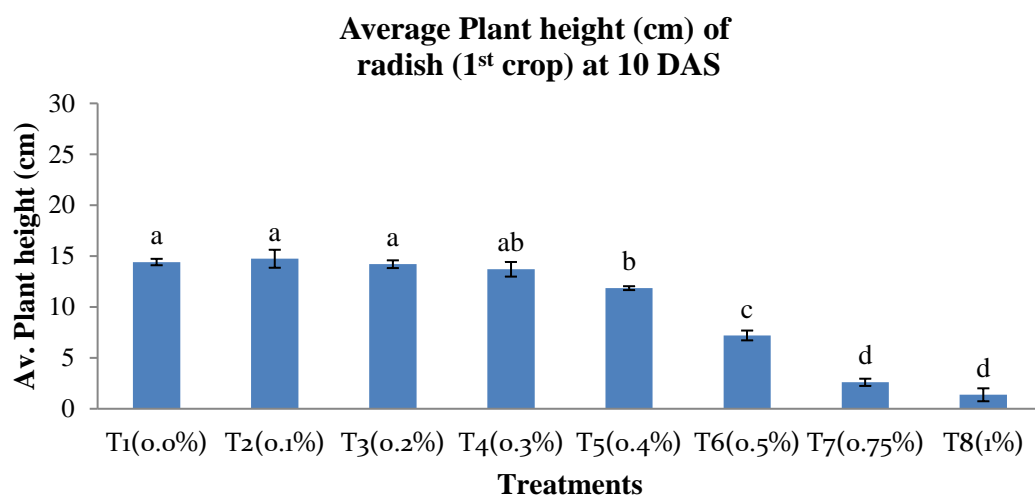
Different doses of organic material (shrimp shell powder) application had shown significant effect on average plant height of radish at harvesting (Figure 4A). According to the experimental findings, the highest plant height (31.57 cm) at harvest was observed in the treatment T₆ (0.5% shrimp shell powder) which was statistically similar with T₄ (29.98 cm) and T₅ (30.19 cm) treatment but had significant difference with control T₁ (0% shrimp shell powder). While the lowest plant height (11.38 cm) at harvest was found in treatment T₈ (1% shrimp shell powder). The increased plant height through the foliar application of chitosan along with N, P, K, and S was also reported Kobayashi *et al.*, (1989).

4.2.4 Residual effect of different levels of organic material (shrimp shell powder) on average plant height (cm) of spinach (2nd crop) at 40 DAS during harvesting

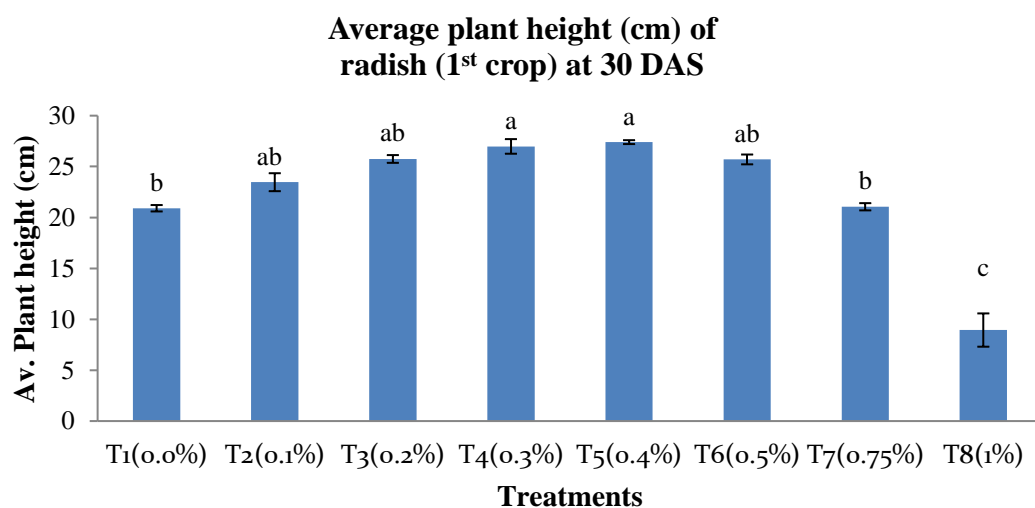
The residual effect of different levels of organic material (shrimp shell powder) application on average plant height (cm) of spinach was found to be significant at harvest (Figure-4B). According to the experimental findings, the lowest plant height (17.8 cm) was observed in control treatment T₂ (0.1% shrimp shell powder) while the highest plant height (34.28 cm) was observed in treatment T₈ (1% shrimp shell powder) which gave 81.76 % more plant height comparable to control treatment. Similar result was also found by the work of Chibu *et al.*, (2000) in rice who reported that plant height increased in chitosan treated plants as compared to control plants. Parvin *et al.*, (2019) results revealed that plant height was greater in chitosan applied tomato (*L. esculentum*) plants than control plants. Mondal *et al.*, (2016) reported the

same that chitosan application increased height of plant. The variation in plant height might be attributed to the optimum and constant supply and availability of nutrients from organic sources, which aid in improved nutrient absorption. Chitosan has been proven to act as a positive factor in enhancing the shoot and root length (Sheikh and Al Malki, 2011). Similarly, with the increasing concentration of shrimp shell powder average plant height (cm) of spinach increased significantly and highest at treatment T₈ (1% shrimp shell powder).

(A)



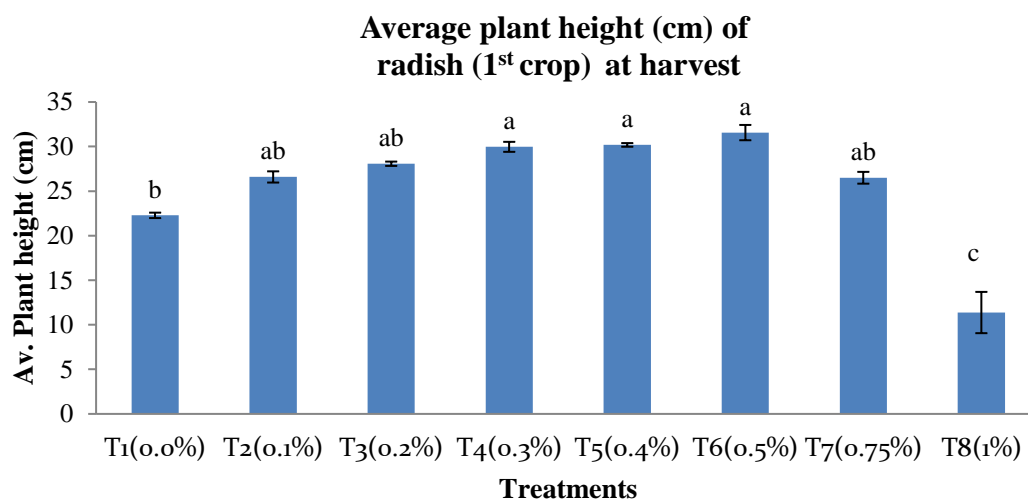
(B)



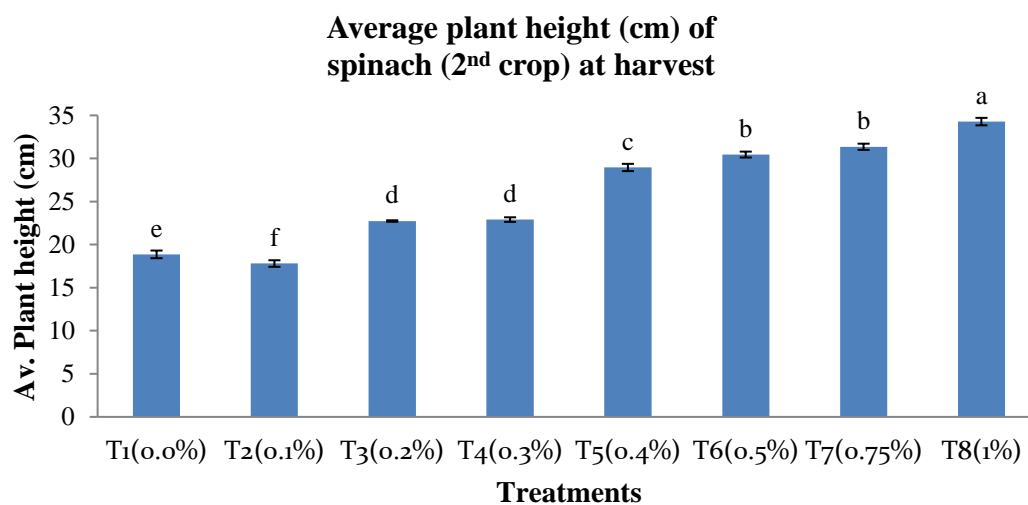
Here, T₁: 0%= 0 gm (SS powder) in 9 kg soil, T₂: 0.1%= 9 gm (SS powder) in 9 kg soil, T₃: 0.2%=18 gm (SS powder) in 9 kg soil, T₄: 0.3%=27 gm (SS powder) in 9 kg soil, T₅: 0.4%=36 gm (SS powder) in 9 kg soil, T₆: 0.5%= 45 gm (SS powder) in 9 kg soil, T₇:0.75%= 67.5 gm (SS powder) in 9 kg soil and T₈: 1%= 90 gm (SS powder) in 9 kg soil.

Figure 3: A) Effect of different levels of organic material (shrimp shell powder) on average plant height (cm) of radish (1st crop) at 10 DAS, B) Effect of different levels of organic material (shrimp shell powder) on average plant height of radish (1st crop) at 30 DAS

(A)



(B)



Here, T₁: 0%= 0 gm (SS powder) in 9 kg soil, T₂: 0.1%= 9 gm (SS powder) in 9 kg soil, T₃: 0.2%=18 gm (SS powder) in 9 kg soil, T₄: 0.3%=27 gm (SS powder) in 9 kg soil, T₅: 0.4%=36 gm (SS powder) in 9 kg soil, T₆: 0.5%= 45 gm (SS powder) in 9 kg soil, T₇:0.75%= 67.5 gm (SS powder) in 9 kg soil and T₈: 1%= 90 gm (SS powder) in 9 kg soil.

Figure 4 A): Direct effect of different levels of organic material (shrimp shell powder) on average plant height (cm) of radish (1st crop) at 40 DAS during harvest, B) Residual effect of different levels of organic material (shrimp shell powder) on average plant height (cm) of spinach (2nd crop) at 40 DAS during harvest

4.3 Fresh weight at harvest (g pot⁻¹)

4.3.1 Effect of different levels of organic material (Shrimp shell powder) on average plant fresh weight (g pot⁻¹) of radish (1st crop)

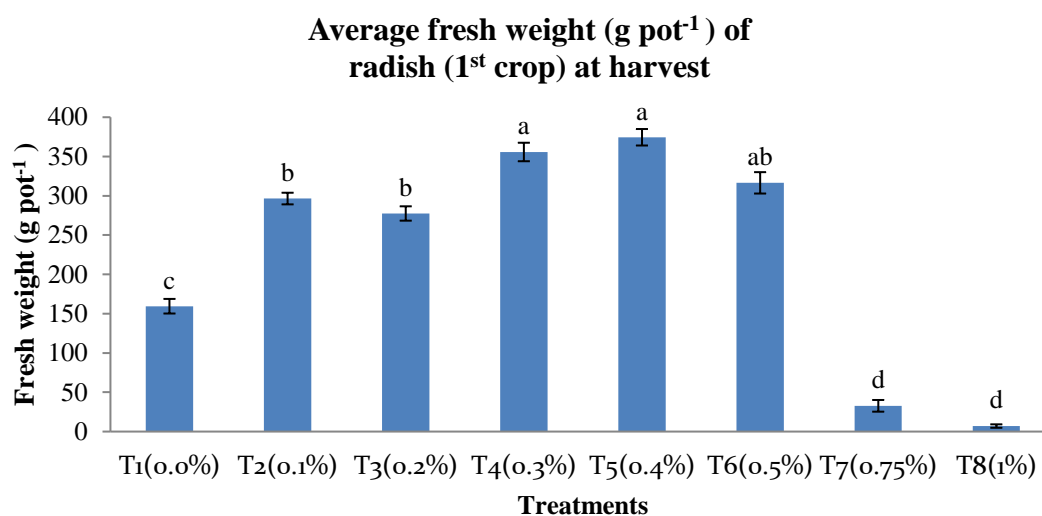
Different levels of organic materials (shrimp shell powder) application significantly affected on average fresh weight of radish (Figure-5A). According to the experimental results, the treatment T₅ (0.4% shrimp shell powder) had the highest average fresh weight of radish (374.5 g pot⁻¹) at harvest which was statistically similar with T₄ (355.8 g pot⁻¹) and T₆ (316.5 g pot⁻¹) treatment. These results were supported by Mondal *et al.*, (2012) who carried out pot and field experiments with the five levels of chitosan concentrations viz., 0 (control), 50, 75, 100 and 125 ppm and observed that foliar application of chitosan at 100 or 125 ppm increased the fresh weight of okra. While the lowest average fresh weight of radish (7g pot⁻¹) was found in treatment T₈ (1% shrimp shell powder). And sudden decrease of average plant fresh weight (g pot⁻¹) of radish was seen at treatments T₇ (0.75 % shrimp shell powder) and T₈ (1% shrimp shell powder). This may be due to some hormonal causes as there was lowest germination percentage and minimum number of plants were recorded in these two treatments. The balanced use of organic sources of nutrients-maintained soil fertility and physical behavior resulting in higher fresh weight of radish.

4.3.2 Residual effect of different levels of organic material (shrimp shell powder) on average plant fresh weight (g pot⁻¹) of spinach (2nd crop)

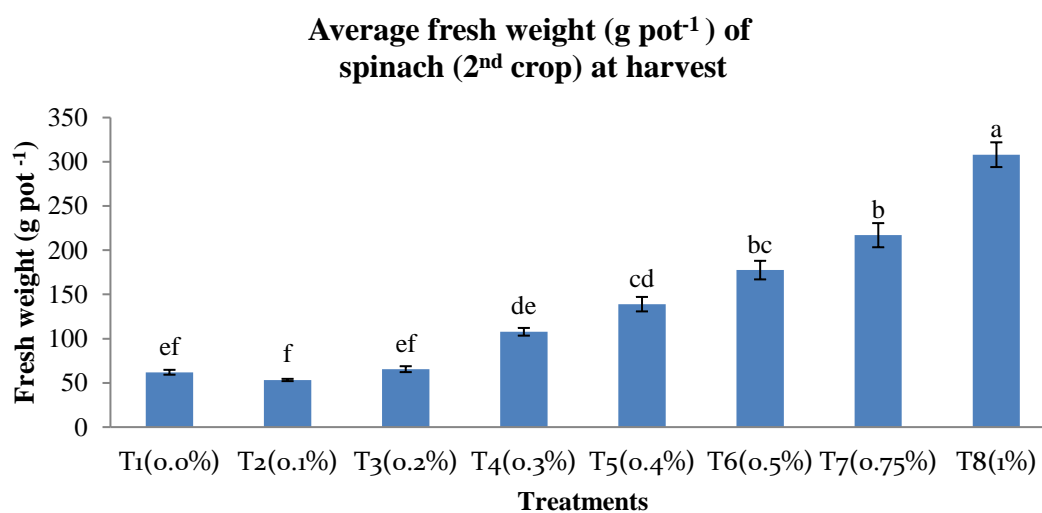
Average fresh weight of spinach at harvest significantly influenced due to the residual effect of different levels of organic material (shrimp shell powder) application (Figure-5B). According to the experimental findings, the highest average fresh weight of spinach (308g pot⁻¹) was observed in T₈ treatment which gave 396.77 % more fresh weight compared to control treatment T₁. While the lowest fresh weight of spinach (53.3 g pot⁻¹) was observed in treatment T₂ which was statistically similar with control treatment T₁ (62 g pot⁻¹) and T₃ (65.5g pot⁻¹) treatment. The significant difference in average plant fresh weight may be attributed to the conducive soil environment created due to residual effect of organic materials. El-Tanahy *et al.*, (2012) also reported that using the highest concentration of chitosan (5%) with the

application of the inorganic fertilizer in cowpea increased the fresh weight of root and shoot of plant and seed yield. This might be due to its nutritional support to the plants, improvement of growth promoting hormonal activity and could improve the biological as well as physio-chemical properties of the pot soils (Tsugita *et al.*, 1993; Rahman., 2015; Islam, 2016;Issak *et al.*, 2017).The term "residual effect of organic materials" typically refers to the beneficial response of crops to supply the nutrients to an earlier crop thereby also provide nutrient for later ones through mineralization which were early immobilized by micro-organism. The residual effect of organic materials application improves soil structure, organic matter content, reduce evaporation, and help fix CO₂ in the soil, when compared to the soil control treatment and thereby influenced plant growth and development. It is simple for chitosan to absorb to plant surface due to its cataionic properties thus prolonging the contact time between plant surface and agrochemical (Divya *et al.*, 2017). Thus, more nutrients may release and absorbed by plant easily. Application of chitosan 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).

(A)



(B)



Here, T₁: 0%= 0 gm (SS powder) in 9 kg soil, T₂: 0.1%= 9 gm (SS powder) in 9 kg soil, T₃: 0.2%=18 gm (SS powder) in 9 kg soil, T₄: 0.3%=27 gm (SS powder) in 9 kg soil, T₅: 0.4%=36 gm (SS powder) in 9 kg soil, T₆: 0.5%= 45 gm (SS powder) in 9 kg soil, T₇:0.75%= 67.5 gm (SS powder) in 9 kg soil and T₈: 1%= 90 gm (SS powder) in 9 kg soil.

Figure 5: A) Effect of different levels of organic material (shrimp shell powder) on average plant fresh weight (g pot⁻¹) of radish (1st crop), B) Residual effect of different levels of organic material (shrimp shell powder) on average plant fresh weight (g pot⁻¹) of spinach (2nd crop) at harvest



Plate 2: Radish plant (First crop) at 40 DAS before harvesting



Plate 5: Spinach plant (second crop) at 40 DAS before harvesting

4.4 Dry weight at harvest (g pot⁻¹)

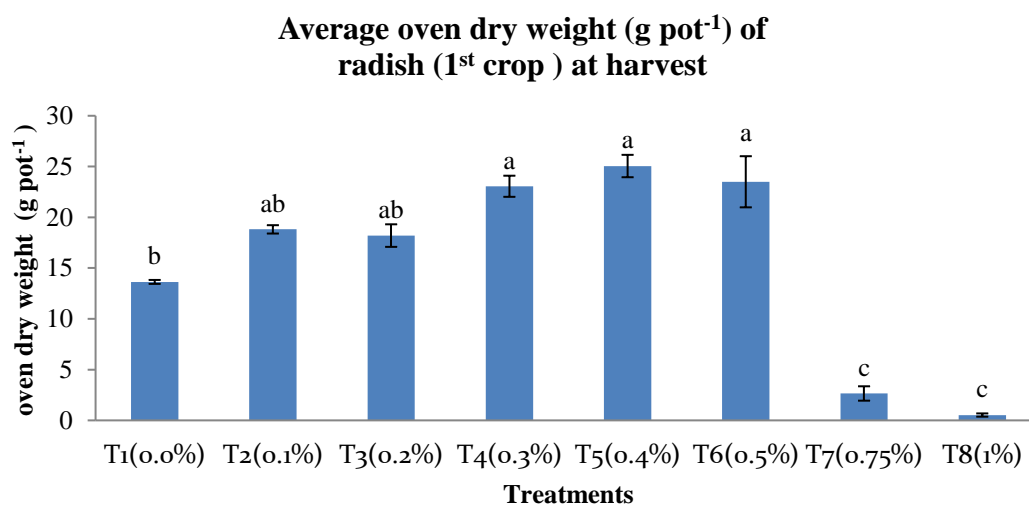
4.4.1 Effect of different levels of organic material (Shrimp shell powder) on average oven dry weight (g pot⁻¹) of radish (1st crop)

Different levels of organic materials (shrimp shell powder) application significantly affected on dry weight of radish at harvest (Figure-6A). According to the experimental results, the treatment T₅ (0.4% shrimp shell powder) had the highest average dry weight of radish (25.05 g pot⁻¹) which was statistically similar with T₄ (23.06 g pot⁻¹) and T₆ (23.5 g pot⁻¹) treatment. While the lowest average dry weight (0.53 g pot⁻¹) was found in T₈ treatment. The variation of dry weight of radish might be attributed to the optimum and constant supply and availability of nutrients from organic sources, which aid in improved nutrient absorption, eventually promoting cell division and therefore increasing all growth features.

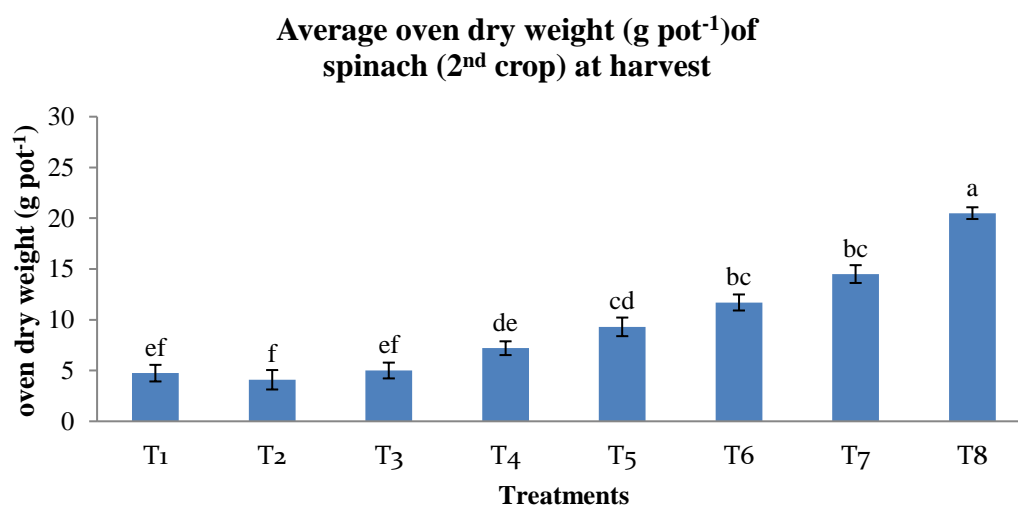
4.4.2 Residual effect of organic material (shrimp shell powder) on average oven dry weight (g pot⁻¹) of spinach (2nd crop)

Different levels of organic material (shrimp shell powder) application significantly affected on dry weight of spinach (second crop) (Figure-6B). According to the experimental results, treatment T₈ (1% shrimp shell powder) showed highest dry weight (20.5g pot⁻¹) which was significantly different from control treatment T₁. While the lowest dry weight (4.09g pot⁻¹) was found at T₂ (0.1% shrimp shell powder).

(A)



(B)



Here, T₁: 0%= 0 gm (SS powder) in 9 kg soil, T₂: 0.1%= 9 gm (SS powder) in 9 kg soil, T₃: 0.2%=18 gm (SS powder) in 9 kg soil, T₄: 0.3%=27 gm (SS powder) in 9 kg soil, T₅: 0.4%=36 gm (SS powder) in 9 kg soil, T₆: 0.5%= 45 gm (SS powder) in 9 kg soil, T₇:0.75%= 67.5 gm (SS powder) in 9 kg soil and T₈: 1%= 90 gm (SS powder) in 9 kg soil.

Figure 6: A) Effect of different levels of organic material (shrimp shell powder) on average oven dry weight (g pot⁻¹) of radish (1st crop), B) Residual effect of organic material (shrimp shell powder) on average oven dry weight (g pot⁻¹) of spinach (2nd crop)

4.5 Available nutrient content in soil

4.5.1 Effect on organic carbon content (%) in soil

4.5.1.1 Effect of different level of organic material (Shrimp shell powder) on soil OC (%) content at postharvest soil of radish (1st crop)

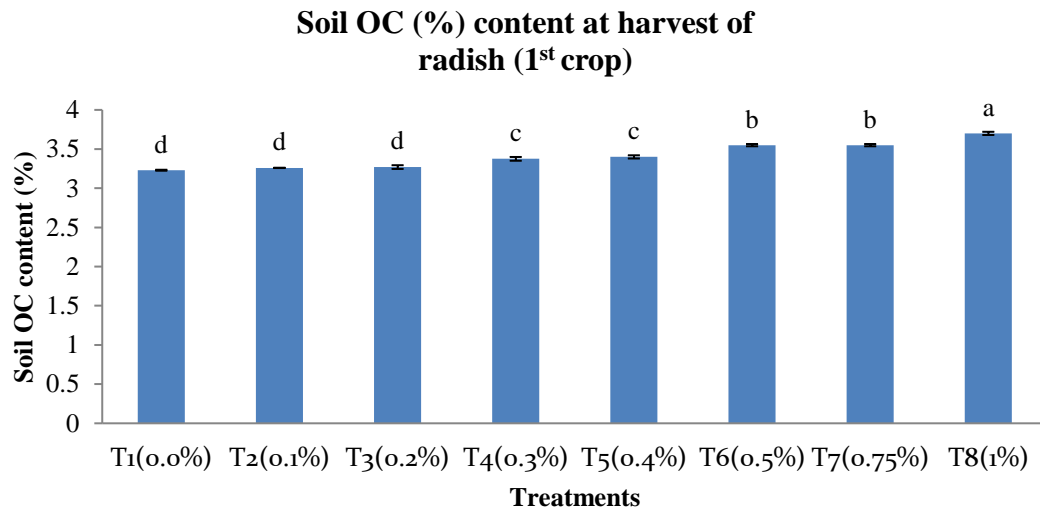
Organic carbon content (%) in post-harvest soil of radish varied significantly due to different doses of organic material (Shrimp shell powder) applications (Figure-7A). It was found that organic carbon content (%) in soil ranges from 3.2 % to 3.7 % after harvest of radish. The maximum organic carbon content in post-harvest soil of radish was observed from T₈ (3.7 %) treatment. While the lowest organic carbon content in radish was found in control treatment T₁ (3.2%) which was statistically similar with T₂ (3.26 %) and T₃ (3.27 %) treatment. Issak and sultana (2017) found in their work that application of chitosan powder in the seedbed soil tends to increment of organic carbon content and the organic carbon content was increased with increasing the level of chitosan powder in the seedbed soils. Maximum organic carbon content (1.18 %) was found in the treatment T₅ having applications of 500 (g m⁻²) of chitosan in the seedbed. Rahman (2015) and Islam (2016) also found that raw material of chitosan powder application increased the organic carbon content in soils. Increasing organic matter content for the sustainable agriculture is a big challenge of soil in Bangladesh. As a result, shrimp shell powder application could play a significant role to increase the organic matter content in soil.

4.5.1.2 Residual effect of different level of organic material (Shrimp shell powder) on soil OC (%) content at postharvest soil of spinach (2nd crop)

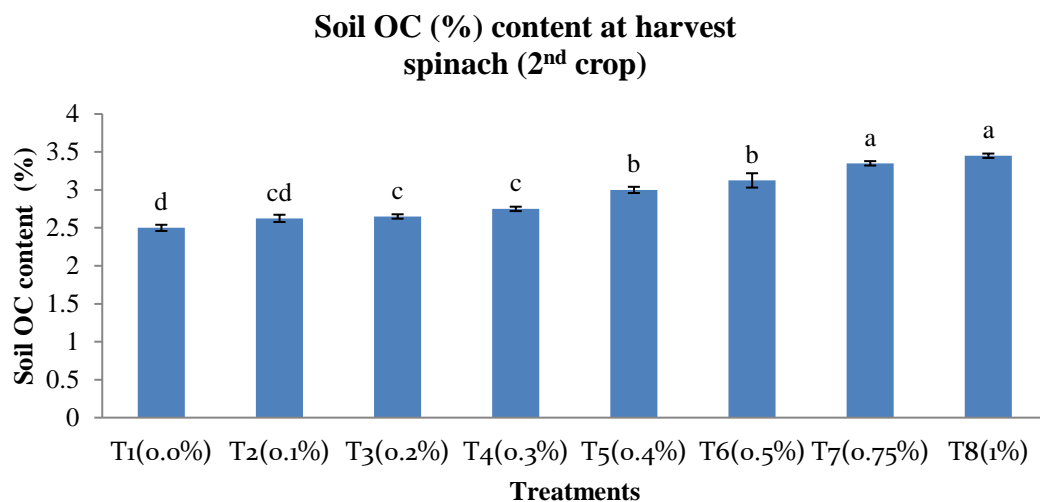
The residual effect of different doses of organic material (Shrimp shell powder) on organic carbon content (%) in soil after harvesting radish and spinach was significant (Figure-7B). The experimental data revealed that the T₈ treatment had the highest organic carbon content (3.45%) in soil, which was statistically similar to the T₇ (3.35%) treatment. The T₁ treatment had the lowest value (2.5%) of organic carbon content in soil which was statistically similar to T₂ treatment (2.62%). health. Sultana *et al.*, (2020) suggested that residual effect of chitosan raw material powder increase the soil organic carbon content. The residual effect of organic materials on organic

carbon is the long-term impact that organic materials have on the amount of organic carbon in soil. Organic materials, such as shrimp shell powder, can increase soil organic carbon by providing a source of food for soil microbes and by improving soil structure. Soil structure is important for water infiltration and retention, as well as for root growth. Increased soil organic carbon can also improve soil fertility and reduce the risk of erosion. The residue effect of organic materials on organic carbon is an important factor to consider when managing soils for long-term sustainability. By increasing soil organic carbon, we can improve soil health and productivity, and help to mitigate climate change.

(A)



(B)



Here, T₁: 0%= 0 gm (SS powder) in 9 kg soil, T₂: 0.1%= 9 gm (SS powder) in 9 kg soil, T₃: 0.2%=18 gm (SS powder) in 9 kg soil, T₄: 0.3%=27 gm (SS powder) in 9 kg soil, T₅: 0.4%=36 gm (SS powder) in 9 kg soil, T₆: 0.5%= 45 gm (SS powder) in 9 kg soil, T₇:0.75%= 67.5 gm (SS powder) in 9 kg soil and T₈: 1%= 90 gm (SS powder) in 9 kg soil.

Figure 7: A) Effect of different level of organic material (Shrimp shell powder) on soil OC (%) content at postharvest soil of radish (1st crop), B) Residual effect of different level of organic material (Shrimp shell powder) on soil OC (%) content at postharvest soil of spinach (2nd crop)

4.5.2 Effect on Organic matter (%) of soil

4.5.2.1 Effect of different level of organic material (Shrimp shell powder) on soil OM (%) content at postharvest soil of radish (1st crop)

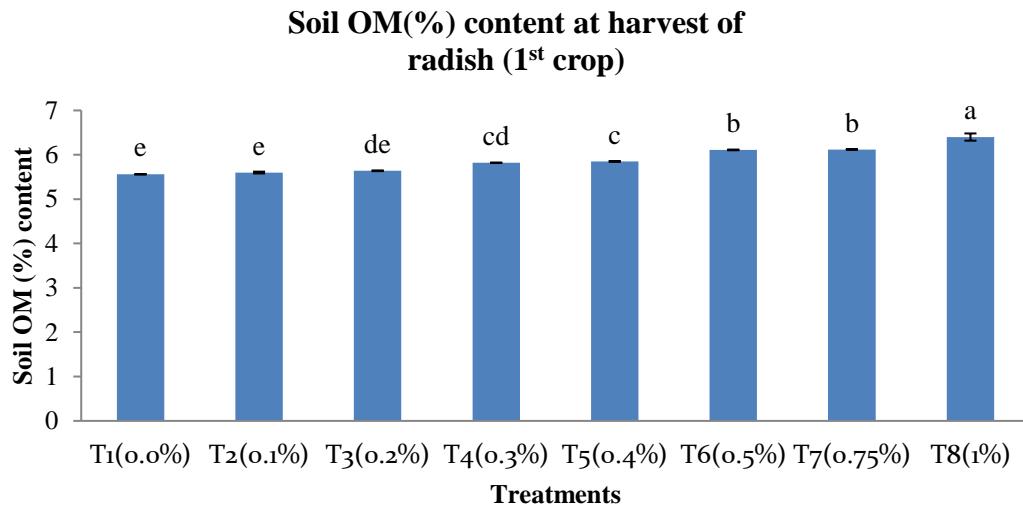
The data on organic matter content (%) of soil after harvesting of radish was significantly influenced by application of different doses of organic material (shrimp shell powder). The highest organic matter content in soil was recorded in T₈ (1% shrimp shell powder) treatment having values of (6.4 %). Whereas the lowest value (5.56%) was noted in T₁ control treatment which was statistically similar with T₂ (5.6 %) and T₃ (5.64 %) treatment. Rahman (2015) reported that chitosan application stimulates organic matter content in soil and maximum organic matter content (1.73 %) was found in the treatment having treated with modified chitosan in the soils and minimum organic matter content (1.54%) was found in control treatment having no modified chitosan application. Organic matter content varied among different treatment might be due to addition of organic source have created environment conducive for formation of humic acid, stimulated the activity of soil microorganism and direct addition and continuous mineralization of organic materials on surface soil layer resulting in an increase in the organic carbon matter of the soil. Prativa and Bhattarai (2011) reported that the soil organic matter content increased only where plots treated with organic fertilizer as compared to inorganic fertilizer treated plot. Increased soil organic matters due to organic inputs sustains soil health for a longer period than chemical fertilization.

4.5.2.2 Residual effect of different level of organic material (Shrimp shell powder) on soil OM (%) content at postharvest soil of spinach (2nd crop)

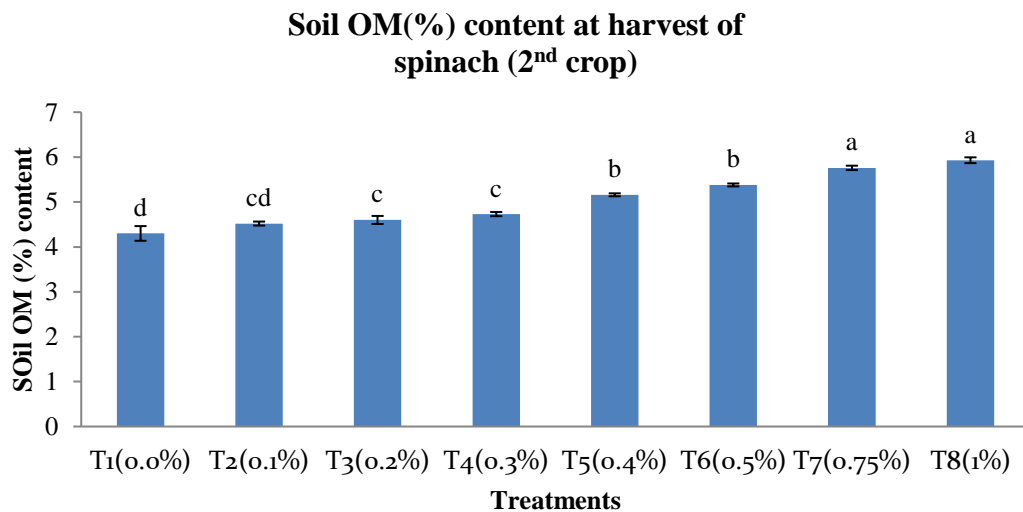
The residual effect of different doses of organic materials (shrimp shell powder) significantly influenced organic matter content in soil after harvest radish and spinach (Figure-8B). Experimental data revealed that the highest the highest organic matter content (5.9%) was observed in T₈ treatment which was statistically similar with T₇ (5.7 %) treatment. Whereas the lowest value (4.30%) was noted in T₁ treatment which was statistically similar with T₂ (4.5%) treatment. Organic matter addition improves nutrient supply and water retention (Kale *et al.*, 1991). These results were supported by Sultana *et al.*, (2020) who carried out an experiment with the raw material of chitosan powder with one control. There were four replications using four different

doses of the raw material of chitosan powder. The results revealed that organic matter status in the post-harvest-soils were increased due to the residual effect of the chitosan powder in rice growing soils. The results were also supported by Kananont *et al.*, (2015) who conducted an experiment and found that fermented chitin waste increase organic matter content in soil. Rahman (2015) and Islam (2016) also observed the same. The application of shrimp shell powder (in the previous experiment) containing higher level of organic carbon. It might be increased the level of organic matter content of the postharvest of spinach (second crop). It's a big challenge to increasing the organic matter content of Bangladesh soils for the sustainable agriculture. So, shrimp shell powder application could play a vital role to increase the organic matter content in soils.

(A)



(B)



Here, T₁: 0%= 0 gm (SS powder) in 9 kg soil, T₂: 0.1%= 9 gm (SS powder) in 9 kg soil, T₃: 0.2%=18 gm (SS powder) in 9 kg soil, T₄: 0.3%=27 gm (SS powder) in 9 kg soil, T₅: 0.4%=36 gm (SS powder) in 9 kg soil, T₆: 0.5%= 45 gm (SS powder) in 9 kg soil, T₇:0.75%= 67.5 gm (SS powder) in 9 kg soil and T₈: 1%= 90 gm (SS powder) in 9 kg soil.

Figure 8: A) Effect of different level of organic material (Shrimp shell powder) on soil OM (%) content at postharvest soil of radish (1st crop) B) Residual effect of different level of organic material (Shrimp shell powder) on soil OM (%) content at postharvest soil of spinach (2nd crop)

4.5.3 Effect on total Nitrogen content of soil

4.5.3.1 Effect on total nitrogen (%) in postharvest soil of 1st crop

Total nitrogen content (%) in post-harvest soil of radish varied significantly due to different doses of organic material (shrimp shell powder) applications (Table 4). It was found that availability of nitrogen varied from 0.056 % to 0.101 % at harvest of radish (first crop). The maximum total nitrogen (.101 %) content in post-harvest soil of radish (first crop) was observed from T₈ treatment which was statistically similar with T₇ (0.096 %) and T₆ (0.099 %) treatment. While the lowest total nitrogen content in post-harvest soil of radish was found in T₁ (0.056%) treatment. In control treatment T₁, plants get some nitrogen from cowdung which was mixed initially. These results indicated that the total nitrogen content is high enough due to the application of shrimp shell powder. Management of nitrogen through organic manures is one of the options to minimize nitrogen loss from soil and improves radish productivity (Delate and Camberdella, 2004). Under the improved nutrient synchrony mechanism proposed by Vanlauwe *et al.* (2006) when organic fertilizers are applied, they supply microbes with energy from the carbon they contain, to drive decomposition processes. This leads to temporal immobilization of soil N to build their body tissues. The immobilized N is made available at a later stage of plant growth when the microbes have decomposed the organic material to make nutrients available and/or some microbes have lysed and released their nutrients to the plant when it needs nutrients most. In effect, the peak of nutrient supply coincides with the peak of crop nutrient demand when crops have matured after harvesting ensuring that nutrients are efficiently utilized and little is lost to the environment.

4.5.3.2 Residual effect on total nitrogen (%) in postharvest soil of 2nd crop

The total N status of the post-harvest soil of second crop was affected by the different treatments of shrimp shell powder and ranged from 0.021% to 0.064% (Table-4). It was found that N status of soil was statistically different among the treatments. The highest N value (0.064%) was recorded in T₈ treatment which was significantly greater than the T₁ treatment. The nitrogen content in soil in soil after harvesting of radish and spinach varied significantly due to the residual effect of organic material

in soil. Organic materials, such as shrimp shell powder, can have a residual effect on the total nitrogen content in soil. The residual chitosan can contribute to increase N content of rice soil as well as to increase long term productivity and enhancement of ecological sustainability (Gill and Meelu, 1982). Similar results were reported by Sultana *et al.*, (2020) who conducted an experiment with the raw material of chitosan powder. There were four replications using four different doses of the raw material of chitosan powder. The treatments were as follows: $T_1 = 0.5 \text{ t ha}^{-1}$, $T_2 = 1.0 \text{ t ha}^{-1}$, $T_3 = 2.0 \text{ t ha}^{-1}$, $T_4 = 4.0 \text{ t ha}^{-1}$ and $T_5 = 0 \text{ t ha}^{-1}$. The second experiment was conducted in the same plot using the following treatments were $T_1 =$ Residual effect of the raw material of chitosan powder @ 0.5 t ha^{-1} (applied in the previous experiment) + 2/3rd of recommended N fertilizer, $T_2 =$ Residual effect of the raw material of chitosan powder @ 1.0 t ha^{-1} (applied in the previous experiment) + 2/3rd of recommended N fertilizer, $T_3 =$ Residual effect of the raw material of chitosan powder @ 2.0 t ha^{-1} (applied in the previous experiment) + 2/3rd of recommended N fertilizer, $T_4 =$ Residual effect of the raw material of chitosan powder @ 4.0 t ha^{-1} (applied in the previous experiment) + 2/3rd of recommended N fertilizer and $T_5 =$ Residual effect of the raw material of chitosan powder @ 0 t ha^{-1} + recommended N (control). The results revealed that total nitrogen content in the post-harvest soils were increased due to the residual effect of the chitosan powder in rice growing soils. This means that the addition of organic materials can increase the soil nitrogen content for a period of time after the organic materials have been added. The residual effect of organic materials on soil nitrogen content is due to the fact that organic materials are slowly decomposed by soil microbes. As the organic materials are decomposed, they release nitrogen into the soil, which can be used by plants.

Table 4: Effect of different doses of organic material (shrimp shell powder) on total nitrogen (%) in post harvested soils of 1st crop and 2nd crop

Treatments	Total nitrogen content (%) in postharvest soil of 1st crop	Total nitrogen content (%) in postharvest soil of 2nd crop
T₁	0.056 d	0.021 d
T₂	0.0693 c	0.025 d
T₃	0.069 c	0.039 c
T₄	0.076 b	0.051 b
T₅	0.086 b	0.051 b
T₆	0.099 a	0.060 a
T₇	0.096 a	0.058 a
T₈	0.101 a	0.064 a
CV (%)	5.14	9.74
LSD_(0.05)	0.006	0.006

Here, T₁: 0%= 0 gm (SS powder) in 9 kg soil, T₂: 0.1%= 9 gm (SS powder) in 9 kg soil, T₃: 0.2%=18 gm (SS powder) in 9 kg soil, T₄: 0.3%=27 gm (SS powder) in 9 kg soil, T₅: 0.4%=36 gm (SS powder) in 9 kg soil, T₆: 0.5%= 45 gm (SS powder) in 9 kg soil, T₇:0.75%= 67.5 gm (SS powder) in 9 kg soil and T₈: 1%= 90 gm (SS powder) in 9 kg soil.

4.5.4 Effect on available phosphorus content (ppm) in soil

4.5.4.1 Effect on available phosphorus content (ppm) in postharvest soil of 1st crop

Experimental data indicated that the available phosphorus content in post harvest soil of radish (first crop) varies significantly among different treatments (Table-5). The highest available phosphorus in post-harvest soil of first crop was recorded from treatment T₈ (55 ppm) which is significantly varies from all other treatments. While the lowest available phosphorus in postharvest soil of first crop was found in T₁ (22 ppm) control treatment. Soil available phosphorus increased in all treatments except absolute control. Increased application of organic material (shrimp shell powder) increased soil available phosphorus. Higher soil available phosphorus observed in T₈ treatment was due to high adsorption and desorption potential of shrimp shell powder.

Phosphorus are limiting nutrient in plant growth and development (Khandaker *et al.*, 2017), any deficiency affect the plant health (Gholizadeh *et al.*, 2009).

4.5.4.2 Residual effect on available phosphorus content (ppm) in post harvest soil of 2nd crop

The available phosphorus content in soil after harvesting of radish and spinach crop varied significantly due to residual effect of organic material (shrimp shell powder) in soil (Table-5). According to the experimental findings, the highest available phosphorus content in postharvest soil of spinach (second crop) was found in treatment T₈ (49 ppm) which is significantly different than all other treatments. While the lowest available phosphorus in content was found in T₁ (17 ppm) control treatment which was statistically similar with treatment T₂ (20 ppm). This might be due to lower amount of residual nutrient in lower doses of shrimp shell powder treated pot. Increased application of chitosan concentration into soil increased the available phosphorus content, which could be due to the release of organic acids during the decomposition of organic matter, which helped in the solubility of native phosphates, resulting in an increase in the available phosphorus content in the soil. The application of organic matter leads to the formation of a coating on sesquioxides, resulting in reduction of phosphate fixing capacity of soil. Residual soil has low phosphorus content because spinach was grown without any organic or inorganic fertilizers.

Table 5: Effect of different doses of organic material (shrimp shell powder) on available phosphorus content (ppm) in in post harvested soils of 1st crop and 2nd crop

Treatments	Available phosphorus content (ppm) in postharvest soil of 1st crop	Available Phosphorus content (ppm) in postharvest soil of 2nd crop
T₁	22 h	17 f
T₂	27 g	20 f
T₃	29 f	24 e
T₄	31 e	27 e
T₅	33 d	30 d
T₆	39 c	34 c
T₇	43 b	40 b
T₈	55 a	49 a
CV (%)	1.07	2.4
LSD(0.05)	1.93	2.57

Here, T₁: 0%= 0 gm (SS powder) in 9 kg soil, T₂: 0.1%= 9 gm (SS powder) in 9 kg soil, T₃: 0.2%=18 gm (SS powder) in 9 kg soil, T₄: 0.3%=27 gm (SS powder) in 9 kg soil, T₅: 0.4%=36 gm (SS powder) in 9 kg soil, T₆: 0.5%= 45 gm (SS powder) in 9 kg soil, T₇:0.75%= 67.5 gm (SS powder) in 9 kg soil and T₈: 1%= 90 gm (SS powder) in 9 kg soil.

4.5.5 Effect on available sulphur content (ppm) in soil

4.5.5.1 Effect on available sulphur content (ppm) in postharvest soil of 1st crop

Sulphur availability in post-harvest soil of first crop (radish) varied significantly due to different doses of organic material (shrimp shell powder) applications (Table-6). At harvest of first crop, the availability of sulphur present in soil ranged from 49.5 ppm at T₁ to 93.94 ppm at T₈. The maximum available of sulphur (93.94 ppm) in post-harvest soil of radish was observed in T₈ treatment and had strong significant difference with control T₁ (0% chitosan). The lowest available sulphur (49.5 ppm) in post-harvest soil of radish was found in control T₁ treatment. While treatment T₈ showed significant difference than all other treatments. Materechera (2012) reported that different organic materials increased the pH of acid soils and improve soil fertility by supplying essential plant nutrients. Practically, it is difficult to supply sulphur organically so in such case shrimp shell powder can be a good source of sulphur for plants.

4.5.5.2 Residual effect on available sulphur content (ppm) in postharvest soil of 2nd crop

The available sulphur content in postharvest soil of spinach (second crop) varied significantly due to the residual effect of organic material (shrimp shell powder) in soil (Table:6). The highest available sulphur content in soil after harvesting of radish and spinach was found in treatment T₈ (46.5 ppm) which was significantly different than all other treatments. This might be due to mineralization of sulphur from organic material and the release of sulphur from sulphur containing amino acids during decomposition of organic material (shrimp shell powder). While the T₁ (0% shrimp shell powder) treatment had the lowest (19.94ppm) available sulphur in the soil after harvesting of the radish and spinach. Available sulphur content of residual soil of all treatments was lower than post harvest soil of radish. Because no other organic or inorganic fertilizer was used after harvesting radish and spinach plant uptook some of nutrient from soil. Organic material usually contains major, secondary and micro nutrients required for plant growth and therefore can be a good source of these plant nutrients. These nutrients need to be mineralized in the soil before being absorbed by plants.

Table 6: Effect of different doses of organic material (Shrimp shell powder) on available sulphur content (ppm) in post harvested soils of 1st crop and 2nd crop

Treatments	Available sulphur content (ppm) in postharvest soil of 1 st crop	Available sulphur content (ppm) in postharvest soil of 2 nd crop
T ₁	49.5 e	19.94 h
T ₂	66.75 c	26.56 e
T ₃	73.06 b	24.56 f
T ₄	53.5 d	22.63 g
T ₅	52.88 d	30.94 d
T ₆	73.69 b	38.39 c
T ₇	73.65 b	43.75 b
T ₈	93.94 a	46.5 a
CV (%)	5.14	2.58
LSD(0.05)	0.004	2.54

Here, T₁: 0%= 0 gm (SS powder) in 9 kg soil, T₂: 0.1%= 9 gm (SS powder) in 9 kg soil, T₃: 0.2%=18 gm (SS powder) in 9 kg soil, T₄: 0.3%=27 gm (SS powder) in 9 kg soil, T₅: 0.4%=36 gm (SS powder) in 9 kg soil, T₆: 0.5%= 45 gm (SS powder) in 9 kg soil, T₇:0.75%= 67.5 gm (SS powder) in 9 kg soil and T₈: 1%= 90 gm (SS powder) in 9 kg soil.

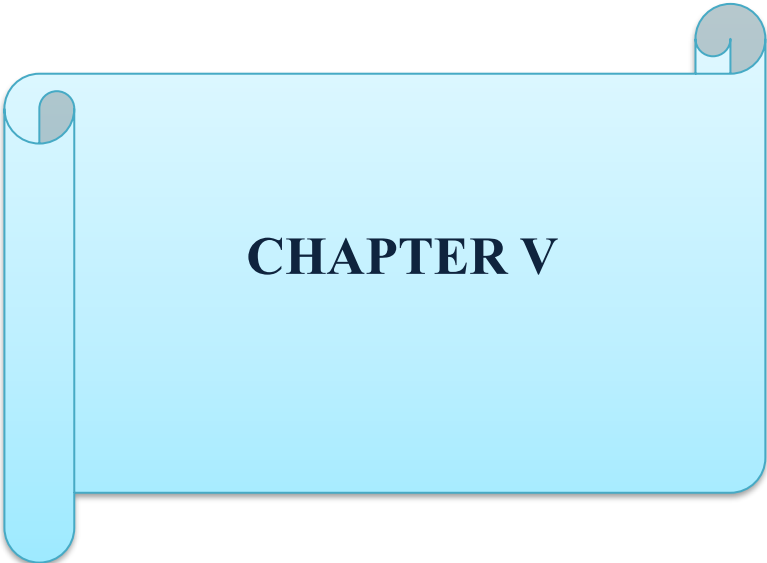
4.5.6 Effect on pH status of soil

A significant variation was found in soil pH value in post-harvest soil of radish (first crop) ranged from 5.9 to 6.9 and post-harvest soil of spinach (second crop) ranged from 5.9 to 7.1. In both postharvest soils, highest pH value was recorded at treatment T₈ (1% shrimp shell powder). From this study it was examined that, the residual effect of shrimp shell powder application in soil increased the pH status of soil. These results was supported by Sultana *et al.*, (2020) who carried out an experiment with the raw material of chitosan powder with one control. So, it is seen that with increasing concentrations of shrimp shell powder pH of acidic soil increases and enhances nutrient availability. Shrimp shell powder (chitosan) is alkaline in nature which was supported by Rahman (2015) who found that modified chitosan powder increased the

soil pH that could be a good point of nutrient mineralization in soil from organic sources. Nutrient supplementation would be increased due to the increment of alkalization levels which will improve the biological and physico-chemical properties of soil (Issak and sultana, 2017). Shrimp shell powder can be a promising soil amendment material for the management of acidic soils and it has the potential to inhibit soil acidification. The raw material of the chitosan powder affect the soil pH, it might be due to the shrimp shells contents most abundantly carbonates of Ca & Mg which was confirmed by Synowiecki *et al.* (2003), Beaney *et al.* (2005) and Mahmoud *et al.* (2007).

Table 7: Direct and residual effect of different doses of organic material (shrimp shell powder) on pH of crop harvested soils

Treatments	Initial pH	pH of the postharvest soil of (1st crop)	pH of the postharvest soil of (2nd crop)
T₁	5.9	5.9	5.9
T₂	5.9	6.1	6.3
T₃	5.9	6.1	6.3
T₄	5.9	6.2	6.5
T₅	5.9	6.5	6.8
T₆	5.9	6.6	6.9
T₇	5.9	6.7	7
T₈	5.9	6.9	7.1



CHAPTER V

CHAPTER V

SUMMARY AND CONCLUSION

Application of organic material (Shrimp shell powder) had a profound influence on germination, plant height, fresh weight and dry weight of leafy vegetables (radish and spinach). It has both morphological and physiological influence on plant. The organic material (shrimp shell powder) enhanced soil characteristics in such a level that induced the growth of radish (first crop) and spinach (residual crop) and boosts up the yield. All the traits were significantly increased by the different treatments. The use of organic material (shrimp shell powder) also helps to maintain soil health and improves soil properties and thus increases soil OC%, total nitrogen %, available sulphur content (ppm) and phosphorus content (ppm).

In case of radish (first crop) different levels of organic material (shrimp shell powder) application significantly influenced germination percentage of radish where the highest germination percentage (98.8%) was observed in T₄ (0.3% shrimp shell powder) treatment which is statistically similar to treatment T₁, T₂ and T₃. The highest average plant height (31.56cm) at harvesting was found at treatment T₆ (0.5% shrimp shell powder) which was statistically similar with T₄ (0.3%) and T₅ (0.4% shrimp shell powder) and lowest plant height (11.38cm) was found in T₈ (1% shrimp shell powder) treatment. Maximum fresh weight (374.5g pot⁻¹) was found in treatment T₅ (0.4% shrimp shell powder) which was significantly different from control T₁ (0%) and statistically similar with T₄ (0.3% shrimp shell powder) and T₆ (0.5%) where minimum fresh weight (7g pot⁻¹) was found in treatment T₈ (1% shrimp shell powder). Similarly, maximum oven dry weight (25.05g pot⁻¹) was recorded at treatment T₅ (0.4% shrimp shell powder) which was significantly different from control T₁ (0% shrimp shell powder) and statistically similar with T₄ (0.3% shrimp shell powder) and T₆ (0.5% shrimp shell powder) and minimum average oven dry weight (0.53g pot⁻¹) was found in T₈ (1% shrimp shell powder).

In case of spinach (second crop), the highest germination percentage (100%) was observed in treatment T₆ (0.5% shrimp shell powder) and T₈ (1% shrimp shell powder) which had significant difference from the control T₁ and lowest germination percentage (89%) was found in treatment T₂ (0.1% shrimp shell powder). The highest average plant height (34.28cm) at 40 DAS during harvesting was found at treatment T₈ (1% shrimp shell powder) which was significantly different from the control treatment T₁ (0% shrimp shell powder) and lowest average plant height (17.8 cm) was found in T₂ (0.1% shrimp shell powder). Maximum average fresh weight (308 g pot⁻¹) was found in treatment T₈ (1% shrimp shell powder) which was significantly different from control treatment T₁ (0% shrimp shell powder) and minimum average fresh weight (53.25g pot⁻¹) was found at treatment T₂ (0.1% shrimp shell powder). Similarly, maximum average oven dry weight (20.5g pot⁻¹) was recorded at treatment T₈ (1% shrimp shell powder) which was significantly different from control T₁ (0% shrimp shell powder) and minimum average oven dry weight (4.09g pot⁻¹) was found in treatment T₂ (0.1% shrimp shell powder). Here, in case of spinach (residual crop) treatment T₈ (1% shrimp shell powder) showed better result and performance and significant difference than all other treatments.

The maximum organic carbon content (3.7%) in post-harvest soil of radish (first crop) was observed from T₈ treatment. While the lowest organic carbon content in post-harvest soil of radish (first crop) was found in control treatment T₁ (3.23%) treatment and the highest organic matter content (6.4 %) in soil was observed in treatment T₈ treatment (1% shrimp shell powder) and lowest (5.56%) organic matter found in control treatment T₁. So, treatment T₈ improves the soil organic matter content and maintains soil health and environment free from pollution. Similarly, the organic carbon content higher (3.45%) at T₈ and lowest organic carbon (2.5%) contained at T₁ of postharvest soil of spinach (second crop) and. So, treatment T₈ (1% chitosan) shows highest organic matter content (5.93%) and treatment T₁ shows lowest organic matter content (4.3%). T₈ treatment also gave highest total nitrogen content%, available sulphur content (ppm) and phosphorus (ppm) in both postharvest soil of first crop and postharvest soil of second crop. Residual value indicates that shrimp shell powder has a slow releasing nitrogen supplementation, soil organic carbon and soil pH.

Based on the experimental results, it can be concluded that,

- i. Application of organic material (Shrimp shell powder) in the pot soil had a significant influence on plant morphological growth and yield attributes in radish (BARI mulashak-1) and spinach (BARI palonshak-1).
- ii. In case of Radish (first crop), the application of organic material (Shrimp shell powder), T₄ (0.3% shrimp shell powder) and T₅ (0.4% shrimp shell powder) treatment is safe and had significant difference over the control treatment T₁ and there is sudden fall of germination % at treatment T₇ and T₈ treatments. So, Treatment T₄ (0.3% shrimp shell powder) showed better performance than other treatments.
- iii. In case of spinach (second crop), the application of organic material (Shrimp shell powder), treatment T₈ (1%) showed better performance than other treatments.
- iv. The organic material (shrimp shell powder) improved chemical properties of soil indicating sustainable soil health improvement.

RECOMMENDATION:

From the findings, it is apparent that the direct and residual effect for application of organic material (Shrimp shell powder) improved yield and yield characters of radish and spinach. And in both soil samples, the total N%, OC%, available Phosphorus and Sulphur content (ppm) are increased with increasing concentration of organic material (shrimp shell powder) with good alkaline properties.

In order to recommend the practices of this direct and residual effect, the following aspects would be considered in future:

- i. Experiments need to be conducted in different locations and field conditions and seasons of Bangladesh to draw a final conclusion regarding residual effect of the organic material (shrimp shell powder).
- ii. varietal trials should be set further at different locations.

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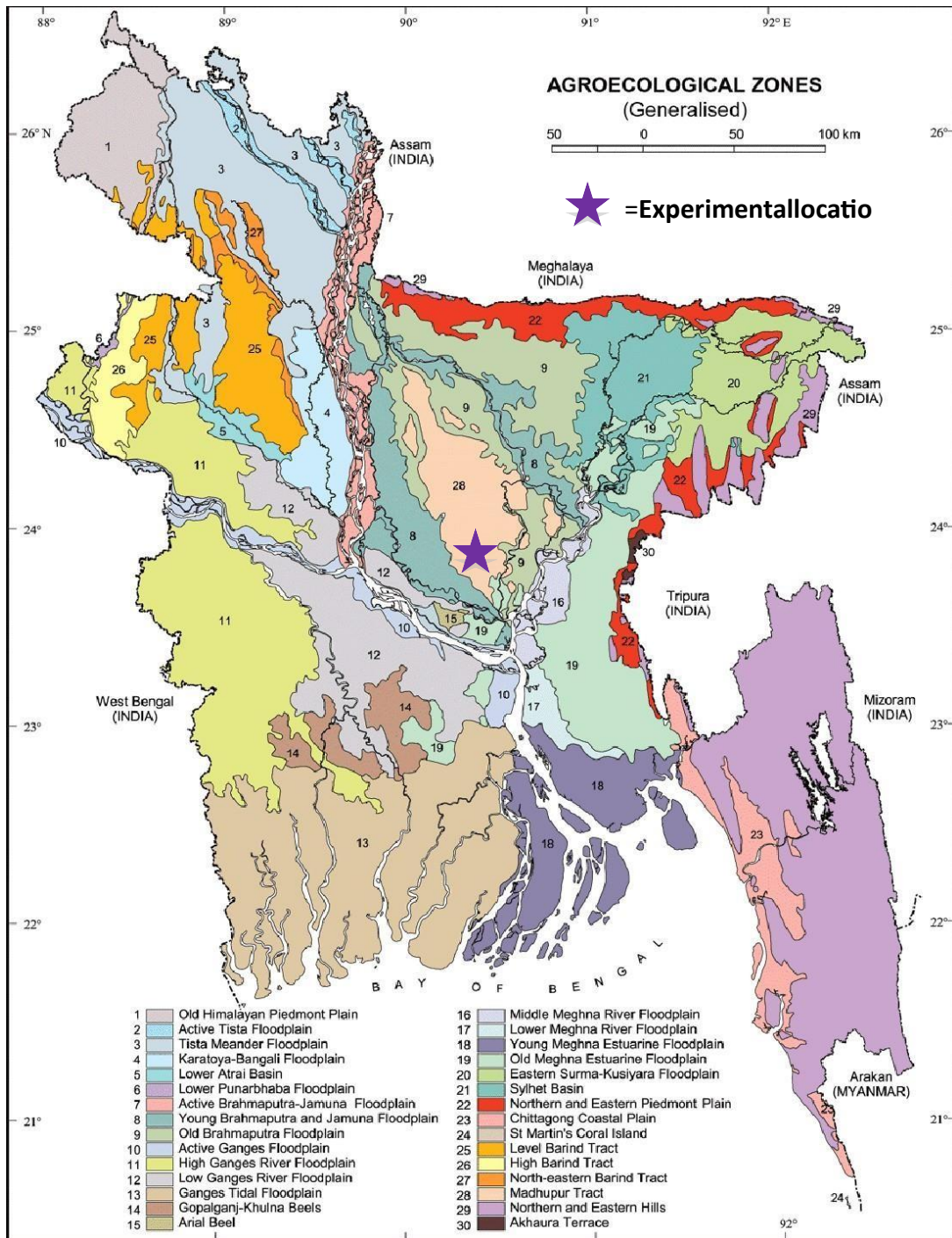
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APPENDIX

Appendix I: Map showing the experimental location under study



Appendix II. Monthly meteorological information during the period from November 2020 to February, 2021.

Year	Month	Air temperature (⁰ C)		Relative humidity (%)	Average rainfall (mm)
		Maximum	Minimum		
2020	November	29.6 ⁰ C	19.8 ⁰ C	53%	00 mm
	December	28.8 ⁰ C	19.1 ⁰ C	47%	00 mm
2021	January	25.5 ⁰ C	13.1 ⁰ C	41%	00 mm
	February	25.9 ⁰ C	14 ⁰ C	34%	7.7 mm

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

Appendix III: Average plant height (cm) at 10 DAS, 30 DAS and 40 DAS of radish (first crop)

Treatments	Av. Plant height (cm) at 10 DAS	Av. Plant height (cm) at 30 DAS	Av. Plant height (cm) at 40 DAS
T ₁	14.4 a	20.9 b	22.28 b
T ₂	14.74 a	23.5 ab	26.59 ab
T ₃	14.2 a	25.75 ab	28.07 ab
T ₄	13.7 ab	26.9 a	30.0 a
T ₅	11.85 b	27.4 a	30.19 a
T ₆	7.2 c	25.7 ab	31.56 a
T ₇	2.6 d	21.05 b	26.5 ab
T ₈	1.4 d	8.94 c	11.38 c
CV (%)	13.52	17.81	20.5
LSD	1.99	5.9	7.61

Appendix IV: Germination (%), average fresh weight (g pot⁻¹) and average oven dry weight (g pot⁻¹) of radish (first crop)

Treatments	Germination (%) of	Av. fresh weight (g pot ⁻¹)	Av. Oven dry weight (g pot ⁻¹)
T ₁	97.5 a	159.5 c	13.6 b
T ₂	96.25 ab	296.5 b	18.8 ab
T ₃	98.7 a	277.5 b	18.2 ab
T ₄	98.8 a	355.75 a	23.06 a
T ₅	91.25 b	374.5 a	25.05 a
T ₆	63.75 c	316.5 ab	23.5 a
T ₇	18.75 d	32.75 d	2.65 c
T ₈	5 e	7 d	0.53 d
CV (%)	6.93	17.49	20.65
LSD	7.26	58.5	7.07

Appendix V: Germination (%), Av. Plant height (cm) at 40 DAS of spinach (second crop)

Treatments	Germination (%)	Av. Plant height (cm) at 40 DAS
T ₁	95 ab	18.86 e
T ₂	89 cd	17.8 f
T ₃	94 bc	22.73 d
T ₄	90.3 bcd	22.9 d
T ₅	95 ab	28.95 c
T ₆	100 a	30.45 b
T ₇	95 ab	31.35 b
T ₈	100 a	34.28 a
CV (%)	3.75	2.74
LSD	5.13	1.04

Appendix VI: Average fresh weight (g pot⁻¹) and average oven dry weight (g pot⁻¹) of spinach (second crop)

Treatments	Av. fresh weight (g pot ⁻¹)	Av. Oven dry weight (g pot ⁻¹)
T ₁	62 ef	4.74 ef
T ₂	53.25 f	4.09 f
T ₃	65.5 ef	5 ef
T ₄	107.75 de	7.2 de
T ₅	139 cd	9.3 cd
T ₆	177.5 bc	11.7 bc
T ₇	217 b	14.5 bc
T ₈	308 a	20.5 a
CV (%)	18.33	15.05
LSD	48.47	3.12

Appendix VII: Analysis of variance (mean square) of plant height(cm) at 10 DAS, 30 DAS and 40 DAS (during harvesting), fresh weight (g pot⁻¹) and oven dry weight (g pot⁻¹) and germination % of radish (first crop)

Source	df	Av. Plant height (cm) at 10 DAS	Av. Plant height (cm) at 30DAS	Av. Plant height (cm) at 40 DAS	Av. Fresh weight (g pot ⁻¹)	Av. Oven dry weight (g pot ⁻¹)	Germination (%)
Replication	3	1.58	29.44	21.59	423.6	7.97	41.47
Treatment	7	122.1	145.34	169.52	82520.5	356.09	5958.93
Error	21	1.83	16.085	26.78	1582.7	23.09	24.4
Total	31						

Appendix VIII: Analysis of variance (mean square) of average plant height (cm) at 40 DAS (during harvesting), fresh weight (g pot⁻¹) and germination (%) of spinach (second crop)

Source	df	Av. Plant height (cm) at 40 DAS	Av. Fresh weight (g pot ⁻¹)	Germination (%)
Replication	3	0.611	1966.1	9.375
Treatment	7	150.866	31855.8	74.91
Error	21	0.504	1086.2	12.16
Total	31			

Appendix IX: Analysis of variance (mean square) of total nitrogen (%) and organic matter (%) of postharvest soil of radish (first crop) and spinach (second crop)

Source	df	OM% on postharvest soil of 1 st crop	OM% on postharvest soil of 2 nd crop	Total N % on postharvest soil of 1 st crop	Total N% on postharvest soil of 2 nd Crop
Replication	3	0.00256	0.0148	4.62E-05	7.62E-06
Treatment	7	0.3562	1.46	1.04E-03	2.22E-04
Error	21	0.0167	0.036	1.99E-05	8.28E-06
Total	31				

Appendix X: Analysis of variance (mean square) of available phosphorus (ppm), sulphur (ppm) and OC (%) of postharvest soil of radish (1st crop) and spinach (2nd crop)

Source	df	P on post harvest soil (1st crop)	P on post harvest soil (2nd Crop)	S on post harvest soil (1st crop)	S on post harvest soil (2nd crop)	OC% on post harvest soil (1st crop)	OC% on post harvest soil (2nd Crop)
Replication	3	0.25	1.88	3.77	0.151	0.025	0.0104
Treatment	7	4322.6	1509.3	879.59	412.41	0.113	0.502
Error	21	1.08	1.14	2.99	0.89	0.002	0.008
Total	31						

PLATES



Plate 1: Radish plant (First crop) at 28 DAS



Plate 2: Radish plant (First crop) at 40 DAS before harvesting



Plate 3: First crop (Radish) for sun drying after harvest



Plate 4: Second crop (Spinach) at 28 DAS



Plate 5: Second crop (Spinach) at 40 DAS before harvesting



Plate 6: Soil sample at shaker for testing soil available phosphorus content



Plate 7: Soil sample weighing before soil test



Plate 8: Picture with my honorable supervisor sir