

ORIGINAL ARTICLE

The Effect of Plant Growth Promoting Bacteria on physiological and Phenological traits of maize (*Zea mays* L.) at different levels of Nitrogen Fertilizer

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ABSTRACT

In order to study the effect of Plant Growth Promoting Bacteria (PGPR) at different levels of nitrogen fertilizer on agronomic traits of maize (SC 704), an experiment was conducted in split plot in randomized complete block designed with three replications at Khash Pyame Nour of University in Iran in 2011-2012 cropping season. Four different levels of nitrogen fertilizer (non-using, 25% (equivalent to 87.5 kg.ha⁻¹), 50% (equivalent to 175 kg.ha⁻¹) and 100% ((equivalent to 350 kg.ha⁻¹)) were chosen as main plot and bio-fertilizer at three levels (non inoculation, inoculation with Azotobacter and Azospirillum) were assigned as subplot. Results of this study indicated that nitrogen fertilizer had significant effect on days to silking emergence and days to maturity. The highest days to silking emergence and days to maturity were obtained from non-using treatment. Also, Results of this study indicated that nitrogen fertilizer had significant effect on days to germination, days to silking emergence and days to maturity. The highest days to germination and days to silking emergence observed in non inoculation treatment, but the highest days to maturity was obtained from inoculation with Azotobacter. In this experiment showed that nitrogen fertilizer and bio-fertilizer had positive effect on growth indices, so that the highest LAI, CGR and NAR observed inoculation with Azotobacter treatment.

Keywords: Maize, PGPR, Growth indices.

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INTRODUCTION

Chemical fertilizers have several negative impacts on environment and sustainable agriculture. Therefore, bio-fertilizers are recommended in these conditions and growth prompting bacteria uses as a replacement of chemical fertilizers [1]. Growth promoting bacteria are bacteria that cause to improving yield as clone in plants root [2&3]. Growth prompting bacteria including *Azotobacter*, *Azospirillum* and *Pseudomonas* [2, 4, 5 &6]. Tilak [7] reported positive effects of double-inoculation of *Azotobacter* and *Azospirillum* on dry matter of maize and sorghum. The high level of leaf area index increases the crop growth rate's mean in the plant's growth period, so this leads to increase in biomass production and yield (8). Stancheva et al., (9) investigation showed that as a result of maize inoculation by bio-fertilizer fertilizers, the dry weight of plant is increased. They concluded that improvement in the foodstuffs' availability and their absorbency in plants are the main causes of this enhancement, and finally this leads to dry matter accumulation in maize. Allen et al., [10] reported that bio-fertilizer fertilizers' existence increases cytokinin and chlorophyll in a plant so, this plant grows more and more. Bath et al., [11] reported that red clover inoculation by Mycorrhiza due to increased absorb of zinc and phosphorus leads to increase in biomass in the plant. The objective of this study was conducted in order to effect of PGPR on physiological and phenological traits in maize at different levels of nitrogen fertilizer at Khash Pyame Nour of University in Iran.

MATERIAL AND METHOD

In order to study the effect of bio-fertilizer application at different levels of nitrogen fertilizer on agronomic traits of maize (SC 704) an experiment was conduct in split plot in randomized complete block designed with three replications in Khash Pyame Nour of University in Iran in 2011-2012 cropping

season. Four different levels of nitrogen fertilizer (non-using, 25% (equivalent to 87.5 kg.ha⁻¹), 50% (equivalent to 175 kg.ha⁻¹) and 100% ((equivalent to 350 kg.ha⁻¹)) were chosen as main plot and bio-fertilizer at three levels (non inoculation, inoculation with *Azotobacter* and *Azospirillum*) were assigned as subplot. The most rainfalls are in winter and early spring, when the amount of evapotranspiration is at minimum (Table 1). Geographical situation was with 61° 12' longitude and 28° 13' latitude and 1400 m height Sea.

| Parameter | May | Jun | July | August | September | December |
|--|--------|-------|-------|--------|-----------|----------|
| The speed of wind (m.s ⁻¹) | 300.15 | 40.14 | 40.11 | 30.12 | 320.14 | 300.09 |
| Temperature (°C) | 29.8 | 32.5 | 34.2 | 35 | 28.1 | 22.6 |

Table 1: The climatic properties of region during performance of study in 2011-2012 cropping season

| Location | Depth (cm) | EC (ds/m) | PH | Organic Matter (%) | Total N (%) | K Available (ppm) | P Available (ppm) | Soil texture |
|----------|------------|-----------|------|--------------------|-------------|-------------------|-------------------|--------------|
| Khash | 0-30 | 1.46 | 7.73 | 0.08 | 0.01 | 200 | 20.4 | Loam Silt |

Table 2: Soil properties of experimental site

Results of soil properties are provided in Table 2. The amount of the required phosphorus and potash fertilizers for all treatments according to soil's test were 200 kg.ha⁻¹ triple super phosphate and 125 kg.ha⁻¹ potassium sulfate. Different amounts of Nitrogen fertilizer base on soil analysis from urea resource were used as soil application in three turns: simultaneously with planting, during the time of eight- leaves emergence and one week before silking emergence. The seed's planting operation was done in 8 June 2012. Each experimental plot is consisted of 6 row spacing with 75 cm distance and 6 m length. Plant spacing per row was 20 cm. The harvesting of produce was done in 13 October 2012 as follows: The four middle lines in all treatments after removing of 0.5 m margin from beginning and ending of each plot were harvested with the floor gathering way and grain yield was determined according to kg / hectare scale. In each experimental plot, two beside rows and 0.5 m from beginning and ending of planting lines were removed as margin and measurements were done on 10 randomized selected plants in the middle lines. Phenological stages dates such as days to germination, days to silking emergence and days to maturity were registered. For studding physiological indices, sampling from each sub plot was done by selecting 4 plants and considering their margin randomly in 30 days after planting and it be continued with 15 days intervals. In sampling, two beside lines and 0.5 m from beginning and ending of the second and the fifth lines were removed, so sampling was done in the second and the fifth lines. In each sapling, in order to sustain the marginal effects, the primary plants were removed and samples were harvested from the second and the fifth lines. Totally, during the growth season from planting to harvest, six stages sampling were done. In order to measure leaf area, at first all leaves were picked, then the length and the largest breadth of each leaf were measured by a ruler. So, leaf area of plants was calculated with $A=L \times W \times 0.75$. In this relation, (A) is leaf's expanse, (L) is leaf Length and (W) is leaf the largest breadth. In all harvests, the dry matter weight was calculated. In this regard, samples were placed in oven with 75 °C temperature for 24 to 48 h, so their dry weights were calculated. By measuring leaf area and dry weight, the amounts of the physiological indices such as leaf Area Index (LAI), Crop Growth Rate (CGR) and Net Assimilation Rate (NAR) were calculated by the numerical calculations with the following equations:

$$LAI = \frac{LA_1 + LA_2}{2} \times \frac{1}{GA} \quad (1) \quad CGR = \frac{W_2 - W_1}{GA(t_2 - t_1)} \quad (2) \quad NAR = \frac{W_2 - W_1}{(t_2 - t_1)} \times \frac{\ln A_2 - \ln A_1}{A_2 - A_1} \quad (3)$$

In the equations 1 to 3, LA₁, A₁, LA₂, A₂, were the primary and the secondary leaf area base on m² respectively. GA was ground area which be occupied with plant base on m², W₁ was the primary dry weight, W₂ was the secondary dry weight base on g.m⁻², t₁ was the primary sampling time and t₂ was the secondary sampling's time base on day. Statically analysis was conducted using MSTAT-c software. Mean comparison was also conducted with Duncan's Multiple Range Test (DMRT). Also their diagrams were drawn by Excel software.

RESULTS AND DISCUSSION

Days to germination

Results of data variance analysis of days to germination showed that nitrogen fertilizer and interaction effect of nitrogen fertilizer and bio-fertilizers had not significant effect on this trait, but this **trait** was influenced by bio-fertilizer (Table 3). By considering the mean comparison of days to germination, it was

appeared that bio-fertilizers such as *Azotobacter*, *Azospirillum* from their influence on days to germination in comparison with check treatment, decreased days to germination in maize (Table 4). These bacteria accelerated seedling grow in farms thought the increasing growth hormone production, secretion of auxin (IAA) and bio-control of the seedling diseases, so, days to germination in maize were decreased. Hafeez et al., [12] reported that by using of seed inoculation with plant growth promoting rhizobacteria (PGPR), speed growth of seedlings up in different cotton plant's races. More speed in growing provides a possibility for seedling to be free from diseases and to be enjoyed from the growth season.

Days to silking emergence

Variance analysis showed that there are a significant difference between the different levels of nitrogen fertilizer and bio-fertilizer on days to silking emergence at 5 and 1% probability levels respectively (Table 3). According to mean's comparison in days to silking emergence it be observed that the most days to silking emergence was gained with mean of 55.4 days in non using nitrogen fertilizer (check treatment), also, using 100 % of nitrogen fertilizer base on soil analysis was equal (350 kg.ha⁻¹) with mean of 47.1 days which showed the least days to silking emergence (Table 4). According to Table 4, maize inoculation with *Azotobacter* and *Azospirillum* showed the least days to silking emergence. Non inoculation treatment showed the most days to silking emergence. Maize inoculation with bio-fertilizer in the different amounts of nitrogen fertilizer on days to silking emergence was significant at 5 % probability level (Table 3). The least days to silking emergence from *Azotobacter* treatment in the level of 100 % of nitrogen fertilizer application were 45 days and the most days to silking emergence from non using bio-fertilizers treatment was belonged to non using nitrogen fertilizer (Table 5).

Days to maturity

Variance analysis about days to maturity showed that there is a significant difference between nitrogen fertilizer application treatments and inoculation treatment with bio-fertilizers at 5 % probability level (Table 3). Studying days to maturity in nitrogen levels showed that the least days to maturity in the levels of 50 and 100 % of nitrogen fertilizer application were gained (Table 4). But, there is no difference between these two levels. The mean's comparison of bio-fertilizers effect on days to maturity showed that maize inoculation with *Azotobacter* and *Azospirillum* in compared with non inoculation treatment (check) has significant increase in days to maturity (Table 4). The prolonged stages of days to maturity in maize C. KSC704 due to using bio-fertilizers such as *Azotobacter* and *Azospirillum*, showed that there are proper and good conditions/opportunities for plant growth. *Azotobacter* and *Azospirillum*, are plant growth promoting rhizobacteria (PGPR) which by producing plant growth promoting hormones and increasing the root's absorbency, cause improvement in plant growth condition and finally cause prolonged maturity in plant. Kenndy [13] in his experiment confirmed this matter. According to results of Table 3, the interaction effect of nitrogen fertilizer and bio-fertilizer on days to maturity was significant in at 1% probability level (Table 3). The most days to maturity were gained from *Azotobacter* and non using nitrogen fertilizer and the least days to maturity were gained from *Azospirillum* with using 100 % of nitrogen fertilizer based on the soil analysis (Table 5).

| S.O.V | d.f | Days after germination | Days to silking emergence | Days to maturity |
|--------------------------|-----|------------------------|---------------------------|------------------|
| Replication | 2 | 3.52 | 80.52 | 28.4 |
| Nitrogen fertilizer (NF) | 3 | 12.07ns | 104.74* | 262.3** |
| Error a | 6 | 2.82 | 18.49 | 10.5 |
| Bio-fertilizer (B) | 2 | 27.02** | 115.10** | 101.86** |
| NF × B | 6 | 3.10ns | 7.96* | 12.71** |
| Error a | 16 | 1.66 | 2.50 | 3.12 |
| C.V (%) | - | 15.9 | 13 | 11.5 |

Table 3: Analysis of variance and mean square for phenological traits

Ns: non-significant, * and **: significant at 5% and 1%, respectively.

| | Days after germination | Days to silking emergence | Days to maturity |
|---------------------|------------------------|---------------------------|------------------|
| Nitrogen fertilizer | | | |
| Witness | 9.7A | 55.4a | 120.6a |
| 25% requirement | 7.8A | 53.2ab | 114.4ab |
| 50% requirement | 7.6a | 51.1ab | 110.2b |
| 100% requirement | 7.1a | 47.1b | 108.5b |
| Bio-fertilizer | | | |
| <i>Azotobacter</i> | 7.4a | 49.6b | 116.6a |
| <i>Azospirillum</i> | 7a | 50.3b | 116.9a |
| Non-inoculation | 9.8a | 55.3a | 111.8b |

Table 4: Comparison of effects of nitrogen fertilizer and PGPR on phenological traits in Maize

Means within each column with a letter in common are not significantly different at $\alpha = 0.05$.

| Treatment | | Days after germination | Days to silking emergence | Days to maturity |
|---------------------|---------------------|------------------------|---------------------------|------------------|
| Witness | <i>Azotobacter</i> | 9b | 53.6c | 126a |
| | <i>Azospirillum</i> | 8.3bc | 51.3cd | 119b |
| | Non-inoculation | 12a | 61.3a | 116cd |
| 25% of requirement | <i>Azotobacter</i> | 8.3bc | 50.6de | 113de |
| | <i>Azospirillum</i> | 6.6 | 523.3cd | 111efg |
| | Non-inoculation | 8.6b | 57.3b | 118bc |
| 50% of requirement | <i>Azotobacter</i> | 7cd | 49.6de | 109fgh |
| | <i>Azospirillum</i> | 6cd | 50.3de | 109fgh |
| | Non-inoculation | 10.8ab | 53.3c | 112ef |
| 100% of requirement | <i>Azotobacter</i> | 5.4d | 45.6f | 108gh |
| | <i>Azospirillum</i> | 7.8c | 47.3ef | 107h |
| | Non-inoculation | 8.1bc | 50.3de | 110efgh |

Table 5: Interaction effect of nitrogen fertilizer and PGPR on phenological traits in Maize
Means within each column with a letter in common are not significantly different at $\alpha = 0.05$.

Leaf Area Index (LAI):

Increased leaf area is plant photosynthesis capacity determiner. Every change in leaf area which is influenced by genotype, Plant density, weather and soil fertility, has some influenced on grain yield. Results showed that changes LAI in response to the different levels of fertilizers during the growth season for treatments had the same process. Different treatments in the primary growth stages had no effect on LAI. At the beginning of the growth season, Maize LAI be increased slowly, so that this process be continued about 40 days after planting. One of the main reasons is that monthly temperature's mean in that time was very high in the experiment area. High temperature prevent may operations in the photosynthesis enzymes, so, this plant after a while has no reserve for investment on the leaf and expense its energy for maintenance. Brich et al., [14] reported that some temperatures below 8 °C and above 40 °C can reduce photosynthesis enzymes' activity such as PEP carboxylase and RUBISCO, so in these conditions plant's growth is very slowly. During this season and by decreasing temperature's mean, increase in this index become linear, and about 75 days after planting of maize, it reaches to the maximum, but, at the end of the season, because of leaves' fall and due to its color which turn yellow, this amount decreased. (Figures 1, 2, 3 and 4). As mentioned above, at the beginning of the growth stages, increasing process of LAI for all treatments was the same. In Figure 1, 2, 3 and 4, leaf area index changes were drawn in four levels of nitrogen fertilizer. The highest amount for leaf area index after 75 days from planting by using 50 and 100 % of nitrogen fertilizer based on soil analysis in inoculation with *Azotobacter* was 4.43 and the lowest amount for leaf area index in this stage (75 days after planting) in check treatment (non using nitrogen fertilizer and non inoculation) was 3.1. This matter shows that in plant's fast growth stages due to the proper environmental conditions such as day's length and temperature, using nitrogen fertilizer is more and the aerial organs' development is more so that LAI is maximum. The highest and the lowest LAI were observed in *Azotobacter* treatment and check treatment for all nitrogen fertilizer levels respectively. High LAI leads to increase in crop growth rate mean in the plant's growth period, and finally biomass production is increased. Kupulnik et al., [15] demonstrated that maize's seed inoculation by *Azospirillum* has been caused increase in leave's numbers and finally some increase in yield as compared with check treatment.

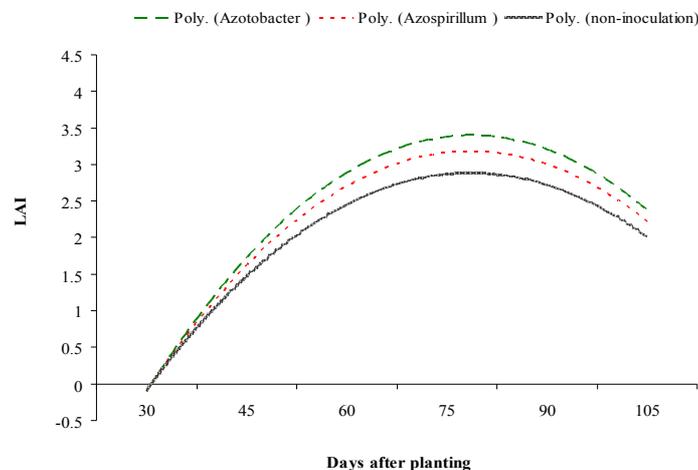


Figure 1: Changes of trend in LAI in non application of nitrogen fertilizer in bio-fertilizer

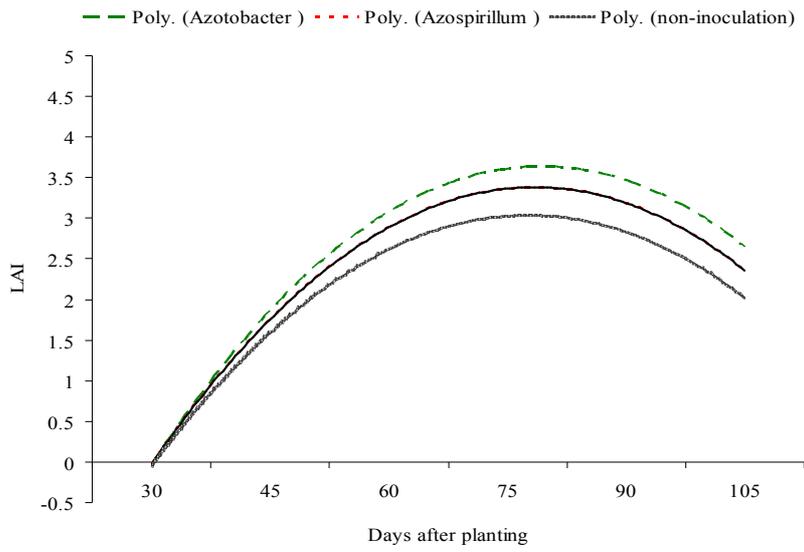


Figure2: Changes of trend in LAI in 25% of nitrogen fertilizer in bio-fertilizer

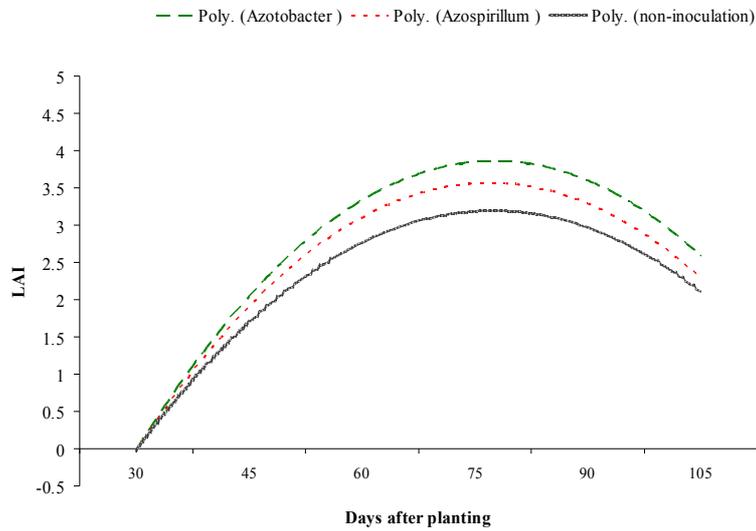


Figure 3: Changes of trend in LAI in 50% of nitrogen fertilizer in bio-fertilizer

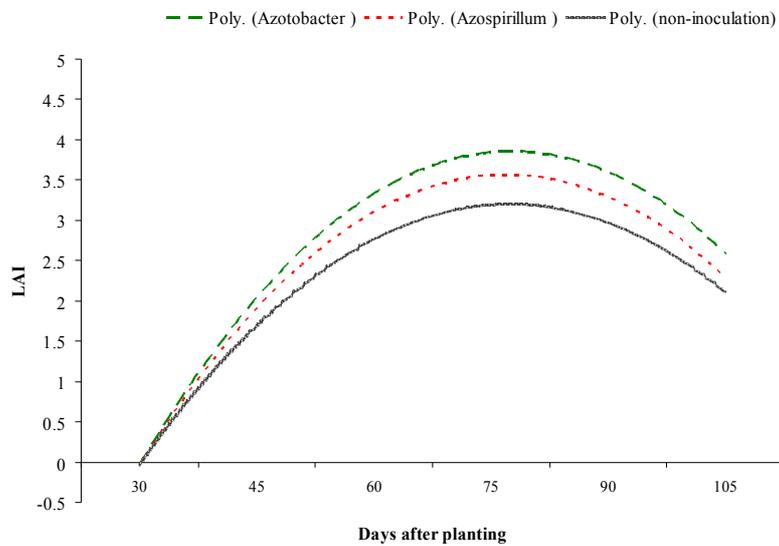


Figure 4: Changes of trend in LAI in 100% of nitrogen fertilizer in bio-fertilizer

Crop Growth Rate (CGR):

Plant growth rate is a quantitative criterion and shows physiological trait of plant. CGR a determined time unit and in a land area unit has a significant correlation with air temperature and sun's radiations. Increased CGR in the beginning of the growth season is related to the fast leaves' development, so this need enough water and nutritious materials for plant growth especially in the growth critical stages. According to Figures 5, 6, 7 and 8, CGR in the primary stages of growth is very low because of incomplete plant canopy, high temperature mean, low assimilation of sunlight after development in leaf area index and less light's penetration to the soil's surface. CGR is increased, during the growth season, about 45 days after planting, CGR changes in the different treatment have a significant difference, so that in *Azotobacter* treatment in all nitrogen fertilizer levels, this difference was great and CGR in 70 days after planting was maximum. The highest and the lowest CGR in *Azotobacter* treatment by using 100 % of nitrogen fertilizer based on the soil analysis and check treatment were registered (Figure 5). CGR changes in respond to bio-fertilizer during the growth season were the same for all treatments, it means that CGR by passing time is increased and after reaching to the maximum amount, it is decreased, so at the end of the growth season because of yellow leaves and their leaves' fall, this CGR become negative. Clarke and Simpson [16] stated that the maximum CGR amount, 71 days after planting is due to new photosynthesis organs, and bio-fertilizer have main role in photosynthesis organs' durability. Many researches have demonstrated that these bio-fertilizers alone couldn't provide all of the needed nitrogen for plant, and the positive effects of bio-fertilizers is that they provide other substances by increasing the solubility of elements such as nitrogen and phosphorous and increasing assimilation in each area unit. Which it is as a result plant growth promoting hormones production. In other word, plant growth promoting hormones especially auxin by provoking root-generator system, increase assimilation in each area unit and by using enough chemical fertilizers, increase their effects, so CGR has increased as well. During the growth season, CGR because of reduced leaf area index and yellow leaves was decreased [17]. Nanda et al., [18] indicated that maize inoculation with bio-fertilizers such as *Azotobacter* and *Azospirillum*, caused a significant increase in plant growth rate. They said that improvement in nutritious materials assimilation rate is the main reason for increasing the plant growth rate. Results of the current researches have shown that using bio-fertilizers, improver's physical structure in soil and increases mineral materials and nitrogen for symbiosis plant [1].

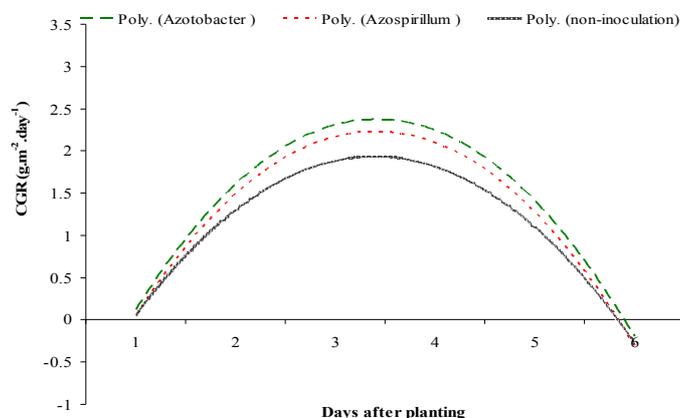


Figure 5: Changes of trend in CGR in non application of nitrogen fertilizer in bio-fertilizer

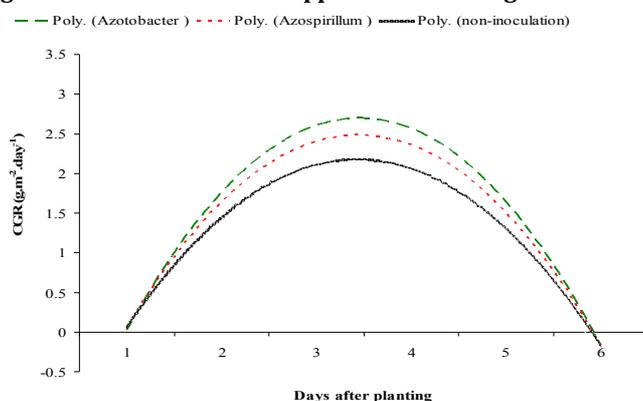


Figure 6: Changes of trend in CGR in 25% of nitrogen fertilizer in bio-fertilizer

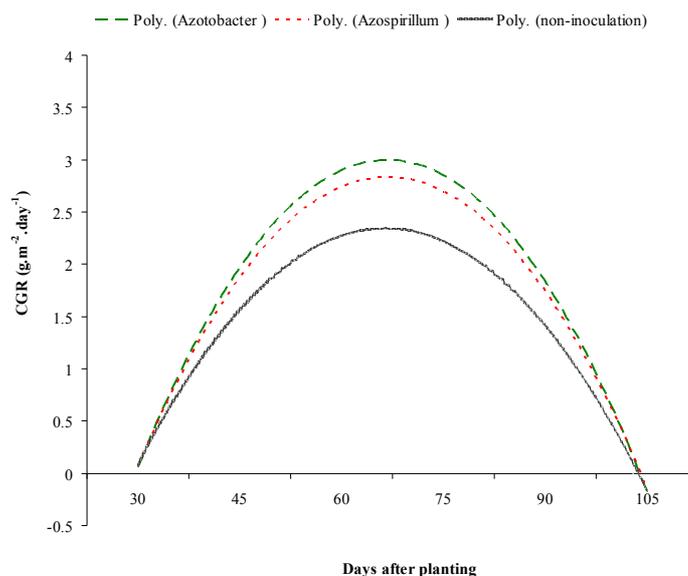


Figure 7: Changes of trend in CGR in 50% of nitrogen fertilizer in bio-fertilizer

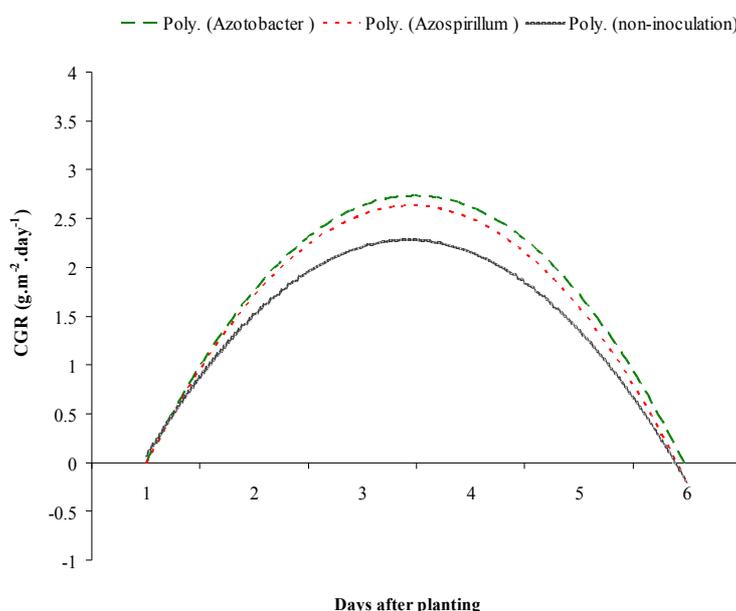


Figure 8: Changes of trend in CGR in 100% of nitrogen fertilizer in bio-fertilizer

Net Assimilation Rate (NAR):

Results showed that changes in net photosynthesis and assimilation with using different amounts of nitrogen fertilizer were decreased too, also, when plants become older, net photosynthesis and assimilation in all levels of nitrogen fertilizers and bio-fertilizers were decreased. Apparently, net photosynthesis and assimilation are influenced by many factors and their measurements are very difficult and not simply distinguishable. However, findings of researchers about assimilation and net photosynthesis are different from each other. For instance Willams et al., [19] concluded that in fixed nitrogen level by increasing density and leaf area index, net photosynthesis and assimilation were decreased. Figures 9, 10, 11 and 12, show changes of NAR in four different levels of nitrogen fertilizer. At the beginning of the growth season treatment with 50% of nitrogen fertilizer in inculcation with *Azospirillum* has the highest NAR about 46.2 g.m⁻².day⁻¹ in each day and treatment with non using nitrogen fertilizer and non inoculation has the lowest NAR about 20.98 g.m⁻².day⁻¹in each day. But at the end of the growth period, treatment with 100 % of nitrogen fertilizer in *Azotobacter* has superiority on other treatments. In treatments with *Azotobacter* and *Azospirillum*, During the growth season, NAR was higher than check treatment and in the end of the growth season, NAR in check treatment has decreased with sever gradient, and this matter is related to earlier leaves' fall in this treatment as compared with other treatments. However, in treatment with *Azotobacter* and *Azospirillum*, with more photosynthesis

during the growth season, because of more green leaves, dry matter accumulation is high. NAR is a good criterion for telling plant's growth properties, in other hand, because leaves or the main photosynthesis organs, so considering the plant growth base on leaf area is desirable. According to the leaf area index, crop growth to LAI, CGR and NAR. Importance in increasing yield of maize, it could be concluded that by using high levels of nitrogen fertilizer (100 %, base on soil analysis is about 350kg.ha⁻¹) and bio-fertilizers, because of plant growth promoting and resending the materials from older leaves to younger leaves, some physiological indices such as LAI, CGR, NAR show their effectiveness by increasing grain yield.

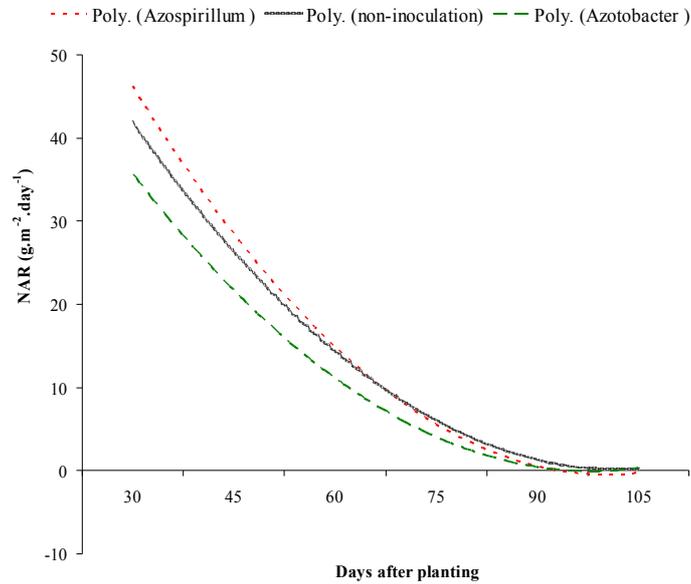


Figure 9: Changes of trend in NAR in non application of nitrogen fertilizer in bio-fertilizer

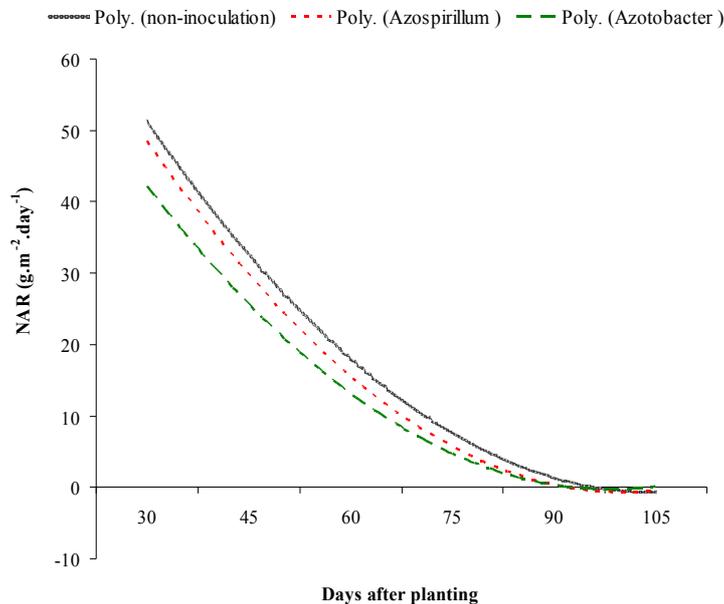


Figure 10: Changes of trend in NAR in 25% of nitrogen fertilizer in bio-fertilizer

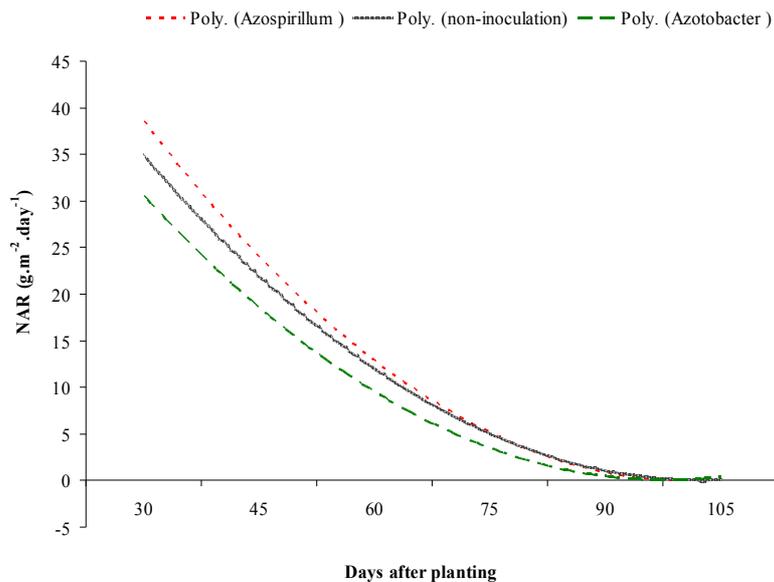


Figure 11: Changes of trend in NAR in 50% of nitrogen fertilizer in bio-fertilizer

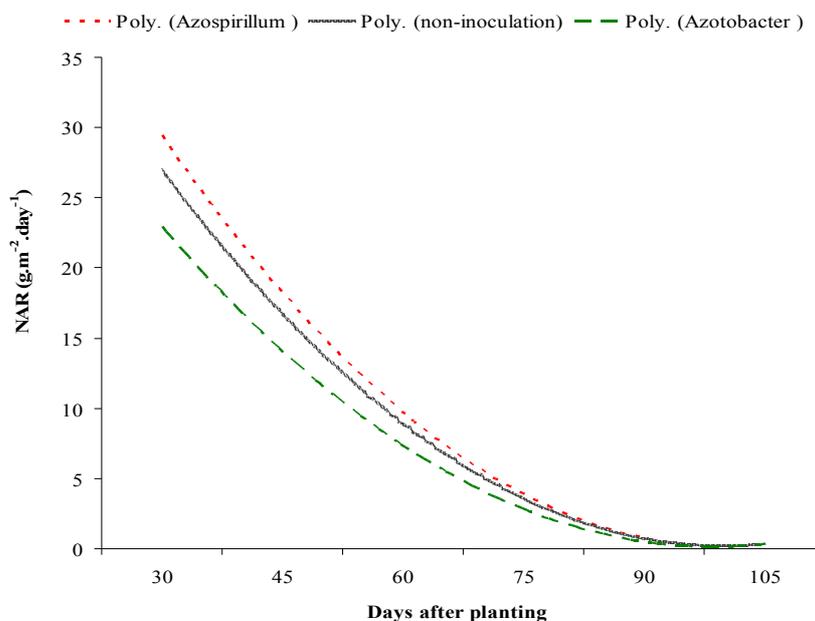


Figure 12: Changes of trend in NAR in 100% of nitrogen fertilizer in bio-fertilizer

CONCLUSION

Therefore, change in growth physiological indices is very important for maize yield. So, by using a proper nutrition planning and providing optimum conditions for plant in order to use environmental capabilities such as sunlight effectively before the generational growth, we can reach the higher grain and quality.

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