Annals of Agrarian Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Annals of Agrarian Science

journal homepage: www.elsevier.com/locate/aasci

Comparative study of *Azotobacter* with or without other fertilizers on growth and yield of wheat in Western hills of Nepal

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A R T I C L E I N F O

Keywords:
Azotobacter
Seed inoculated
Chemical fertilizer NPK
FYM
Grain yield

A B S T R A C T

A pot experiment was conducted 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 (T1, T2, T3, T4, T5, T6, and T7) each replicated three times. The treatments were control (T1), 120:80:80 kg NPK ha^{-1} (T2), *Azotobacter* seed inoculated (T3), *Azotobacter* soil application (T4), *Azotobacter* + 120:80:80 kg NPK ha^{-1} (T5), *Azotobacter* + 10 t FYM ha^{-1} (T6), *Azotobacter* + 120:80:80 kg NPK ha^{-1} + 10 t FYM ha^{-1} (T7). 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 (T2) 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).

Introduction

Nitrogen (N) is a major macronutrient often limiting growth and yield of wheat crop [1]. Nitrogen is found in chlorophyll, the green coloring matter of leaves that enables the plant to transfer energy from sunlight by photosynthesis and hence influences cell size, leaf area, and photosynthetic activity and is a key element in amino acids, proteins including grain protein, chlorophyll and root development [2]. Wheat is very sensitive to insufficient N. Insufficient nitrogen results in the yellowing (chlorosis) of leaves due to a drop in chlorophyll content (symptoms first become visible on the old leaves which turn yellow and wither at progressing deficiency), reduced tillering, and disturbance of normal cell growth division, and a decrease in rate, and extent of protein synthesis [3]. Because of this, crop yields may also be greatly reduced. Nitrogen leaches easily and is required in relatively large amounts by the wheat crop. Nitrogen is one of the most expensive nutrients to supply, and may also have an environmental impact through nitrate leaching pollution of groundwater, eutrophication of rivers and lakes and global warming and nitrous oxide emissions associated with denitrification by soil bacteria [4].

Biofertilizers are the live formulation of microorganisms which have the ability to mobilize plant nutrients in the soil and offer a cheap, low capital intensive, non-bulk and eco-friendly source to boost productivity [5–7]. Among biofertilizers, *Azotobacter* strains play a key role in the nitrogen cycle in nature that binds atmospheric nitrogen inaccessible to plants and releasing it in the form of ammonium ions available to plants in the soil fixing an average 20 kg N/ha per year. It is able to grow at a pH range of 4.8–8.5 and fixes N at optimum pH of 7.0–7.5 [8]. *Azotobacter* can fix at least 10 µg of nitrogen per gram of glucose consumed. Inoculation effect of free-living *Azotobacter* species are largely associated with nitrogen fixation [9], formation of various physiologically active growth hormones like gibberellin, auxin and cytokinin [10,11], ammonia, vitamins and growth substances responsible for seed germination [12,13], protection against root pathogens [14,15], stimulation of beneficial rhizospheric microorganisms and enhancement of plant yield [16]. However, the exact mode of action by which *Azotobacter* enhances the growth in the plant is not fully understood. The abundance of *Azotobacter* in soil depends upon many factors such as soil physicochemical (e.g. organic matter, pH, temperature, soil moisture) and microbiological properties. However, the abundance varies as per the depth of the soil profile.

Azotobacter is generally applied to soil, seeds or seedlings, with or

Peer review under responsibility of Journal Annals of Agrarian Science.
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https://doi.org/10.1016/j.aasci.2018.04.004
Received 4 November 2017; Received in revised form 2 April 2018; Accepted 11 April 2018
1512-1887/

without some carrier for the microorganisms. Regardless of methods, the number of cells reaching the soil from commercial products is smaller than the existing numbers of soil or rhizosphere microorganisms; these added cells are unlikely to have a beneficial impact on the plant unless multiplication occurs. Seed inoculation method will provide a maximum number of populations [17]. Seeds having less germination percentage if inoculated with *Azotobacter* can increase germination by 20–30%. Seeds inoculated by *Azotobacter* helps in the uptake of N, P along with micronutrients like Fe and Zn, in wheat.

Azotobacter inoculation replaced up to 50% of urea-N for wheat grown in greenhouse trial under aseptic conditions [18]. *Azotobacter* inoculation in wheat results in increased plant height, tillers, and ear length and grain yield of wheat over non inoculated control [19]. The grain and straw yield of wheat markedly increased from 39.4 q/ha to 41.8 q/ha and 54.3 q/ha to 57.2 q/ha, respectively because of seed inoculation with *Azotobacter* [20]. In view of the facts discussed above, this experiment was carried out to study the effect of biofertilizer (*Azotobacter* inoculation) on growth and yield of wheat.

Materials and methods

Location of the experimental site

This experiment was carried out in the premises of Institute of Agriculture and Animal Science, Lamjung Campus, Sundarbazaar in the western mid hills of Nepal during December 2016–April 2017 growing seasons. The site is located at an elevation of 610 masl with the latitude of 28° 8′ 41″N and longitude of 84° 24′ 43″ E.

Soil analysis

The soil sample of the experimental site was collected and the pH, nitrogen, organic matter, phosphorus, and potash content were evaluated.

Design of experiment

The experiment was carried out in the pot with seven treatments and three replications. There were 21 pots, each having four wheat plants. To maintain suitable moisture condition in the pot, the hole was drilled into the pot. For pot filling, the soil was collected from the horticultural farm of IAAS, Sundarbazaar, Lamjung Nepal. The soil was mixed thoroughly and the pot was filled with it. Then, eight wheat seeds were placed in each pot. Gautam variety of wheat seeds was used as planting material which was collected from a commercial seed trader of Sundarbazaar, Lamjung, Nepal. The treatments were control (T1), only inorganic fertilizer 120:80:80 kg NPK ha^{-1} (T2), *Azotobacter* seed inoculated (T3), *Azotobacter* soil application (T4), *Azotobacter* + 120:80:80 kg NPK ha^{-1} (T5), *Azotobacter* + 10 t FYM ha^{-1} (T6), *Azotobacter* + 120:80:80 kg NPK ha^{-1} + 10 t FYM ha^{-1} (T7). For control, no manures and fertilizers were applied.

The amount of fertilizer and FYM for one plant was calculated using a formula for the estimation of plant population per hectare (Pp). The total amount of fertilizer and FYM required for one hectare was divided by plant population per hectare. Thus, the need of amount per plant was obtained.

$$Pp = \frac{10{,}000\ m^2\ X\ number\ of\ seeds\ per\ stand}{Product\ of\ spacing\ (m^2)}$$

The product of spacing used was 18 cm × 6 cm while the number of seed per stand was 1. This resulted in plant population of 925926 per hectare.

$$Fertilizer\ per\ plant = \frac{Amount\ of\ fertilizer\ per\ hectare}{Plant\ population\ per\ hectare}$$

Azotobacter (33×10^7 cfu/ml) was used to coat 24 wheat seeds while 43.2 gm of FYM (Farmyard manure) per pot was used as for 4 plants. As inorganic fertilizer 120 kg of urea, 80 kg of MOP and 80 kg of DAP were used for nitrogen, phosphorous and potassium (NPK) source for a hectare. So for a single pot which contained 4 wheat plant was supplied with 0.715 gm of urea, 0.28 gm of MOP and 0.56 gm of DAP.

Selection of wheat seed and seed treatment for sowing

Well developed, bold and healthy seeds were collected. The seeds were treated with mercurial fungicides like vitavex @ 2 gm/kg seed. The treatment was given to prevent diseases like flag smut, foot rot, wilt etc. Wheat seeds were soaked in hot water at 53 °C temperature for 10 min before sowing in order to control diseases like alternaria blight, smut etc [35].

Seed and soil inoculation

Seed and soil inoculation technique was used. 10% sugar solution was boiled and, then, cooled. This slurry was uniformly applied to the seed. Then the seed was coated with the powder of *Azotobacter* and sown. For soil inoculation, *Azotobacter* powder was directly placed into the soil above which the wheat seeds were sown.

Sowing, irrigation, weed control and harvesting

Sowing was done on December 26, 2016. Following seed sowing, light irrigation was done. After the complete germination, the wheat plants were thinned out leaving only four wheat plants in each pot. Plant to plant distance of 6 cm was maintained. Irrigation with 250 ml of water was done on an interval of two days which subsequently decreased to once a week when neared to harvest.

Hand weeding was done for the first time on 35th days of sowing followed by second weeding on 55th days. Aphid infestation was controlled by spraying detergent water (2 teaspoon detergent per litre of water) to wheat plants for two weeks on alternate days. When the aphid infestation was not controlled, Rogohit (Dimethoate 30% EC) were applied. Harvesting was done manually on April 17, 2017 (113 days after sowing).

Data collection

Plant height, leaf number, leaf length and width, number of tillers per plant, panicle length and weight, number of grains per plant, root length, straw yield, total biomass, yield per plant, and biological yield were taken.

Data analysis

MS-Excel worksheet version 13 was used to record the data. Table, charts, graph and simple statistical analysis were performed using Excel. The significance of difference among the treatment combinations was estimated at 5% level of significance.

Results

Maximum plant height (cm), root length (cm), leaf number on 39 days after sowing, leaf length and width, tiller number, panicle number per plant, panicle weight per plant (gm), number of grains per plant, grain weight per plant (gm), grain yield (ton/hectare), straw yield per plant (gm), and biological yield were recorded with treatment T7 (*Azotobacter* + 120:80:80 NPK ha^{-1} + FYM). Root weight, panicle number per plant, panicle length, and total biomass were found to be maximum in treatment T2 (120:80:80 NPK ha^{-1} only).

The lowest plant height (cm), root length (cm), leaf number on 39 days after sowing, leaf width, tiller number, panicle number per plant,