EFFECT OF COMBINED APPLICATION OF BIOFERTILIZER AND NITROGENOUS FERTILIZER ON GROWTH AND YIELD OF WHEAT (*Triticum aestivum* L.)

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CERTIFICATE

*This is to certify that thesis entitled, "**EFFECT OF COMBINED APPLICATION OF BIOFERTILIZER AND NITROGENOUS FERTILIZER ON GROWTH AND YIELD OF WHEAT (Triticum aestivum L.)**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.) in SOIL SCIENCE**, embodies the result of a piece of bona-fide research work carried out by **MD. MONIRUL ISLAM**, Registration no. **19-10052** 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.

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Dedicated

To

My beloved

Parents

EFFECT OF COMBINED APPLICATION OF BIOFERTILIZER AND NITROGENOUS FERTILIZER ON GROWTH AND YIELD OF WHEAT (*Triticum aestivum* L.)

ABSTRACT

The experiment was conducted at the research field, Sher-e-Bangla Agricultural University during the period from November, 2019 to March, 2020 to study the effect of combined application of biofertilizer and nitrogenous fertilizer on growth and yield of wheat (*Triticum aestivum* L.). The experiment comprised of single factor comprising eight treatments. *Azotobacter* was incorporated with wheat seed as per treatment. This experiment was laid out in a randomized complete block design (RCBD) with three (3) replications. The results revealed that treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] exhibited its superiority compared to that of other biofertilizer and nitrogenous fertilizer treatments in terms of grain yield. Treatment T_5 out-yielded over T_8 (No *Azotobacter* + 120 kg N ha^{-1}) by 5.26%, T_3 [*Azotobacter* (6 kg ha^{-1}) + 60 kg N ha^{-1}] by 7.57%, T_6 [No *Azotobacter* + 90 kg N ha^{-1}] by 8.64%, T_7 [*Azotobacter* + 120 kg N ha^{-1}] by 13.69%, T_4 [No *Azotobacter* + 60 kg N ha^{-1}] by 15.18% and T_2 [*Azotobacter* (8 kg ha^{-1})] by 25.00% higher grain yield. Treatment T_5 also showed the tallest plant at 90 DAS (107.53 cm), the highest number of spikelets per spike (19.47), the highest number of grains per spike (75.93), the longest spike (15.70 cm), the maximum weight of 1000-grain (48.90 g), the highest straw yield (7.68 t ha^{-1}) and the maximum harvest index (38.76%) than other treatments in this experiment. On the other hand, the treatment T_1 [Control (No *Azotobacter*, No N as urea)] returned with 69.88% lower yield than treatment T_5 which was significantly the lowest compared with other treatments under study. In case of soil properties, the highest soil pH (6.21) and the highest soil organic matter (1.58%) in post-harvest soil was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}]. Considering the soil nutrients, the maximum total N (0.11%), the maximum available phosphorous (25.96 ppm), the maximum available sulphur (20.73 ppm) and the highest exchangeable K (0.129 me%) in post-harvest soil was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}]. It can be said that *Azotobacter* along with nitrogenous fertilizer improved soil chemical properties along with increased availability of essential plant nutrients in soil solution. *Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1} application seemed promising for producing higher grain yield of wheat and sustaining soil productivity.

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LIST OF ABBREVIATIONS

ABBREVIATION	ELLABORATION
AEZ	Agro-Ecological Zone
Agric.	Agriculture
Agra.	Agricultural
Annu.	Annual
Appl.	Applied
Biol.	Biology
Cfu	Colony forming unit
Chem.	Chemistry
cm	Centi-meter
CV	Coefficient of Variance
DAS	Days After Storage
DAP	Days After Planting
Dev.	Development
Ecol.	Ecology
Environ.	Environmental
etci	and others
Exptl.	Experimental
g	Gram (s)
i.e.	that is
J.	Journal
kg	Kilogram (s)
LSD	Least Significant Difference
M.S.	Master of Science
m^2	Meter squares
mg	Milligram
Nutr.	Nutrition
Physiol.	Physiological
Progress.	Progressive
Res.	Research
SAU	Sher-e-Bangla Agricultural University
Sci.	Science
T	Tuber size
Soc.	Society
SRDI	Soil Resource Development Institute
t ha^{-1}	Ton per hectare
UNDP	United Nations Development Programme
Viz	*videlicet* (L.), Namely
%	Percentage
@	At the rate of
μMol	Micromole

LIST OF ABBREVIATIONS (CONT'D)

AEZ	Agro-Ecological Zone
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
CV %	Percent Coefficient of Variance
cv.	Cultivar (s)
DAS	Days After Sowing
eds.	editors
et al.	et alia (and others)
etc.	et cetera (and other similar things)
FAO	Food and Agricultural Organization
L.	Linnaeus
i.e.	id est (that is)
MoP	Muriate of Potash
SAU	Sher-e-Bangla Agricultural University
SRDI	Soil Resources and Development Institute
TDM	Total Dry Matter
TSP	Triple Superphosphate
UNDP	United Nations Development Programme
var.	variety
viz.	namely

CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important cereal crop throughout the world, it is the essential food for over 35% of the global population and provides calories and proteins in the diet for the human (Sary *et al.*, 2009; Campuzano *et al.,* 2012; Abedi *et al.,* 2010). Wheat is the main food of people in many countries and about 70% calories and 80% protein of human diet is supplied from its consumption (Taregh *et al.*, 2011). It is used as a source of both food and income and considered as the most important strategic cereal crop not only in Bangladesh but also in the world. Wheat is the second most important cereal crop in Bangladesh. It can be a good supplement of rice and can play a vital role to feed the teeming millions of hungry people in this country. About two-thirds of the world's population lives on wheat grains. It is superior to rice for its higher protein content, vitamins and minerals. The annual production of wheat grain in Bangladesh in the year 2018–2019 was 10.16 lakh metric tons obtained from 3.30 lakh hectares of land and in the year 2019–2020 it was 10.29 lakh metric tons obtained from 3.32 lakh hectares of land (BBS, 2020).

Bangladesh is not self-sufficient to feed her ever increasing population, but there is a possible scope to minimize food deficit by means of increasing wheat production with adoption of modern scientific technology for cultivation. Wheat has a definite prospect for growing as rabi crop in the northern districts where a vast area remains fallow during the rabi season. Because, it can be grown in low soil moisture condition even without irrigation which is not possible in case of boro rice. The climate and soil of Bangladesh is quite favorable for the cultivation of wheat. Wheat can be used as good substitute for rice in respect of protein supplement which contains about 11% as against 6.4% in rice. The wheat research center (WRC) of Bangladesh Agricultural Research Institute (BARI) released 31 varieties of spring wheat for commercial cultivation. The average yield of these varieties hardly approached to 3 tons per hectare in research field. But in some of the developed countries yield is much higher which even reach about 8 tons per hectare. So, the production of wheat needs to be increased annually as to meet the over increasing demand of food grain on a per with population growth.

Every increasing population of the world demands the increase in food production which intern depends upon the improved agricultural practices. More recently a real challenge faces the workers in the agricultural research field to stop using the high rates of agro-chemicals which negatively affect human health and environment. Farmers use chemical fertilizers to increase production to meet their needs, but the excessive use of fertilizers leads to contamination of soil and groundwater and reduce soil fertility. Annually more than 200 million tons of chemical fertilizers are used worldwide to increase the yield of crop plants. (FAO, 2022). Despite their efficiency in promoting crop yield, they have proved to be hazardous for soil health as well as for the well-being of human and animal populations. The worldwide spread of inflation, initiated by several fold rises in Petroleum price thereby depicting its striking influence on the prices of chemical nitrogenous fertilizers. The prices of nitrogenous fertilizers have nearly doubled during the last 4–5 years. On the other hand, fertility of Bangladesh soils has been declining due to extensive use of land and chemical fertilizers in quest of producing more food for ever-increasing population. The organic matter content of most soils is below the critical level mainly due to rapid decomposition under high temperature and high humidity prevailing in Bangladesh. In addition, the use of urea accelerates further decomposition of organic matter. As a result, yield stagnancy has arisen as a national problem.

Since Bangladesh soils are very deficient in nitrogen, but this nutrient is required in larger quantities for obtaining a good crop yield and therefore, urea is being used extensively for all crops. Its extensive use has been inflicting an adverse effect on environment causing pollution of drinking water and damaging beneficial soil flora and fauna. Under such situation, to address the issue of soil fertility and crop productivity the overall management system of crop culture needs to be improved. On the other hand, for marginal farmers in Bangladesh, the purchase of chemical fertilizers is difficult and expensive. This has necessitated searching for cheaper source of nitrogen to meet the needs of crops. So, biofertilizers can replace partially chemical fertilizers. Hence, there is a need to search for alternative strategies to improve soil health without causing damage to environment as well as soil. Therefore, biofertilizers are gaining importance as they are ecofriendly, non-hazardous and nontoxic products (Sharma *et al.,* 2007).

Biofertilizers include mainly the nitrogen fixing, phosphate solubilizing and plant growth promoting microorganisms. Biofertilizers benefiting the crop production are *Azotobacter*, *Azospirillum*, blue green algae, *Azolla*, P-solubilizing microorganisms, mycorrhizae and sinorhizobium (Selvakumar *et al.*, 2009). Among the biofertilizers, *Azotobacter* represents the main group of heterotrophic, non-symbiotic, gram negative, free living nitrogen-fixing bacteria. It can successfully grow in the rhizospheric zone of wheat, maize, rice, sorghum, sugarcane, cotton, potato, tomato, brinjal, cabbage and many others and fix 10–20 kg N ha^{-1} cropping seasion^{-1} (Jadhav *et al.*, 1987). The genus *Azotobacter* includes 6 species, with *A. chroococcum* most commonly inhabiting in various soils all over the world (Mahato *et al.*, 2009). Besides nitrogen fixation, *Azotobacter* synthesizes and secretes considerable amounts of biologically active substances like B vitamins, nicotinic acid, pentothenic acid, biotin, heteroauxins, gibberellins etc. which enhance root growth of plants (Rao, 1986). Another important characteristic of *Azotobacter* association with crop improvement is excretion of ammonia in the rhizosphere in the presence of root exudates, which helps in modification of nutrient uptake by the plants (Narula and Gupta, 1986). When *Azotobacter* is applied to seeds, seed germination is improved to a considerable extent, so also it controls plant diseases due to above substances produced by *Azotobacter*. The exact mode of action by which *Azotobacter* enhances plant growth is not yet fully understood. Three possible mechanisms have been proposed: N_2 fixation; delivering combined nitrogen to the plant; the production of phytohormone-like substances that alter plant growth and morphology, and bacterial nitrate reduction, which increases nitrogen accumulation in inoculated plants (Mrkovacki and Milic, 2001).

In Bangladesh, very limited studies were carried out on the effect of *Azotobacter* on wheat growth and productivity. Therefore, a field experiment was carried out to study the effect of *Azotobacter* inoculant on wheat growth and productivity. The present investigation is to study the effect of nitrogenous fertilizer and *Azotobacter* applied alone and in various combinations on the yield of wheat. So as to select the appropriate dose of fertilizers in wheat production, saving as much fertilizer nitrogen as possible.

Based on the above points the present study was undertaken with the following objectives:

1. To examine the impact of combined use of *Azotobacter* and nitrogen fertilizer on growth and yield of wheat.

2. To identify the appropriate combined dose of biofertilizer and nitrogen for wheat cultivation.

CHAPTER II

REVIEW OF LITERATURE

A large number of research-works on growth and yield of wheat and its response to biofertilizer and nitrogen have been carried out in different wheat growing countries of the world. Research activities on the effect of combined application of biofertilizer and nitrogenous fertilizer on growth and yield of wheat are few in Bangladesh. Some of the literatures pertinent to this study are reviewed below:

Singh *et al*. (2019) carried out an experiment to study the effect of different organic, inorganic and biofertilizer on the yield and yield components of Wheat (*Triticum aestivum* L.). The experiment concluded that among the treatments T_4 (Poultry manure) has performed better in parameter like plant height (101.70cm) and number of spikes plant^{-1} (7.4). T3 (FYM + *Azotobacter*) has better performed in parameter like dry matter (27.90g) and number of tillers plant^{-1} (7.6). T_5 (Poultry manure + *Azotobacter*) has better performed in parameter like grain/spike (52.0), 1000 grain weight (40.1g), yield (21.7qt/acre), and harvest index (53.8%). So, it can be concluded that inoculation with *Azotobacter chroococcum* in combination with organic manure (poultry manure) gave better yield in comparison to other treatments.

Patra *et al.* (2019) conducted a field experiment at CCSHAU, Hisar to determine the effect of seed priming, biofertilizer inoculations and nitrogen levels on late sown wheat. The experiment comprised of 5 seed priming treatments i.e. no seed priming, seed priming with water, seed priming with water + *Azotobacter*, seed priming with water + AM fungi, seed priming with water + Biomix in main plot and 4 nitrogen levels viz. 120, 135, 150 and 165 kg ha^{-1} in sub plot was carried out in split plot design with three replications. Seed priming with water along with either of biofertilizer inoculations i.e., *Azotobacter*, AM fungi and Biomix significantly improved grain yield, straw yield and yield attributes except 1000 -grain weight as compared to non-primed-uninoculated treatment. However, harvest index was not significantly influenced by seed priming as well as inoculation treatments. The net returns (Rs.44041 ha^{-1} ha) and B:C ratio (2.07) were highest under the treatment water primed seeds inoculated with biomix closely followed by water primed seeds inoculated with AM fungi with returns of Rs. 43471 ha^{-1} and B:C of 2.06. Application of 150 kg N ha^{-1} significantly improved yield attributes except 1000 grain weight; yields and economic returns over both 120 and 135 kg N ha^{-1} levels, however, it was at par to 165 kg N ha^{-1} dose. Integrated use of 135 kg N ha^{-1} and water primed seed inoculated with biomix gave yield and economic returns comparable to highest dose of 165kg N ha^{-1} alone.

Mahato and Kafle (2018) conducted a pot experiment to evaluate the effects of *Azotobacter* inoculant on the growth and yield of wheat (variety Gautam) at the premise of Lamjung Krishi Campus, Nepal during the winter season of 2016–17. A completely randomized design was chosen with seven treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) each replicated three times. The treatments were control (T_1), 120:80:80 kg NPK ha^{-1} (T_2), *Azotobacter* seed inoculated (T_3), *Azotobacter* soil application (T_4), *Azotobacter* + 120:80:80 kg NPK ha^{-1} (T_5), *Azotobacter* + 10 t FYM ha^{-1} (T_6), *Azotobacter* + 120:80:80 kg NPK ha^{-1} + 10 t FYM ha^{-1} (T_7). Root length, root weight, shoot weight, plant height, panicle weight, grain weight, grain yield, total biomass, and biological yield were significantly affected by treatments. Inoculation of *Azotobacter* only increased 16.5%–19.42% grain yield over control i.e. non inoculated treatments while with other fertilizers increase was of range 19.42%–63.1%. The increase in yield was 23.3% with only chemical fertilizer NPK (T_2) over control. So *Azotobacter* can be used as a biofertilizer for greater yield and the yield is highest with *Azotobacter* combined with farmyard manure and inorganic fertilizer (NPK).

Patra and Singh (2018) conducted a field experiment during rabi season 2015–16 at research farm of CCSHAU, Hisar to study the impact of priming, biofertilizers with different nitrogen levels on growth, yield and nutrient uptake of late sown wheat. The experiment comprised of 5 treatments i.e. no seed priming, seed priming with water, seed priming with water + *Azotobacter*, seed priming with water + AM fungi, seed priming with water + Biomix in main plot and 4 nitrogen levels viz. 120, 135, 150, 165 kg ha^{-1} in sub plot was carried out in split plot design with three replications. Priming with biofertilizer inoculations significantly improve yield and nutrient uptake. Yield and nutrient uptake is highest at biomix treated plot than others. The growth and yield and nutrient uptake showed an increase with increase in nitrogen dose up to 150 kg ha^{-1}.

Sharifi and Amiryusefi (2017) carried out in a field factorial experiment based on randomized complete block design with three replications in a silty loam soil. Experimental factors were including two levels of *Azotobacter chroococcum* (seed inoculation with *Azotobacter* at a concentration of 107 bacteria per gram and un-inoculation) and five levels of nitrogen (0, 50, 100, 150 and 200 kg N ha^{-1} from urea source). *Azotobacter* effect was significant on grain yield, number of grains per spike, grain protein content ($P \leq 0.05$), SPAD, number of spikes per m^2 and thousand grain weight ($P \leq 0.01$). Effect of nitrogen was significant on all of the traits except of number of grains per spike ($P \leq 0.01$). Interaction effect of two factors was significant on grain yield, SPAD ($P \leq 0.05$), plant height, number of spikes per m^2 and number of grains per spike ($P \leq 0.01$). Grain yield was 4899 kg ha^{-1} in combination of seed inoculation with *Azotobacter* and nitrogen (100 kg ha^{-1}), which there was no significant differences with 150 and 200 kg N ha^{-1}. This fertilizer combination increased seed yield by 13.76%, compared to un-innoculation and nitrogen (100 kg ha^{-1}). They concluded inoculation with *Azotobacter* bacteria and nitrogen (100 kg ha^{-1}) could achieve grain yield potential, reduce the adverse environmental impacts and save the N-fertilizer utilization.

Hussen *et al.* (2017) carried out an experiment at Baqubah nursery, Directorate of Diyala agriculture to evaluate the response of wheat (*Triticum aestivum* L.) to inoculation of biofertilizers (*Azotobacter chroococcum* and *Pseudomonas fluorescens*) and chemical fertilizers and their interactions on some growth and yield parameters of wheat. The result showed that the chemical treatments had a significant effect on plant height (47.30 cm), grain yield (.82 g), biological yield (2.18 g), harvest index (37.36%), average of seeds weight/10 spikes (0.81 g), grain yield per m^2 (162 g) and grain yield per hectare (1620 kg). The biofertilizer treatments had a significant effect on the area of flag leaf (14.0 cm^2), while the interaction treatments had a significant effect on spike length (8 cm) and number of seeds per spike (29.90). According to these results, the chemical fertilizers were the most efficacy in effect on growth traits of wheat followed by the combined of chemical fertilizers and biofertilizers then treatment of biofertilizers alone as compared with control treatment. The result of this study revealed that inoculation with biofertilizers (*Azotobacter chroococcum* & *pseudomonas fluorescens*) combined with chemical fertilizers led to improved growth traits and yield of wheat more than using biofertilizers alone, due to the biofertilizers was developed the activity of roots system and producing of hormones for stimulating plant growth subsequently increasing the ability of a plant to access nutrients.

El-Samie *et al*. (2017) conducted two field experiments at the Experimental Farm of the Faculty of Agriculture Fayoum, University in Demo Farm, during 2011–2012 and 2012–2013 seasons. The combination treatments were Control, F_1 = 75 kg N/fed., F_2= 30 m^3 /fed organic manure + biofertilizer, F_3 = 30m^3 /fed organic manure + biofertilizer + 37.5 kg N/fed., F_4= 30m^3 /fed organic manure + biofertilizer + 18.75 kg N/fed., F_5= 30m^3 /fed organic manure + 37.5 kg N/fed., F_6 = 15m^3 /fed organic manure + 37.5 kg N/fed and F_7 =biofertilizer + 37.5 kg N/fed. The tallest plants were obtained from F_3 treatment, and F_4 treatment followed by F_2 treatment gave the largest number of spike plant^{-1} (5.17 and 5.00, respectively). The two treatments F5 and F4 gave higher number of grains per spike (63.67, 63.33) in 1st season and (68.67, 68.50) in 2nd season and weight of grains/spike in the second season (3.53, 3.17) respectively, also F_7 and F_5 followed by F_3 treatment (in 1st season) gave higher weight of grains per spike than the other fertilizer treatments. F_3 treatment alone gave higher weight for 1000-weight (48.03 g), grains yield/fed (2.23 and 2.59 t in 1st and 2nd seasons, respectively) and high percentage for harvest index (44.90% in 2nd season) and protein percentage (14.38 and 14.79% in 1st and 2nd seasons, respectively) than the other fertilizer treatments.

Singh *et al*. (2016) conducted a field experiment during the Rabi season of 2010–2011 at Crop Research Centre of S.V.P.U.A & T Meerut to study the impact of Biofertilizers with different NPK levels on growth and yield of wheat (*Triticum aestivum* L.). Four NPK fertilizer levels (150:75:50, 120:60:40, 90:45:30, 60:30:20 kg NPK ha^{-1} corresponding to control, 125%, 100%, 75%, 50% RDF) with and four biofertilizers treatments {control, *Azotobacter*, Phosphate Solubilizing bacteria (PSB) and co-inoculation of *Azotobacter* +PSB} were replicated three times in Randomized Block Design. The growth and yield attributes showed an increase with increase in the NPK fertilizer levels. 125% RDF recorded significantly highest grain yield (54.10 q ha^{-1}) and straw yield (79.69 q ha^{-1}). *Azotobacter* and PSB inoculation, being at par caused significant improvement in the growth and yield attributes over control. It is concluded on the basis of above investigation the highest grain yield (54.10) was obtained under T_4 (125% NPK of RDF + *Azotobacter* + PSB) followed by (53.13) 100% NPK of RDF + *Azotobacter* + PSB (T_8). Nutrient uptake by grains (N, P & K) recorded highest with the maximum levels of NPK (125% NPK of RDF + *Azotobacter* + PSB) which was significantly higher than all other treatments. On the basis of the investigation they recommended (125% NPK of RDF + *Azotobacter* + PSB) for wheat cultivation under irrigated farming situations.

Yadav *et al*. (2014) conducted a field experiment on late sown wheat at C.S. Azad University of Agriculture and Technology, Kanpur (U.P.), India; during rabi season of 2008–09. The treatments consisted of alone-application of FYM 5 t ha^{-1} or biofertilizer (*Azotobacter* or *Azospirillium*) 5 kg ha^{-1} and their individual or combined integration with 40 kg N ha^{-1} against alone-application of 40, 80 or 120 kg N ha^{-1} along with one control. The results revealed that integrated use of 40 kg N ha^{-1} + 5 t ha^{-1} FYM + 5 kg ha^{-1} biofertilizer (*Azotobacter*) produced highest grain yield of 36.29 q ha^{-1} and earned maximum net income of Rs. 24641 ha^{-1}. It was followed by integration of 40 kg N ha^{-1} + 5 t ha^{-1} FYM + 5 kg ha^{-1} *Azospirillium* with 35.66 q ha^{-1} grain yield and Rs. 23864 ha^{-1} net income and by lone-application of 120 kg N ha^{-1} with 34.89 q ha^{-1} grain yield and Rs. 23173 ha^{-1} net income. These 3 treatments remained significantly at per with each other. All fertility treatments produced significantly higher yield and earned more income than control treatment. It was also observed that integration of FYM or biofertilizer with 40 kg N ha^{-1} recorded yield and income at per with 80 kg N ha^{-1}. They proved that application of inorganic N fertilizer may be reduced by 66.7% with integrated use of 40 kg N + 5 t FYM + 5 kg biofertilizer per hectare in late sown wheat crop.

Mane *et al*. (2014) reported that the application of 125% RDF (80:40:40 kg N:P: K ha^{-1}) + *Azotobacter* + PSB recorded significantly higher panicle length, dry matter per plant, number of spikelets per panicle, number of grains per panicle, weight of grains per panicle, grain yield, straw yield and biological yield of wheat than all other treatments.

Soleimanzadeh and Gooshchi (2013) studied an experiment to find out the effect of seed inoculation by *Azotobacter* and different levels of nitrogen fertilizer on growth and yield of sunflower (Azargol cultivar) in the farm of Islamic Azad University, Par Abad Mogha Branch during 2010–2011 growing seasons. The experiment treatments were arranged in factorial based on a complete randomized block design with three replications. Four nitrogen fertilizer levels of 25, 50, 75 and 100% N recommended with two levels of *Azotobacter*: with and without *Azotobacter* (control) were assigned in a factorial combination. Results showed that plant height, grains number per ear and biological yield were significantly higher in inoculated plants than in non-inoculated plants. Plant height, ear length, grains number per ear, biological yield and grain yield were increased with increasing N level above 75% N recommended in non-inoculated plants, whereas no significant difference was observed between 50 and 100% N recommended. The positive effect of *Azotobacter* inoculation was decreased with increasing N levels. According to the results of this experiment, they concluded that application of (*Azotobacter* + 50% N) had an appropriate performance and could increase grain yield to an acceptable level.

Singh *et al.* (2013) studied the effect of different combinations of nitrogen (urea and/or farmyard manure) doses with *Azotobacter* strain (Azo8) for a dry land wheat variety GW273. It was observed that the combination of *Azotobacter* strain (Azo8) along with urea (60Kg N ha^{-1}) and farm yard manure (40Kg N ha^{-1}) gave the best response. It resulted in more than 23% and 36% increase in shoot fresh weight and dry weight, 26% and 38% increase in root fresh weight and dry weight, 39% increase in test weight of seeds and 27% increase in yield over control.

Milosevic *et al.* (2012) carried out an investigation to study the effect of wheat seed inoculation (the cultivars Renesansa and Zlatka) with *Azotobacter chroococcum,* strain 86 (2–5x CFU 108 ml^{-1}). Experiments were conducted under field conditions, on a chernozem soil. The effectiveness of inoculation on wheat seed quality (germination energy and percentage), yield and 1000-seed weight was shown to depend on the amount of applied urea (50, 100 and 200 kg ha^{-1} and non-fertilized control) and wheat cultivar. *A. chroococcum* treatment, depending on variety and fertilizer treatment increased the energy of germination by 1 to 9% and seed viability by 2 to 8%. Negative effects on seed germination energy and percentage were found in the case of the cultivar Zlatka, in the variant with 100 kg urea ha^{-1}. The largest increase in 1000-seed weight was obtained in the case of the cultivar Renesansa, in the variant without N application (16%). *A. chroococcum* inoculation increased the seed yield of both cultivars in the variant without urea application and with 50 kg ha^{-1} of urea. The highest yield increase (74%) was registered in the case of the cultivar Zlatka when *Azotobacter* inoculated and treated with 50 kg ha^{-1} of urea.

Agamy *et al*. (2012) carried out a field experiment at the Experimental Farm of Faculty of Agriculture, Fayoum University to study the effect of the application of biofertilizer (Bio) (namely, *Azotobacter*, *Azospirillium*, *Rhizobium* and

Pseudomonas), farmyard manure (FM) (0, 15 ton fed^{-1}) and mineral fertilizer (NPK) (0, 25, 50 and 100% of the recommended dose) singly or in combination on growth, yield, anatomical structure and physiological analysis of wheat plant. The results showed that, the application of Bio or FM in combination with NPK significantly increased all growth characters i.e., plant height, number of spikes plant^{-1}, leaf area and fresh and dry weights of both shoot and spikes plant^{-1}. The enhanced growth characters were reflected in yield and yield components, mainly the increase in grains yield fed^{-1} which reached 173.7% by the combined treatment of Bio + FM + 50% NPK. The highest increase in the yield and yield components was obtained by the combined three types of fertilizers, specially the treatment of bio + FM + 50% NPK which led to increases at about 142% in spike length, 137% in number of spikelets spike^{-1}, 18% in weight of 1000 grains and 174% in grains weight fed^{-1} as compared with the control. They concluded that the combined effect of 50% recommended chemical fertilizer (NPK) + organic fertilizer + biofertilizer gave the best results than any other treatment.

Sharma and Chauhan (2011) reported that integrated application of chemical fertilizers in conjugation with manures and biofertilizers was found to be the best nutrient management.

Ahmed *et al.* (2011) concluded that inoculation of wheat plants with biofertilizers (*Azotobacter*, Yeast and *Azotobacter* + Yeast) resulted in significantly higher values of most of growth and yield parameters. Yeast inoculated plants showed superiority over *Azotobacter* inoculation. Further, they reported that mixed inoculums (*Azotobacter* + Yeast) were found to be better than single inoculums.

Rokhzadi and Toashish (2011) found from their experiment that in chickpea, the grain yield, biomass dry weight and N and P uptake of grains were statistically improved due to inoculation with each PGPR such as *Azospirillum*, *Azotobacter chroococcum* + *Mesorhizobium ciceri* + *Pseudomonas fluorescens*. However, group comparison between the treatments showed that, when *Azospirillum* or

Azotobacter was found in the treatment composition there was an expressive improvement in grain yield and plant biomass.

Singh and Prasad (2011) conducted a field experiment during the rabi season to study the efficacy of biofertilizers on growth and productivity of wheat (Triticum aestivum). Leaf and shoot dry weight increased up to 90 days after sowing (DAS) and then gradually declined. Shoot dry weight also gradually increased up to 120 DAS and then decreased. However, dry matter in panicle increased rapidly from 120 DAS till harvesting. At early stage (30–60 DAS), dry matter production was slow and the rate of dry matter accumulation increased from 60 DAS onwards. About 21.5 per cent of dry matter of whole plant was observed at 60 DAS, 61.2 per cent at 90 DAS and 94.4 per cent at 120 DAS. The result showed significant response of biofertilizers on growth and productivity of wheat. Combined application of biofertilizers caused considerable increase in plant height over all the treatments. Tillering enhanced significantly due to application of biofertilizers either alone or in combination. Greater tillering was noticed when the crop received combined treatments than other treatments. Similar trend of results was also observed in case of yield components of wheat i.e. ears/$m^{2,}$ grains/ear and 1000 grain weight increased significantly when the crop received biofertilizers either alone or combined. The highest grain yield was recorded when the crop received combined biofertilizers.

Behera and Rautaray (2010) conducted a field experiment at Indore, India, from 2000 to 2002 in a Vertisol having clay loam texture. The objective was to evaluate the effect of nitrogen-fixing bacteria, phosphorus-solubilizing bacteria, vesicular *Arbuscular mycorrhizae* (VAM) (*Glomus fasciculatum*), and chemical fertilizers on yield performance and quality parameters of durum wheat (*Triticum turgidum* var. durum Desf.). The grain (5664 kg ha^{-1}) and straw yields were higher under recommended fertilizer dose (100% NPK) than 50% NPK (4674 kg ha^{-1}). Compared to the 50% NPK, biofertilizers + 50% NPK increased grain yield marginally (2–6%).

Kushare *et al.* (2009) carried out a field experiment to study the effect of N and P levels and biofertilizers on the growth and yield of wheat under late sown irrigated conditions. Four NP fertilizer levels (0:0, 40:20, 60:30 and 80:40 kg N:P ha^{-1} corresponding to control, 50%, 75%, and 100% RDF) in main plots and four biofertilizer treatments (control, *Azotobacter*, Phosphate Solubilizing bacteria (PSB) and co-inoculation of *Azotobacter* + PSB) in subplot were replicated four times in split plot design. The growth and yield attributes showed an increase with increase in the NP fertilizer levels. 100% RDF recorded significantly highest grain yield (32.40 q ha^{-1}) and straw yield (39.78 q ha^{-1}). *Azotobacter* and PSB inoculation, being at par caused significant improvement in the growth and yield attributes over control. Co-inoculation of both the biofertilizers further increased the growth and yield attributes over individual inoculation. Combined inoculation yielded maximum grain yield (26.06 q ha^{-1}) and straw yield (33.69 q ha^{-1}). Interaction effect showed that application of 60:30 kg N:P ha^{-1} (75% RDF) coupled with combined inoculation registered significantly higher grain yield (30.96 q ha^{-1}) of wheat with higher net profit, B:C ratio than those with 80:40 kg N:P ha^{-1} (100% RDF) (30 q ha^{-1}) without biofertilizer inoculation. Thus 25% saving in nitrogen and phosphorus application could be possible with combined inoculation of *Azotobacter* + PSB.

Reda *et al.* (2006) found that inoculation with nitrogen-fixers lead to increase in wheat grain yield and its nutrient contents particularly in the presence of 50% of nitrogen fertilizer recommended dose.

Khan and Zaidi (2007) found that the triple inoculation with *Azotobacter chroococcum*, Bacillus sp. and *Glomus fasiculatum* increased significantly the dry mater and grain yield by 2.6 and 2-fold respectively than that of non-inoculated wheat plants.

Pattanayak *et al*. (2007) reported that integration of inorganic NPK fertilizers with biofertilizers produced maximum rice yield. Application of 40 kg N ha^{-1} (50% of N dose) + 17.5 kg P_2O_5 ha^{-1} + 32 kg K_2O ha^{-1}, integrated with biofertilizers (*Azotobacter*, *Azospirillum* and *Azolla*) resulted in highest grain yield (3.57 t ha^{-1}) and straw yield (4.32 t ha^{-1}) of rice. Further when peanut crop was grown on this residual soil, it produced the highest pod yield (2.38 t ha^{-1}). This shows that biofertilizers applied to one crop in the cropping sequence have beneficial effect on the next crop also. With this a total of 60 kg inorganic N ha^{-1} was saved in the rice-peanut cropping sequence with apparent P and K recovery of 20 and 76%, respectively.

Malik and Shah (2005) conducted an experiment to find out the efficiency of W-5 and DA-2 strains of *Azotobacter chroococcum* with 6 wheat (*Triticum aestivum* L.) varieties. The data on grain yield, biomass, grain yield of $1m^2$, harvest index, 1000-grain weight, grains/ear and tillers/m^2 were generated from 2 crop seasons, i.e. 2002–2003 and 2003–2004. The grain yield, its attributing traits, varieties and the treatments (W-5 and DA-2) differed significantly, indicating diversity in varieties and the inoculant used in the experiment. The inoculant enhanced grain yield and biomass significantly over the control. The grain yield of the inoculated plots was increased due to increase in tillering capacity and the ear size producing higher number of grains.

An experiment was conducted by Dileep and Ravinder (2004) at Jammu to study the effect of biofertilizers on wheat crop. The findings highlighted that, *Azotobacter* + *Azospirillum* in 1:1 ratio was found to be effective in increasing the growth, yield attributes and yield of wheat crop to significant levels. It also resulted in higher NUE (Nitrogen Use Efficiency).

Azotobacter sp. is free-living aerobic bacteria dominantly found in soils. A large number of experiments have been performed to investigate the effects of inoculation of cereals with *Azotobacter* sp. Results of these studies showed that in many cases grain, yield and N concentration in plants increased by inoculation with *Azotobacter* sp. (De Freitas 2000, Kumar et al. 2001 and Emtiazi et al. 2004).

Agrawal *et al*. (2004) reported that at 80 DAS, about 72.03% increase in nitrogen uptake over the control was recorded due to *Azotobacter* inoculation and it was at par with the addition of 20 kg N ha^{-1} alone. Nitrogen uptake ranged from 0.73 to 1.66% and 0.93 to 1.85% in straw and grain, respectively. *Azotobacter* alone and 20 kg N ha^{-1} were statistically at par in affecting the nitrogen content in straw as well as in grain. Inoculation alone increased about 37.97%, 39.17% and 37.37% phosphorus uptake over the control in the yields of straw, grain and total yield respectively, whereas, potassium uptake was 95.25%, 43.23% and 44.81%. On the basis of results obtained, it was concluded that inoculation of *Azotobacter* could save about 20 kg fertilizer nitrogen in wheat crop.

Kader *et al*. (2002) conducted an experiment to evaluating the effects of *Azotobacter* inoculant on the yield of wheat (cv. Kanchan). They reported that application of 168 kg N ha^{-1} as urea + cow dung + *Azotobacter* have resulted in 84% higher grain yield per plant of wheat as compared to control. The treatments were T_0= (control), T_1= (240kg N ha^{-1} as urea), T_2= (*Azotobacter*), T_3= (192kg N ha^{-1} as urea + *Azotobacter*), T_4= (168 kg N ha^{-1} as urea + Cow dung) and T_5= (168 kg N ha^{-1} as urea + Cow dung + *Azotobacter*). Except 1000 grain weight, all the yield components of wheat viz. plant height, filled spikelets $spike^{-1}$, spike length and the number of grain $spike^{-1}$ were influenced significantly by the treatments. The highest grain yield of 780 mg $plant^{-1}$ i.e. 84% increase over the control (425 mg $plant^{-1}$) was obtained due to the treatment of T_5 which did not differ significantly from the yield obtained (687 mg $plant^{-1}$, 732 mg $plant^{-1}$ and 715 mg $plant^{-1}$) with the application of T_1, T_3 and T_4 respectively. There was 18% increase in grain yields due to using *Azotobacter* inoculant only over the control, which was not statistically significant. The straw yields showed a similar pattern. *Azotobacter* inoculation also influenced the root growth significantly. Total N uptake in grain, straw and root increased significantly due to different treatments. The highest N uptake (23.17 mg $plant^{-1}$) was recorded with the treatment T_5 and the lowest with the T_0 (control), (11.03 mg $plant^{-1}$). The total N uptake was increased by 89, 36, 101, 88 and 109% over the control due to T_1,

T_2, T_3, T_4 and T_5 respectively. *Azotobacter* either alone or in combination with urea N had some beneficial effects on the yield of wheat, which amounted to saving about 20% of urea N. Plant height, spikelets spike^{-1} and spike length were significantly influenced due to inoculation with *Azotobacter,* but 1000 grain weight was not influenced significantly. Wheat grain and straw yields were increased by 16% and 18% respectively over control. *Azotobacter* inoculation increased N uptake in grain and straw. They also reported the increase in total N uptake by 36% over control due to application of *Azotobacter*.

Kumar *et al.* (2001) inoculated cowpea with *Glomus fasciculatum* (a vesicular *Arbuscular mycorrhiza*), Rhizobium (CP-3) and *Azotobacter* Mac-21) in nutrient sterilized soil using earthen pots. Plant inoculated with mycorrhiza plus Rhizobium and *Azotobacter* exhibited highest nodule number and dry weight. Vesicular *Arbuscular mycorrhiza* or *Azotobacter* or *Rhizobium* or their combinations can have important effects on nodulation.

Nehra *et al.* (2001) conducted a field experiment during winter season of 1997–98 and 1998–99 on sandy-loam soil of Hisar, Haryana, India. They concluded that increasing dose of nitrogen increased the dry-matter accumulation, leaf-area index, effective tiller number, grains/ear, grain and straw yields, photosynthetic pigments (chlorophyll ‘a’, ‘b’ and carotenoids) and photosynthesis of wheat (*Triticum aestivum* L.). Maximum values were recorded with the recommended dose of 120 kg N + 60 kg P_2O_5 + 60 kg K_2O ha^{-1}. However, nitrogen at 120 and 90 kg ha^{-1} + *Azotobacter* were statistically at par with recommended dose of NPK. Inoculation of wheat seeds with *Azotobacter chroococcum* strain HT-56 also improved growth, yield, photosynthetic pigments and photosynthesis of wheat during both the years.

Swedrzynska (2000) reported that inoculation of wheat with active strain of *Azospirillum brasilense* led to increase in yield up to 27% comparing with uninoculated plants.

Several studies have shown that *A. chroococcum* as soil inoculant is not only effective in N fixation but also has other properties such as production of growth hormones (Remus *et al.,* 2000), production of fungicidal substances (Lakshminarayana, 1993), siderophore production (Suneja *et al*., 1994) and the property to solubilize phosphate (Kumar and Narula, 1999; Narula *et al*., 2000).

Barden (2000) compared four mineral N fertilizer levels (0, 40, 80 and 120 kg feddan^{-1}) in the presence and absence of biofertilizer (HALEX, a mixture of 2 *Azotobacter* strains) on three local maize cultivars during the 1996 and 1997 summer seasons. Maize cultivars differed significantly in all studied characters except the number of surviving plants and the shelling percentage. The cultivar was (single cross 10) gave the highest yield in both seasons. Increase N fertilizer rate from 0 to 120 kg feddan^{-1} in the absence of biofertilizer (HALEX) increased all the studied characters except the number of surviving plants and the shelling percentage. Application of 80 kg N feddan^{-1} in the presence of biofertilizer gave the highest means in all the studied characters except the number of surviving plants and the shelling percentage, where there were no significant differences among the eight treatments. [1 feddan= 0.42 ha].

Singh *et al*. (2000) conducted a field experiment to determine the nitrogen requirement of oat cv. HFO 114 with and without *Azotobacter* inoculation. The green-forage yield of increased significantly with each increment of N dose up to 90 kg ha^{-1}. The yield response to N decreased with *Azotobacter* inoculation, but the decrease was less at lower N levels compared with that at higher levels. The nitrogen-utilization efficiency (NUE) decreased with *Azotobacter* inoculation as well as with increasing level of N. optimum dose of N gave higher NUE compared with maximum dose. The optimum doses of N for oat were 95.3 and 112.6 kg ha^{-1} with and without *Azotobacter*. In *Azotobacter* inoculated oat, N fertilization at optimum rate was economically superior to the maximum rate.

Yadav *et al.* (2000) carried out a pot culture study and observed that wheat yield attributed like plant height, biomass and grain yield increased due to inoculation with *Azotobacter*.

Jatasara *et al*. (2000) conducted a field experiment at CCS Haryana Agricultural University, Hisar, India during the winter season for three consecutive years from 1995–96 to 1997–98 to study the effect of different nitrogen levels and *Azotobacter* inoculation on the growth, grain yield and nitrogen utilization efficiency of two varieties of oat. Increasing levels of nitrogen up to 60 kg ha^{-1} significantly increased the grain and straw yields over the lower doses. Nitrogen application also had a beneficial effect on the yield attributing traits of oat. Nitrogen utilization efficiency was highest at 60 kg N ha^{-1} and declined thereafter. *Azotobacter* inoculation was found beneficial in terms of increased grain and straw yield and higher nitrogen utilization efficiency over the uninoculated treatments.

Pandey *et al.* (1998) reported *A. chroococcum* showed good aptitude in grain and straw yield of wheat in pot and field experiment compared to the control treatment. Increase in the grain and straw yield and N concentrations due to inoculations with *Azotobacter* have been also reported by Meshram and Shende (1982), Lin et al. (1983) and Fulchieri and Frioni (1984).

Bedaiwi *et al.* (1997) found that biofertilization could save 40% of nitrogen requirements for wheat in the new lands.

Soliman *et al.* (1995) found that the inoculation with *Azotobacter chroococcum* and *Azospirillum brasiliense* either each applied singly or both in combination, under various levels of ammonium sulfate had increased straw, grain yield and N-uptake by wheat plants with increasing of nitrogen levels. The highest wheat yield was achieved by using a mixture of *Azotobacter chroococcum* and *Azospirillum brasiliense*. They reported that application of *Azotobacter* contributed up to 50% of wheat nutrient requirements under greenhouse conditions and increased rice yields by 20% in the field (Yanni and El-Fattah 1999).

Lippmann *et al.* (1995) reported that application of a composite of N_2 fixers (*Azospirillum* spp., *Azotobacter* spp. and *Bacillus* spp.) cause significant increases in plant dry weight, N-content and yield of wheat.

Beshir and Ghany (1995) carried out a pot experiments in the greenhouse, during the winter season to study the effects nodulations and growth parameters, as well as protein percentages in nodules and seeds. Plants from seeds treated with *P. fluorescens* had the highest *P. fluorescens* count as well as the highest weight of nodules per plant and N% in seeds.

Kloepper and Beauchamp (1992) reported increased wheat yield up to 43% and 30% with the inoculation of *Azotobacter* and Bacillus respectively.

Zambre *et al*. (1984) conducted a field experiment to study the effect of *Azotobacter chroococcum* and *Azospirillum brasilense* inoculation under graded levels of fertilizer nitrogen (0, 30, 60, 90, 120 kg ha^{-1}) on wheat (*Triticum durum* Desf. var. HD2189). The results revealed that both the inoculants significantly increased the growth parameters *viz*. tillers, dry-matter accumulation, grain yield and protein content of wheat gains. Fertilizer-N @ 90 kg ha^{-1} with both inoculations was found to be the most responsive, however 120 kg ha^{-1} with *Azospirillum* inoculation recorded maximum number of tillers (3.5 $plant^{-1}$), dry-matter accumulation (13.5 g $plant^{-1}$), grain yield (41.9 g ha^{-1}) and protein content in grains (13.3%). Maximum percent increase in grain yields with *Azospirillum* (25.8%) and *Azotobacter* (19.2%) over uninoculated control was observed at 90 kg N ha^{-1} level. *Azospirillum* inoculation, *Azotobacter* inoculation and uninoculated control significantly differed between each other.

Brown (1974) reported that root exudation of tryptophan and related substances may cause indole 3-acetic acid synthesis by the inoculum in the root zone. He also stated that the production of plant growth hormones by *Azotobacter* in pure cultures (used as inoculant) and by these bacteria in the root zone has caused significant plant growth responses. Similarly, Iruthayaraj (1981) observed that inoculation with *Azotobacter* could increase yield by 15–28% due to N fixation, production of antibacterial and antifungal compounds, growth regulators and siderophores.

CHAPTER III

MATERIALS AND METHODS

A field experiment was conducted in rabi season to study the effect of combined application of Biofertilizer and nitrogenous fertilizer on growth and yield of wheat. This chapter presents a brief description of the experimental site, soil, climate, experimental design, treatments, cultural operations, collection and analysis of different parameters. The details of these are described below.

3.1 Experimental Site

The experiment was conducted at the research field of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, during the period from November 2019 to March 2020. The experimental area was situated at 23˚75'N latitude and 90˚31'E longitude at an altitude of 8.6 meter above the sea level. The experimental site belongs to the agro-ecological zone of "Modhupur Tract", AEZ-28 (Anon., 1981). The experimental site is shown in the map of AEZ of Bangladesh in Appendix I.

3.2 Soil

The soil of the experimental area belongs to the Madhupur Tract. The analytical data of the soil sample collected from the experimental area were determined in SRDI, Soil Testing Laboratory, Dhaka. The texture of the top soil was silty clay, olive-gray in color with fine to medium noticeable dark yellowish-brown mottles. The pH of the soil was 6.0 with 0.69% organic carbon. The test area is flat, with an irrigation and drainage system in place, and was above flood levels. The chosen plot was a medium-high piece of land. The details are presented in Appendix II.

3.3 Climate of the experimental site

The climate of the experimental site is subtropical, characterized by heavy rainfall during the months from April to September (Kharif season) and scanty rainfall during rest of the year (Rabi season). The total rainfall of the experimental site was 70.80 mm during the period of the experiment. The average maximum and the minimum temperatures were 29.80°C and 20.00°C respectively. Plenty of sunshine and moderately low temperature prevails during experimental period, which is suitable for wheat growing in Bangladesh. The maximum and minimum temperatures, humidity and rainfall during the study period were collected from the Bangladesh Meteorological Department (climate division) and have been presented in Appendix I

3.4 Plant Materials

BARI Gom-33 was used as the test crop in this experiment. Seeds were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

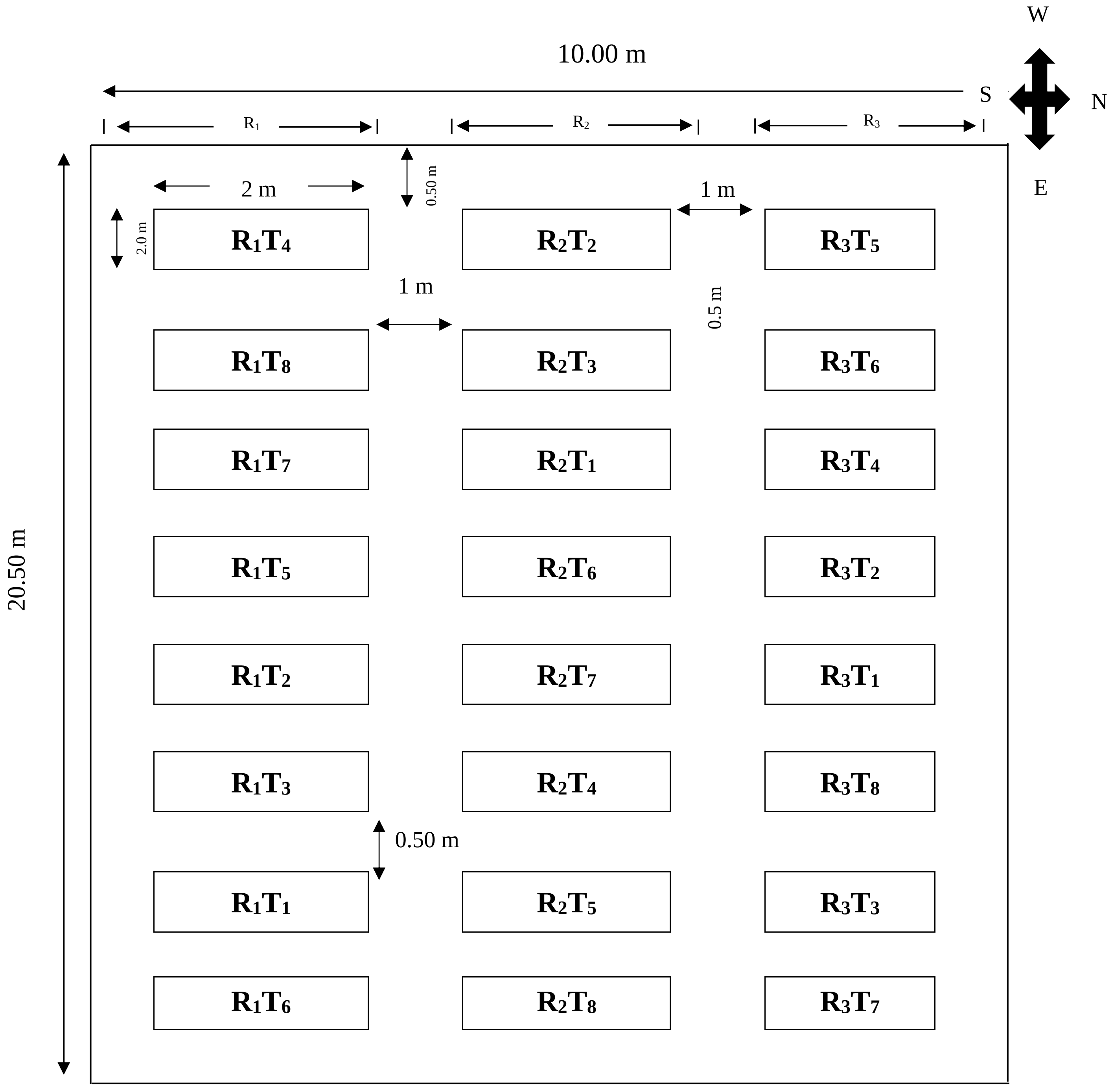
3.5 Land preparation

The experimental plot was opened first on the last week of November 2019 by a power tiller for growing the desired crop. It was then thoroughly prepared by ploughing and cross ploughing was provide several times with a power tiller followed by laddering to bring until good tilth suitable for sowing the seed. Then the land was leveled and the corners of the experimental plot were shaped and the clods were broken into pieces. The land was cleaned of weeds and stubbles and was finally leveled.

3.6 Treatments of the experiment

The experiment consisted of one factor. The treatments were as follows:

T_1 = Control (No *Azotobacter*, No N as urea)
T_2 = *Azotobacter* (8 kg ha^{-1})
T_3 = *Azotobacter* (6 kg ha^{-1}) + 60 kg N ha^{-1}
T_4 = No *Azotobacter* + 60 kg N ha^{-1}
T_5 = *Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}
T_6 = No *Azotobacter* + 90 kg N ha^{-1}
T_7 = *Azotobacter* (2 kg ha^{-1}) + 120 kg N ha^{-1}
T_8 = No *Azotobacter* + 120 kg N ha^{-1}



Each plot area = 2 m × 2 m = 4 m²
Plot to plot distance = 0.50 m
Block to block distance = 1 m
Length of field = 20.50 m
Breadth of field = 10.00 m

Treatments: Combinations of fertilizer

T_1 = Control (No *Azotobacter*, No N as urea)
T_2 = *Azotobacter* (8 kg ha^{-1})
T_3 = *Azotobacter* (6 kg ha^{-1}) + 60 kg N ha^{-1}
T_4 = No *Azotobacter* + 60 kg N ha^{-1}
T_5 = *Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}
T_6 = No *Azotobacter* + 90 kg N ha^{-1}
T_7 = *Azotobacter* (2 kg ha^{-1}) + 120 kg N ha^{-1}
T_8 = No *Azotobacter* + 120 kg N ha^{-1}

Figure 1. Field layout of the single-factor experiment in Randomized Complete Block Design (RCBD

3.7 Experimental design and layout

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. An area of 20.5 × 10 m^2 was divided into three blocks. Each block consisted of 8 plots. Thus, the total number of plots was 24. The 8 treatment combinations were assigned in each plot of each block. The size of each unit plot was 2m × 2m. The space between two blocks and two plots were 1m and 0.5 m, respectively. The layout of the experiment is shown in figure 1.

3.8 Application of fertilizers

Recommended doses of P, K, S, Zn and B (30 kg P ha^{-1} from TSP, 60 kg K ha^{-1} from MoP, 5 kg ha^{-1} from ZnO, 15 kg ha^{-1} from gypsum and 1 kg ha^{-1} from boric acid, respectively) were applied. The whole amount of TSP, MoP, ZnO, Gypsum and boric acid and one-third of the recommended dose of urea fertilizer (as per treatment) were applied as basal dose during final land preparation for the application of P, K, Zn, S, B and N respectively. The remaining amount of N was applied in two split doses at crown root initiation stage (25 DAS) and prior to spike initiation stage (60 DAS) as top dressing. *Azotobacter* biofertilizer was collected from Department of Soil Science, Bangladesh Agricultural Research Institute (BARI). The population of *Azotobacter* biofertilizer was around 10^8 cfu/g. Seeds were inoculated with *Azotobacter* as per treatment. Acacia gum was used to inoculate the seed with *Azotobacter*.

3.9 Sowing of seed

At good tilth condition, furrows were made with hand rakes for sowing. Seeds were sown continuously in line on 3 December, 2019. The line to line distance was maintained 20 cm. After sowing, seed were covered with the soil and slightly pressed by hand.

3.10 Intercultural operation

3.10.1 Weeding

Weed infested the experimental plots. So, two weedings were done manually at 30 and 50 DAS. During weeding the weeds identified were kakpaya ghash (*Dactlyoctenium aegyptium* L.), Durba (*Cynodon dactylon* L.), Arail (*Leersia hexandra*), Chelaghash (*Parapholis incurve* L.), Mutha (*Chenopodium album* L.), Foskabegun (*Physalis beterophylls*) and Titabegun (*Solanum torvum*).

3.10.2 Irrigation

Three irrigations were applied at crown root initiation stage, tillering stage and heading stage at 22, 45 and 60 DAS respectively. Excess water was drained out from the field.

3.10.3 Pest management

At 72 DAS the experimental plots were sprayed with Melethion 57 EC @ 2m/L to control aphid. No infestation of diseases was found. The wheat ground field was covered by a net to protect the wheat grain from bird especially parrots from mid-February to harvest.

3.11 Harvesting and sampling

On March 29, 2020; the crop was harvested when it was fully mature. Samples were taken from various locations throughout each plot, keeping the main three rows in the center and the boundary rows intact. The chosen sample plants were then tagged and transported to the agronomy field laboratory for data collection. Plants from the central $1m^2$ were harvested plot wise, bundled and labelled. The harvest bundles were stretched out on the threshing floor to dry in the sun. By pounding the bundles with wooden stick, the grains were separated from the plants. The grain and straw were re-dried to consistent moisture content, and the weight was recorded and converted to t ha^{-1} basis.

3.12 Data collection parameters

1. Plant height at 40 DAS, 65 DAS and 90 DAS
2. Spike length
3. Number of spikelets spike^{-1}
4. Number of Grains spike^{-1}
5. Weight of 1000 grains
6. Grain yield
7. Straw yield
8. Harvest index

3.13 Procedure of data collection

3.13.1 Plant height

Plant height was measured in centimeter (cm) by a meter scale at 40, 65 and 90 days after sowing. Three plants per plot were randomly selected during earlier growth stage of the crop. The height was measured taking base to the leaf/ spike and the mean height was recorded.

3.13.2 Spike length

Spike length was recorded from the basal node of the rachis to the apex of each spike. Spike length was measured by measuring scale in cm.

3.13.3 No. of spikelets spike^{-1}

No. of spikelets was counted from the spike, which has at least one visible, spike.

3.13.4 No. of Grains spike^{-1}

Number of grains was counted from the hills, which has at least one visible, spike.

3.13.5 Weight of 1000-grains

Thousand grains were randomly selected from each seed stock obtained from each plot and it was dried in an oven up to 14% moisture content. Then weight was taken on an electric balance.

3.13.6 Grain yield

After threshing and adequate drying, the yield of each sample plot was assessed (14% moisture level). Cleaning was performed and the results were converted to tons ha^{-1}.

3.13.7 Straw yield

Straw weight of each sample plot was measured after threshing, drying and converted into ton ha^{-1}.

3.13.8 Harvest index

Harvest index (%) was determined by dividing the economic (Grain) yield from the harvest area by the total biological yield (grain + straw) of the same area.

$$\text{Harvest index (\%)} = \frac{\text{Grain yield (t ha}^{-1}\text{)}}{\text{Biological yield (t ha}^{-1}\text{)}} \times 100$$

3.14 Post harvest soil sampling

After harvest of crop soil samples were collected from each plot at a depth of 0 to 15 cm. Soil samples of each plot was air-dried, crushed and passed through a two mm (10 meshes) sieve. The soil samples were kept in plastic container to determine the chemical properties of soil.

3.15 Post harvest soil analysis

Soil samples were analyzed for chemical characteristics viz. organic matter, pH, total N and available P, available sulphur and exchangeable K contents. The soil samples were analyzed by the following standard methods as follows:

3.15.1 Soil pH

Soil pH was measured with the help of a glass electrode pH meter, the soil water ratio being maintained at 1:2.5 as described by (Page *et al.*, 1982).

3.15.2 Organic matter

Organic carbon in soil sample was determined by wet oxidation method. The underlying principle was used to oxidize the organic matter with an excess of 1N $K_2Cr_20_7$ in presence of conc. H_2SO_4 and conc. H_3PO_4 and to titrate the excess $K_2Cr_20_7$ solution with 1N $FeSO_4$. To obtain the content of organic matter was calculated by multiplying the percent organic carbon by 1.73 (Van Bemmelen factor) and the results were expressed in percentage (Page *et al.*, 1982).

3.15.3 Total nitrogen

Total N content of soil were determined followed by the Micro Kjeldahl method. One gram of oven dry ground soil sample was taken into micro 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 200°C and added 3 ml H_2O_2 and then heating at 360°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 (Page *et al.*, 1982). Then 20 ml digest solution was transferred into the distillation flask, then 10 ml of H_3BO_3 indicator solution was taken into a 250 ml conical flask which is marked to indicate a volume of 50 ml and placed the flask under the condenser outlet of the distillation apparatus so that the delivery end dipped in the acid. Add sufficient amount of 10 N-NaOH solutions in the container connecting with distillation apparatus. Water runs through the condenser of distillation apparatus was checked. Operating switch of the distillation apparatus collected the distillate. The conical flask was removed by washing the delivery outlet of the distillation apparatus with distilled water. Finally, the distillates were titrated with standard 0.01 N H_2SO_4 until the color changes from green to pink. The amount of N was calculated using the following formula:

$$\% \text{ N} = \frac{(\text{T} - \text{B}) \times \text{N} \times 0.014 \times 100}{\text{S}}$$

Where, T = Sample titration (ml) value of standard H_2SO_4

B = Blank titration (ml) value of standard H_2SO_4

N = Strength of H_2SO_4

S = Sample weight in gram

3.15.4 Available phosphorus

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 colorimetrically at 660 nm wavelength and readings were calibrated with the standard P curve (Page *et al.,* 1982).

3.15.5 Available sulphur

An Alkaline Oxidation Method for Determination of Total Sulfur in Soils. The method consists of extracting the sulfur with 0.5 M $NaHCO_3$ at pH 8.5 and determining sulfur in the extract by the Johnson-Nishita procedure (Tabatabai and Bremner, 1970).

3.15.6 Exchangeable potassium

Exchangeable K was determined by 1 N NH_4OAc (pH 7) extraction methods and by using flame photometer and calibrated with a standard curve (Page *et al.,* 1982).

3.16 Statistical analysis

The data obtained for different characters were statistically analyzed following the analysis of variance techniques by using MSTAT-C computer package programme. The significant differences among the treatment means were compared by Least Significant Different (LSD) at 5% level of probability (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS AND DISCUSSION

The experiment was conducted to find out the effect of combined application of biofertilizer and nitrogenous fertilizer on growth and yield of wheat. The results have been presented and discussed with the help of tables and graphs and possible interpretations given under the following headings. The analytical results have been presented in Table 1 through Table 2 and Figure 1 through Figure 5.

4.1 Effect of biofertilizer and nitrogenous fertilizer on growth attributes of wheat

4.1.1 Plant height

Plant height of wheat showed significant variation due to effect of biofertilizer and nitrogenous fertilizer at different data recording intervals (Figure 2 and Appendix IV). The tallest plant at 40 DAS (51.20 cm), 65 DAS (86.77 cm) and at 90 DAS (107.53 cm) was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}]. On the other hand, the shortest plant at 40 DAS (40.23 cm), 65 DAS (72.20 cm) and at 90 DAS (90.63 cm) was observed in control treatment (T_1). Application of biofertilizer enhanced height of plant when used in combination with regular nitrogenous fertilizer. These results were in conformity with the findings of Patra *et al.* (2019), Mahato and Kafle (2018), Sharifi and Amiryusefi (2017), Kader *et al*. (2002) and Yadav *et al.* (2000) who also reported increase in plant height of wheat due to application of biofertilizer.

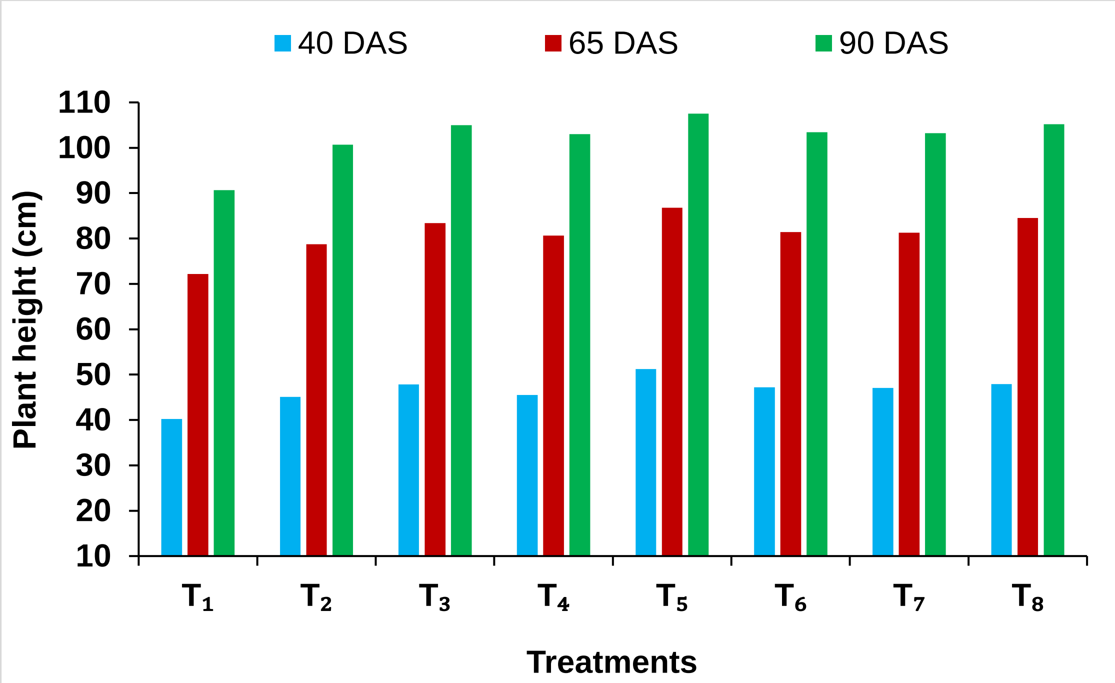


Figure 2. Effect of biofertilizers and nitrogenous fertilizers on plant height of wheat (LSD = 2.95, 4.37 and 3.08 at 40, 65 and 90 DAS, respectively). Here, T_1 = Control (No *Azotobacter*, No N as urea), T_2 = *Azotobacter* (8 kg ha^{-1}), T_3 = *Azotobacter* (6 kg ha^{-1}) + 60 kg N ha^{-1}, T_4 = No *Azotobacter* + 60 kg N ha^{-1}, T_5 = *Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}, T_6 = No *Azotobacter* + 90 kg N ha^{-1}, T_7 = *Azotobacter* (2 kg ha^{-1}) + 120 kg N ha^{-1}, T_8 = No *Azotobacter* + 120 kg N ha^{-1}

4.2 Effect of biofertilizer and nitrogenous fertilizer on yield attributes of wheat

4.2.1 Spikelets per spike

Spikelets per spike (no.) of wheat showed significant variation due to effect of biofertilizer and nitrogenous fertilizer application (Table 1 and Appendix V). Significantly the highest number of spikelets per spike (19.47) was recorded from treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] which was statistically similar to treatment T_8 (19.33), T_3 (19.20) and T_6 (19.13). On the other hand, the lowest number of spikelets per spike (13.53) was obtained from the treatment T_1 (No *Azotobacter*, No N as urea). Application of biofertilizer increased number of spikelets per spike of wheat plant when used in combination with regular nitrogenous fertilizer. Mane *et al*. (2014), Agamy *et al*. (2012) and Kader *et al*. (2002) found similar kind of results regarding impact of biofertilizer on spikelets per spike of wheat plant.

4.2.2 Grains per spike

Biofertilizer and nitrogenous fertilizer application exerted significant effect on number of grains per spike of wheat (Table 1 and Appendix V). The highest number of grains per spike (75.93) was recorded from treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] which was statistically similar to treatment T_8 (75.33), T_3 (72.87) and T_6 (71.87). On the other hand, the lowest number of grains per spike (53.13) was reported from control treatment (T_1). Application of biofertilizer increased number of grains per spike of wheat plant when used in combination with regular nitrogenous fertilizer. Singh *et al*. (2019), Sharifi and Amiryusefi (2017), Soleimanzadeh and Gooshchi (2013) and Hussen *et al.* (2017) reported that number of grains per spike of wheat increased when *Azotobacter* was used as biofertilizer.

4.2.3 Spikes length

Length of spike of wheat showed significant variation due to effect of biofertilizer and nitrogenous fertilizer application (Table 1 and Appendix V). The longest spike (15.70 cm) was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] while the shortest length of spike (10.70 cm) was obtained from control treatment (T_1). Use of *Azotobacter* as biofertilizer showed positive effect on spike length of wheat. These results were in conformity with the findings of Mane *et al*. (2014), Soleimanzadeh and Gooshchi (2013), Hussen *et al.* (2017) and Kader *et al.* (2002) who observed that spike length of wheat was significantly influenced by application of biofertilizer.

4.2.4 1000-grains weight

Biofertilizer and nitrogenous fertilizer application exerted significant effect on 1000-grains weight of wheat (Table 1 and Appendix V). The maximum weight of 1000-grain (48.90 g) was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] which was statistically similar to treatment T_8 (47.17 g), T_3 (47.07 g) and T_6 (46.93 g). On the other hand, the minimum weight of 1000-grains (38.43 g) was obtained from the treatment T_1 (No *Azotobacter*, No N as urea). Use of *Azotobacter* as biofertilizer showed positive effect on 1000-grains weight of wheat. This result was supported by the findings of Sharifi and Amiryusefi (2017), El-Samie *et al*. (2017), Agamy *et al*. (2012) and Singh and Prasad (2011) who mentioned that *Azotobacter* as biofertilizer had positive effect on 1000-grain weight of wheat.

Table 1. Effect of biofertilizers and nitrogenous fertilizers on yield attributes of wheat

Treatments	Spikelets per spike (no.)	Grains per spike (no.)	Spikes length (cm)	1000-grains weight (g)
T_1	13.53 c	53.13 d	10.70 e	38.43 d
T_2	17.80 b	66.00 c	11.55 d	44.03 c
T_3	19.20 a	72.87 abc	13.89 b	47.07 ab
T_4	18.60 ab	67.73 bc	12.67 c	45.27 b
T_5	19.47 a	75.93 a	15.70 a	48.90 a
T_6	19.13 a	71.87 abc	13.87 b	46.93 ab
T_7	18.60 ab	71.47 abc	12.75 c	45.33 b
T_8	19.33 a	75.33 ab	13.91 b	47.17 ab
LSD (0.05)	**1.08**	**7.73**	**0.79**	**2.54**
CV (%)	**3.38**	**6.37**	**3.31**	**3.18**

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability.

Here, T_1 = Control (No *Azotobacter*, No N as urea), T_2 = *Azotobacter* (8 kg ha^{-1}), T_3 = *Azotobacter* (6 kg ha^{-1}) + 60 kg N ha^{-1}, T_4 = No *Azotobacter* + 60 kg N ha^{-1}, T_5 = *Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}, T_6 = No *Azotobacter* + 90 kg N ha^{-1}, T_7 = *Azotobacter* (2 kg ha^{-1}) + 120 kg N ha^{-1}, T_8 = No *Azotobacter* + 120 kg N ha^{-1}.

4.3 Effect of biofertilizer and nitrogenous fertilizer on yields and harvest index of wheat

4.3.1 Grain yield

Grain yield of wheat showed significant variation due to effect of biofertilizer and nitrogenous fertilizer application (Figure 3 and Appendix VI). The highest grain yield (4.40 t ha^{-1}) was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] while the lowest grain yield (2.59 t ha^{-1}) was obtained from the treatment T_1 (No *Azotobacter*, No N as urea). Application of biofertilizer showed increment in grain yield of wheat when used alone or in combination with regular nitrogenous fertilizer. Mahato and Kafle (2018), Patra and Singh (2018), Hussen *et al.* (2017), Yadav *et al.* (2014), Soleimanzadeh and Gooshchi (2013), Kader *et al.* (2002) and Nehra *et al.* (2001) observed similar kind of results in grain yield of wheat when biofertilizer was used.

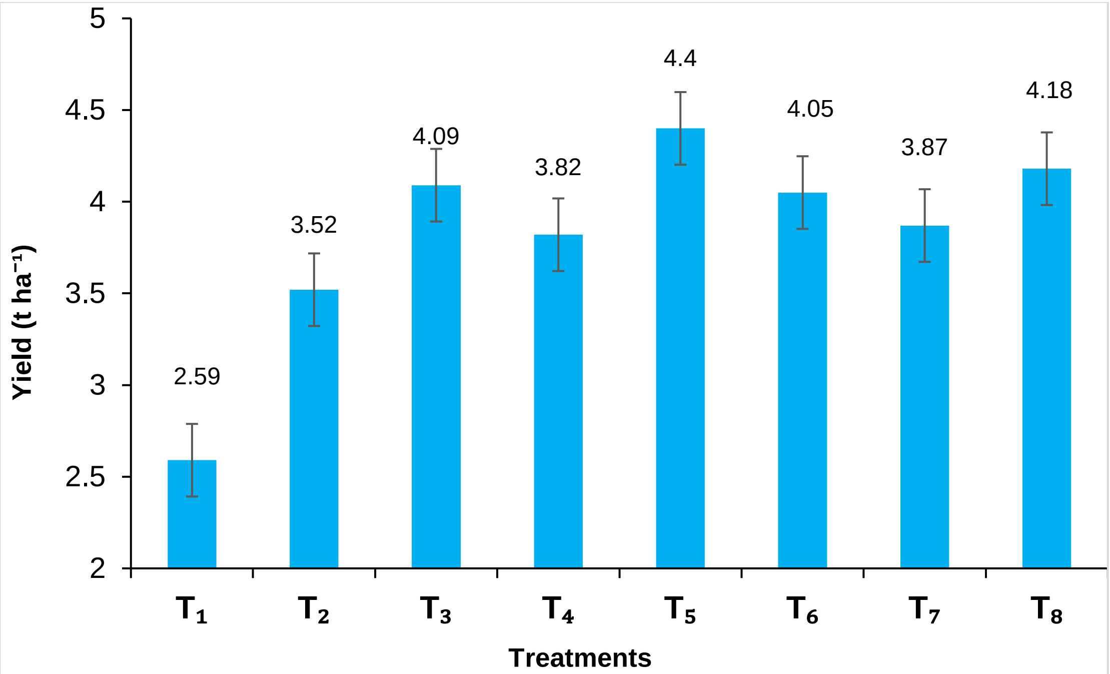


Figure 3. Effect of biofertilizers and nitrogenous fertilizers on grain yield of wheat (LSD = 0.31). Here, T_1 = Control (No *Azotobacter*, No N as urea), T_2 = *Azotobacter* (8 kg ha^{-1}), T_3 = *Azotobacter* (6 kg ha^{-1}) + 60 kg N ha^{-1}, T_4 = No *Azotobacter* + 60 kg N ha^{-1}, T_5 = *Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}, T_6 = No *Azotobacter* + 90 kg N ha^{-1}, T_7 = *Azotobacter* (2 kg ha^{-1}) + 120 kg N ha^{-1}, T_8 = No *Azotobacter* + 120 kg N ha^{-1}

4.3.2 Straw yield

Biofertilizer and nitrogenous fertilizer application exerted significant effect on straw yield of wheat (Figure 4 and Appendix VI). The maximum straw yield (7.68 t ha^{-1}) was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] while the minimum straw yield (4.68 t ha^{-1}) was obtained from the treatment T_1 (No *Azotobacter*, No N as urea). Use of *Azotobacter* as biofertilizer showed positive effect on straw yield of wheat. This result was supported by the findings of Mane *et al*. (2014) and Behera and Rautaray (2010) who also observed increase in straw yield of wheat when biofertilizer was used along with conventional fertilizers.

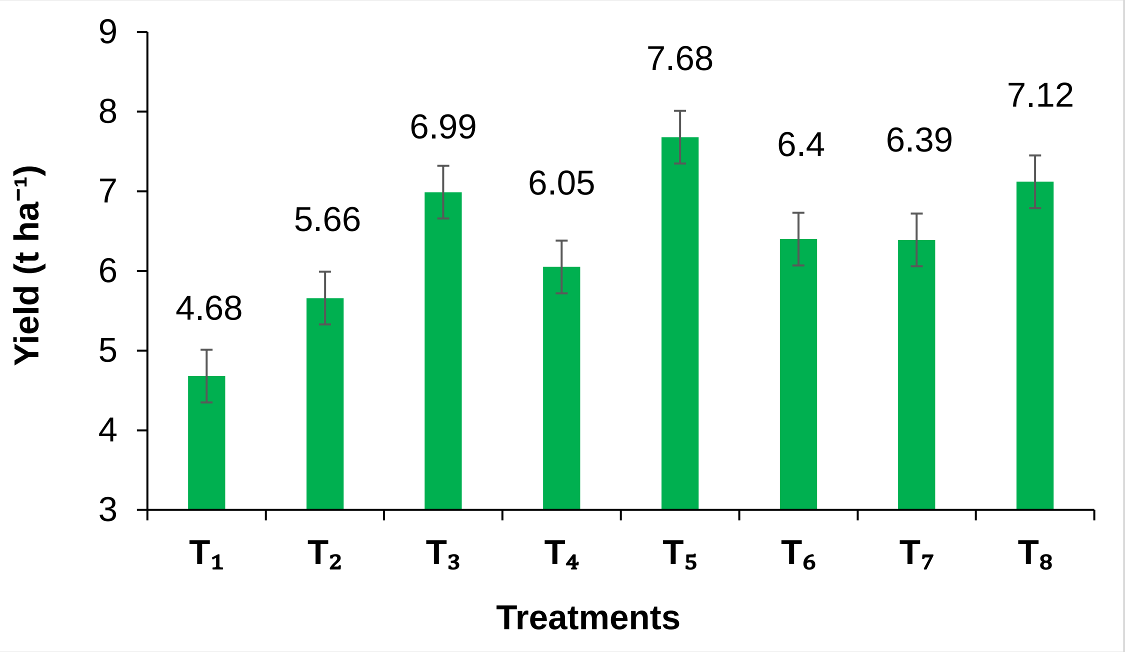


Figure 4. Effect of biofertilizers and nitrogenous fertilizers on straw yield of wheat (LSD = 1.37). Here, T_1 = Control (No *Azotobacter*, No N as urea), T_2 = *Azotobacter* (8 kg ha^{-1}), T_3 = *Azotobacter* (6 kg ha^{-1}) + 60 kg N ha^{-1}, T_4 = No *Azotobacter* + 60 kg N ha^{-1}, T_5 = *Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}, T_6 = No *Azotobacter* + 90 kg N ha^{-1}, T_7 = *Azotobacter* (2 kg ha^{-1}) + 120 kg N ha^{-1}, T_8 = No *Azotobacter* + 120 kg N ha^{-1}

4.3.3 Harvest index

Harvest index of wheat showed significant variation due to effect of biofertilizer and nitrogenous fertilizer application (Figure 5 and Appendix VI). The highest harvest index (38.76%) was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] which was statistically similar (38.70%) to T_8 (No *Azotobacter* + 120 kg N ha^{-1}) whereas the lowest harvest index (35.63%) was obtained from the treatment T_1 (No *Azotobacter*, No N as urea). Application of biofertilizer showed increment in harvest index of wheat when used alone or in combination with regular nitrogenous fertilizer. These results were in conformity with the findings of El-Samie *et al*. (2017), Mane *et al*. (2014) and Soleimanzadeh and Gooshchi (2013) who also stated that application of biofertilizer had significant impact on harvest index of wheat.

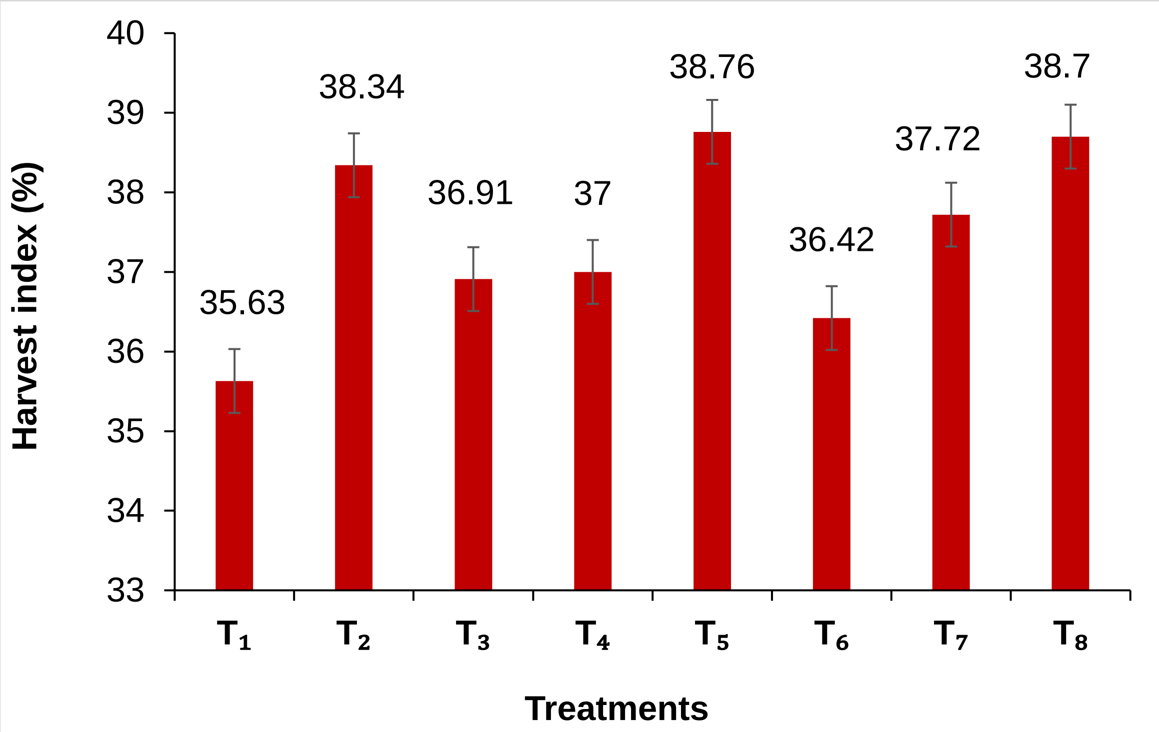


Figure 5. Effect of biofertilizers and nitrogenous fertilizers on harvest index of wheat (LSD = 0.24). Here, T_1 = Control (No *Azotobacter*, No N as urea), T_2 = *Azotobacter* (8 kg ha^{-1}), T_3 = *Azotobacter* (6 kg ha^{-1}) + 60 kg N ha^{-1}, T_4 = No *Azotobacter* + 60 kg N ha^{-1}, T_5 = *Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}, T_6 = No *Azotobacter* + 90 kg N ha^{-1}, T_7 = *Azotobacter* (2 kg ha^{-1}) + 120 kg N ha^{-1}, T_8 = No *Azotobacter* + 120 kg N ha^{-1}

4.4 Effect of biofertilizer and nitrogenous fertilizer on soil properties and nutrient contents at wheat field

4.4.1 Soil pH

No statistically significant variation was recorded for soil pH in post-harvest soil due to biofertilizer and nitrogenous fertilizer application (Table 2 and Appendix VII). Numerically, the highest soil pH (6.21) was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] whereas the lowest soil pH (5.89) was obtained from the treatment T_1 (No *Azotobacter*, No N as urea).

4.4.2 Soil Organic matter

Biofertilizer and nitrogenous fertilizer application did not result in any statistically significant variation among organic matter percentage in post-harvest soil (Table 2 and Appendix VII). The highest soil organic matter (1.58%) was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] which was statistically similar to T_8 (1.23%) whereas the lowest soil organic matter (1.10%) was obtained from the treatment T_1 (No *Azotobacter*, No N as urea).

4.4.3 Total Nitrogen in post-harvest soil

Statistically significant variation was recorded for total N in post-harvest soil due to biofertilizer and nitrogenous fertilizer application (Table 2 and Appendix VII). The maximum total N (0.11%) was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] which was statistically similar to T_8 (0.11%) whereas the minimum total N (0.05%) was obtained from the treatment T_1 (No *Azotobacter*, No N as urea).

Table 2. Effect of biofertilizers and nitrogenous fertilizers on soil properties and nutrient contents at wheat field

Treatments	pH	Organic matter (%)	Total N (%)	Available P (ppm)	Available S (ppm)	Exchangeable K (meq/100 g soil)
T_1	5.89	1.23	0.05 d	19.32 h	17.98 g	0.110
T_2	5.91	1.27	0.06 d	20.29 g	18.28 f	0.113
T_3	6.13	1.49	0.10 b	24.21 c	19.52 b	0.122
T_4	5.98	1.39	0.07 c	20.63 f	18.82 e	0.113
T_5	6.21	1.58	0.11 a	25.96 a	20.73 a	0.129
T_6	6.03	1.43	0.08 c	22.12 d	18.93 c	0.122
T_7	6.08	1.31	0.08 c	21.11 e	18.90 d	0.115
T_8	6.17	1.53	0.11 a	24.24 b	20.72 a	0.123
LSD (0.05)	**NS**	**NS**	**0.01**	**0.02**	**0.02**	**NS**
CV (%)	**0.25**	**0.84**	**16.07**	**0.06**	**0.32**	**7.42**

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability.
Here, T_1 = Control (No *Azotobacter*, No N as urea), T_2 = *Azotobacter* (8 kg ha^{-1}), T_3 = *Azotobacter* (6 kg ha^{-1}) + 60 kg N ha^{-1}, T_4 = No *Azotobacter* + 60 kg N ha^{-1}, T_5 = *Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}, T_6 = No *Azotobacter* + 90 kg N ha^{-1}, T_7 = *Azotobacter* (2 kg ha^{-1}) + 120 kg N ha^{-1}, T_8 = No *Azotobacter* + 120 kg N ha^{-1}
NS = Non-significant

4.4.4 Available Phosphorous in post-harvest soil

Biofertilizer and nitrogenous fertilizer application showed statistically significant variation among available phosphorous value in post-harvest soil (Table 2 and Appendix VII). The maximum available phosphorous (25.96 ppm) was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] whereas the minimum available phosphorous (19.32 ppm) was obtained from the treatment T_1 (No *Azotobacter*, No N as urea).

4.4.5 Available Sulphur in post-harvest soil

Statistically significant variation was recorded for available sulphur in post-harvest soil due to biofertilizer and nitrogenous fertilizer application (Table 2 and Appendix VII). The maximum available sulphur (20.73 ppm) was observed from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] which was statistically similar to T_8 (20.72 ppm) whereas the minimum available sulphur (17.98 ppm) was obtained from the treatment T_1 (No *Azotobacter*, No N as urea).

4.4.6 Exchangeable potassium in post-harvest soil

Biofertilizer and nitrogenous fertilizer application did not show any statistically significant variation among exchangeable K (potassium) value in post-harvest soil (Table 2 and Appendix VII). Numerically, the highest exchangeable K (0.129 me%) was recorded from the treatment T_5 [*Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}] whereas the lowest exchangeable K (0.110 me%) was obtained from the treatment T_1 (No *Azotobacter*, No N as urea).

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted at the research field, Sher-e-Bangla Agricultural University during the period from November, 2019 to March, 2020 to study the effect of combined application of biofertilizer and nitrogenous fertilizer on growth and yield of wheat (*Triticum aestivum* L.). The experiment comprised of single factor comprising eight treatments viz. T_1 = Control (No *Azotobacter,* No N as urea), T_2 = *Azotobacter* (8 kg ha^{-1}), T_3 = *Azotobacter* (6 kg ha^{-1}) + 60 kg N ha^{-1}, T_4 = No *Azotobacter* + 60 kg N ha^{-1}, T_5 = *Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1}, T_6 = No *Azotobacter* + 90 kg N ha^{-1}, T_7 = *Azotobacter* (2 kg ha^{-1}) + 120 kg N ha^{-1} and T_8 = No *Azotobacter* + 120 kg N ha^{-1}. This experiment was laid out in a randomized complete block design (RCBD) with three (3) replications. Data were collected on different aspects of growth, yield attributes, yield and harvest index of wheat including soil properties and nutrient contents.

The results revealed that treatment T_5 exhibited its superiority compare to that of other biofertilizer and nitrogenous fertilizer treatments in terms of grain yield. Treatment T_5 out-yielded over T_8 by 5.26%, T_3 by 7.57%, T_6 by 8.64%, T_7 by 13.69%, T_4 by 15.18% and T_2 by 25.00% higher grain yield. Treatment T_5 also showed the tallest plant at 90 DAS (107.53 cm), the highest number of spikelets per spike (19.47), the highest number of grains per spike (75.93), the longest spike (15.70 cm), the maximum weight of 1000-grain (48.90 g), the highest straw yield (7.68 t ha^{-1}) and the maximum harvest index (38.76%) than other treatments in this experiment. On the other hand, the treatment T_1 [Control (No *Azotobacter*, No N as urea)] returned with 69.88% lower yield than treatment T_5 which was significantly the lowest compare with other treatments under study.

Considering the soil nutrients, the maximum total N (0.11%), the maximum available phosphorous (25.96 ppm), the maximum available sulphur (20.73 ppm) and the highest exchangeable K (0.129 me%) in post-harvest soil was recorded from the treatment T_5.

CONCLUSION

From the above result it was revealed that T_5 treatment gave higher yield along with higher values in all the growth and yield attributing parameters. It can be said that higher amount of *Azotobacter* along with traditional nitrogenous fertilizer improved soil chemical properties along with increased availability of essential plant nutrients in soil solution. From the result of the experiment, it may be concluded that *Azotobacter* (4 kg ha^{-1}) + 90 kg N ha^{-1} application seems promising for producing higher grain yield of wheat and sustaining soil productivity.

RECOMMENDATION

Considering the results of the present experiment, further studies in the following areas are suggested:

- Different doses and strains of biofertilizers including *Azotobacter* may be used with different level of nitrogenous fertilizer for getting variety specific biofertilizer recommendations.

- Studies of similar nature could be carried out in different agro-ecological zones (AEZ) of Bangladesh for the evaluation of zonal adaptability.

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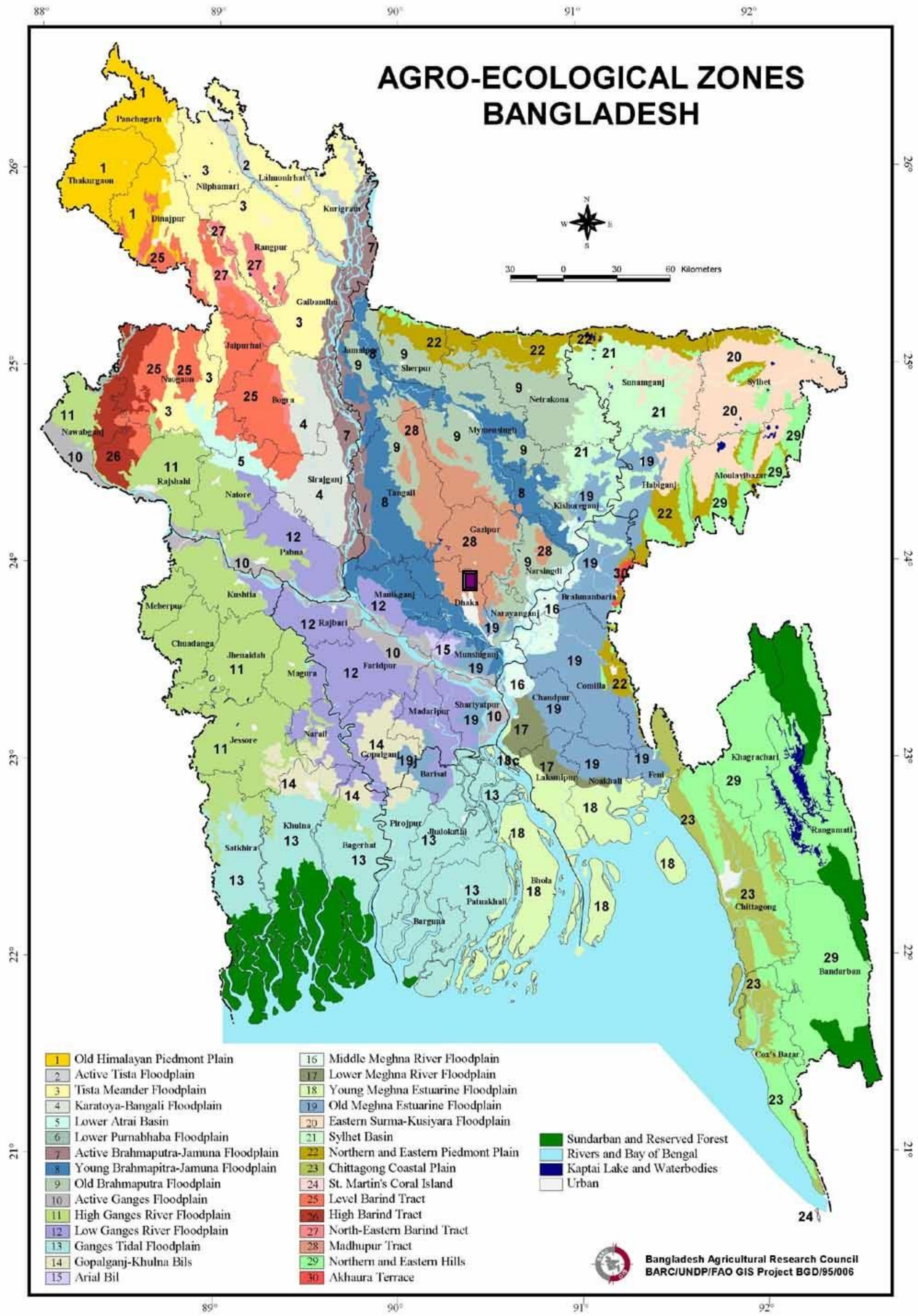
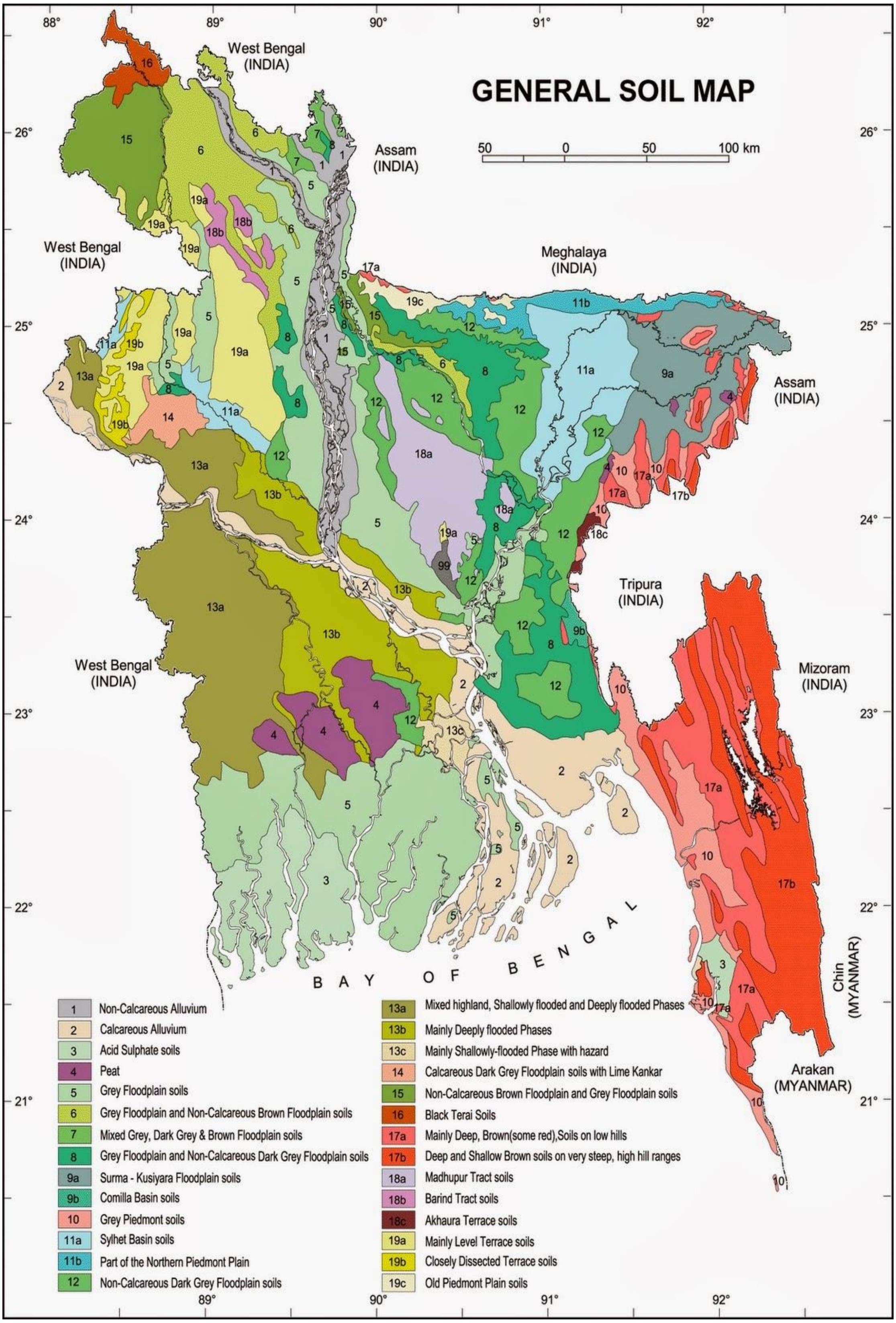
APPENDICES

Appendix I (A): Map showing the experimental sites under study



The experimental site under study

Appendix I(B): Map showing the general soil sites under study



Appendix II: Characteristics of soil of experimental site analyzed by Soil Resources Development Institute (SRDI), Khamarbari, Farmgate, Dhaka

A. Morphological characteristics of the experimental field

Morphological features	**Characteristics**
Location	Experimental field, SAU, Dhaka
AEZ	Madhupur Tract (28)
General Soil Type	Shallow Red Brown Terrace Soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled
Flood level	Above flood level
Drainage	Well drained

B. Physical and chemical properties of the initial soil

Characteristics	**Value**
% Sand	27
% Silt	43
% clay	30
Textural class	Silty-clay
pH	6.0
Organic carbon (%)	0.69
Organic matter (%)	1.10
Total N (%)	0.06
Available P (ppm)	20.00
Exchangeable K (meq/100 g soil)	0.10
Available S (ppm)	22

Source: SRDI, 2019

Appendix III: Monthly average of Temperature, Relative humidity, total Rainfall and sunshine hour of the experiment site during the period from November 2019 to March 2020

Year	Month	Temperature			Relative Humidity (%)	Total Rainfall (mm)	Sunshine (Hour)
		Max (°C)	Min (°C)	Mean (°C)			
2019	November	32	24	29	65	42.8	349
	December	27	19	24	53	1.4	372
2020	January	25	14	23	50	3.9	364
	February	30	19	26	38	3.1	340
	March	35	24	31	38	19.6	353

Appendix IV. Mean square value of plant height of wheat from Analysis of variance (ANOVA)

Source of variation	Degree of freedom	Mean square value of Plant height		
		40 DAS	65 DAS	90 DAS
Replication	2	0.639	9.550	2.645
Fertilizer combinations	7	21.177*	39.326*	54.501*
Error	14	2.834	6.216	3.086

* = Significant at 0.05 level of probability

Appendix V. Mean square value of yield attributes of wheat from Analysis of variance (ANOVA)

Source of variation	Degree of freedom	Mean square value of			
		Spikelets per spike	Grains per spike	Spike length	1000-grain weight
Replication	2	0.281	5.541	0.086	0.948
Fertilizer combinations	7	11.579*	162.476*	2.016*	27.567*
Error	14	0.379	19.479	0.204	2.108

* = Significant at 0.05 level of probability

Appendix VI. Mean square value of yields and harvest index of wheat from Analysis of variance (ANOVA)

Source of variation	Degree of freedom	Mean square value of		
		Grain yield	Straw yield	Harvest index
Replication	2	0.016	0.046	-1.77636E-15
Fertilizer combinations	7	0.938*	2.625*	3.868*
Error	14	0.0319	0.609	0.019

* = Significant at 0.05 level of probability

Appendix VII. Mean square value from Analysis of variance (ANOVA) of soil properties and nutrient contents of soil from wheat field

Source of variation	**Degree of freedom**	**Mean square value of**					
		pH	**Organic matter**	**Total Nitrogen**	**Available phosphorus**	**Available Sulphur**	**Exchangeable potassium**
Replication	2	0.0000845	0.0001	0.000085	0.0001	0.0000845	0.0001
Fertilizer combinations	7	0.814^{NS}	0.316^{NS}	0.001*	16.256*	3.165*	0.127^{NS}
Error	14	0.00018	0.000181071	0.0001811	0.000181071	0.000181	0.00018

* = Significant at 0.05 level of probability
NS = Non-significant

PLATES

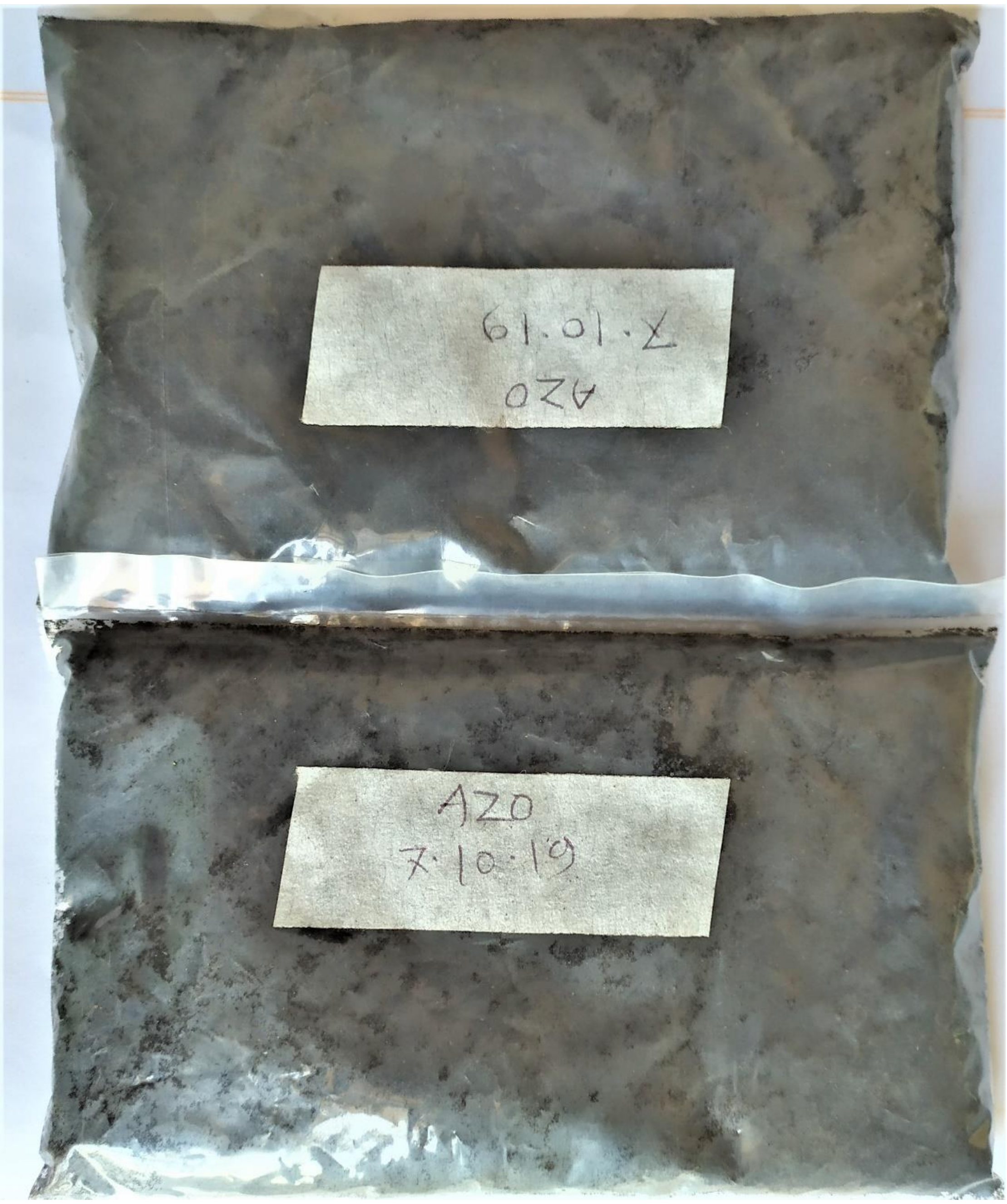


Plate 1. *Azotobacter* biofertilizer

Plate 2. Collection of soil samples

Plate 3. Sowing of seeds

Plate 4. Netting the experiment plots

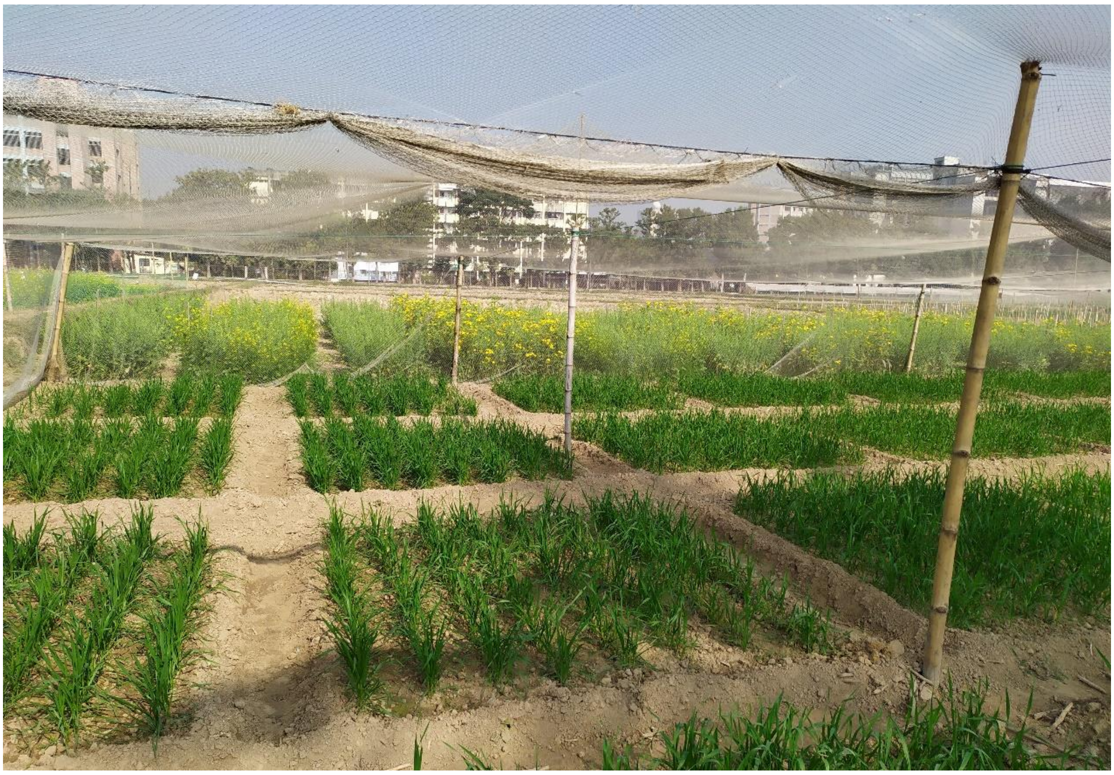


Plate 5. Seedlings of wheat plant

Plate 6. Aphid infestation on wheat spike

Plate 7. Experiment title at the field