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Evaluation Effect of Growth-stimulating Bacteria and Salicylic Acid on Growth Physiological Indices and Grain Yield of Corn (*Zea mays* L.) under Non-irrigation Conditions at Different Growth Stages

Gholam Esmaeilvandi[1], Kamran Mohsenifar[2]*

1. *MSc. Graduated, Department of Agronomy, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.*
2- *Department of Soil Science, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.*

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ABSTRACT

BACKGROUND: Under water deficit conditions, use of growth-promoting bacteria and salicylic acid as a plant growth stimulant is very useful.

OBJECTIVES: This research was conducted to assess effects of biological fertilizers and salicylic acid under non-irrigation conditions at different growth stages on growth indices and crop production of corn during 2016 cropping season.

METHODS: The study was done via split-plot experiment, based on Randomized Complete Block Design with three replications. The treatments included full irrigation (control), non-irrigation from flowering stage to the end of growth period, non-irrigation from the beginning of the grain filling stage to the end of the growth period, belonged to main plots and biological fertilizers and salicylic acid treatment were placed in sub-plots [control treatment, 1 mM salicylic acid, nitroxin + biophosphorus bacteria grain rub, nitroxin + biophosphorus bacteria grain rub along with salicylic acid spraying].

RESULT: The results showed that the full irrigation treatment significantly improved the growth physiological indices such as the leaf area index. The combination of nitroxin, biophosphorus and salicylic acid had a positive and significant effect on the examined indicators. The effects of non-irrigation treatments, biological fertilizers, salicylic acid and their interaction effect on grain yield were significant. Non-irrigation from flowering stage reduced the grain yield by 58.3% compared to the control. The combined application of biological fertilizers + salicylic acid in treatments of non-irrigation and full irrigation significantly increased the grain yield. In the treatment of non-irrigation from flowering and early grain filling, the nitroxin + biophosphorus + salicylic acid bacteria treatment increased the grain yield by 22.7% and 19.3%, respectively, compared to the control. The maximum grain yield (6552 $kg.ha^{-1}$) belonged to the full irrigation treatment and nitroxin + biophosphorus + salicylic acid foliar application.

CONCLUSION: In case of non-irrigation during grain filling stage, by use Nitroxin + biophosphorus bacteria grain rub treatment along with salicylic acid foliar application, also to provide suitable conditions for corn growth, a significant amount of drought stress effect can be reduced and obtained yield under normal conditions.

KEYWORDS: *Biofertilizer, Growth curve, Maize, Nitroxin, Seed yield.*

***Corresponding Author:** Mohsenifar ✉ Mohsenifar@live.com

1. BACKGROUND

Plant growth is a set of biochemical and physiological processes that have mutual effects on each other and are affected by all environmental factors, especially air temperature and humidity. Knowing and investigating the physiological indices of growth is very important in the analysis of factors affecting yield and its components. The purpose of studying these indices is to explain how the plant reacts to the environmental conditions. One of the reasons for researchers' special attention to physiological indices is that the analysis of growth indices only requires the measurement of leaf area and plant dry weight. In corn, as a fast-growing plant, in a relatively short period of growth, the effect of different treatments on growth indices can be well studied. One of these treatments is the irrigation because the development of the leaf surface and the dry weight of the plant is effectively affected by the said treatment. Due to the special characteristics of this plant, the corn cultivation belt in the world is closely matched with arid and semi-arid regions, due to being C_4 and especially thermophilic characteristics; it is closely compatible with arid and semi-arid regions (Lak *et al.*, 2006). Lack of water as an important factor in most stages of the growth of crops in arid and semi-arid regions by limiting growth makes it difficult to achieve high yield, so that the reduction in growth due to water shortage is far more than other environmental stresses (Liu *et al.*, 2005). The corn plant needs a lot of water during the growth period and does not have much tolerance to water deficit (Jensen *et al.*, 1996). Nesmith and Ritchie (1992) stated that there is a positive correlation between leaf development and water in the plant corn. Stegma and Lemert (1981) in an experiment aimed at investigating the effects of drought on corn, concluded that drought stress reduced the leaf area index, but this stress effect depended on its time and intensity, so that water stress soon while it prevents the development of the leaves and causes the maximum reduction of the leaf area index, water stress after silking did not have a significant effect on the maximum reduction of this index, but it accelerated the aging and yellowing of the leaves. In sustainable agricultural systems, the use of biological fertilizers, especially in soils poor in nutrients, is particularly important in increasing production and maintaining soil quality (Sharma, 2003 and Abraham *et al.*, 2007). According to the reports available in water shortage conditions, the use of biological fertilizers can also be effective in reducing the negative effects of water shortage (Ehteshami *et al.*, 2013). Researchers showed that the inoculation of agricultural plants with growth-promoting bacteria under environmental stress conditions stimulates plant growth by reducing the absorption of toxic ions, increasing the production of auxin hormone and stress-specific proteins (Han and Lee, 2005). One of the most sensitive physiological stages of a plant is cell growth and development. Cell growth can occur only when the turgor pressure exceeds the threshold of the cell wall. Plant hormones increase cell development and the relative speed of plant growth (Mckersie and Leshem, 2004). Salicylic acid leads to the improvement of the leaf surface by preventing the fall of leaves and delaying senescence and increasing the greenness and vigor of the plant (Hopkins and Huner, 2008). Several studies have shown that the effect of Si is negligible in suitable growth conditions, while it is obvious and significant in stress conditions. Plants deprived of Si are usually weaker and more sensitive than other plants (Al-Aghabary *et al*., 2004).

2. OBJECTIVES

This research was conducted with the aim of assess the effect of biological fertilizers on improving the physiological indices of corn growth and yield under non-irrigation conditions, as well as investigating the possibility of increasing drought resistance.

3. MATERIALS AND METHODS

3.1. *Research Farm information*

This research was carried out in the beginning of August of the agricultural year 2016 in Izeh city. Izeh city is located at 31 degrees and 50 minutes north latitude and 49 degrees and 52 minutes east longitude compared to Greenwich and a height of 760 meters above sea level.

3.2. *Weather condition*

Izeh city is located in Khuzestan province and is considered one of the semi-arid regions in terms of climate. The average annual rainfall in this province is very low and at the same time very irregular. In terms of climate, Izeh is the coolest city in Khuzestan province. The temperature of Izeh city in winter sometimes reaches below $0^{o}C$ and its average temperature in summer is $24^{o}C$.

3.3. *Physical and chemical properties of farm soil*

In order to check the physical and chemical characteristics of the field soil, before planting, six parts of the field soil were sampled from a depth of 0-30 cm and after crushing clods, samples were passed through a two mm sieve and finally a composite sample was obtained. The samples were evaluated in the soil science laboratory in terms of some physical and chemical traits, the results are presented in table 1.

Table 1. Physiochemical characteristics of field soil

Soil depth (cm)	**Soil texture**	**Clay (%)**	**Silt (%)**	**Sand (%)**	**EC ($dS.m^{-1}$)**
0-30	Clay loam	44	38	18	1.1
pH	**OC (%)**	**N (%)**	**K (ppm)**	**P (ppm)**	**SP (%)**
6.7	0.4	0.09	233	12.2	47

3.4. *Statistical model of research*

The study was consisted of a split-plot experiment, using Randomized Complete Block Design with three replications. The investigated treatments include irrigation conditions at different growth stages in the main plots [Including full irrigation (I_1, control), non-irrigation from the flowering stage to the end of the growth period (I_2), non-irrigation from the beginning of the grain filling stage to the end of the growth period (I_3)] and biological fertilizers and salicylic acid treatment were placed in sub-plots [Including control treatment (F_1), 1 mM salicylic acid, nitroxin (F_2) + biophosphorus bacteria grain rub (F_3), nitroxin + biophosphorus bacteria grain rub along with salicylic acid spraying (F_4)].

3.5. *Research implementation steps*

3.5.1. *Land preparation operations*

Before the planting process, the land was initially irrigated, and in August, fertilization was carried out. The land preparation operations involved plowing the soil to a depth of 30 cm, followed by the use of two perpendicular disks. Basic fertilizers were applied to the soil before the second disk passed and they were thoroughly mixed with the soil by the action of the disk. Finally, the field was leveled using a trowel.

3.6. *Required fertilizer consumption*

The required amount of fertilizer was determined based on the soil test. All the phosphorus, potassium, and half of the nitrogen fertilizer were added to the field. The used fertilizer included 150 $kg.ha^{-1}$ of pure phosphorus from the triple superphosphate source, 120 $kg.ha^{-1}$ of pure potassium from the potassium sulfate source, and 250 $kg.ha^{-1}$ of nitrogen from the urea source. To minimize leaching and wastage, the nitrogen fertilizer was applied in two stages. Fifty percent of the nitrogen was applied at the time of planting, and the remaining fifty percent was applied at the 6-leaf stage. This approach was adopted to ensure the efficient utilization of the nitrogen fertilizer and to reduce the risk of nutrient loss from the soil.

3.7. *Planting operations and applying treatments*

In the treatments involving biological fertilizer, the grains were inoculated with the specified biological compounds before planting, and the grain planting process was carried out manually. Before planting, the selected grains were soaked in 1 liter per hectare of nitroxin biological fertilizer, which contained Azotobacter and Azospirillum for 20 minutes. Subsequently, the grains were spread on a flat surface and left to dry for ten minutes before planting. After the soaking process, the grains were immediately inoculated with 1 liter per hectare of biophosphorus biological fertilizer (Pseudomonas) (all these steps were conducted in the shade). Subsequently, the treated grains were planted. Each experimental plot consisted of six cultivation rows, each 5m in length. The distance between the rows was 75 cm, and the spacing between grains within the same row was 20 cm. The planting depth for the grains was set at 5 cm. A distance of 2m was maintained between each experimental block (repetition). Moreover, the distance between the main plots and subplots was set to be four no-till lines apart. The first irrigation was performed after grain planting and continued until the emergence of male inflorescences in all treatments. Additionally, foliar application of salicylic acid (1 mM) was carried out at the 8-10 leaf stage. Subsequently, from the stage of the appearance of male inflorescences and the beginning of the grain filling stage until the stage of full ripening, the specific irrigation treatment was implemented. However, in the control treatment, irrigation continued as usual until the ripening stage. Irrigation intervals for the different treatments were determined based on the customary practices of the region and in accordance with the control treatment. Manual weeding was diligently performed to control weed growth in all plots. Throughout the entire growth period, necessary precautions were taken to prevent and manage pests and diseases effectively. Fortunately, no specific pests or diseases were observed, indicating that the plants remained relatively free from such issues during the course of the study.

3.8. *Studied traits*

3.8.1 *Leaf Area Index* (*LAI*)

Leaf area index was measured in three stages (tasseling, beginning of flowering and grain filling). At each stage, the surface of each leaf was calculated using the following equation (Sobhani, 2000):

Equ. 1. $S = 0.46 (L\times W) + 0.00046 (L\times W)2\ R2 \geq 0.98^{**}$

Where S is the surface of the leaf, L and W are the maximum length and width of the green corn leaf, respectively. After measuring the surface of all the leaves, the leaf area index was calculated at different experimental treatments.

3.8.2. *Total Dry Matter* (*TDM*)

To measure total dry matter accumulation, sampling was conducted in three stages. In each stage, 5 plants were selected from each plot and then transferred to the laboratory. The plants were subsequently divided into four parts: stem, leaf, cob, and male inflorescence. Each part was dried individually inside an oven for 48 hours at 72^{o}C. The dry weight of each plant part was then measured using a digital scale with an accuracy of 0.01 grams. The total dry weight of the organs was considered as the accumulation of dry matter at each stage.

3.8.3. *Crop Growth Rate* (*CGR*)

The crop growth rate in grams per square meter per day was calculated using the following formula:

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

TDM = dry weight of the whole plant at each stage of sampling (g. m^{-2})

T_2-T_1 = time interval between two samplings (days)

GA = area occupied by plant (square meters)

3.8.4. *Net assimilation rate* (*NAR*)

Net assimilation rate was calculated in grams per square meter of leaf surface per day using following formula:

Equ. 3. $NAR\ (g.\ m^{-2}.d^{-1}) = (Ln\ (LAI_2) - Ln\ (LAI_1)/(LAI_2 - LAI_1) \times CGR_2$

CGR = crop growth rate (g. m^{-2}. d^{-1})

LAI = leaf area index

3.8.5. *Relative water content of cob leaves*

At silking stage, the relative water content of cob leaves was measured to investigate how the plant reacts to water deficit stress. For this purpose, the cob leaves of three plants from the second and sixth rows of each sub-plot were separated before irrigation at 12 noon and transferred to the laboratory. In the laboratory, discs were immediately prepared from these leaves and weighed, and their fresh weight (FW) was recorded. After that, the discs were placed in containers containing distilled water at a temperature of four degrees Celsius for 24 hours until they reached full saturation. At the end of this stage, the surface moisture of the leaf discs was moistened with paper towels and weighed again. The said weight was recorded as the saturated weight (SW). To calculate the dry weight (DW), the samples were placed in an oven at a temperature of 72 degrees Celsius for 48 hours. The relative water content of cob leaves was calculated using the following equation (Alizadeh, 1995):

Equ. 4. The relative water content cob leaves (%) = (FW- DW/SW-DW) × 100

3.8.6. *Grain yield*

The ripeness of the grains was determined by the formation of a black layer at the base of the grains and the final harvest was done manually. In order to calculate the grain yield, after fully ripening and after removing the marginal lines, all the plants were picked from the two middle lines in the center of each plot from an area of 3 square meters and using 10 ears that were randomly separated from each plot.

3.9. *Statistical analysis*

The statistical analysis of the data was done by SAS software (Ver.8) and the mean comparison was also done by LSD test at 5% probability level and the required graphs were drawn by Excel software (Ver.2010).

4. RESULT AND DISCUSSION

4.1. *Leaf Area Index (LAI)*

The leaf area index was significantly affected by the irrigation treatment and the combination of growth-stimulating bacteria and salicylic acid at different growth stages (Table 2). With the non-irrigation from the flowering stage to the end of the growth period, the leaf area index decreased significantly compared to full irrigation. The lowest leaf area index in the two stages of beginning of flowering and grain filling, with an average of 2.7 and 1.4, respectively, was attributed to the treatment of non-irrigation from the flowering stage to the end of the growth period (Table 3). The use of salicylic acid and growth-stimulating bacteria significantly increased the leaf area index. The highest leaf area index at three stages of tasseling, beginning of flowering and grain filling was obtained with an average of 5.7, 4.6 and 3.8 from nitroxin + biophosphorus + salicylic acid treatment, respectively. The lowest leaf area index at stages of tasseling, beginning of flowering and grain filling was assigned to the control treatment with an average of 4.6, 3.5 and 2.7, respectively (Table 3). The leaf area index was significantly affected by the interaction effect of the treatment of non-irrigation and the use of salicylic acid and growth-promoting bacteria (Table 2). The treatment of non-irrigation at different growth stages caused a significant decrease in the leaf area index, on the other hand, the treatment combination of growth-promoting bacteria and salicylic acid improved the leaf area index significantly. In the treatment of non-irrigation from the flowering stage to the end of the growth period (I2), the leaf area index at three stages of tasseling, beginning of flowering and grain filling was 4.6, 2.8, and 1.4, respectively and with nitroxin+ biophosphorus + salicylic acid increased with an average of 5.5, 3.5 and 2.9, respectively. Until the stage of flowering, no significant difference was observed between treatments of complete irrigation and non-irrigation from the grain filling stage in terms of leaf area index. At grain filling stage, the maximum leaf area index (with an average of 3.85) was related to the full irrigation treatment (control) and the application of nitroxin + biophosphorus + salicylic acid (Table 4). The decrease in leaf area index under drought stress was consistent with the results of researchers in the same field (Islam *et al*., 2011; Mao *et al*., 2011; Jalilian and Mohsennia, 2013). Salicylic acid in combination with growth bacteria led to the improvement of the leaf area at different stages of sampling compared to the control by preventing the falling of leaves, delaying senescence, increasing greenness and vigor of the plant. Similarly, the decrease in the leaf area index in the treatment of non-irrigation from the flowering stage to the end of the growth period (I_2) compared to full irrigation (control) (I_1) and non- irrigation from the stage of grain filling to the end of the growth period (I_3) may be due to the reduction cell division, accelerating the aging and shedding of plant leaves and preventing its spread in vegetative growth stages (Cakir, 2004). The reason for the increase in the leaf area index in the F_4 treatment may be due to the increase in the development and expansion of the plant roots, followed by the increase in access to water and nutrients and the increase in the leaf area index. Treatment with salicylic acid and growth-promoting bacteria in normal conditions and in drought stress conditions has increased plant tolerance and the ability to absorb water and elements to its highest level. The researchers also found that the drought stress during the vegetative period led to the shrinking of the leaves and reduced the leaf area index during the ripening period and the amount of light absorption by the plant. During periods of severe stress, the plant's stomata close to prevent excessive water loss through transpiration. So, this closure also hinders entry of essential carbon dioxide (CO_2) required for photosynthesis. Consequently, photosynthesis is hampered, leading to reduce in dry matter production. If stress persists, photosynthesis intensity sharply decreases, further impacting overall growth and development (Gardner *et al*., 1984).

Table 2. Results of analysis of variance of studied traits

S.O.V	df	Total dry weight			Leaf Area Index		
		Tasselling stage	beginning of flowering stage	Grain filling stage	Tassel-ling stage	beginning of flowering stage	Grain filling stage
Replication (R)	2	73.14 ^{ns}	125.64 ^{ns}	112.98 ^{ns}	0.98 ^{ns}	1.43^{ns}	1.26^{ns}
Irrigation (I)	2	1910.23**	2354.13**	7652.36**	19.12**	21.82**	11.35**
E_a	4	218.24	359.06	346.03	4.19	2.37	3.28
Fertilizer (F)	3	1412.15**	2933.25**	6426.52**	14.75**	17.47**	10.81**
I×F	6	1248.12 **	1983.62 **	4571.91 **	11.95 **	13.7 **	5.14 **
E_b	18	143.22	273.89	194.25	2.94	1.66	2.37
CV (%)		10.25	8.57	11.45	11.28	12.02	7.85

^{ns}: Non significant, * and ** indicate significance at the level of 5% and 1%, respectively.

Continue table 2.

S.O.V	df	Crop growth rate		Net assimilation rate	
		Tasseling until beginning of flowering stage	beginning of flowering until grain filling	Tasseling until beginning of flowering stage	beginning of flowering until grain filling
Replication (R)	2	11.54 ^{ns}	10.02 ^{ns}	9.11 ^{ns}	4.17 ^{ns}
Irrigation (I)	2	324.17**	244.32**	67.47**	52.28**
E_a	4	16.54	14.46	10.25	12.47
Fertilizer (F)	3	285.52**	191.09**	52.77 *	44.93**
I×F	6	208.29 **	168.76 **	49.55**	36.28 **
E_b	18	11.74	10.85	7.45	7.34
CV (%)		9.57	8.42	7.61	10.14

^{ns}: Non significant, * and ** indicate significance at the level of 5% and 1%, respectively.

4.2. *Total dry matter* (*TDM*)

The effect of non-irrigation at different growth stages and the combined treatment of growth-stimulating bacteria and salicylic acid significantly affected the accumulation of total dry matter at the 1% of probability level (Table 5). The highest accumulation of dry matter was observed at the grain filling stage, with the most substantial amount recorded in the full irrigation treatment, while the lowest amount was observed in the treatment with non-irrigation from the flowering stage to the end of the growth period (Table 6). The accumulation of total dry matter significantly increased at the stages of tasseling, beginning of flowering and grain filling in the treatment involving growth-stimulating bacteria and salicylic acid. In these stages, the highest accumulation of dry matter (with average values of 837.7, 1377 and 1617 g.m^{-2}, respectively) was observed in the treatment with nitroxin + biophosphorus + salicylic acid.

Table 3. Mean comparison effect of treatments combination on studied traits

Treatment	Total dry weight ($gr.m^{-2}$)			Leaf area index	
	Tasselling stage	beginning of flowering stage	Grain filling stage	beginning of flowering stage	Grain filling stage
Irrigation					
I_1	553.6* a	913.32 a	1355.61 a	3.8 a	2.7 a
I_2	558.5 a	690.92 b	81.55 c	2.7 b	1.4 b
I_3	554.2 a	911.15 a	1070.08 b	3.8 a	2.8 a
Fertilizer					
F_1	577.2 d	946.4 c	1291 c	3.5 b	2.7 c
F_2	654.5 c	1241.6 b	1454 b	3.5 b	3.2 b
F_3	732.1 b	1234 b	1472 b	4.4 a	3.2 b
F_4	837.7 a	1377 a	1617 a	4.6 a	3.8 a

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT). I_1: full irrigation (control), I_2: non-irrigation from the flowering stage to the end of the growth period, I_3: non-irrigation from the grain filling stage to the end of the growth. F_1: control, F_2: 1 mM salicylic acid, F_3: Nitroxin bacteria grain rub + Biosphere, F_4: Nitroxin bacteria grain rub + Biosphere along with salicylic acid foliar application.

Continue table 3.

Treatment	Crop growth rate ($gr.m^{-2}.d^{-1}$)		Net assimilation rate ($gr.m^{-2}.d^{-1}$)	
	Tasseling until beginning of flowering stage	beginning of flowering until grain filling	Tasseling until beginning of flowering stage	beginning of flowering until grain filling
Irrigation				
I_1	32.7a*	25.5 a	8.8 a	6.7 a
I_2	21.4b	12.3 b	6.7b	3.9 b
I_3	32.9a	24.2 a	8.07 a	6.4 a
Fertilizer				
F_1	31.8 c	22.5 c	8.8 c	6.6c
F_2	35.2 b	24.3 bc	9.5 bc	7.4 b
F_3	37.1 ab	26.7 b	10.1 b	7.5 b
F_4	39.4 a	29.5 a	11.5 a	9.4 a

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT). I_1: full irrigation (control), I_2: non-irrigation from the flowering stage to the end of the growth period, I_3: non-irrigation from the grain filling stage to the end of the growth. F_1: control, F_2: 1 mM salicylic acid, F_3: Nitroxin bacteria grain rub + Biosphere, F_4: Nitroxin bacteria grain rub + Biosphere along with salicylic acid foliar application.

Furthermore, the lowest dry matter accumulation in these stages (with average values of 577.2, 964.4 and 1291 $g.m^{-2}$, respectively) was found in the control treatment (Table 6). The interaction effect of treatments on the accumulation of total dry matter was also significant at the 1% of probability level (Table 5). At the stages of tasseling and beginning of flowering, there was no significant difference in dry matter accumulation between the full irrigation treatment and the non-irrigation from the grain filling stage at various levels of salicylic acid and growth-stimulating bacteria treatments. The lowest dry matter accumulation was associated with the non-irrigation from the flowering stage to the end of the experiment (I_2) and the non-application of growth-stimulating bacteria and salicylic acid treatments. The application of salicylic acid and growth-stimulating bacteria significantly increased the accumulation of dry matter in the non-irrigation treatments (Table 7). The results obtained from this experiment were consistent with the findings of Memar and Mojadam (2015) and Jalilian and Mohsennia (2013). Jalilian and Mohsennia (2013) studied the effect of drought stress on barley seedling growth and found that drought stress led to a reduction in dry matter accumulation throughout the growth period compared to the control treatment. The application of salicylic acid and growth-stimulating bacteria not only improved plant tolerance and nutrient release around the root zone but also resulted in enhanced plant growth and increased dry matter accumulation. In the treatment with nitroxin + biophosphorus + salicylic acid, due to improved root growth, enhanced water and nutrient uptake occurred, leading to an increase in hydrocarbon content and dry matter accumulation in the plant. Despite significant reductions in dry matter accumulation at various growth stages due to non-irrigation from the flowering stage, the application of salicylic acid and growth-stimulating bacteria (especially in the combination of nitroxin + biophosphorus + salicylic acid treatment) not only improved plant growth but also significantly increased dry matter accumulation by improving the plant's access to water. The increase in dry matter accumulation under the application of growth-stimulating bacteria and salicylic acid may be attributed to the provision of better conditions for photosynthesis, improved accumulation of photosynthetic substances and increased dry matter (Li *et al*., 2014).

4.3. *Crop Growth Rate (CGR)*

The effect of non-irrigation, growth stimulating bacteria and salicylic acid on CGR at different growth stages was significant at 1% probability level (Table 2). Full irrigation and non-irrigation treatments from grain formation stage to grain filling showed the highest CGR. The lowest CGR belonged to non-irrigation from tasseling to grain formation with an average of 3.9 and from the grain formation stage to grain filling with an average of 12.3 $g.m^{-2}.day$ (Table 3). The combination of growth-stimulating bacteria and salicylic acid caused a significant increase in the crop growth rate.

Table 4. Mean comparison of interaction effects of treatments combination on studied traits

Treatment		Total dry weight ($gr.m^{-2}$)			Leaf area index		
Irrigation	Fertilizer	Tassel-ling stage	beginning of flowering stage	Grain filling stage	Tassel-ling stage	beginning of flowering stage	Grain filling stage
I_1	F_1	553.5* c	1017 cd	1373 bc	4.95 cd	4.11 c	3.24 cd
	F_2	610 b	1068 c	1426 b	5.43 b	4.81ab	3.40 bc
	F_3	683.5 ab	1169 b	1415 b	5.31 bc	4.82 ab	3.63ab
	F_4	720.7 a	1278 a	1781 a	5.82 a	4.95 a	3.85a
I_2	F_1	543.2 c	672 g	811.5 h	4.65 d	2.83 f	1.41 g
	F_2	599.5 bc	713 fg	894 g	4.85 cd	3.22 ef	2.42 f
	F_3	672.5 ab	814 e	991 f	5.28bc	3.43 de	2.81e
	F_4	726.1a	943 d	1172 e	5.55 ab	3.2de	2.95 e
I_3	F_1	538.5 c	998 cd	1100 fg	4.85 cd	4.31 bc	2.41 f
	F_2	595.2 bc	1072 c	1283 d	5.45 b	4.93 a	2.83 e
	F_3	670.5 ab	1152 b	1381 bc	5.28 bc	4.84 ab	3.45 bc
	F_4	720.5 a	1271 a	1412 b	5.83 a	4.93 a	3.65 ab

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT). I_1: full irrigation (control), I_2: non-irrigation from the flowering stage to the end of the growth period, I_3: non-irrigation from the grain filling stage to the end of the growth. F_1: control, F_2: 1 mM salicylic acid, F_3: Nitroxin bacteria grain rub + Biosphere, F_4: Nitroxin bacteria grain rub + Biosphere along with salicylic acid foliar application.

Continue table 4.

Treatment		Crop growth rate ($gr.m^{-2}.d^{-1}$)		Net assimilation rate ($gr.m^{-2}.d^{-1}$)	
Irrigation	Fertilizer	Tasseling until beginning of flowering stage	beginning of flowering until grain filling	Tasseling until beginning of flowering stage	beginning of flowering until grain filling
I_1	F_1	32.55 d	23.24 de	8.1 cd	6.5de
	F_2	36.32 bc	24.32 cd	10.5 b	8.6ab
	F_3	37.81 b	37.51 b	10.1 b	7.4cd
	F_4	39.92 a	31.11a	12.1a	9.6a
I_2	F_1	20.54 h	9.41h	6.5 e	4.8g
	F_2	23.33 g	13.52g	6.5 e	5.1fg
	F_3	26.75 f	13.09 g	7.5 de	5.6efg
	F_4	27.85 ef	15.12 f	8.5cd	6.5de
I_3	F_1	32.13 d	22.25 e	8.2cd	6.1ef
	F_2	35.05 c	23.56 de	10.2 b	7.1cd
	F_3	37.22 b	25.17 bc	11.1 ab	7.5cd
	F_4	39.23 a	25.32 bc	12.5 a	7.8bc

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT). I_1: full irrigation (control), I_2: non-irrigation from the flowering stage to the end of the growth period, I_3: non-irrigation from the grain filling stage to the end of the growth. F_1: control, F_2: 1 mM salicylic acid, F_3: Nitroxin bacteria grain rub + Biosphere, F_4: Nitroxin bacteria grain rub + Biosphere along with salicylic acid foliar application.

By the usage of salicylic acid and growth-promoting bacteria, the crop growth rate increased at the stages of the tasseling until grain formation and the stage of grain formation until grain filling, by 19.3 and 23.72%, respectively. The maximum crop growth rate was obtained with 39.5 and 29.5 $g.m^{-2}.day$, respectively, from nitroxin + biophosphorus + salicylic acid treatment. The lowest rate of crop growth belonged to the control treatment (Table 3). The interaction effect of irrigation and salicylic acid treatment and growth promoting bacteria on the crop growth rate in the examined growth stages was significant at the 1% of probability level (Table 2). The highest growth rate belonged to the full irrigation treatment at growth stages and biophosphorus consumption along with salicylic acid foliar application with an average of 39.93$g.m^{-2}.day$. The lowest crop growth rate with an average of 20.54 and 9.41$g.m^{-2}.day$, respectively, was attributed to the treatments of non-irrigation from the flowering stage to the end of the growth period and non-irrigation from the grain formation stage to grain filling and not using salicylic acid and stimulating bacteria (Table 4). In this experiment, under drought stress conditions, the crop growth rate decreased significantly, which was consistent with the results of researchers in the same field (Nazarli *et al*., 2010 and Fazeli Rostampur *et al*., 2010). Li *et al*. (2014) as well as Mao *et al*. (2011) found that drought stress significantly reduced the growth rate of grain corn compared to the control treatment. The rate of photosynthesis and the crop growth rate were directly affected by drought stress, and the application of the combined treatment of growth-promoting bacteria and salicylic acid reduced the effects of drought stress to some extent and improved the above traits. In the conditions of drought stress, the use of stress reducers such as salicylic acid and growth-promoting bacteria increases the growth of vegetative and reproductive organs and especially the crop growth rate, so that by improving the internal condition plant, increasing the relative crop growth rate (Valadabadi *et al*., 2009). The reason for the decrease in the crop growth rate in the treatment of non-irrigation and lack of use of salicylic acid and growth-promoting bacteria compared to the control irrigation may be due to the reduction of photosynthetic activity, and related enzymes. Drought stress may have affected the crop growth rate of the corn through the reduction of chlorophyll, leaf area index, followed by the reduction of photosynthesis and the accumulation of photosynthetic materials. Also, in the combined treatment of growth-promoting bacteria and salicylic acid, while improving plant tolerance, it may have contributed to an increase in the crop growth rate through the expansion of roots, increased absorption and retention of moisture and nutrients, as well as increasing the leaf area index.

4.4. *Net Assimilation Rate* (*NAR*)

The net assimilation rate was significantly affected at the 1% of probability level by the treatments of non-irrigation and the salicylic acid and growth-promoting bacteria (Table 2).

Table 5. Summary of the results of analysis of variance of the data in which the mean square is shown

S.O.V	df	Relative water content	Grain yield
Replication (R)	2	43.7	3465
Irrigation (I)	2	1255.9 **	65372 **
E_a	4	55.7	6527.7
Fertilizer (F)	3	834.4 **	41297**
I*F	6	495.7**	37588 **
E_b	18	25.7	3815.29
CV (%)		8.3	11.43

ns: Non significant, * and ** indicate significance at the level of 5% and 1%, respectively.

The highest rate of net assimilation rate belonged to the complete irrigation treatment (Table 3). Non-irrigation caused a significant decrease in the net assimilation rate. Non-irrigation from the flowering stage to the end of the growth period had the most adverse effect on growth indices and especially on the net assimilation rate. The maximum net assimilation rate in the stages of the tasseling stage until grain formation and the stage of grain formation until grain filling was 8.8 and 6.5 $gr.m^{-2}.day$, respectively, due to complete irrigation and non-irrigation from the grain filling stage. The lowest amount of this trait was related to the non-irrigation from the flowering stage (Table 3). The maximum rate of net absorption in the stage of emergence of male inflorescence to grain formation and the stage of grain formation to grain filling was related to nitroxin + biophosphorus + salicylic acid treatment with an average of 11.5 and 9.4 $g.m^{-2}.day$, respectively. The minimum net assimilation rate belonged to the control treatment with an average of 8.8 and 6.6, respectively (Table 3). The net assimilation rate at the stages of tasseling to grain formation and the stage of grain formation to grain filling was significantly affected at the level of 1% by the interaction effect of the treatment of non-irrigation and the combination of growth-stimulating bacteria and salicylic acid treatment (Table 2). By non-irrigation at different growth stages, a significant decrease in the net assimilation rate was observed. The use of salicylic acid and growth-promoting bacteria at different treatments of non-irrigation and complete irrigation increased the net assimilation rate. In the non-irrigation treatment from the flowering stage to the end of the growth period (I_2), the net assimilation rate in the stage of tasseling until grain formation with nitroxin + biophosphorus + salicylic acid treatment from 6.5 to 8.5 and in the grain formation stage until grain filling from 4.8 to 6.5 $g.m^{-2}.day$ increased. Also, in the treatment of non-irrigation from the grain filling stage to the end of the test period (I_3), nitroxin + biophosphorus + salicylic acid treatment caused a significant increase in the net assimilation rate (Table 4). Treatments of non-irrigation from the flowering stage to the end of the growth period (I_2) and non-irrigation from the grain filling stage to the end of the growth period (I_3) significantly reduced the NAR, which is consistent with the results of researchers in the same field (Islam *et al.*, 2017 and Tohidi Moghadam, 2016). The increase in the NAR in the treatment with growth-promoting bacteria and salicylic acid may be due to the increase in the activity of photosynthetic enzymes and antioxidant enzymes of the plant, which increases the tolerance of the plant, the leaf area index and the optimal use of light to improve the conditions of photosynthesis and, as a result, increase the NAR. These results were consistent with the results of researchers in the same field (Fazeli Rostampur *et al*., 2010). If it is possible to prevent the occurrence of drought stress in the stages of reproductive growth, the rate of net assimilation will increase and the decline of net photosynthesis will be slower. Since the reproductive growth stage coincides with the high rate of photosynthesis and plant growth, therefore, reducing the effect of drought stress in these stages improves photosynthesis and, as a result, increases the rate of net assimilation. The NAR does not remain constant with the passage of time and shows a downward trend in the growth and development of the plant as the plant ages. This drop is accelerated in an unsuitable environment and drought stress, so when new leaves are added due to the shading of the leaves on each other, the dry weight per leaf area decreases.

4.5. *Relative leaf water content* (*RWC*)

The effect of non-irrigation and the combination of growth-promoting bacteria and salicylic acid on relative leaf water content was significant at the 1% of probability level (Table 5). Drought stress by non-irrigation at different growth stages caused a sharp decrease in the relative water content of leaves. The minimum relative leaf water content with an average of 39.4% was obtained from the treatment with non-irrigation from the flowering stage to the end of the growth period. Applying non-irrigation from the grain filling stage (with an average of 53.5%) showed a significant decrease compared to the control treatment. The maximum relative leaf water content with an average of 68.7% was related to the treatment of full irrigation (Table 6). The relative water content of the leaf was significantly increased in the treatment with salicylic acid and growth-promoting bacteria. The highest RWC with an average of 73.8% was assigned to nitroxin + biophosphorus + salicylic acid treatment. The lowest amount with an average of 65.5% was related to the control treatment. No significant difference was observed between salicylic acid treatment and nitroxin + biophosphorus treatment in terms of relative water content (Table 6). Also, the interaction effect of irrigation and the combination of growth-promoting bacteria and salicylic acid on RWC was significant (Table 2). The highest RWC with an average of 75.8% was obtained from the interaction effect of full irrigation and nitroxin + biophosphorus + salicylic acid treatment. The lowest RWC among different treatments with an average of 31.1% belonged to the treatment of non-irrigation from the flowering stage to the end of the growth period (I_2) and not using salicylic acid and growth stimulating bacteria. Application of salicylic acid and growth-promoting bacteria in the treatments of non-irrigation significantly increased the RWC (Table 7).

Table 6. Mean comparison effect of different level of irrigation and fertilizer combination on studied traits

Treatment	Relative water content (%)	Grain yield ($kg.ha^{-1}$)
Irrigation		
I_1	68.7 a	5214a
I_2	39.4 c	2173c
I_3	53.5 b	3348b
Fertilizer		
F_1	65.5c	5190d
F_2	70.3b	5640c
F_3	70.1 b	5982b
F_4	73.8 a	6433a

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT). I_1: full irrigation (control), I_2: non-irrigation from the flowering stage to the end of the growth period, I_3: non-irrigation from the grain filling stage to the end of the growth. F_1: control, F_2: 1 mM salicylic acid, F_3: Nitroxin bacteria grain rub + Biosphere, F_4: Nitroxin bacteria grain rub + Biosphere along with salicylic acid foliar application.

With the simultaneous application of nitroxin + biophosphorus + salicylic acid, the relative water content increased, salicylic acid by affecting some enzymes to increase plant tolerance and growth-stimulating bacteria, by releasing nitrogen and phosphorus nutrients around the roots, causes more root expansion than the control treatment, as a result, in the condition of non-irrigation, due to the greater ability of the plant roots to absorb and retain moisture, it can prevent waste on water and increase the RWC by increasing the plant's access to water (Li *et al*., 2014). The relative water content of leaves in the treatment of non-irrigation at different growth stages showed a statistically significant difference compared to the control, which was consistent with the findings of other researchers (Erickson *et al*., 2009; Smith, 2008). It seems that the rate of water loss by the plant is higher in the treatments of non-irrigation due to the lack of humidity, which causes a decrease in the relative water content of the leaves. But with use of stress reducing agents such as salicylic acid and growth-promoting bacteria, because plants have more and better access to water around roots to transfer it to aerial organs, rate of water loss is lower, and as result, plants have a higher relative water content. Erickson *et al*. (2009) showed that drought stress by non-irrigation at different growth stages caused a decrease in RWC of leaves in corn. The high RWC and reduction of lost water in the treatment with growth-promoting bacteria and salicylic acid are characteristics of this treatment combination, which can be used as an important factor in reducing damage of drought stress and maintaining the growth and development of the plant (Mc Caig and Rumugosa, 2009). In the treatment of not using salicylic acid and growth-promoting bacteria, it seems that the RWC decreases due to high transpiration and its replacement is due to lack of access to sufficient water by the roots of corn at different treatments, especially in drought stress conditions, which is consistent with other results researchers (Erickson *et al*., 2009; Smith, 2008; Atteya, 2003).

4.6. *Grain yield*

Drought stress resulted in a decrease in the yield components, after which the grain yield also decreased significantly.

Table 7. Mean comparison of interaction effects of different levels of irrigation and fertilizer combination on studied traits

Irrigation	Fertilizer	Relative water content (%)	Grain yield (kg. ha^{-1})
I_1	F_1	65.6 de	5304 d
	F_2	67.1 cd	5672 c
	F_3	71.2 b	5913 b
	F_4	75.8 a	6552 a
I_2	F_1	35.1 n	2010 k
	F_2	37.7 mn	2254 j
	F_3	45.5 jk	2341 ij
	F_4	49.5 hi	2598 h
I_3	F_1	48.4 ij	3375 g
	F_2	52.5 gh	3862 f
	F_3	58.2 f	3798 f
	F_4	62.8 e	4182 e

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT). I_1: full irrigation (control), I_2: non-irrigation from the flowering stage to the end of the growth period, I_3: non-irrigation from the grain filling stage to the end of the growth. F_1: control, F_2: 1 mM salicylic acid, F_3: Nitroxin bacteria grain rub + Biosphere, F_4: Nitroxin bacteria grain rub + Biosphere along with salicylic acid foliar application.

Non-irrigation from the flowering stage to the end of the growth period (I_2) caused the maximum decrease in grain yield. The highest grain yield with an average of 5214 kg.ha^{-1} belonged to the full irrigation treatment (control) (I_1). The lowest grain yield with an average of 2173 kg.ha^{-1} belonged to the treatment of non-irrigation from the flowering stage to the end of the growth period (I_2) (Table 6). The highest and lowest grain yield with an average of 6433 and 5190 kg.ha^{-1} belonged to nitroxin + biophosphorus + salicylic acid treatment and control treatment, respectively (Table 6). The interaction effect of irrigation and combination of growth-promoting bacteria and salicylic acid had a significant effect on grain yield. The decrease in the 1000 grain weight and other yield components, including the number of rows per ear and the number of grains per row, caused a significant decrease in grain yield by non-irrigation and creating drought stress. The maximum grain yield was obtained with an average of 6552 kg.ha^{-1} in full irrigation treatment (control) (I_1) and using nitroxin +biophosphorus + salicylic acid. Non-irrigation at the stages of flowering and grain filling showed a significant decrease in grain yield. The minimum grain yield with an average of 2010 kg.ha^{-1} was achieved by non-irrigation from the flowering stage to the end of the growth period (I_2) and not using salicylic acid and growth-stimulating bacteria. However, with nitroxin + biophosphorus + salicylic acid treatment, grain yield increased from 2010 kg.ha^{-1} to 2600 kg.ha^{-1}. Also, in the treatment of non- irrigation from the stage of grain filling to the end of the growth period (I_3) and the use of nitroxin + biophosphorus + salicylic acid, the grain yield increased by 907kg.ha^{-1} (Table 7). Following the reduction of the yield components such as the 1000 grain weight, the length and diameter of the cob, the number of rows and the number of grains per row in the condition of non-irrigation, the grain yield also decreased significantly, which was consistent with the results of other researchers in the same direction (Gardner *et al*., 1984 and Shoae Hoseini *et al*., 2008). Under the conditions of non-irrigation at different growth stages and full irrigation, nitroxin + biophosphorus + salicylic acid treatment showed a significant superiority in terms of grain yield compared to other treatments. The drought stress before flowering and during the flowering period caused non-synchronism of the emergence of male and female organs of corn and increased the interval of the emergence of corolla and silks, and as a result, with the decrease in the number of rows, the number of grains and the weight of grains, the yield of corn decreased. The occurrence of drought stress at the same time as the meiotic division in the corolla caused the pollen grains to become sterile, it prevented the elongation of the silks, and by reducing the number of rows per ear, the number of grains per row and the 1000 grain weight, it caused a sharp decrease in yield (Rashidi, 2005), as a result of any stress in these periods by reducing the number of grains per row, the number of rows per cob and the weight of the grain, it causes a sharp decrease in the grain yield. Grain yield potential at the time of flowering is a function of the previous growth of the plant, so the plants that faced drought stress from the flowering stage with nitroxin + biophosphorus + salicylic acid treatment have grown in less competitive conditions for moisture absorption than the control. As a result, by improving yield components, they had more yield potential. Drought stress and non- irrigation in corn causes a sharp decrease in egg fertilization and as a result, the number of grains per cob and then the grain yield decreases. Basically, in limited growth species such as corn, reproductive growth stages show great sensitivity to drought stress (Shoae Hoseini *et al*., 2008). The researchers reported that drought stress by non-irrigation reduces corn yield in three major ways. First, it reduces the total absorption of photo synthetically active radiation by the plant canopy due to the limitation of leaf development, temporary wilting, tuberization or premature senescence during periods of severe drought stress. Second, it reduces the efficiency of light consumption, which is an important limiting factor in corn yield under stress. Third, it lowers grain corn yield by reducing harvest index (the proportion of crop dry matter allocated to grain) (Earl and Davis, 2003). The increase in grain yield with the usage of salicylic acid and the combination with growth-promoting bacteria can be due to the increase in the 1000 grain weight, the reduction of disturbance in pollination and the photosynthetic stages of the plant and the transfer of materials to the grains (Kuiper *et al*., 1990). The increase in the 1000 grain weight in the combined treatment of bacteria and salicylic acid may be due to one of two reasons: 1- increasing the amount of photosynthetic substances introduced into the cob or 2- increasing the length of the grain filling period. One of the goals of plant hormones spraying is to increase the green period and photosynthetic activity of leaves to transfer more photosynthetic materials to grains (Garsia and Honowy, 2006). Poneleit *et al*. (2008) reported that corn grain weight is one of the effective components in yield regulation, but it has less sensitivity than other yield components. According to the report of Gardner *et al*. (1984) the hormones resulting from the activity of biological fertilizers and their foliar application by affecting the formation, growth and destruction of flowers and grains have an important effect on the relationship between the origin and destination of plants and it is possible through the effect indirectly affect the transfer speed on the need of the sink. According to the physiological yield of these treatments, there is no doubt that these compounds can increase the activity of the target and reduce the aging process and reduce the plant sensitivity (Zai *et al*., 2003).

5. CONCLUSION

The results showed that the full irrigation treatment effectively and significantly improved the growth physiological indices, such as the leaf area index. On the other hand, non-irrigation had a significant negative effect on these indices. The combination of nitroxin, biophosphorus and salicylic acid had a positive and significant effect on the examined indicators. The combined use of fertilizers improved the physiological indices in all irrigation levels, especially in full irrigation. Non-irrigation from the flowering stage had the most negative effect on the yield and yield components of grain corn, reducing the grain yield by 59% compared to the control treatment. All components of the yield exhibited a significant decrease when irrigation was interrupted from the flowering stage, leading to a severe reduction in grain yield. The treatment involving Nitroxin + biophosphorus + salicylic acid demonstrated the highest grain yield. The increase in grain yield observed in the combined treatment under full irrigation conditions can be attributed to the improved photosynthesis conditions, increased material accumulation, resulting in a higher number of grains per row, more rows per cob and a higher 1000 grain weight. Severe reduction in leaf area index and yield components was observed in the non-irrigation treatment from the flowering stage, coupled with the absence of salicylic acid and growth-stimulating bacteria application, resulting in a significant decrease in grain yield. Under favorable irrigation conditions, the treatment involving nitroxin + biophosphorus + salicylic acid exhibited the highest grain yield.

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