






Growth and yield response of hybrid maize to arbuscular mycorrhizal fungi inoculation and zinc fertilizer management

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ABSTRACT

An experiment was conducted at the Agronomy Field Laboratory in Bangladesh Agricultural University to evaluate the effect of arbuscular mycorrhizal fungi (AMF) inoculation and zinc (Zn) fertilizer management on growth and yield of hybrid maize Kohinoor 1820. The experiment consisted of two levels of AMF inoculation (AMF-inoculated and non-inoculated) and five levels of Zn fertilizer management. The Zn management regimes were NoZinc = no Zn fertilizer (control); Basal100 = 100% recommended dose (RD) of Zn fertilizer (i.e. 15 kg ZnSO₄.7H₂O ha⁻¹) added during final land preparation; Foliar100@EV = 100% RD of Zn was applied as foliar spray during early vegetative (EV) stage; Foliar100@Rp = 100% RD of Zn was applied as foliar spray during reproductive (Rp) stage; Foliar@50EV+50Rp = 100% RD of Zn fertilizer was applied as foliar spray by equal split during EV and Rp stages. ZnSO₄.7H₂O @15 kg ha⁻¹ was used for basal application and 0.1% of the same fertilizer was used as foliar spray. The experiment was laid out a factorial randomized complete block design (RCBD) with 3 replications. ‘Serakinkon’, a commercially available AMF inoculum collected from Japan was used in the experiment. The inoculum mainly consisted of *Gigaspora margarita* species of AMF. It was found that both AMF inoculation and Zn fertilizer management significantly affected leaf greenness (SPAD value), number of cobs plant⁻¹, number of seeds cob⁻¹, weight of 1000 grains, and grain yield (all p<0.05). The highest maize grain yield was obtained from the AMF inoculated plots when Zn was foliar applied during both early vegetative and reproductive stages (50%EV + 50% Rp) or foliar applied (100% RD) during reproductive stage. It appeared that only AMF inoculation boosted 15% maize yield in comparison to non-inoculated crops. Zn fertilizer management increased 16% grain yield over control (no Zn applied). Further research should be conducted on the screening of naturally occurring AMF strain suitable for maize crop.

Keywords: AMF, hybrid maize, yield, zinc, foliar spray



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1 Introduction

Maize (*Zea mays*) is one of the most important cereal crops of the world and hence it may be accepted as the second most important cereal crop in Bangladesh for its higher productivity. The total annual production of maize in Bangladesh is 35.69 lac tons from 10.99 lac acres of land in the fiscal year 2018-2019 (BBS, 2020) and the production is increasing year by year. Though the maize revolution came hand in hand with the rise of poultry and fish feed industry, it can also be a potential grain crop for nutritional support to the country population. Therefore, the government is now trying to promote maize not just as a feed crop, but also as a food crop. According to a recent US Department of Agriculture's (USDA, 2016) report, farmers in Bangladesh earn over \$2,275 by investing \$1,421 for every hectare of maize which is greater than comparing to both rice and wheat cultivation. Despite increased production, Bangladesh has to import maize to meet domestic consumption requirements of 38 lac tons estimated for fiscal 2016-17 (USDA, 2017). Grain imports are projected to increase by 28% from 10 lac tons in the fiscal 2017-18 for its increased demand from feed mills and mixing with wheat flour for human consumption (USDA, 2017). The ministry of agriculture (MoA) has been considering converting all lands under tobacco cultivation into maize fields to raise the production of the cereal up to 6.0 million tons by 2021. In Bangladesh, maize is mostly grown in the winter (*rabi*) season (November-March) after the harvest of transplant *aman* rice. Additionally, more area is coming under maize production in the post winter (*kharif*) season (February-May), mainly after the harvest of potato (Ali et al., 2009). In the *rabi* season, the crop suffers from severe soil moisture stress unless irrigation is provided. Due to the uncertainty of the season and crop, they are often reluctant to use recommended dose of fertilizers. Therefore, the yield of maize is less than the potential value in Bangladesh. In this circumstance, a future increase in the production of maize must come from higher yield per unit area per unit time.

Zinc deficiency is a major limiting factor that reduced the yield of maize severely. In agricultural soils, Zn status varies from area to area as it is not evenly distributed. For overcoming Zn deficiency issue, the fertilization of Zn is gaining much interest. Zhang et al. (2013) concluded from their two years successive experiment on maize that $ZnSO_4 \cdot 2H_2O$ (soil placement) increases soil DTPA-Zn concentration. Zinc fertilization improves root growth, and increases shoot Zn concentration up to 102%-305% during the first year of fertilization. In the second year, Zn concentration in grains increased up to 51%, and the grain harvest index increased up to 50% compared with control. Studies also focused on Zn foliar application effect of maize yield and improving Zn contents. Liu et al. (2016) also observed the im-

proved growth and Zn contents in maize through $ZnSO_4 \cdot 7H_2O$ application. Increased Zn concentration improved net photosynthesis rate, transpiration rate, stomatal conductance, chlorophyll-a and chlorophyll-b contents, which alternatively improved maize productivity and yield. Zinc fertilization result depends upon crop species and method of fertilization (Mao et al., 2014).

A small fraction (3–5%) of applied Zn is available for plant uptake, so Zn fertilizer solubility in water is also an interesting theme for study. Highly Zn soluble fertilizers proved effective in Zn-supply compared to low and medium soluble fertilizers. Even low soluble fertilizers did not increase the bioavailability of Zn over time. The input of phosphatic fertilizers and fertilizers which raise soil pH sometimes decreased the bioavailability of Zn (Shaver et al., 2007). Another method of Zn application is foliar fertilization (Potarzycki and Grzebisz, 2009), which is cost-effective. Liu et al. (2016) applied 7.5 kg $ZnSO_4 \cdot 7H_2O$ of Zn, which improved the photosynthesis rate and yield of maize. Rodinpuia et al. (2019) also performed foliar application on maize and observed significantly improved plant dry weight, height and other growth attributes. Zinc raised the synthesis of plant growth hormones, increase cell elongation, plant metabolism and N-accumulation. Researches also focused on the combined soil and foliar fertilization effect on maize growth. Wang et al. (2017) found that along with Zn and K foliar application increased Zn accumulation in wheat and decreased grain phytic acid: Zn molar ratio up to 63% compared to control.

Arbuscular mycorrhizal fungi (AMF) can create a symbiotic association with plant roots forming a hyphal network which enables the plant harvest soil moisture and nutrient from a larger rhizosphere. As a result, an AMF symbiotic plant suffers less from nutrient deficiency and moisture stress than a non-symbiotic plant growing in the same condition. Therefore, the use of AMF symbiosis with plants has become popular lately. AMF has been used in many crops to cope with drought (Sarkar et al., 2016), salinity (Estrada et al., 2013), disease tolerance (Talukder et al., 2019), nutrient and heavy metal stress (Sarkar et al., 2015a,b, 2017, 2018), to cut the fertilizer use in nutrient-deficit soils as well as to increase the quality of crop (Sarkar et al., 2020). AMF and nutrient solubilizing bacteria increase nutrient absorption in plants (Chungopast et al., 2021). The most common AMF species used in agriculture are belong to the genera *Gigaspora*, *Rhizophagus*, and *Glomus* (Almagrabi and Abdelmoneim, 2012; Basu et al., 2021; Coccina et al., 2019). External mycelium of AMF significantly increased the nutrient uptake and increase moisture contents. Except of extraradical mycelium, AMF alter the biochemical activities such as stimulate dehydrogenase and phosphatase enzymes activity, increased carbon biomass in soil and glomalin-related

soil protein secretion, all these activities are responsible for Zn uptake regulation (Wamberg et al., 2003). Isotopic studies of ^{65}Zn revealed that AMF can contribute up to 24.3% of total Zn uptake by plants. AMF lower the rhizospheric pH, thus released the bonded Zn for plant uptake (Subramanian et al., 2009). The siderophore production increased in AMF inoculated plants compared to non-inoculated plants of maize Balakrishnan and Subramanian (2012); Moreira et al. (2020). An increase in Zn concentration of plants was also reported in AMF inoculated plants, irrespective of soil condition (Ercoli et al., 2017; Bhantana et al., 2021). There is a negative correlation between Zn concentration and phytic acid concentration, thus increasing Zn uptake to suppress the phytic acid concentration in plants (Gibson et al., 2018).

After thorough reviewing of previous research, we hypothesized that AMF-inoculated maize will uptake more Zn from soil and therefore its application will increase maize yield. To test these hypotheses, we have designed a research with the following specific objectives: (i) to assess the effect of AMF inoculation on growth and yield of hybrid maize, and (ii) to find out appropriate dose and method of application of Zn fertilizer for AMF-inoculated hybrid maize crop.

2 Materials and Methods

2.1 Study location

The experiment was conducted at the Agronomy Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh during the period from December 2020 to June 2021 to determine the effect of arbuscular mycorrhizal fungi and zinc fertilizer management on the growth, yield and quality of Hybrid Maize 'Kohinoor 1820'. Geographically, the study site is located at $24^{\circ}75'$ N latitude and $90^{\circ}50'$ E longitude at an elevation of 18 m above the sea level. The soil was non-calcareous dark grey floodplain soil under Old Brahmaputra Floodplain Agro-ecological zone-AEZ 9. The region occupies a large area of Brahmaputra sediments which were laid down before the river shifted into its present Jamuna channel about 200 years ago. The soil was silt loam in texture having pH 6.8, 0.15% total N, 4 ppm available P, 15.9 ppm available S, 0.08 cmol kg^{-1} available K, 0.90 ppm available Zn, and 2.72% soil organic matter (Islam et al., 2016). The experimental was conducted in *rabi* season (October-March) which is characterized by scanty rainfall associated with moderately low temperature.

2.2 Experimental treatment and design

The experiment comprised two factors, *viz.* AMF inoculation and Zn fertilizer management. AMF inoculation had two levels; AMF inoculation and

non-inoculation. The Zn management practices were no zinc application (control), only basal application [100% recommended dose (RD)], foliar application at early vegetative (EV) stage [100% of RD], foliar application at reproductive (tasseling and silking) stage [100% RD], and foliar application 50% at EV stage + 50% at TS stage. The experiment was laid out in a factorial randomized complete block design (RCBD) with 3 replications. The size of each unit plot was 4 m \times 3 m. Row to row and plant to plant distances were 75 cm and 25 cm, respectively. Hybrid maize 'Kohinoor 1820' (Ispahani Agro Limited, Bangladesh) was used as planting materials for this experiment.

2.3 Application of treatments

AMF spores were applied in the form of a commercial inoculant namely, 'Serakinkon' powder (The Central Glass Company, Tokyo, Japan). The inoculant was composed of 50 *Gigaspora margarita* Becker and Hall (BEG 34) spores per gram powder. Mycorrhizal inocula were used in the field where necessary according to the treatment @ 15 kg ha^{-1} at 3 cm depth of the soil surface and then the soil was saturated with water. AMF inoculum was incorporated into the soil 21 days after sowing (DAS). The plots were spaded one day before planting and the whole amount of fertilizers (except zinc) were incorporated thoroughly before planting according to fertilizer recommendation guide (FRG, 2018). For basal application of zinc, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ was used @ 15 kg ha^{-1} . For foliar application, 0.1% aqueous solution was prepared. The solution was prepared by dissolving $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ powder with water. The foliar application was done by evenly spraying until the whole plants were wet and the solution just began to drip from the leaves, in the morning around 10 AM. The rate of application was 900~1000 L ha^{-1} .

2.4 Agronomic practices

The experimental lands were prepared with a tractor drawn rotavator followed by a power tiller. Then it was exposed to the sunshine for 7 days prior to the next ploughing. The land was then ploughed and cross-ploughed to obtain good tilth. Deep ploughing was done to produce a good tilth, which was necessary to get better yield of maize. Proper laddering was done to break the clods and human labours were provided to make the soil fine. All the weeds and stubbles were also removed from the experimental field by them. The soil was treated with insecticides at the time of final ploughing. Insecticides Furadan 5G was used @ 8 kg ha^{-1} to protect young plants from the attack of mole cricket, ants, and cutworms. Maize seeds were collected from local seed market. Before sowing in the main field, the seed was sown in two pots for germination test. Dry, clean and ho-

mogenous air-dried seeds with about 12% moisture content were used. Seeds were treated with Vitavex at the rate of 0.2% of seed weight. Seeds were sown on 02 December 2020. Care was taken to protect the seedlings from birds and rodents up to 20 days after sowing. Emergence of seedling was completed within 10 days after sowing. Over crowded seedlings were thinned out two times. First thinning was done after 15 days of sowing which was done to remove unhealthy and weak seedlings. The second thinning was done 10 days after first thinning. Twice manual weeding were done at the early stage of crop. Two irrigation were given after first and second weeding. There was no major incidence of insects or diseases. So, no other pest control measure was adopted in the experiment. The experimental crop was grown with proper care and agronomic management to ensure satisfactory yield.

2.5 Harvesting and data collection

Leaf greenness was recorded by the help of SPAD 502 Plus Chlorophyll Meter. The leaf greenness was measured at vegetative stage in term if SPAD value and it is considered a relative measure of leaf chlorophyll content. The crop was harvested plot-wise for each sowing date when cobs became bright straw color and grains showed black scar at the base when separated from the rachis of cob and when 75-80% cobs matured. The plants were harvested manually by cutting plants with sickles near the ground level. The crop was harvested on 13 April 2020. For collecting necessary data on yield contributing characters, the same plants as used for collection of growth parameters, were used. The harvested crop of each plot was bundled separately, tagged and taken to threshing floor for data collection. Grain yield was determined from the central area of 6 m² from each plot and expressed as t ha⁻¹ and adjusted with 12% moisture basis. Grain moisture content was measured after shelling and drying of grains by using a digital moisture tester. The stover yields were taken at fresh condition and representative sample was taken from there and dried to oven thoroughly plot by plot to record dry weights and then converted to t ha⁻¹.

2.6 Data analysis and visualization

The recorded data were statistically analyzed using open source statistical environment 'R' (R Core Team, 2021). For the Analysis of Variance (ANOVA) were conducted using 'agricolae' package of 'R'. The differences among treatment means were adjudged by Tukey's post hoc test. Plots presenting growth and yield of maize were prepared by 'ggplot2' library (Wickham, 2016) of 'R'.

3 Results and Discussion

3.1 Leaf greenness (SPAD value)

SPAD value is the measure of leaf greenness of plant leaves. It was found that SPAD value of maize leaves were significantly ($p < 0.05$) affected by AMF inoculation (Fig. 1). Leaves of AMF inoculated plants gave higher reading of SPAD value than those of non-inoculated plants. Kandel (2020) reported that there is strong positive correlation between different values of SPAD with grain yield in maize plant. AMF inoculation enable plants to acquire more soil moisture and micronutrients, especially Mg from soil (Sheng et al., 2008) Thus, AMF inoculated plants might have benefited and produced more photosynthetic pigments. Improved photosynthetic efficacy of maize with AMF under high temperature stress was reported by Mathur et al. (2018). Zinc fertilizer management also had significant effect ($p < 0.05$) on the SPAD values of maize leaves (Fig. 2). Split foliar application of Zn (50% at early vegetative stage and 50% at reproductive stage) produced the highest SPAD value in maize leaves. Foliar application, both at early vegetative stage (100%) and reproductive stage (100%) produced statistical similar result. The lowest value was measured in plants treated with no zinc fertilizer. Samreen et al. (2017) reported that Zn application increases the leaf chlorophyll content in *Vigna radiata*. Zn fertilization in plant also enables antioxidant mechanisms in plant under stress condition and increase leaf greenness (Arough et al., 2016). The interaction between AMF inoculation and zinc fertilizer management didn't have any significant effect on leaf greenness (data not shown here).

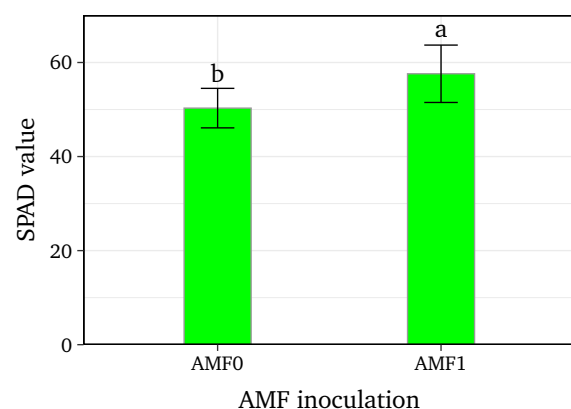


Figure 1. Effects of AMF on leaf greenness (SPAD value) of hybrid maize Kohinoor 1820. AMF0 = non-inoculated, and AMF1 = inoculated by arbuscular mycorrhizal fungi; vertical bar represents mean \pm standard deviation; values of bars with different letters are significantly different at $P=0.05$.

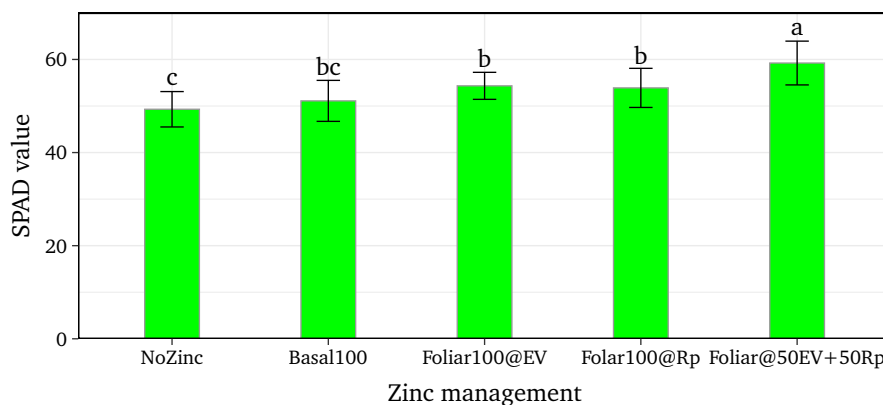


Figure 2. Effects of zinc fertilizer management on leaf greenness (SPAD value) of hybrid maize Kohinoor 1820. NoZinc = no Zn fertilizer (control); Basal100 = 100% recommended dose (RD) of Zn added during final land preparation; Foliar100@EV = 100% RD of Zn was applied as foliar spray (0.1% ZnSO₄) during early vegetative (EV) stage; Foliar100@Rp = 100% RD of Zn fertilizer was applied as foliar spray during early reproductive (Rp) stage; Foliar@50EV+50Rp = 100% RD of Zn was applied as foliar spray by equal split during EV and Rp stages; vertical bar represents mean \pm standard deviation; values of bars with different letters are significantly different at $P=0.05$.

3.2 Number of leaves plant⁻¹

AMF inoculation, zinc fertilizer management and their interactions did not show any significant effect (all $p>0.05$) on the number of leaves plant⁻¹ in maize (Table 1, Table 2). However, it was found that the highest number of leaves (12.78) were recorded from plots with AMF inoculation with foliar Zn application 100% at early vegetative stage (Table 3). The fewest leaf numbers plant⁻¹ (10.68) was recorded in plots where no AMF was inoculated and the Zn was foliar applied at early vegetative stage (100%).

3.3 Plant height

Plant height was significantly affected by AMF inoculation ($p<0.05$). AMF inoculated plots produced significantly taller plants than those non-inoculated plots (Table 1). Both zinc fertilizer management and its interaction with AMF inoculation also showed significant difference in maize plant height (both $p<0.05$) (Table 2, Table 3). The tallest plant (184.36 cm) was produced when Zn was applied as foliar spray (100% RD) during early vegetative stage in inoculated plants. This treatment produced 9.1 cm taller plants than the control treatment (no AMF inoculation and no Zn). However, the shortest plants (161.37 cm) were recorded in AMF non-inoculated plots when Zn was foliar applied (100%) at reproductive stage. This trend might be due to the ability of Zn to synthesize plant growth regulators such as auxins, which play an important role in cell enlargement and elongation in meristems (Ehsanullah et al., 2015). Zhao et al. (2015) reported that AMF inoculation in coal mine soils under drought condition promotes growth of maize. Growth of maize plant was also reported to be

enhanced when humic substances was supplemented with AMF inoculation in maize (Pinos et al., 2019).

3.4 Cob length

AMF inoculation, zinc fertilizer management and their interactions did not show any significant effect (all $p>0.05$) on the cob length in maize (Table 1, Table 2). However, it was found that the largest cobs (19.94 cm) were recorded from plants with AMF inoculation and foliar split Zn application at 50% at early vegetative stage and 50% at reproductive stage (Table 3).

3.5 Number of cobs plant⁻¹

AMF inoculation, zinc fertilizer management and their interactions did not show any significant effect (all $p>0.05$) on number of cobs plant⁻¹ in maize (Table 5, Table 4). However, it was found that the maximum number of cobs were recorded from plants with AMF non-inoculation and foliar Zn application 100% at reproductive stage (Table 6). The lowest number of cobs (0.94) were recorded in AMF non-inoculated plots when foliar Zn application was done 100% at early vegetative stage and the highest number were recorded in 'AMF0 \times Foliar100@Rp' treatment. Singh et al. (2016) reported that the number of cob was significantly increased with the application of AMF along with chemical fertilizers.

3.6 Weight of 1000 seeds

Both AMF inoculation and zinc management significantly affected the weight of 1000 seeds (WTS) of maize (both $p<0.05$). Inoculated maize produced

Table 1. Effect of arbuscular mycorrhizal fungi (AMF) inoculation on growth characters of hybrid maize Kohinoor 1820

AMF inoculation	Plant height (cm)	No. of leaves plant ⁻¹	Cob length (cm)
Non-inoculated	167.36 ± 9.52 b	11.43 ± 0.71	17.05 ± 1.87
Inoculated	172.6 ± 9.99 a	11.93 ± 0.8	17.5 ± 1.87
p-value	0.047	0.024	0.075

Values and mean ± standard deviation; values in a column with different letters differ significantly at P = 0.05.

Table 2. Effect of zinc fertilizer management on growth characters of hybrid maize Kohinoor 1820

Zn management	Plant height (cm)	No. of leaves plant ⁻¹	Cob length (cm)
NoZinc	170.15 ± 8.4 b	11.16 ± 0.26	16.23 ± 0.84
Basal100	174.13 ± 4.36 a	12.26 ± 0.77	16.39 ± 1.57
Foliar100@EV	174.93 ± 13.65 a	11.44 ± 1.06	18.12 ± 2.2
Folar100@Rp	167.5 ± 12.17 c	11.79 ± 0.76	16.49 ± 1.61
Foliar@50EV+50Rp	163.21 ± 6.17 d	11.76 ± 0.63	19.14 ± 1.09
p-value	0.037	0.612	0.081

Values and mean ± standard deviation; values in a column with different letters differ significantly at P = 0.05; NoZinc = no Zn fertilizer (control); Basal100 = 100% recommended dose (RD) of Zn added during final land preparation; Foliar100@EV = 100% RD of Zn was applied as foliar spray (0.1% ZnSO₄) during early vegetative (EV) stage; Folar100@Rp = 100% RD of Zn fertilizer was applied as foliar spray during early reproductive (Rp) stage; Foliar@50EV+50Rp = 100% RD of Zn was applied as foliar spray by equal split during EV and Rp stages.

Table 3. Effect of interaction of arbuscular mycorrhizal fungi (AMF) and zinc fertilizer management on growth characters of hybrid maize Kohinoor 1820

Combination	Plant height (cm)	No. of leaves plant ⁻¹	Cob length (cm)
AMF0 × NoZinc	175.26 ± 9.3 b	11.07 ± 0.22	16.07 ± 0.73
AMF0 × Basal100	172.37 ± 3.8 b	11.75 ± 0.39	15.83 ± 1.34
AMF0 × Foliar100@EV	165.50 ± 8.04 c	10.68 ± 1.01	18.75 ± 2.49
AMF0 × Folar100@Rp	161.37 ± 14.8 d	12.11 ± 0.63	16.25 ± 1.84
AMF0 × Foliar@50EV+50Rp	162.31 ± 4.53 d	11.57 ± 0.2	18.35 ± 0.99
AMF1 × NoZinc	165.03 ± 3.42 c	11.25 ± 0.3	16.40 ± 1.07
AMF1 × Basal100	175.88 ± 4.89 b	12.78 ± 0.75	16.94 ± 1.85
AMF1 × Foliar100@EV	184.36 ± 11.6 a	12.21 ± 0.21	17.49 ± 2.17
AMF1 × Folar100@Rp	173.64 ± 6.1 b	11.47 ± 0.87	16.72 ± 1.72
AMF1 × Foliar@50EV+50Rp	164.10 ± 8.5 c	11.95 ± 0.91	19.94 ± 0.31
p-value	0.025	0.840	0.131

Values and mean ± standard deviation; values in a column with different letters differ significantly at P = 0.05; AMF0 = AMF non-inoculated, and AMF1 = AMF inoculated; NoZinc = no Zn fertilizer (control); Basal100 = 100% recommended dose (RD) of Zn added during final land preparation; Foliar100@EV = 100% RD of Zn was applied as foliar spray (0.1% ZnSO₄) during early vegetative (EV) stage; Folar100@Rp = 100% RD of Zn fertilizer was applied as foliar spray during early reproductive (Rp) stage; Foliar@50EV+50Rp = 100% RD of Zn was applied as foliar spray by equal split during EV and Rp stages.

Table 4. Effect of arbuscular mycorrhizal fungi (AMF) inoculation on yield contributing characters of hybrid maize Kohinoor 1820

AMF inoculation	No. of cobs plant ⁻¹	No. of seeds cob ⁻¹	WTS (g)
Non-inoculated	1.1 ± 0.16	513.44 ± 38.4 b	273.1 ± 14.29 b
Inoculated	1.11 ± 0.17	554.78 ± 26.26 a	282.44 ± 11.51 a
p-value	0.118	<0.01	<0.01

Values and mean ± standard deviation; values in a column with different letters differ significantly at P = 0.05;

Table 5. Effect of zinc fertilizer management on yield contributing characters of hybrid maize Kohinoor 1820

Zn management	No. of cobs plant ⁻¹	No. of seeds cob ⁻¹	WTS (g)
NoZinc	1.1 ± 0.11	499.39 ± 35.36	261.85 ± 11.31 d
Basal100	1.06 ± 0.19	511.4 ± 43.27	272.73 ± 11.23 c
Foliar100@EV	1.05 ± 0.17	553.32 ± 28.81	277.3 ± 10.01 b
Folar100@Rp	1.19 ± 0.16	538.72 ± 24.38	286.14 ± 7.59 ab
Foliar@50EV+50Rp	1.14 ± 0.19	567.75 ± 13.58	290.83 ± 6.14 a
p-value	0.145	<0.024	<0.039

Values and mean ± standard deviation; values in a column with different letters differ significantly at P = 0.05; NoZinc = no Zn fertilizer (control); Basal100 = 100% recommended dose (RD) of Zn added during final land preparation; Foliar100@EV = 100% RD of Zn was applied as foliar spray (0.1% ZnSO₄) during early vegetative (EV) stage; Folar100@Rp = 100% RD of Zn fertilizer was applied as foliar spray during early reproductive (Rp) stage; Foliar@50EV+50Rp = 100% RD of Zn was applied as foliar spray by equal split during EV and Rp stages.

Table 6. Effect of interaction of arbuscular mycorrhizal fungi (AMF) and zinc fertilizer management on yield contributing characters of hybrid maize Kohinoor 1820

Combination	No. of cobs plant ⁻¹	No. of seeds cob ⁻¹	WTS (g)
AMF0 × NoZinc	1.14 ± 0.03	470.00 ± 13.1	258.39 ± 14.43
AMF0 × Basal100	1.17 ± 0.03	487.95 ± 46.2	263.75 ± 3.91
AMF0 × Foliar100@EV	0.94 ± 0.18	528.80 ± 4.9	269.57 ± 3.74
AMF0 × Folar100@Rp	1.26 ± 0.13	519.58 ± 10.0	284.35 ± 9.58
AMF0 × Foliar@50EV+50Rp	1.02 ± 0.18	560.88 ± 17.3	289.46 ± 5.75
AMF1 × NoZinc	1.07 ± 0.16	528.78 ± 19.0	265.32 ± 8.69
AMF1 × Basal100	0.95 ± 0.22	534.84 ± 29.9	281.71 ± 7.60
AMF1 × Foliar100@EV	1.16 ± 0.02	577.83 ± 15.7	285.02 ± 7.59
AMF1 × Folar100@Rp	1.12 ± 0.19	557.86 ± 16.9	287.93 ± 6.52
AMF1 × Foliar@50EV+50Rp	1.25 ± 0.11	574.61 ± 4.4	292.19 ± 7.46
p-value	0.391	<0.048	0.053

Values and mean ± standard deviation; values in a column with different letters differ significantly at P = 0.05; NoZinc = no Zn fertilizer (control); Basal100 = 100% recommended dose (RD) of Zn added during final land preparation; Foliar100@EV = 100% RD of Zn was applied as foliar spray (0.1% ZnSO₄) during early vegetative (EV) stage; Folar100@Rp = 100% RD of Zn fertilizer was applied as foliar spray during early reproductive (Rp) stage; Foliar@50EV+50Rp = 100% RD of Zn was applied as foliar spray by equal split during EV and Rp stages.

larger grain size with average WTS of 282.44 g, while the same in non-inoculated plants were 273.1 g (Table 4). For zinc management, the largest grains (WTS: 290.83 g) were produced when the maize crop was foliar sprayed with Zn solution 50% (of RD) at early vegetative growth and 50% at reproductive stage (Table 5). However, statistically similar result was obtained when 100% RD of Zn was foliar applied at reproductive stage. Though the interaction between AMF inoculation and Zn management showed no significant difference of WTS, the the largest grains (WTS: 292.19 g) were produced when the maize crop was foliar sprayed with Zn solution 50% (of RD) at early vegetative growth and 50% at reproductive stage (Table 6). Though 1000-seed weight of rice did not responded significantly to AMF inoculation (Zhang et al., 2016), Saboor et al. (2021) reported a significant increase in 1000-seed weight in AMF inoculated maize grown on Zn-deficit soil. Maize crop grown under rainfed condition gave significantly higher WTS when Zn was applied as foliar spray in comparison to basal application as reported by Wasaya et al. (2017).

3.7 Grain yield

Grain yield of maize was significantly effected by AMF inoculation, zinc fertilizer management and their interaction (all $p < 0.5$). The highest maize grain yield was obtained from AMF inoculated crops (6.81 t ha^{-1}) while non-inoculated plots gave on an average 5.91 t ha^{-1} yield (Fig. 3). For Zn management, the highest grain yield (6.75 t ha^{-1}) was recorded in plots where it was foliar applied during both early vegetative and reproductive stages (50%EV + 50% Rp) (Fig. 4). However, statistically similar yield (6.52 t ha^{-1}) was obtained when Zn was foliar applied (100% RD) during reproductive stage. The lowest yield (5.82 t ha^{-1}) was recorded in plots where no Zn was applied, i.e. control plots. From the combination of both factors, it can be seen that AMF inoculated plots gave best yield performance in maize when Zn was foliar applied during both early vegetative and reproductive stages (50%EV + 50% Rp) or foliar applied (100% RD) during reproductive stage (Fig. 5). It appeared that only AMF inoculation boosted 15% maize yield in comparison to non-inoculated crops. Zn fertilizer management increased 16% grain yield over control (no Zn applied). The increased grain yield of maize with AMF inoculation and foliar Zn application was the cumulative positive results of yield contributing characters (number of cobs plant⁻¹, number of seeds cob⁻¹, and weight of 1000 seeds). These parameters were also favored by AMF inoculation and foliar Zn application. Other studies have also reported increased grain yield of wheat Habbasha2015, mungbean (Haider et al., 2018), rice (Wang et al., 2020) and maize (Ehsanullah et al., 2015) wiht foliar Zn ap-

plication in comparison to no Zn or soil applied Zn treatments.

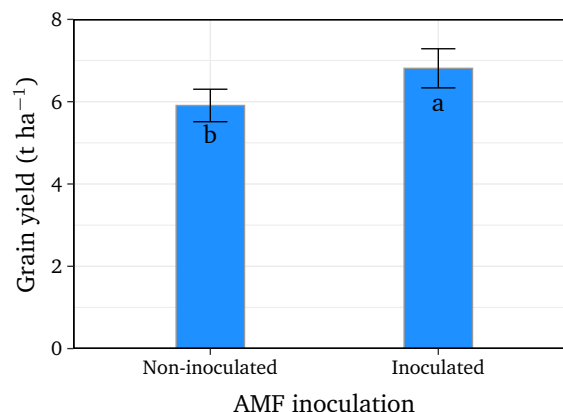


Figure 3. Effects of arbuscular mucorrhizal fungi (AMF) inoculation on grain yield of hybrid maize Kohinoor 1820. AMF0 and AMF1 denote AMF inoculated and AMF non-inoculated, respectively. Vertical bar represents mean \pm standard deviation; values of bars with different letters are significantly different at $P=0.05$

3.8 Stover yield

AMF inoculation, zinc fertilizer management and their interactions did not show any significant effect (all $p > 0.05$) on number of cobs plant⁻¹ in maize (data not shown). However, it was found that the highest stover yield (12.2 t ha^{-1}) was recorded from plants with AMF non-inoculation and foliar Zn application 100% at early vegetative stage. The lowest stover yield (8.1 t ha^{-1}) of maize was recorded in AMF inoculated plots when foliar Zn application was done 100% at reproductive stage. Stover yield of maize increased by the application of AMF and Zn solubilizing bacteria in a study reported by Ayyar and Appavoo (2017). However, in our study, though inoculated plots produced the tallest plants, it was not translated into higher stover yield.

4 Conclusion

This study revealed that the highest grain yield of hybrid maize Kohinoor 1820 can be obtained by application of arbuscular mycorrhizal fungi and foliar Zn ($0.1\% \text{ ZnSO}_4 \cdot 7\text{H}_2\text{O}$ solution) either 100% @ reproductive (silking and tasseling) stage or by equal split during early vegetative growth and reproductive stage. However, since foliar Zn application during these two managements gave statistically similar yield, considering the labor cost of spray application, we recommend 100% foliar application during reproductive stage.

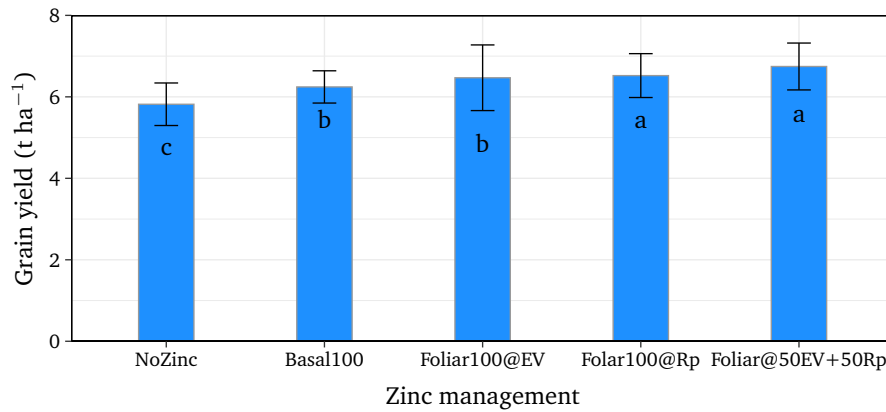


Figure 4. Effects of Zn management on grain yield of hybrid maize Kohinoor 1820. NoZinc = no Zn fertilizer (control); Basal100 = 100% recommended dose (RD) of Zn added during final land preparation; Foliar100@EV = 100% RD of Zn was applied as foliar spray (0.1% ZnSO₄) during early vegetative (EV) stage; Foliar100@Rp = 100% RD of Zn fertilizer was applied as foliar spray during early reproductive (Rp) stage; Foliar@50EV+50Rp = 100% RD of Zn was applied as foliar spray by equal split during EV and Rp stages. Vertical bar represents mean ± standard deviation; values of bars with different letters are significantly different at P=0.05.

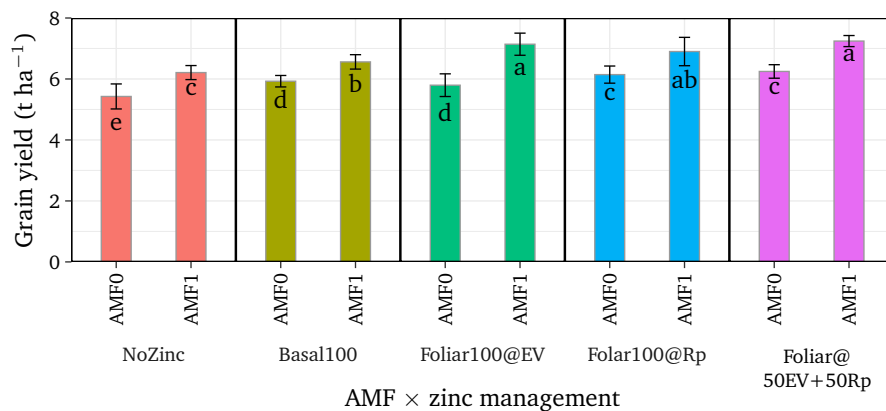


Figure 5. Effects of interaction of AMF inoculation and Zn management on grain yield of hybrid maize Kohinoor 1820. AMF0 and AMF1 denote AMF inoculated and AMF non-inoculated, respectively. NoZinc = no Zn fertilizer (control); Basal100 = 100% recommended dose (RD) of Zn added during final land preparation; Foliar100@EV = 100% RD of Zn was applied as foliar spray (0.1% ZnSO₄) during early vegetative (EV) stage; Foliar100@Rp = 100% RD of Zn fertilizer was applied as foliar spray during early reproductive (Rp) stage; Foliar@50EV+50Rp = 100% RD of Zn was applied as foliar spray by equal split during EV and Rp stages. Vertical bar represents mean ± standard deviation; values of bars with different letters are significantly different at P=0.05

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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