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EFFECT OF IRRIGATION INTERVAL AND BIOLOGICAL AND NITROGEN FERTILIZERS ON GRAIN YIELD AND YIELD COMPONENTS OF RICE CULTIVARS

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ABSTRACT

The effects of different irrigation regimes and the application of biological and chemical nitrogen (N) fertilizers on yield and yield components of rice cultivars in a field experiment at the Rice Research Institute of Iran, Rasht in 2018-2019 crop years were studied. The study was split-split plots based on the complete randomized block design with three replications. The main experimental factor was irrigation interval at three levels (flood irrigation and irrigation at intervals of 10 and 15 days), the second factor was fertilizer type at three levels [seedling root inoculation with Nitroxin biofertilizer, seedling root inoculation with Nitroxin biofertilizer + chemical N fertilizer to meet 50% of plant N requirement, and chemical N fertilizer (as urea) to meet 100% of plant N requirement], and the third factor was cultivar at two levels (rice varieties 'Hashemi' and 'Gilaneh'). The results showed that the combined application of the biological and chemical fertilizer (100% N) did not cause any significant differences in the yield of the rice cultivars. The flood irrigation produced 27.7% and 41.6% higher grain yield than the irrigation at intervals of 10 and 15 days, respectively. The number of unfilled grains per panicle was 20% higher in the treatment of 100% chemical N fertilizer than in the combined application of the biological and chemical fertilizers. The irrigation interval of 15 days with the combined application of the fertilizers consumed less water in both cultivars than the other treatments. The combined application of Nitroxin and chemical fertilizer not only was appropriate for yield and yield components, but it also decreased the effect of chemical N fertilizer application by 50%.

Keywords: irrigation management, nitrogen, Nitroxin, rice.

INTRODUCTION

Rice is one of the most important grains and the staple food of over 50% of the world population and plays a key role in economic and social stability (Dass et al., 2016; Jabran and Chauhan, 2015; Nithya and Ramamoorthy, 2015; Rajput et al., 2017). Water is the main factor of sustainable production in rice-producing regions. Almost three-fourth of the global rice is produced in flooded paddies accounting for nearly half of the whole paddies of the world (Carmelita et al., 2011). Rice has the highest water requirement among all crops. To produce 1 kg of rice, farmers have to consume 2-3 times as much water in their paddies as they use for other grains (Taghizadeh et al., 2008). Until achieving full maturity, rice needs about 8000-11000 m^3 water per ha and it needs 700 liters of water to produce 1 kg of dry matter (Rezaei and Nahvi, 2007). Given water scarcity and low water use efficiency, the optimal use of water seems to be imperative for preserving food security, especially when it comes to rice, which is a strategic crop whose production level should be increased by over 70% versus the status quo by 2025. Therefore, to resolve future challenges, water experts in the world are looking for ways for optimal management of water in a region-specific manner (Asadi et al., 2016).

Water stress is a major abiotic stress that not only reduces water use but also influences all aspects of growth and development so that it hinders the mobilization of minerals and nutrients inside

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the plant and reduces photosynthesis, thereby decreasing the number of fertile tillers, spike length, biomass, filled grain percentage, 1000-grain weight, and finally, the yield of rice. Rice cultivars vary in their resistance to water deficit conditions under aerobic culture (Asadi et al., 2016; Jaleel et al., 2009; Rezaei et al., 2017). Various research studies in different parts of the world, including Iran, have proven the positive effect of non-flood irrigation management on grain yield and water efficiency of rice.

The sound application of other inputs, e.g. fertilizers, can partially offset the impact of deficit irrigation and alleviate its inhibitory effects. In other words, the interaction of deficit irrigation and biofertilizer application can influence soil fertility management, agricultural products, and ecosystem sustainability (Carmelita et al., 2011; Mandal et al., 2007). Although the application of chemical fertilizers in suitable dosage can increase the quantitative and qualitative yield of plants in the short run, their over-use not only reduces plant responses to them but also results in their accumulation in soil and the elimination of soil-borne organisms, the degradation of soil structure, the closure and reduction of soil porosity, the loss of soil fertility, and their infiltration into groundwater tables, which pollutes them. Excessive use of chemical fertilizers ruins the natural linkage between decomposing bacteria and root zone, thereby reducing soil dynamism and turning it into an abiotic substrate (Kochaki et al., 2005; Salehifar et al., 2018). The optimal use of fertilizers plays a key role in enhancing crop yield, including rice (Hosseinzadeh et al., 2012).

Nitrogen is a major highly-consumed nutrient with a decisive role in crop growth and yields. The limitation of nutrients at the vegetative growth stage reduces nutrient storage and yield. Although the application of mineral fertilizers appears to be the fastest way of supplying soil fertility, the high costs of fertilization, as well as its impacts on environmental pollution and degradation and water and soil resources and even the loss of yield, are a matter of concern (Davoudi et al., 2014; Hassegawa et al., 2008; Pampolino et al., 2007).

One way to improve nitrogen fertilizer use efficiency and reduce its wastage is to consume it concurrently with organic and biological fertilizers. Biofertilizers are natural fertilizers that play an essential role in plant nutrition and soil health and can guarantee the production sustainability of agricultural systems (Board, 2004; Han et al., 2006). Biofertilizers are composed of beneficial microorganisms, each is used for a certain purpose such as nitrogen fixation and the release of the ions of phosphate, potassium, iron, and so on. These microorganisms usually reside around roots and help plants take up nutrients (Vessey, 2003; Wu et al., 2005).

The biofertilizer Nitroxin contains some of the most effective nitrogen-fixing bacteria from the genera *Azotobacter* and *Azospirillum*. In addition to fixing atmospheric nitrogen and making a balance in the uptake of macronutrients and micronutrients required by the plant, the bacteria contained in Nitroxin contribute to root and shoot growth and development of the plant through synthesizing and secreting growth-promoting compounds, e.g. growth-regulating hormones (Asadi Kopal and Eesazadeh Lzrjan, 2009). The use of Nitroxin not only allows avoiding the application of chemical nitrogenous fertilizers, but it can also contribute to increasing crop yields by the various impacts it has (Hamidi et al., 2006).

By applying biofertilizers in a correct manner, reliance on chemical fertilizers to improve sustainable and organic production can be decreased through reducing nutrient wastage in the environment, and nutrient use efficiency can be increased (Ye et al., 2011; Zhao et al., 2010). Given the importance of appropriate water and nutrient management for rice, the present research aimed to shed light on the effect of biofertilizer and chemical N fertilizer application method on yield and yield components of two rice cultivars including 'Hashemi' (the dominant cultivar in the area) and 'Gilaneh' (a newly introduced cultivar) under different irrigation conditions.

MATERIAL AND METHODS

The research was conducted at the research farm of Iranian Rice Research Institute in Rasht to explore the effect of drought stress and biological and chemical N fertilizers on yield and yield components of rice cultivars in a split-split-plot experiment based on a randomized complete block design with three replications, in two years. The study site (lat. 37°16' N, long. 49°36' E, elevation 36 m from sea level) has a temperate and humid climate with an annual precipitation rate of 1330 mm based on the 10-year average (Guilan Meteorological Quarterly, 2019). The soil texture in the study site was loam-clay with a pH of 7.4. The electrical conductivity of the soil was 1.2 and 1.12 dS/m in the first and second year. Its absorbable P and K content was 17.8 and 0.184 ppm in the first year and 17 and 0.155 ppm in the second year, respectively. The percentage of N was 0.184 in the first year and 0.155 in the second year (Table 1).

Rainfall and sunny hours during the growing season in the second year were higher than the first year (Table 2).

The experimental treatments included the main plot assigned to different irrigation regimes (flood irrigation and irrigation intervals of 10 and 15 days), the sub-plot assigned to biofertilizer and chemical N fertilizer (seedling root inoculation with Nitroxin, seedling root inoculation with Nitroxin + chemical N fertilization to meet 50% of plant requirement, and chemical N fertilization to meet 100% of plant requirement), and the sub-sub-plot assigned to rice cultivars ('Hashemi' and 'Gilaneh').

Table 1. Soil physical and chemical analysis

Year	K	P	N	Organic matter	PH	EC	Soil texture
	ppm	ppm	%	%		ds.m^{-1}	g kg^{-1}
2017	280	17.8	0.184	1.4	7.4	1.2	Loam clay
2018	290	17	0.155	1.3	7.4	1.12	

Table 2. Historical monthly in 2017 and 2018 growing seasons climatic data of the experimental area

Year	Month	21 Mar - 20 Apr	21 Apr - 16 May	17 May - 16 Jun	17 Jun - 18 Jul	19 Jul - 18 Aug	20 Aug - 19 Sep
2017	Tmean (°C)	13.6	19.3	23.6	26	28.2	26.9
	Rainfall (mm)	86.2	27.8	18.6	13.8	0	61
	Humidity (%)	78	78	75	74	71	75
	Sunny hours (h)	140	169.2	229.1	232.5	293.7	245.8
2018	Tmean (°C)	13.7	19.4	23.1	28.1	27	25.1
	Rainfall (mm)	20.4	37.2	48.7	30.8	68.4	13.8
	Humidity (%)	76	74	75	73	77	74
	Sunny hours (h)	145.9	170.4	230.3	295.4	164.9	209.7

The farm was plowed three times - first in February, second in early-May concurrent with the establishment of a nursery, and third (puddling) concurrent with transplanting. The seeds were grown in the nursery under a plastic cover and when the seedlings grew to a height of 20-25 cm (2-4-leaf stage), the healthy and uniform seedlings were picked and after they were inoculated with Nitroxin in the relevant treatments, they were all transplanted at the main farm. Nitroxin contains some N-fixing bacteria, e.g. *Azotobacter* and *Azospirillium*, each at a population of 108 live cells per ml of Nitroxin. The seeds were inoculated with the biofertilizer in darkness due to the sensitivity of the bacteria to light and heat (Taleie and Amini, 2015). Based on the results of soil analysis, the farm was fertilized with 100 kg/ha potash at two stages (50% as the base fertilizer and the remaining 50% in the middle of tillering) and 50 kg/ha phosphorous as the base fertilizer during farm plowing. The chemical N fertilizer was applied to the relevant treatments as heading at two stages of maximum tillering and flower initiation. The N, P, and K fertilizers were supplied from urea, triple superphosphate, and potassium chloride sources, respectively. The rice seedlings were planted in a 20 cm × 20 cm arrangement. Each experimental plot was 3 × 6 m with 15 × 30 planting rows. To facilitate sampling and commuting inside the plots, the treatments were spaced by 50 cm. To uniformly transplant the rice seedlings, the farm was marked with a marker at distances of 20 × 20 cm. No herbicide, insecticide, or fungicide was applied during the growth period. To measure the target traits, 10 rice hills were harvested from each experimental plot.

At harvest time, the number of panicles per m^2, total, filled and unfilled grains per panicle, 1000-grain weight, and economic yield were recorded. To determine the total number of grains per unit area, the spikes of 30 hills were randomly selected from each plot, they were cut from the crown, and the total number of grains, as well as the number of filled and unfilled grains, were counted for each spike separately. To calculate 1000-grain weight, 30 panicles were selected from each plot randomly and harvested from their crown. Then, 1000 grains were counted and weighed. The number of panicles per unit area was determined on 25 randomly-selected hills for which the fertile and infertile panicles were first separated and the final number of fertile spikes per unit area was calculated. To determine the economic yield, after eliminating the margins, 50 plants were harvested from each plot. They were, then, threshed, cleaned, and weighed. Their moisture content was, then, measured with a moisture-meter, and the grain weight was estimated based on 14% moisture. Harvest index = Total grain weight (dry weight)/Total above ground biomass (dry weight). The statistical analysis of the data, data conversion, and graph drawing were performed in the SAS9.2 and MS-Excel 2010 software packages. Means were compared by LSD test at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Yield and yield components

The results of the combined ANOVA revealed that the effect of year and the interactive effect of irrigation, fertilizer, and cultivar were significant on grain yield (Table 3). The comparison of data means revealed that the modified cultivar 'Gilaneh' produced about 17% higher yield than the local cultivar 'Hashemi'. The increase in the irrigation interval resulted in a decline in the grain yield. This decline amounted to about 27.7% when irrigation was decreased from flood irrigation to 10-day irrigation interval, while further increase in irrigation interval to 15 days resulted in its decline by 41.6%. Drought stress reduced the paddy yield of both cultivars in all fertilization treatments significantly. Any limitation on water resources restricts the translocation of some nutritional resources, making the plant shorten its vegetative phase, terminate it earlier, and initiate its reproductive phase. Consequently, yield and yield components are decreased (Tarigholeslami et al., 2012). The highest grain yield was obtained from the combined application of bioferitlizer and chemical fertilizer and from 100% chemical N fertilizer (Figure 1).

These two fertilization treatments were categorized in the same statistical class, which underlined the significance of biofertilizers in reducing the application of chemical N fertilizers. Based on the results, it can be stated that the biofertilizer Nitroxin is capable of reducing chemical urea in paddies of 'Hashemi' and 'Gilaneh' by up to 50%. A research study has reported that the application of Nitroxin resulted in the highest harvest index and grain yield. Biofertilizers increase grain yield by improving leaf area index and light interception by plants (Rahmani et al., 2014). Since biofertilizers release nutrients and make them available to the plants gradually and the chemical N fertilizer was applied at different growth stages, the plants had access to this nutrient throughout their growth period and this eventually enhanced their grain yield (Moslehi et al., 2016). Hatamifar et al. (2013)

stated that chemical N and K fertilizers, applied at different irrigation regimes, significantly influenced yield, the number of fertile tillers per m^2, and panicle length. The grain yield of crops is determined by various ratios of their yield components. Studying the yield components of rice is a method to determine its yield-limiting factors and figure out ways to enhance its yield (Taghizadeh et al., 2008).

'Hashemi' had nearly 19% fewer panicles than 'Gilaneh' (225 versus 267). As irrigation interval was increased, the number of panicles per unit area, the number of grains per main panicle, and the number of filled grains per panicle were decreased, but the number of unfilled grains per panicle was increased. In stressful conditions, the number of effective tillers decreases, probably due to the increased number of tillers and their more severe competition, and the number of spikes per unit area decreases too (Hazra and Chandra, 2014). The most number of panicles was related to the treatment of 100% chemical N fertilizer and the treatment of seedling root inoculation with Nitroxin + 50% chemical N fertilizer (11 and 10 panicles), respectively. An increase in panicle number per unit area is the cause of the higher grain yield of the plants treated with nitrogen-containing fertilizers (Bindra et al., 2000). The interaction of irrigation interval and N fertilizer was significant for panicle number per unit area (Taghizadeh et al., 2008).

Drought stress increases grain shedding dramatically. If the stress happens during the grain filling period, most produced grains will fall and the grains will be unfilled. The grain filling period is a sensitive stage at which all photosynthates are mobilized towards the grains. Then, plants exposed to stress may not have adequate carbohydrates to fill all formed grains and produce more unfilled grains. In other words, the plants will be unable to produce adequate assimilates (source limitation) to fill the grains (Saeidi and Abdoli, 2015). The treatment of 100% chemical N fertilizer and the treatment of root inoculation with Nitroxin + 50% chemical N fertilizer increased the number of grains per panicle and the number of filled grains versus the treatment of seedling root inoculation with Nitroxin. The highest number of grains per panicle was related to the combined application of N and *Azospirillum* (Moslehi et al., 2016). The application of N fertilizer + *Azotobacter* biofertilizer increased the grain number of wheat plants per spike significantly (Maleki et al., 2010). The number of unfilled grains was 20% and 30% higher in the treatment of 100% chemical N fertilizer than in the treatments of Nitroxin + 50% chemical N fertilizer and seedling root inoculation with Nitroxin biofertilizer, respectively. Esfahani et al. (2005) reported an increase in the number of unfilled grains when N-containing fertilizers were applied excessively. They stated that the higher levels of N fertilization increased the number of spikelets per spike and this augmented the competition over carbohydrates, resulting in an increase in the number of unfilled grains per spike. Similar to our findings, Nikonejad et al. (2016) reported that unfilled grains were more at higher N levels, which can be ascribed to the production of more grains owing to excessive use of N fertilizer, but these more grains are left unfilled due to the assimilate limitation and the inter-plant competition increases the number of unfilled grains.

Thousand-grain weight is a key component of rice yield. This is a genetic and most stable varietal trait. The two studied cultivars differed in 1000-grain weight significantly ($P < 0.01$) so that 'Hashemi' had a nearly 5% higher 1000-grain weight than 'Gilaneh'. The treatments did not influence this trait significantly (Table 3). Nikonejad et al. (2016) who studied rice line 8615 reported that the effect of N rate was insignificant on 1000-grain weight. The effect of irrigation treatment (continuous flooding and irrigation intervals of 5, 8, and 11 days) was found to be insignificant on yield and yield components (Rezaei and Nahvi, 2007).

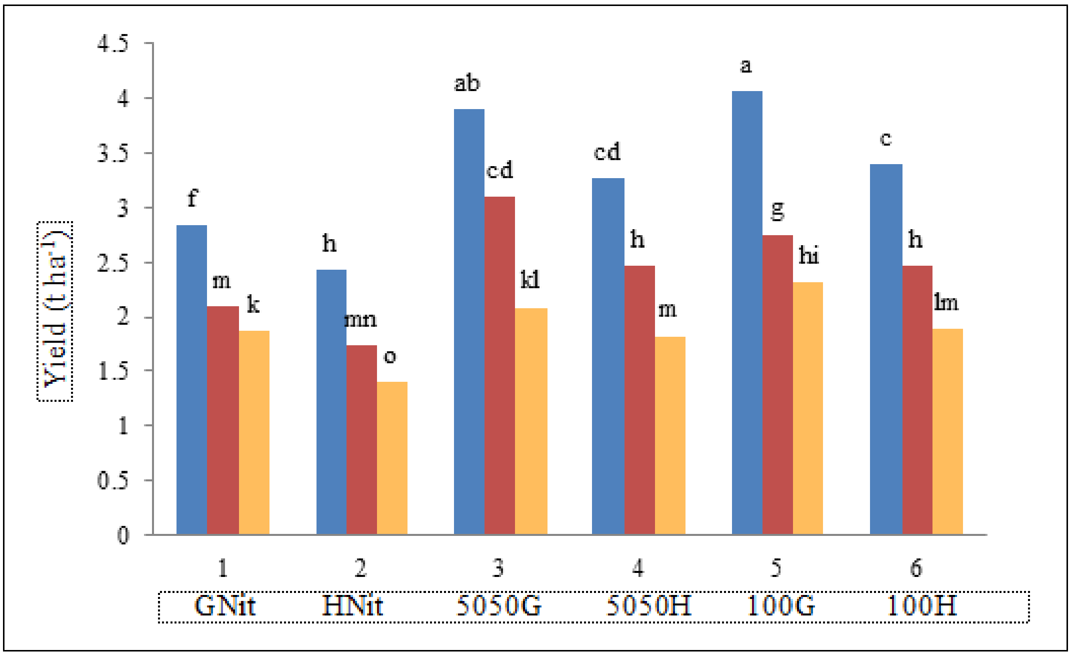

Figure 1. The effect of irrigation, fertilizer and cultivar on grain yield of two rice cultivars
(🟦submerged, 🟥10 days, 🟧15 days)
Inoculation of Gilaneh seedlings in nitroxin fertilizer (GNit); Inoculation of Hashemi cultivar seedlings in nitroxin fertilizer (HNit); Inoculation of seedling roots with nitroxin biofertilizer + 50% nitrogen chemical fertilizer in Gilaneh cultivar (5050G); Inoculation of seedling roots with nitroxin biofertilizer + 50% nitrogen chemical fertilizer in Hashemi cultivar (5050H); Consumption of 100% nitrogen chemical fertilizer in Gilaneh cultivar (100G); Consumption of 100% nitrogen chemical fertilizer in Hashemi cultivar (100H).

Table 3. Combined analysis of variance for effect of experimental treatments yield and yield components in two cropping years

Mean squares								
Source of variance	df	Grain yield (t ha^{-1})	Panicle No. (pan/m^2)	Grain No. (pan^{-1})	1000 grain weight (g)	Filled Grains	Unfilled Grain	Harvest index (kg kg^{-1})
Year	1	0.187*	2623.4*	47.7	0.0057	7.78	3.48	21.18
Block (year)	4	0.018	400.39	133.39	0.056	118.9	3.7	2.75
Irrigation	2	17.76**	8135**	2604.7**	0.342	1611.4**	25.1	2373.9**
Year×Irrigation	2	0.031	716.8	171.5	0.695	366.3	8.81	17.86
Irrigation×Block (year)	8	0.0086	242.5	903.25	0.155	287.8	27.4	3.05
Fertilizer	2	6.37**	33518.7**	1987.87**	1.111	955.3*	29.8	102.44**
Irrigation×Fertilizer	4	0.732**	5554.05**	878.83*	0.089	1024.2**	16.8	19.06
Year×Fertilizer	2	0.0081	1545.3	41.67	0.201	177.8	0.83	8.09
Fertilizer×Block (year)	8	0.011	501.03	398.89	0.304	270.3	12.6	9.04
Year×Fertilizer×Irrigation	4	0.124**	2707.47*	117.19	0.161	54.8	7.97	29.2
Cultivar	1	5.628**	48012.65**	4776.03**	42.75**	2196**	336.3**	497.6**
Year×Cultivar	1	0.099*	4979*	2.93	14.58**	135.5	0.507	53.06
Year×Cultivar×Irrigation	2	0.109*	325.6	289.7	0.668	88.7	3.43	4.73
Year×Fertilizer×Cultivar	2	0.104*	134.31	41.9	0.176	72.8	17.6	6.09
Irrigation×Fertilizer×Cultivar	4	0.189**	1454.57*	199.57	0.535	40.18	7.73	200.36**
Year×Cultivar×Fertilizer×Irrigation	4	0.81*	1381.8	66.99	0.31	37.8	12.89	38.64*
Error	52	0.017	544	223.2	1.18	167.7	10.77	8.02
% CV		5.17	9.49	14.75	4.43	13.7	51.1	7

* and ** Significant at 1 and 5% probability level, respectively.

Harvest index

Harvest index is defined as the ratio of grain yield to total aboveground biomass. Otherwise, harvest index define as a ratio between economical yield and biological yield (Elkheir et al., 2018). Based on the results of combined variance analysis, harvest index (HI) was significantly ($P < 0.01$) affected separatelly by the irrigation, fertilizer, and cultivar and the interractions of irrigation × fertilizer, year × irrigation × fertilizer, year × cultivar × irrigation, irrigation × fertilizer × cultivar, and year × cultivar × fertilizer × irrigation (Table 3). The comparison of average of HI revealed that HI was lower at more severe moisture stresses. This may be related to the adverse impacts of drought stress on the growth, biological yield, and grain yield. The loss of rice biomass due to water stress has been reported by many researchers (Rizwan et al., 2018; Pandey and Shukla, 2015). Cv. ‘Hashemi’ had lower HI than cv. ‘Guilaneh’. It seems that this difference in HI between the two cultivars is related to the decline of grain yield of ‘Guilaneh’ versus ‘Hashemi’. The highest HI was 51.7% observed in the treatment of ‘Guilaneh’ seedlings inoculated with Nitroxin, fertilized with chemical N to meet 50% of plant N requirement, and irrigated by the flooding technique. The lowest HI was 30.47% related to the treatment of ‘Hashemi’ seedlings inoculated with Nitroxin and irrigated at 15-day intervals. Similar to our findings, Zargari et al. (2014) asserted the effectiveness of biofertilizer on increase of HI at rice. In a study on the effect of biofertilizer on wheat growth and yield, Bakhshaei et al. (2014) reported that Nitroxin application enhanced HI. Also, a study reported the effect of Nitroxin and *Azotobacter* biofertilizers on rice growth and yield, according to which the biofertilizers were effective in improving HI (Rahmani et al., 2014). They found that Nitroxin produced the highest HI. A study on the interactive effect of *Azotobacter* and Pseudomonas biofertilizers and chemical N fertilizer on rice growth and yield revealed that HI was increased with an increase in N level, which could be associated with the increase in plant weight, growth, and biomass. The decline in the HI of the control treatment is likely to be related to the decline in grain and straw yield (Ghaffari et al., 2018), which is consistent with our findings.

CONCLUSIONS

The application of Nitroxin along with the chemical fertilization with N was partially effective in alleviating the adverse impacts of drought stress on rice and prevented the sharp decline of yield-related traits.

Seedling root inoculation with Nitroxin + 50% chemical fertilizer (N) increased grain yield versus seedling root inoculation with Nitroxin by 27% whereas 100% chemical fertilizer (N) increased it by 22%.

Out of the two studied cultivars, the improved cultivar 'Gilaneh' outperformed the local variety 'Hashemi' in grain yield.

In conclusion when the rice plants are exposed to deficit irrigation conditions, the application of Nitroxin along with N fertilizers improves the plants' nutritional status and growth and this can partially alleviate the negative effects of water deficit on growth and yield of rice.

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