

Full Length Research Paper

A study on the effects of different biofertilizer combinations on yield, its components and growth indices of corn (*Zea mays* L.) under drought stress condition

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In order to study the combined effects of chemical phosphorus fertilizer, Phosphate-solubilising bacteria and mycorrhizal fungus they were determined on reducing drought stress damages of grain corn (SC704) under field conditions at College of Abouraihan, University of Tehran. The experiment was conducted as split plot RCBD with three replications. Experimental factors were drought stress as the main-plot (irrigation after 50 mm [without drought stress], 100 mm [low drought stress] and 150 mm [severe drought stress] evaporation from pan class (A), and fertilizer compounds as the sub-plot. Result of variance analysis showed that drought stress had significantly affected on most measured traits. All the measured traits in compounds phosphate-solubilising bacteria and Mycorrhiza fungies treatment were higher than other treatments under drought stress conditions except root colonization. Furthermore, grain yield in super phosphate triple treat under severe drought stress condition (3.55 ton/ha) was significantly less than normal irrigation (12.08 ton/ha) and low stress conditions (9.97 ton/ha). According to this experiment result, under drought stress condition, all calculated growth indices (such as LAI, CGR and NAR) decreased. Accordingly this experiment showed that phosphate-solubilising microorganisms can positively have effect on the increase of plant growth and phosphorus absorption in maize plant, leading to plant tolerance improving under drought stress conditions.

Key words: Mycorrhiza fungies, phosphate-solubilising bacteria, super phosphate triple, ear length.

INTRODUCTION

Intensive agriculture entails the risk of excessive fertilization. Microorganisms are important in agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers as much as possible. Organic agriculture is one of the ways that can produce high quality crops (Higa, 1994). Phosphorus (P) is an essential macronutrient for plant growth. Despite phosphorus being widely and abundantly distributed in the soil in both its inorganic and organic forms, many

soils throughout the world are deficient in phosphorus. Phosphorus can be tightly bound with calcium, iron, or aluminium, leading to precipitation of phosphorus (Li et al., 2003). Use of phosphorus fertilizers has become an expensive practice. The use of cheap, alternative sources of phosphorus, such as rock phosphate (RP) and microorganisms. Therefore, has received considerable attention in recent years (Rajan et al., 1996) Many bacteria (Rodriguez and Fraga, 1999) and fungi (Whitelaw, 2000) are able to improve plant growth by solubilising sparingly soluble inorganic and organic phosphates in the soil.

Sanders and Tinker (1971) and Hayman and Mosse (1972) indicated mycorrhizal plants take up phosphorus

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Table 1. Soil properties of experimental field.

| PH | Ec ds.m-1 | Clay % | Silt % | Sand % | Total N | P (mg/kg) | K (mg/kg) | soil texture |
|----|-----------|--------|--------|--------|---------|-----------|-----------|--------------|
| 8 | 2.62 | 28 | 37 | 35 | 0.071 | 13 | 224 | C.L |

from the same source of readily soluble phosphorus as nonmycorrhizal plants. Production and release of organic acids is an important mechanism involved in inorganic phosphorus solubilisation (Richardson, 2001). Moreover, the use of bio stimulators in condition of environmental stress can decrease effects of stress and enhance soil water holding capacity, root growth and yield (Li and Ni, 1996). Drought is one of the most important abiotic stress factor (Bruce et al., 2002), which affects almost every aspects of plant growth (Aslam et al., 2006). Drought, or more generally, limited water availability is the main factor limiting crop production (Seghatoleslami et al., 2008). Drought is a permanent constraint to agricultural production in many developing countries, and an occasional cause of losses of agricultural production in developed ones (Ceccarelli and Grando, 1996). No exact figures on yield and economic losses in maize due to drought are available. In maize, grain yield reduction caused by drought ranges from 10 to 76% depending on the severity and stage of occurrence (Bolaos et al., 1993). Sivasubramaniawn (1992) related the drought resistance of plants to the chlorophyll stability index that has been employed to determine the thermo stability of chlorophyll. Obviously, combined application of organic fertilizer and urea fertilizer or combination urea fertilizer and polyamines significantly increased yield, vegetative growth and chlorophyll index (Oad et al., 2004; Zeid, 2008). This experiment was conducted to investigate the effects of applications of biological fertilizer and chemical fertilizer on yield and component, yield, leaf area index, Crop Growth Rate (CGR), Net Assimilation Rate (NAR) of corn under drought stress.

MATERIALS AND METHODS

Combined effects of phosphorus fertilizer, phosphate-solubilising bacteria and mycorrhizal fungus were determined on reducing drought stress damages of grain corn (KSC704 commercial hybrid) under field conditions in experimental farm College of Abouraihan, University of Tehran (35°28'N, 42.51°17'E, altitude 1024 m). The experiment was planted in 2009 as a randomized complete block design with split-plot arrangement and three replications. The soil texture was clay loam and the result of soil analysis presented in Table 1.

Treatment consisted of three levels of drought stress: without stress (irrigation after 50 mm evaporation from pan class A), low drought stress (irrigation after 100 mm evaporation from pan class A) and severe drought stress (irrigation after 150 mm evaporation from pan class A). In sub-plots five compound fertilizer such as: (b1) phosphate-solubilising bacteria with Mycorrhiza fungies, (b2) phosphate-solubilising bacteria and Mycorrhiza fungi with 50% super phosphate triple, (b3) phosphate-solubilising bacteria with

50% super phosphate triple, (b4) Mycorrhiza fungi with 50% super phosphate triple and (b5) phosphate chemical fertilizer (100% super phosphate triple) were used.

Phosphate solubilising microorganisms used in this experiment were included Mycorrhiza fungi (*Glomus mosseae*) (with 65-70% colonization rate). Also biofertilizer phosphate-solubilising bacteria (biophosphor ®) was included *Bacillus* and *Pseudomonas* with CFU=10⁷.

Plants were grown in five-row plots with 5 m length and 0.75 cm spacing between rows. The plant density was 66000 plant/ha. Fertilizer was used based on soil test. Irrigation was performed on class A evaporation pan for each treatment. Data was recorded on 10 competitive plants of each plot and grain yield (kg ha⁻¹) was calculated for the entire plot. Each plot was harvested at maturity for yield and yield components and leaf area index and dry matter were measured each 15 days to calculate Crop Growth Rate (CGR), Net Assimilation Rate (NAR) and Leaf Area Index (LAI) according to below equations:

$$(1) LAI = LA / SA$$

$$(2) CGR = (W_2 - W_1) / SA(t_2 - t_1) \text{ g.m}^{-2}.\text{day}^{-1}$$

$$(3) NAR = (W_2 - W_1) / (t_2 - t_1) * (\ln LA_2 - \ln LA_1) / (LA_2 - LA_1) \text{ mg.mm}^{-2}.\text{day}^{-1}$$

In above abbreviations: LA = Leaf Area, SA = Ground area that occupied a plant. W = Dry matter, t = Day after planting.

Data was subjected to Analysis Of Variance (ANOVA) and the treatment means was compared using Duncan's multiple range test (alpha = 5%). The analysis was done by MSTATC and SAS (Ver. 9.1) software. Microsoft office Excel was used for figures drawing and indices calculation.

RESULTS

Analysis of variance showed that there are significant differences among most traits (Table 2). A significant effect of stress levels treatments on grain yield of maize. The highest grain yield of 12.08 ton/ ha was obtained for the normal irrigation treatment and lowest (3.55 ton/ha) for the severe Drought stress treatment (Table 3). The results showed that stress treatment significantly reduced (P 0.05) grain yield, percent colonization and harvest index. There were no significant differences in stress levels for row number in ear but there was a significant difference in fertilizer compounds. The other researcher showed that drought stress declined seed yield and its components (Reca et al., 2001; Seghatoleslami et al., 2008).

All the assessed traits in b2 compound inoculate treatment were of higher values than other treats under drought stress condition. Furthermore, the investigated traits of b5 treat under severe drought stress were significantly less pronounced than normal irrigation and low stressed conditions. This finding was in agreement

Table 2. Analysis of variance of measured traits of corn under different fertilizer treatments and drought stress conditions.

| S.O.V | df | Row No./ Ear | Kernel No./Row | 300 Kernel weight | Ear length | Total Yield | Percent colonization | Harvest index |
|-------------------|----|--------------|----------------|-------------------|------------|-------------|----------------------|---------------|
| Replication | 2 | 6.22ns | 214.60ns | 247.83ns | 27.75ns | 10.0** | 14.04* | 61.79* |
| Stress | 2 | 18.52ns | 2473.51** | 2679.04* | 193.86* | 296.42** | 638.77** | 2329.07** |
| Main error | 4 | 4.25 | 79.45 | 157.70 | 15.02 | 3.27 | 1.02 | 21.81 |
| Fertilizer | 4 | 8.18** | 158.2** | 164.58** | 14.11** | 20.49** | 2842.30** | 199.00** |
| Stress*Fertilizer | 8 | 0.55ns | 15.76ns | 67.12ns | 1.52ns | 1.00ns | 44.69** | 10.39ns |
| Sub error | 24 | 1.23 | 23.20 | 31.11 | 1.99 | 0.82 | 3.60 | 16.51 |
| Mean | | 13.72 | 40.17 | 66.93 | 18.47 | 8.53 | 40.42 | 42.00 |

*, **, ns : significant at 5%, 1% level and not significant, respectively.

Table 3. Means comparison of measured traits of corn under different fertilizer treatments and drought stress conditions using Duncan's multiple range test.

| | Row No./ Ear | Kernel No./ Row | 300 Kernel Weight (gr) | Ear Length (cm) | Total Yield (ton/ha) | Percent colonization (%) | Harvest index (%) |
|-----------------------|--------------|-----------------|------------------------|-----------------|----------------------|--------------------------|-------------------|
| Irrigation | | | | | | | |
| Normal irrigation | 14.456a | 49.22a | 78.004a | 21.222a | 12.087a | 45.47a | 52.42a |
| Low drought stress | 14.26a | 45.82a | 70.712a | 19.807a | 9.9712b | 43.03b | 45.37b |
| Severe drought stress | 12.444a | 25.479b | 52.089b | 14.41b | 3.5507c | 32.75c | 28.20c |
| Fertilizer | | | | | | | |
| b1 | 14.445a | 42.95ab | 69.858ab | 18.9263ab | 9.3667b | 57.93a | 45.68a |
| b2 | 14.6303a | 45.25a | 72.44a | 20.2215a | 10.352a | 23.39b | 46.72a |
| b3 | 13.746a | 39.39b | 65.239bc | 18.121bc | 7.815c | 27.07d | 38.08b |
| b4 | 13.574a | 39.013b | 65.644bc | 18.3509b | 8.7463b | 47.07c | 43.44a |
| b5 | 12.21b | 34.27c | 61.487c | 16.7778c | 6.4014d | 16.63e | 36.07b |

Means with same phrases in each columns not significant at 5% probability.

with the results of Ehteshami et al. (2009).

A combination of Mycorrhiza fungi and phosphate-solubilising bacteria had effects on these traits although, there were no significant differences among b2 and b5 for traits except for grain yield and percent colonization. Our results concur partly with observations made by Ehteshami et al. (2009) who reported that Mycorrhiza fungi and phosphate-solubilising bacteria increased traits.

According to this experiment result, under drought stress condition, seed inoculums with b2 treatment significantly affected the reduction of plant damages and therefore increased the total yield. Results of this experiment showed that phosphate-solubilising micro-organisms can positively interact with promoting plant growth as well as with phosphorus uptake in maize plant, leading to plant tolerance improved under drought stress conditions.

Leaf area index with the use of b1 was 3.8, in addition to normal irrigation maximum LAI was obtained among other levels (Figure 1). Application chemical fertilizer

treatment alone in different levels of irrigation observed that LAI in this treatment was decreased (Figure 2). This result showed that biological fertilizer to obtain relative resistance opposes the drought stress in corn. The lowest LAI at flowering stage was in b5 (2.85) and drought stress treatment (2.49).

The other researcher showed that the highest LAI was in 8 days period of irrigation (5.1) and drought stress in this treatment decreased 11% in LAI (Jafari et al., 2010).

Crop growth rate in b2 during the experiment was increased compared to other treatment (Figure 3). Probably in this experiment the crop growth rate is related to leaf area index, for the reason that crop growth changing rate is depended on two parameters: Namely leaf area index and net assimilation rate. This finding was in agreement with the results of Brogeham (2000). At flowering stage (75-90 day after planting), Severe drought stress condition had decreased CGR index compared to other irrigation treatments (Figure 4). Net assimilation rate with the use of b2 was increased compared to other treatments (Figure 5). Other fertilizer

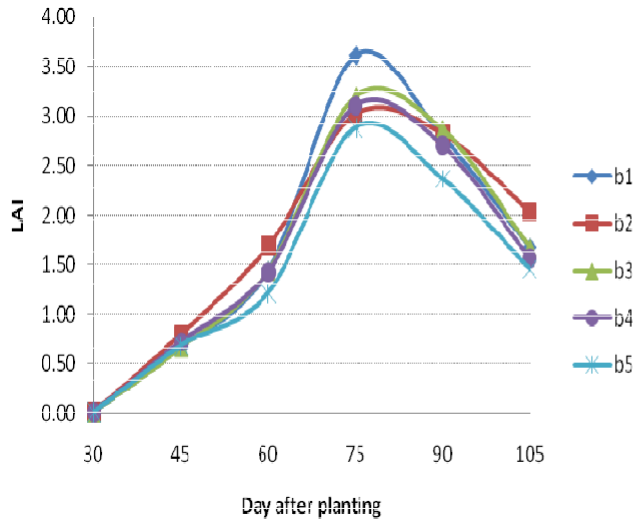


Figure 1. Leaf area index in five fertilizer treatment in during period of growth.

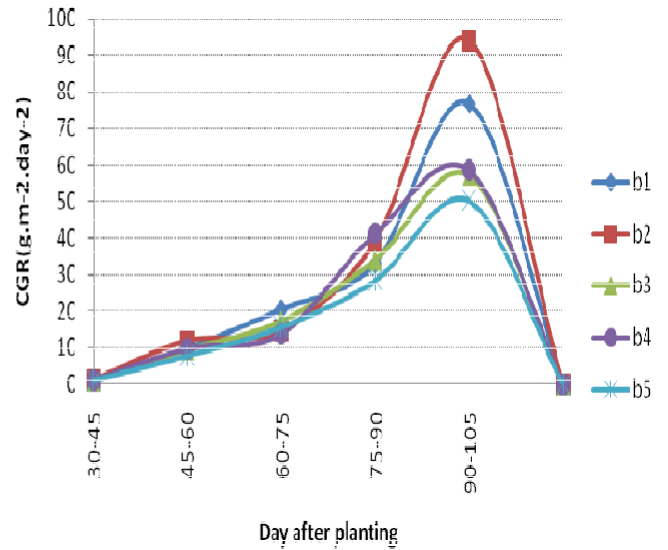


Figure 3. Crop growth rate in five fertilizer treatment in during period of growth.

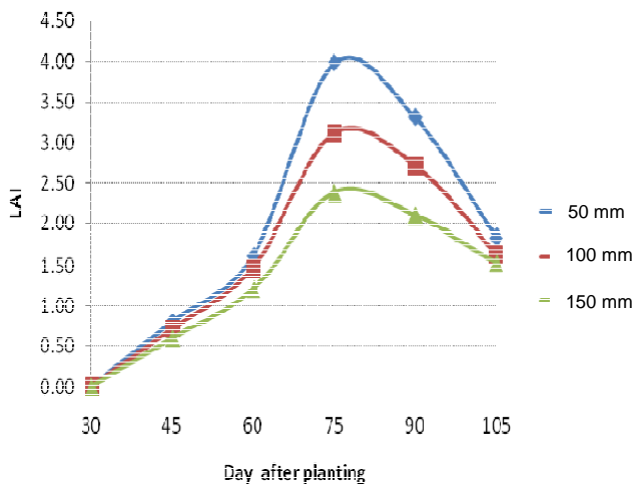


Figure 2. leaf area index in different levels irrigation in during period of growth.

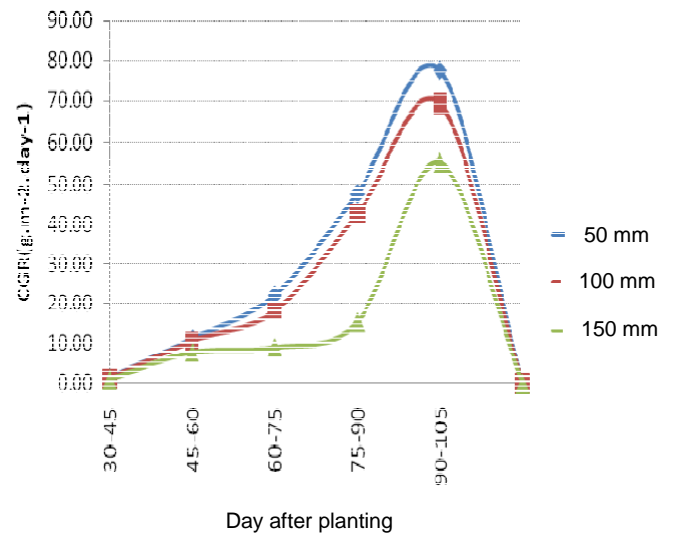


Figure 4. Crop growth rate in different levels irrigation in during period of growth.

compounds were not different from each other (Figure 5). The normal irrigation and low drought stress had overlapped on each other (Figure 6).

In biological fertilizer compounds the highest NAR was $0.055 \text{ (mg.mm}^{-2}\text{.day}^{-1}\text{)}$. Result of this experiment showed that chemical fertilizer used under drought stress condition can decrease NAR index more than biological fertilizer. Balak (1993) showed that NAR would decrease with the increase of LAI. This trend of decrease will continue from the beginning till the end of growth season.

Conclusion

Results of this study showed that, drought stress caused

decreases of yield and its component and measured indices such as LAI, CGR and NAR. Drought stress is a major abiotic constraint responsible for heavy production losses (Khan et al., 2007).

Application of biological fertilizer (b2) has given the highest grain yield in this study. El-Karmany (2001) showed that integrated chemical and biological fertilizer obtained highest kernel number per ear compared to sole application of them (4). Also our study showed that chemical fertilizer combined with biological fertilizer was beneficial to the environment because with decreasing the use of chemical fertilizer and use of organic inputs we

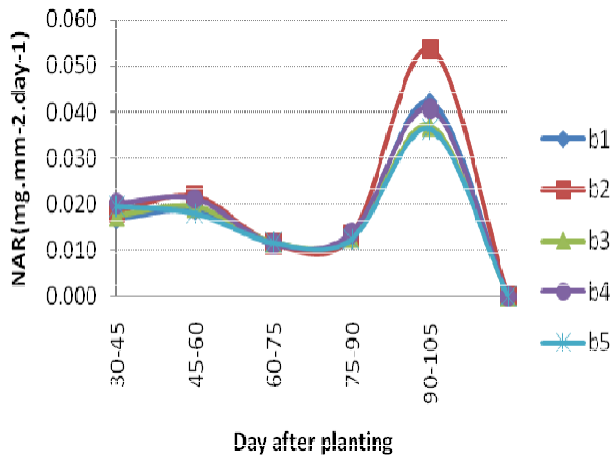


Figure 5. Net assimilation rate in five fertilizer treatment in during period of growth.

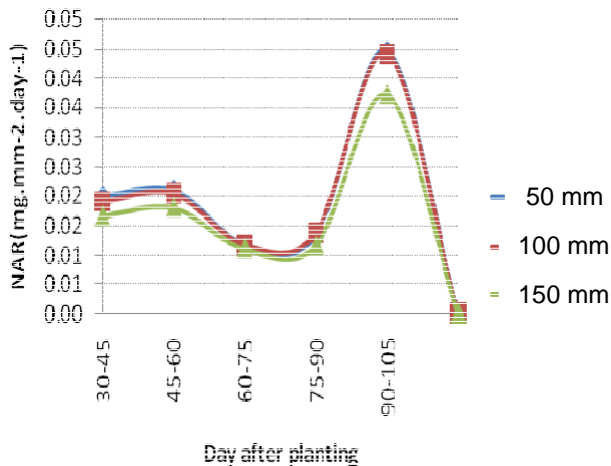


Figure 6. Net assimilation rate in different levels irrigation in during period of growth.

can side with sustainable agriculture, and increase the efficiency of water.

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