Journal of Crop Nutrition Science
ISSN: 2423-7353 (Print) 2538-2470 (Online)
Vol. 5, No. 4, 2019
http://JCNS.iauahvaz.ac.ir
OPEN ACCESS

Assess Effect of Biological Phosphorous Fertilizers and Micro Elements (Boron and Manganese) on Alfalfa Growth Curve Indices

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RESEARCH ARTICLE

ARTICLE INFO.
Received Date: *28 Sep. 2019*
Received in revised form: *29 Oct. 2019*
Accepted Date: *30 Nov. 2019*
Available online: *30 Dec. 2019*

To Cite This Article: Maryam Khirkhah, Hamid Madani, Ghorban Normohammadi, Mani Mojadam. Assess Effect of Biological Phosphorous Fertilizers and Micro Elements (Boron and Manganese) on Alfalfa Growth Curve Indices. ***J. Crop. Nutr. Sci.,*** 5(4): 12-22, 2019.

ABSTRACT

BACKGROUND: Fertilizer management is one of the most important factors in successful cultivation of crops affecting yield quality and quantity. Growth analysis is still the most simple and precise method to evaluate the contribution of different physiological processes in plant development.

OBJECTIVES: Current research was conducted to evaluate effect of different level of Phosphorous biofertilizer and micro elements (Boron and Manganese) on physiological parameters of Alfalfa.

METHODS: This research was carried out in three farm (One-year, three-year and five-year hay fields) according combined analysis factorial split plot experiment based on randomized complete blocks design during two year (2012-2013) with three replications. This experiment was performed on alfalfa planted at different years (one year, three years and five years). The main factor included phosphorous biofertilizers (P_0: control, P_1: Adding the bacteria at the rate of 100 g per 400 liters of water and the sub factor consisted Manganese fertilizer (Mn_0: control, Mn_1: Foliar application of chelate at the rate of 1 $L.ha^{-1}$, Mn_2: soil application in source of manganese sulfate at the rate of 10 $kg.ha^{-1}$) and Boron fertilizer (B_0: control, B_1: Foliar application at the rate of 1 $L.ha^{-1}$, B_2: soil application at the rate of 10 $kg.ha^{-1}$).

RESULT: Compare different level of treatments revealed the highest amount of physiological parameters belonged to TR_{14}: $P_1B_1Mn_1$ and TR_{15}: $P_1B_1Mn_2$ and the lowest one were for TR1: $P_0B_0Mn_0$ treatment in three farms (One-year, three-year and five-year hay fields).

CONCLUSION: Finally according result of current research revealed applying studied elements significantly increases crop production and can be suggested as the best nutritional recommendation for alfalfa so consume phosphorus biofertilizer, Foliar application of boron with soil application and foliar application of manganese had highest amount of physiological parameters such as LAI, CGR, NAR and RGR.

KEYWORDS: *Biofertilizer, Forage, Leaf area, Nutrition, Physiological parameters.*

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1. BACKGROUND

Alfalfa is a flowering plant in the pea family, cultivated widely throughout the world as forage for cattle, and is most often harvested as hay, but can also be made into silage, grazed, or fed as green chop. It is the most productive legume, with potential yields exceeding twelve tons of hay per ha/yr or more (Ghasemi Mobtaker, 2012). Overuse of different chemical fertilizers is one of the causes for the degradation of environment and soil. Bio fertilizers are the newest and most technically advanced way of supplying mineral nutrients to crops. Compared to chemical fertilizers, their supply nutrient for plant needs, minimizes leaching, and therefore improves fertilizer use efficiency (Subbarao *et al.,* 2013). Fertilizer management is one of the most important factors in successful cultivation of crops affecting yield quality and quantity (Tahmasbi *et al.,* 2011). Chemical fertilizers are significant to succor nutrients in soil. Heavy doses of chemical fertilizers and pesticides are commonly used in order to enhance corn yields. Excessive nitrogen content in soil causes an inappropriate high uptake of this macronutrient by plants, which may result in inadequate growth and development due to the accumulation of nitrogen compounds in plant tissue (Szulc, 2013). Organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food and long term sustainability and concerns on environmental pollution associated with indiscriminate use of agrochemicals. Though the use of chemical inputs in agriculture is inevitable to meet growing demand for food in world, there are opportunities in selected crops and niche areas where organic production can be encouraged to tape the domestic export market (Venkatash-Warlu, 2008). The growth and yield of a crop can be adversely affected by deficient or excessive supply of any one of the essential nutrients. However, in intensive agriculture nitrogen is the major nutrient which determining crop yield. Plant growth is affected more due to deficiency of nitrogen than that of any other nutrient. Nitrogen fertilization influences dry matter yield by influencing leaf area index, leaf area duration and photosynthetic efficiency (Mohan *et al*., 2015). Yield is a complex trait resulting from interaction of morphological, physiological and environmental parameters on the growth of plants. Identification of the variations of morphological and physiological traits influencing the yield of a plant in a certain environment is an essential tool for selecting and breeding of yield (Azarpour *et al*., 2014). Growth analysis is a way to assess what events occurs during plant growth. Total dry matter trend (TDM), Crop growth rate (CGR) and relative growth rate are, the most important traits in plant growth analysis. Growth analysis is a way to assess what events occurs during plant growth. Total dry matter trend (TDM), Crop growth rate (CGR) and relative growth rate are, the most important traits in plant growth analysis. Growth analysis is a suitable method for plant response to different environmental conditions during plant life (Hokmalipour and Hamele Darbandi, 2011a).

Growth analysis is still the most simple and precise method to evaluate the contribution of different physiological processes in plant development. It provides a considerable insight into the functioning of a plant as depends on genotype or environment. The purpose of growth analysis is the determination of the increase in dry matter referred to a suitable basis for photosynthetically active tissue, leaf area and amount of leaf protein (Alam and Haider, 2006). Identification of growth physiological indices in analysis of factors affecting yield and its components has a great importance and its stability determines the dry matter production which is a criterion of yield components and in this regard leaf area index (LAI), total dry weight (TDW) and leaf dry weight (LDW) should be measured in periodic intervals during the growing season (Gardner *et al*., 1985). The above indices plus crop growth rate (CGR), relative growth rate (RGR), net assimilation rate (NAR), leaf area duration (LAD), leaf area rate (LAR), leaf weight rate (LWR) and specific leaf area (SLA) are indices which often use for evaluation of plant productivity capability and environmental efficiency (Anzoua *et al*., 2010). Leaf area index (LAI) and dry matter production is the main growth factor which may directly reflect to cotton yield. Growth analysis parameters like crop growth rate (CGR) are product of LAI. Relative growth rate (RGR) measures the increase in dry matter with a given amount of assimilatory material at a given point of time (Rajput *et al*., 2017). The amount of growth and photosynthetic translocation is related to nutrients availability (Munir *et al*., 2012). Dwyer and Tewart (1986) reported that leaf area index is major factor determining photosynthesis and dry matter accumulation. CGR is related to LAI, for this reason that crop growth rate changes is depended to two parameters: namely leaf area index and net assimilation rate. LAI is the component of crop growth analysis that accounts for the ability of the crop to capture light energy and is critical to understanding the function of many crop management practices. Leaf area index can have importance in many areas of agronomy and crop production through its influence on: light interception, crop growth weed control, crop-weed competition, crop water use, and soil erosion. To measure LAI, scientists have cut a number of plants at the soil surface, separated leaves from other plant parts, and measured the area of individual leaves to obtain average leaf area per plant. The product of leaf area per plant and the plant population gives the LAI. Alternatively, LAI could be measured none destructively with this procedure if area of individual leaves was determined by some combination of leaf length and width measurements (Shirkhani and Nasrolahzadeh, 2016). Growth analysis is verify crops ecological adaptation to new environments, competition between species, crops management effects and identification of productive capacity of different genotypes. Dynamics of dry matter distribution to various plant organs, their yielding and productivity may be characterized by using growth indices (Zajac *et al*., 2005).

2. OBJECTIVES

Current research was conducted to evaluate effect of different level of Phosphorous biofertilizer and micro elements (Boron and Manganese) on physiological parameters of Alfalfa.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research was carried out in three farm (One-year, three-year and five-year hay fields) according combined analysis factorial split plot experiment based on randomized complete blocks design during two year (2012-2013) with three replications. This experiment was performed on alfalfa planted at different years (one year, three years and five years). The main factor included biological phosphorous fertilizers (P_0: control or non use of phosphorous biologic fertilizer, P_1: Adding the suspension containing bacteria in the soil at the rate of 100 g per 400 liters of water in source of fertile fertilizer 2 in the alfalfa field made of Green Biotechnology Company) and the sub factor consisted Manganese fertilizer (Mn_0: control or non use of Manganese fertilizer, Mn_1: Foliar application of manganese chelate source at the rate of 1 $L.ha^{-1}$ from Sepehr Parmis Company, Mn_2: consumption as soil application in source of manganese sulfate at the rate of 10 $kg.ha^{-1}$ made of Kimia Kood Company) and Boron fertilizer (B_0: control or non use of Boron fertilizer, B_1: Foliar application of boric acid source at the rate of 1 $L.ha^{-1}$ made of Gorgan Golsam Company, B_2: Consumption of boron in the form of soil application from borax source at the rate of 10 $kg.ha^{-1}$ made of Kimia Kood Company). Place of study was located in Marvdasht city at longitude 52°48'N and latitude 29°52'S in the Pars province (South of Iran). The soil properties were mentioned in table 1 and 2.

Table 1. Physical and chemical properties of studied field of first year

Year	Depth (cm)	Soil texture	K (ppm)	P (ppm)	N (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)
5	30-60	Silty clay loam	308.5	17.01	0.2	15.4	1.36	9
3	0-30	clay	295.2	18	0.18	11.6	1.3	7.9
1	0-30	clay loam	257.9	12.83	0.1	13.4	1.14	5.4

Continue table 1.

Year	Depth (cm)	Cu (ppm)	B (PPM)	OC (%)	TNV (%)	SP (%)	EC ($ds.m^{-1}$)	pH
5	30-60	3.78	0.97	1.95	38.5	61.5	1.18	7.85
3	0-30	1.8	0.75	1.82	39.3	60	0.082	8.01
1	0-30	2.3	0.42	1.01	40.7	56	0.96	7.9

Table 2. Physical and chemical properties of studied field second year

Year	Depth	Soil texture	K (ppm)	P (ppm)	N (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)
5	30-60	clay loam	297.2	16.05	0.17	16.7	1.08	8.4
3	0-30	Silty clay loam	291.2	17.5	0.19	15.9	1.42	9.3
1	0-30	clay loam	288.1	15	0.19	15.1	1.36	6.1

Continue table 2.

Year	Depth	Cu (ppm)	B (ppm)	OC (%)	TNV (%)	SP (%)	EC ($ds.m^{-1}$)	pH
5	30-60	2.95	0.67	1.49	40.3	62.3	0.98	7.67
3	0-30	3.78	0.93	1.9	42.1	60.8	1.11	7.88
1	0-30	2.87	0.67	1.65	40	61.2	0.93	8.03

3.2. Farm Management

Experimental treatments were applied at the beginning of the growth of each plot and then irrigated by sprinkler method. In all three farms, the size of the plots is 5×6m. According to the treatments, the number of one-plot plots in all farms is 18, of which there were 54 plots in each farm, in total there were 162 experimental plots in three farms. No serious incidence of insect or disease was observed, so no pesticide or fungicide was applied.

3.3. Measured Traits

The leaf area was determined by leaf area meter. The leaf area index was calculated from leaf area ratio to ground level. Crop growth rate, net assimilation rate and relative growth rate were measured according fallowing formula (Buttery, 1970; Enyi, 1962):

Equ.1. CGR ($g.m^{-2}.day^{-1}$) =
TDM_2- TDM_1/T_2-T_1

TDM_1= Primary dry weight (g), TDM_2= Secondary dry weight (g)

T_1= initial sampling time,

T_2= Secondary sampling time

Equ.2. NAR ($g.m^{-2}.day^{-1}$) =
CGR× $LnLA_2$-$LnLA_1/LA_2$-LA_1

CGR = Growth rate in grams per day per square meter

LA_1 = Initial leaf area,

LA_2 = Secondary leaf area

Equ.3. RGR ($g.g^{-1}.day^{-1}$) =
[Ln (TDM_2) – Ln (TDM_1)]/T_2-T_1

RGR= relative growth rate in gram per gram per day

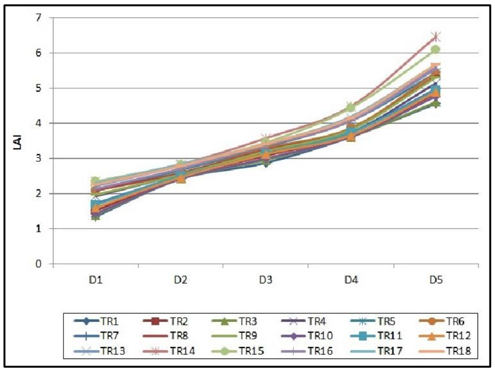
3.4. Statistical Analysis

Analysis of variance and mean comparisons were done via SAS software (Ver.8) and Duncan multiple range test at 5% probability level.

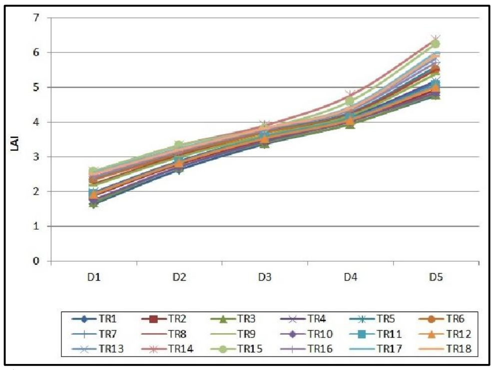
4. RESULT AND DISCUSSION

4.1. Leaf area index (*LAI*)

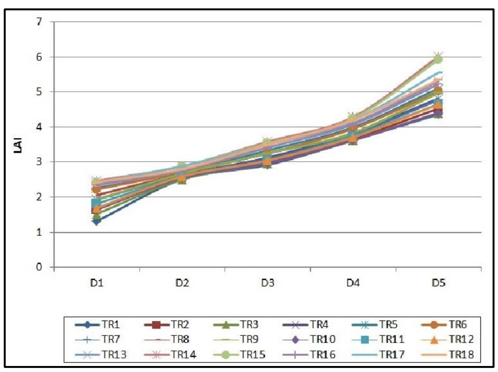
At the beginning of growth, the leaf area of the plant is made up by the young leaves with high photosynthetic capacity, i.e., high efficiency of fixation of atmospheric CO_2. As the plant develops, leaf senescence enhances, reducing the photosynthetic efficiency of the leaves besides increasing respiratory losses, compromising the NAR and LAR, and consequently the RGR (Wilson, 1981). Leaf area index (LAI) is the main physiological determinant of crop yield. It describes the surface growth and light use during the crop period (Ullah *et al.*, 2013). Compare different level of treatments revealed the highest amount of Leaf area index (LAI) belonged to the TR_{14}: $P_1B_1Mn_1$ and TR_{15}: $P_1B_1Mn_2$ and the lowest one were for TR1: $P_0B_0Mn_0$ treatment in the three planted farms (One-year Fig.1a, three-year Fig.1b and five-year Fig.1c hay fields).



1a



1b

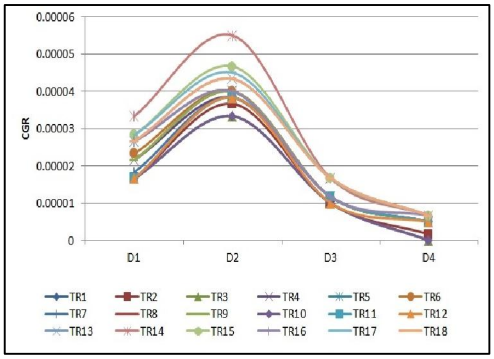


1c

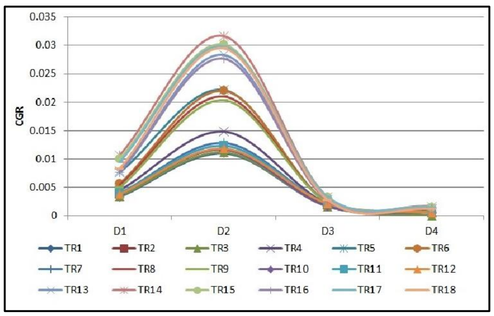
Fig 1a, 1b, 1c. Compare LAI affected interaction effect of treatments.
D_1: 21 June, **D_2**: 21 July, **D_3**: 22 August, **D_4**: 22 September, **D_5**: 21 October.
TR1: $P_0B_0Mn_0$, **TR2**: $P_0B_0Mn_1$, **TR3**: $P_0B_0Mn_2$, **TR4**: $P_0B_1Mn_0$, **TR5**: $P_0B_1Mn_1$, **TR6**: $P_0B_1Mn_2$, **TR7**: $P_0B_2Mn_0$, **TR8**: $P_0B_2Mn_1$, **TR9**: $P_0B_2Mn_2$, **TR10**: $P_1B_0Mn_0$, **TR11**: $P_1B_0Mn_1$, **TR12**: $P_1B_0Mn_2$, **TR13**: $P_1B_1Mn_0$, **TR14**: $P_1B_1Mn_1$, **TR15**: $P_1B_1Mn_2$, **TR16**: $P_1B_2Mn_0$, **TR17**: $P_1B_2Mn_1$, **TR18**: $P_1B_2Mn_2$.

4.2. Crop growth rate (*CGR*)

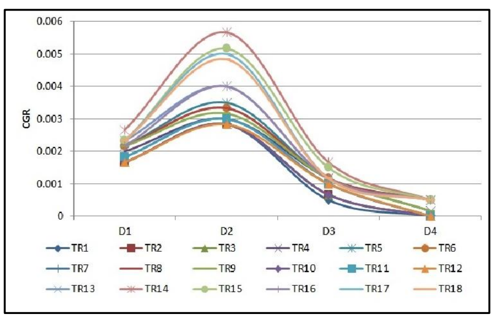
Compare the different level of treatments revealed the highest amount of crop growth rate belonged to TR_{14}: $P_1B_1Mn_1$ and TR_{15}: $P_1B_1Mn_2$ and the lowest one were for TR1: $P_0B_0Mn_0$ treatment in the three farms (One-year Fig.2a, three-year Fig.2b and five-year Fig.2c hay fields). Wu *et al*. (2005) also reported that the inoculation of the corn grains with the biological fertilizers increased the growth rate of crops. The researchers reasoned this by increasing the availability of the nutrients and improving the absorption of nutrients by the plant. Some researchers such as Hokm Alipour and Hamele Darbandi (2011b) reported negative values of crop growth rate and relative growth rate are due to loss of leaves at the end of the growing season. So with increasing nitrogen levels at all of the corn cultivars plant height was significantly increased. Also Clarke and Simpson (1978) stated that the simultaneously the maximum growth rate of the product was due to the increase in durability of photosynthetic organs, which increased in the presence of the biological fertilizers. Many researchers have stated that the biological fertilizers alone cannot provide the total nitrogen element needed by the plant, and the positive effects of biological fertilizers on the availability of the other nutrient elements such as phosphorus through increased solubility and absorption and the production of the various growth promoting hormones (Vessy, 2003).



2a



2b



2c

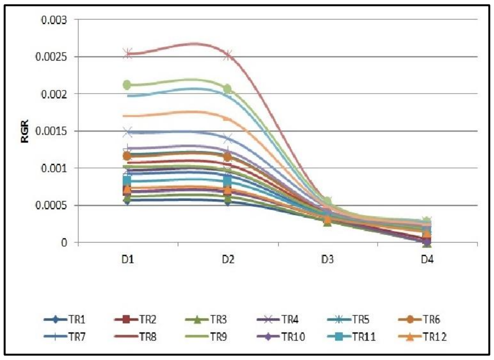
Fig 2a, 2b, 2c. Compare CGR affected interaction effect of treatments.
D_1: 21 June, **D_2**: 21 July, **D_3**: 22 August, **D_4**: 22 September. **TR1**: $P_0B_0Mn_0$, **TR2**: $P_0B_0Mn_1$, **TR3**: $P_0B_0Mn_2$, **TR4**: $P_0B_1Mn_0$, **TR5**: $P_0B_1Mn_1$, **TR6**: $P_0B_1Mn_2$, **TR7**: $P_0B_2Mn_0$, **TR8**: $P_0B_2Mn_1$, **TR9**: $P_0B_2Mn_2$, **TR10**: $P_1B_0Mn_0$, **TR11**: $P_1B_0Mn_1$, **TR12**: $P_1B_0Mn_2$, **TR13**: $P_1B_1Mn_0$, **TR14**: $P_1B_1Mn_1$, **TR15**: $P_1B_1Mn_2$, **TR16**: $P_1B_2Mn_0$, **TR17**: $P_1B_2Mn_1$, **TR18**: $P_1B_2Mn_2$.

4.3. Relative growth rate (*RGR*)

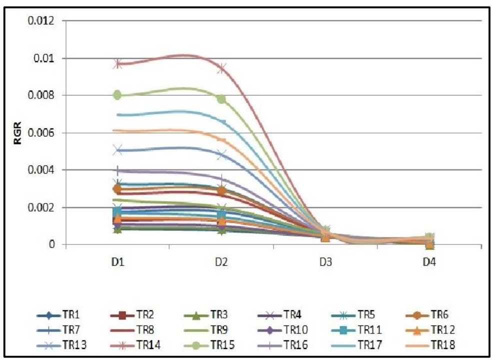
Compare the different level of treatments revealed the highest amount of relative growth rate belonged to TR_{14}: $P_1B_1Mn_1$ and TR_{15}: $P_1B_1Mn_2$ and the lowest one were for TR1: $P_0B_0Mn_0$ treatment in three farms (One-year Fig.3a, three-year Fig.3b and five-year Fig.3c hay fields). Relative growth rate due to change in photosynthesis and respiration crop situation. As a result, by increase age of lower, stay leaves in shade and fall, dry matter accumulation, increase in respiration and reduce CGR led to become negative RGR at the end of the growing season (Tadayon and Emam, 2007).

4.4. Net assimilation rate (*NAR*)

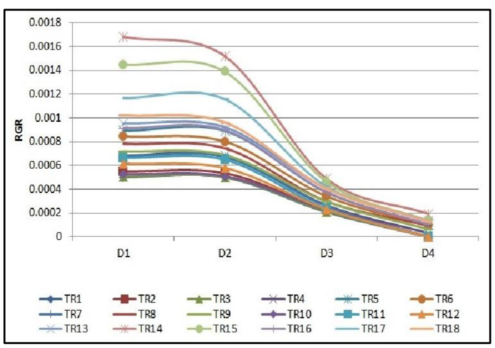
Compare the different level of treatments revealed the highest amount of net assimilation rate belonged to TR_{14}: $P_1B_1Mn_1$ and TR_{15}: $P_1B_1Mn_2$ and the lowest one were for TR1: $P_0B_0Mn_0$ treatment in three farms (One-year Fig.4a, three-year Fig.4b and five-year Fig.4c hay fields). Increase in net assimilation rate (NAR) is attributed to increased photosynthetic capacity of the leaves with improved nutrition of the plants (Ahmad *et al*., 1990). The NAR and relative growth rate (RGR) improve with the increasing levels of N (Warraich *et al*., 2002). Watson *et al*. (1966) reported NAR reduction is mainly due to the drop in the photosynthetic rate that occurs with increased respiratory losses of the plant.



3a

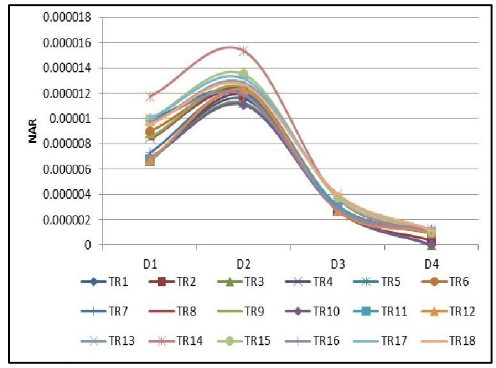


3b

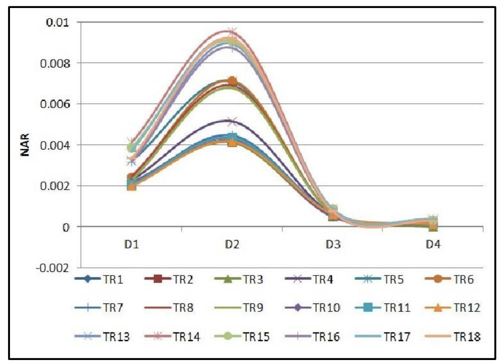


3c

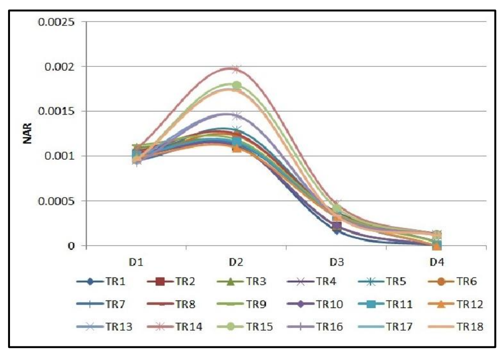
Fig 3a, 3b, 3c. Compare RGR affected interaction effect of treatments.
D_1: 21 June, **D_2**: 21 July, **D_3**: 22 August, **D_4**: 22 September. **TR1**: $P_0B_0Mn_0$, **TR2**: $P_0B_0Mn_1$, **TR3**: $P_0B_0Mn_2$, **TR4**: $P_0B_1Mn_0$, **TR5**: $P_0B_1Mn_1$, **TR6**: $P_0B_1Mn_2$, **TR7**: $P_0B_2Mn_0$, **TR8**: $P_0B_2Mn_1$, **TR9**: $P_0B_2Mn_2$, **TR10**: $P_1B_0Mn_0$, **TR11**: $P_1B_0Mn_1$, **TR12**: $P_1B_0Mn_2$, **TR13**: $P_1B_1Mn_0$, **TR14**: $P_1B_1Mn_1$, **TR15**: $P_1B_1Mn_2$, **TR16**: $P_1B_2Mn_0$, **TR17**: $P_1B_2Mn_1$, **TR18**: $P_1B_2Mn_2$.



4a



4b



4c

Fig 4a, 4b, 4c. Compare NAR affected interaction effect of treatments.
D_1: 21 June, **D_2**: 21 July, **D_3**: 22 August, **D_4**: 22 September. **TR1**: $P_0B_0Mn_0$, **TR2**: $P_0B_0Mn_1$, **TR3**: $P_0B_0Mn_2$, **TR4**: $P_0B_1Mn_0$, **TR5**: $P_0B_1Mn_1$, **TR6**: $P_0B_1Mn_2$, **TR7**: $P_0B_2Mn_0$, **TR8**: $P_0B_2Mn_1$, **TR9**: $P_0B_2Mn_2$, **TR10**: $P_1B_0Mn_0$, **TR11**: $P_1B_0Mn_1$, **TR12**: $P_1B_0Mn_2$, **TR13**: $P_1B_1Mn_0$, **TR14**: $P_1B_1Mn_1$, **TR15**: $P_1B_1Mn_2$, **TR16**: $P_1B_2Mn_0$, **TR17**: $P_1B_2Mn_1$, **TR18**: $P_1B_2Mn_2$.

Importantly, NAR is not only determined by the photosynthetic rate, but also by the size of leaf area, in addition to the duration of vegetative period, architecture of upper part, translocation, and assimilate partitioning.

5. CONCLUSION

Finally according result of current study revealed consume phosphorus biofertilizer, Foliar application of boron with soil application and foliar application of manganese had the highest amount of physiological parameters such as LAI, CGR, NAR and RGR.

ACKNOWLEDGMENT

The authors thank all colleagues who took part in the study.

FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

FUNDING/SUPPORT: This study was done by the scientific support of Department of Agronomy, Islamic Azad University, Ahvaz Branch.

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