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Nitrate pollution reduction using biological fertilizers in paddy fields, the South Caspian Sea basin, Guilan Province, Iran

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ABSTRACT

Application of chemical fertilizers, especially urea, causes groundwater pollution. Therefore, to decrease environmental pollution, biological fertilizers may be employed. In order to investigate the effect of irrigation levels, Azospirillum and nitrogen levels on rice yield and its components, an experiment was conducted in the north of Iran during the crop season in 2013. The experiment was arranged in a split-split plot based on a completely randomized block design with 3 replications in which irrigation level was the main factor. The levels of factors used were: I_1= continuous submergence and I_2 = 11 days' irrigation interval; A_1 = application and A_2= no application of Azospirillum as the sub factor; and nitrogen fertilizer levels as a sub-sub factor including$N_1 = 0$, $N_2 = 30$, $N_3 = 60$ and $N_4 = 90$ kg ha^{-1}. Continuous submergence and 11 days' irrigation interval produced 4247 and 2720 kg ha^{-1}, while the application of Azospirillum and the treatment without it produced 4064 and 2903 kg ha^{-1}, respectively. Moreover, N_4, N_3, N_2 and N_1 produced 3950, 3800, 3225 and 2700 kg ha^{-1}, while $I_1A_1N_4$ and $I_1A_1N_3$ had maximum and $I_2A_2N_1$ had minimum mean values with 5381, 5330 and 1882 kg ha^{-1}, respectively. Therefore, it is concluded that the application of a balanced nitrogen fertilizer with Azospirillum could prevent the indiscriminate use of chemical fertilizers and reduce nitrate pollution, leading towards more sustainable agriculture in the north of Iran.

Key words: biological fertilizer, nitrate pollution, paddy fields, rice.

INTRODUCTION

Water and nutrients may interact with each other to produce a coupling effect. Some studies indicated that there was a significant interaction between nitrogen application and water management on nitrogen absorption and utilization, and grain yield in rice (Sun *et al*. 2010, 2014). Water and nitrogen are important inputs for rice production. The scarcity of fresh water resources now threatens rice production in China (Yao *et al*. 2014).

Chemical fertilizers have been used for over 50 years in Iran. Over 61% of the chemical fertilizers used are nitrogen fertilizers, of which urea constitutes 90.9%. The average application rate of urea is 33.3 kg ha^{-1} in Iran, which are 2.55, 3.58 and 2.64 times of those in Spain, Australia, and Canada, respectively. Despite the very heavy application of urea, the average grain yield of cereal is much lower than in other countries. Furthermore, there is not a positive and significant relationship between urea application and grain yield in Iran. Nitrogen fertilizer application has increased so the possibility of nitrate contamination of groundwater is high in paddy fields in the north of Iran (Mashayekhi & Lashkari 2010). In one study, the content of nitrate in a well near the paddy fields was measured and it was found that there was a positive correlation between nitrogen fertilizer application and groundwater pollution. Some studies have also been carried out in the north of Iran to determine the nitrate concentration in the soil, as well as surface and shallow ground waters (Mashayekhi & Lashkari 2010). In 1996, sampling from surface and groundwater, including water in rice fields, rivers, drains, domestic wells and semi deep wells, was performed in Guilan and Mazandaran provinces in Iran. The results showed that the most nitrate fluctuations were related to domestic wells. In the wet season, 13% of samples and, in a dry season, 3% of samples had concentrations which exceeded the standard level of 10 mg L^{-1} (Malakutim 1996).

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Agricultural activities have a serious impact on nitrate contamination of groundwater (Macquarie *et al*. 2001; Buda *et al*. 2014).

Using nitrogen as a fertilizer and leakage of nitrate from livestock have decreased the ground water quality (Sweeten *et al.* 1995). Other studies have indicated that there was a close relation between nitrate contamination in groundwater and agricultural management (Hinkle & Tesoriero 2014). The utilization of biological nitrogen fixation technology can decrease the use of urea; prevent the depletion of soil organic matter and reduce environmental pollution. Nitrogen fixation by cyanobacteria helps to minimize the over-dependence on chemicals, particularly urea, in rice farming and also enhance the efficiency of nitrogen by releasing ammonia constantly to the rice crop (Geetha Lakshmi *et al*. 2012). Bio-fertilizers are becoming increasingly popular in many countries and for many crops, but very few studies on their effect on grain yield have been conducted in rice (Meynard Banayo *et al*. 2012).

The Azospirillums are soil bacteria capable of producing associative symbiosis in the roots of various plants including grain crops such as rice. The genus Azospirillum has several N-fixing species, which are rhizobacteria associated with monocots and dicots such as grasses, wheat, maize and *Brassica chinensis* L (Sing *et al*. 2011). The Azospirillums are the most famous microorganisms that can produce colonies in the rhizospher around cereals roots and lead to nitrogen fixation. Azospirillum strains have been isolated from rice repeatedly, and recently the strain *Azospirillum* sp. B510 has been sequenced (Kaneko *et al*. 2010).

Inoculation of plants with Azospirillum has been found to cause significant increases in growth and yield which is equivalent to that attainable by application of 15-20 kg N ha^{-1} (Rodrigues *et al*. 2008).

Phytohormone production and a beneficial effect on plant growth were also shown for a range of other microorganisms (Fernando *et al*. 2010).

Although in recent years the application rate of fertilizers in Iran has increased sharply and a large amount of fertilizers, in addition to that produced domestically, have been imported. Nevertheless, during this period, not only has the yield of crops not increased in accordance with the increased application rate of fertilizers, but also the yield of crops has declined. Reasons for this include water shortage, different irrigation methods, lack of scientific knowledge by farmers and methods of fertilizer application. Noteworthy, employment of nitrogen fertilizer is a very significant factor in the growth of rice. Since obtaining a higher grain yield is an important experimental goal, it has been suggested that 120 kg N ha^{-1} should be the basic fertilizer application model for further experiments of rice hybrids (Ashouri *et al*. 2013). A yield-increasing effect on rice by inoculation with *Azospirillum* sp. strain B510 has been shown but the experiment was conducted in pots only (Isawa *et al*. 2010). Results indicated that the influences of nitrogen on grain yield, straw yield, plant height, fertile tiller number/m^2 and panicle length were significant. Maximum yield and yield components were achieved by applying 90 kg ha^{-1} and maximum mean of grain yield equal to 4343 kg ha^{-1} was related to this treatment (Hatamifar *et al*. 2013). The objectives of the present study were to investigate the effect of irrigation, nitrogen fertilizer management and the application of nitrogen fixation bacteria on grain yield of rice. The effect of Azospirillum employment was assessed on reducing the application of nitrogen fertilizer in rice fields and reducing the pollution of groundwater.

MATERIALS AND METHODS

In order to investigate the effect of irrigation levels, Azospirillum and nitrogen fertilizer levels on yield and yield components of rice, an experiment was conducted in the north of Iran during crop season 2013. The experiment was arranged in split-split plot based on completely randomized block design with 3 replications in which water levels were the main factor including I_1 = continuous submergence and I_2 = 11 days' irrigation interval; A_1= application and A_2= no application of Azospirillum as was the sub factor; and nitrogen fertilizer levels were the sub-sub factor comprising N_1= 0, N_2 = 30, N_3 = 60 and N_4 = 90 kg ha^{-1}. Moreover, Nitrogen fertilizer was split into three important growth stages, transplanting (50%), tillering (25%) and booting (25%). For all treatments, drainage basins were mounted from which waste water belonging to each replication treatment was excluded. Each experimental plot had 15 lines with five meters in length and the planting scheme was 25 × 25 cm. For bacteria inoculation, roots of seedlings were inoculated with bacteria for at least 12 hours. The nursery construction was performed in April and transplanted to the field in early May. Fertilizer application was based on the soil test and instructions of the technicians of the Rice Research Organization in Iran and the amount of P and K was calculated and applied to every plot.

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The soil texture of the study area was clay-loam with a pH of 5.9., total organic matter, 3.08%, electrical conductivity, 0.68 ds m^{-1}, total nitrogen, 0.43%, available phosphorus, 19.2 ppm and available potassium, 95 ppm. Grain yield was measured with 6 m^2 harvesting of every plot. The yield and yield components were analyzed using MSTATC software. The Duncan's multiple range test was used to compare the means at 1% significance.

RESULTS AND DISCUSSION

Grain yield

The results showed that the irrigation levels, application of Azospirillum and nitrogen levels, had significant relationships with grain yield (Table 1). Continuous submergence and 11 days' irrigation interval produced 4247 and 2720 kg ha^{-1}, respectively (Table 2).

The Application of Azospirillum compared to treatment without it yielded 4064 and 2903 kg ha^{-1}, respectively (Table 2). N_4, N_3, N_2 and N_1 created 3950, 3800, 3225 and 2700 kg ha^{-1}, respectively (Table 2). Noteworthy, $I_1A_1N_4$ and $I_1A_1N_3$ had maximum while $I_2A_2N_1$ had minimum mean values with 5381, 5330 and 1882 kg ha^{-1}, correspondingly (Fig. 1).

Furthermore, N_2 fixing efficiency of Azospirillum isolates were positively correlated with the grain yield which were showed by increasing the plant growth parameters such as number of roots, length of roots, the number, length and width of leaves, length of stem, number of tiller and grains weight (Kanimozhi & Panneerselvam 2010). Fallah Amoli *et al*. (2014) showed that the treatments of Azospirillum and pseudomonas as well as 100% recommended N showed the importance of the role of PGPRs in appropriate transferring of nutrient and low pollution of environment, as well as the quality of rice and effective factors on quality based on genetic and breeding factors. In this study, local Tarom (rice) as a qualified cultivar was affected by N + PGPRs positively.

Number of tiller/m^2

The effect of irrigation levels, application of Azospirillum and nitrogen fertilizer as well as interaction of irrigation levels, application of Azospirillum and nitrogen fertilizer on Number of tiller/m^2 were significant (Table 1). Continuous submergence and 11 days' irrigation interval produced 407 and 288 tiller/ m^2 respectively (Table 2). The application of Azospirillum compared to treatment without it produced 406 and 290 tiller m^{-2} respectively (Table 2). N_4, N_3, N_2 and N_1 produced 427, 370, 324 and 268 tiller/m^2 respectively (Table 2). $I_1A_1N_4$ had maximum while $I_2A_2N_1$ had minimum mean values with 510 and 142 tiller/m^2 respectively (Table 3).

Number of panicle/m^2

The effect of irrigation levels, application of Azospirillum and nitrogen fertilizer as well as interaction of irrigation levels, application of Azospirillum and nitrogen fertilizer on Number of panicle/m^2 were significant (Table 1). Continuous submergence and 11 days' irrigation interval produced 347 and 245 panicle/m^2 respectively (Table 2). The applic- ation of Azospirillum compared to treatment without it produced 354 and 237 panicle/m^2 respectively (Table 2). N_4, N_3, N_2 and N_1 produced 373, 313, 280 and 225 panicle/m^2, respectively (Table 2).

$I_1A_1N_4$ had maximum, while $I_2A_2N_1$ had minimum mean values with 420 and 170 panicle/m^2 respectively (Table 3). Results indicated that the influence of nitrogen on grain yield, straw yield, plant height, fertile tiller number/m^2 and panicle length was significant. Maximum yield and yield components achieved by applying 90 kg ha^{-1} and maximum mean of grain yield equal to 4343 kg ha^{-1} were related to this treatment (Hatamifar *et al.* 2013).

Number of grains in panicle

The effect of irrigation levels, application of Azospirillum and nitrogen fertilizer along with interaction of irrigation levels, the application of Azospirillum and nitrogen fertilizer on the number of grains in panicle were recognized significant (Table 1). Continuous submergence and 11 days' irrigation interval produced 107 and 80 grains in the panicle, respectively (Table 2).

The application of Azospirillum and the treatment without it yielded 102 and 84 grains in the panicle, respectively (Table 2). N_4, N_3, N_2 and N_1 produced 100, 97, 90 and 84 grains in the panicle, respectively (Table 2). $I_1A_1N_4$ had maximum, whereas $I_2A_2N_1$ had minimum mean values of 116 and 60 grains in the panicle, respectively (Table 3). Consequently, the significant increase in the grain yield by Azospirillum application at 75 and 100% of the

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recommended dose of nitrogen (RDN) is mainly due to the higher number of panicles per m^2, the increase in the mean panicle weight and also 1000-grain weights (Nayak *et al*. 2003).

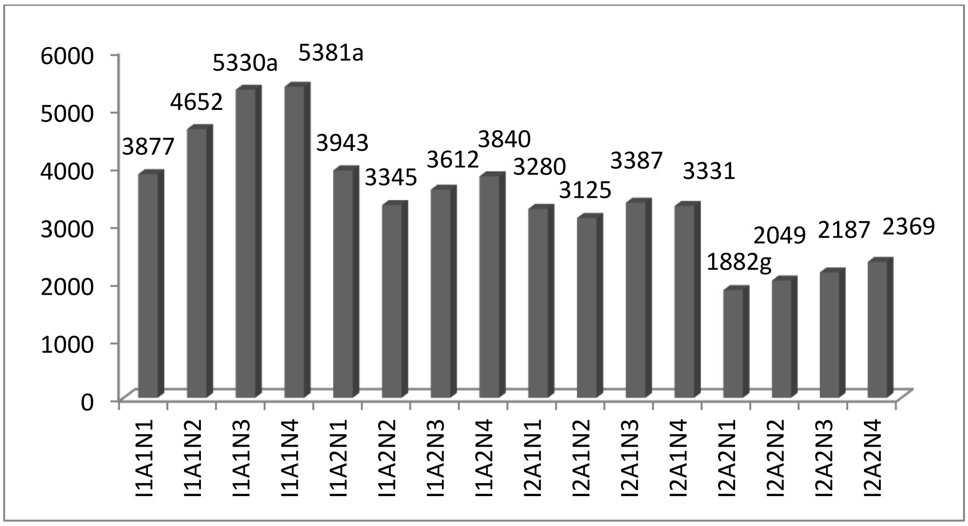

Fig. 1. Interaction of irrigation levels, application of Azospirillum and nitrogen fertilizer on grain yield.

Unfilled grain percentage

The effects of irrigation levels, application of Azospirillum and nitrogen fertilizer, as well as interaction of irrigation levels, application of Azospirillum and nitrogen fertilizer on unfilled grain percentage were significant (Table 1). Continuous submergence and 11 days' irrigation interval produced 7 and 11% unfilled grain, respectively (Table 2). The application of Azospirillum and the treatment without it produced 2 and 5% unfilled grain, respectively (Table 2). N_4, N_3, N_2 and N_1 produced 5, 6, 7 and 11% unfilled grain, respectively (Table 2). $I_1A_1N_4$ had maximum whereas $I_2A_2N_1$ had minimum mean values of 5.5 and 16.7% unfilled grain, respectively (Table 3).

1000-grains weight

The effects of irrigation levels, application of Azospirillum and nitrogen fertilizer along with interaction of irrigation levels, application of Azospirillum and nitrogen fertilizer on 1000-grains weight were significant (Table 1). Continuous submergence and 11 days' irrigation interval produced 28.5 and 22.3 g, respectively (Table 2). The application of Azospirillum and the treatment without it produced 26.5 and 24.4 g, respectively (Table 2). N_4, N_3, N_2 and N_1 produced 26.2, 25.8, 24.5 and 23.5 g, respectively (Table 2). $I_1A_1N_4$ had maximum while $I_2A_2N_1$ had minimum mean values of 29.5 and 20.5 g, respectively (Table3). It has been reported that application of N fertilizer increased 1000-grain weight of rain-fed lowland rice even when the rice crop was encountered to water deficit (Castillo *et al*. 1992).

Biomass

The effects of irrigation levels, application of Azospirillum and nitrogen fertilizer as well as interaction of irrigation levels, application of Azospirillum and nitrogen fertilizer on biomass were significant (Table 1). Continuous submergence and 11 days' irrigation interval produced 9103 and 6442 kg ha^{-1}, respectively (Table 2). The application of Azospirillum and treatment without it produced 8883 and 6602 kg ha^{-1} respectively (Table 2). N_4, N_3, N_2 and N_1 produced 8813, 8516, 7681 and 6860 kg ha^{-1} respectively (Table 2). $I_1A_1N_4$ had maximum whereas $I_2A_2N_1$ had minimum mean values of 12080 and 3931 kg ha^{-1} respectively (Table 3). Grain yield and biomass have been shown to increase as the applied N rate was increased (Ashouri 2015).

Harvest index

The effects of irrigation levels, the application of Azospirillum and nitrogen fertilizer along with interaction of irrigation levels, application of Azospirillum and nitrogen fertilizer on harvest index were significant (Table 1). Continuous submergence and 11 days' irrigation interval produced 47 and 42% respectively (Table 2).

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Table 1. Analysis of variance on yield and yield components of rice.

Harvest index	Biomass	1000-grains weight(g)	Unfilled grain (%)	Number of grains in panicle	Number of panicles/m^2	Number of tiller/m^2	Grain yield	df	S.O.V
1.573	20381ns	0.019ns	0.008^{ns}	2^{ns}	1028^{ns}	995^{ns}	289996^{ns}	2	R
8**	122985622**	470**	254**	8721**	125052**	168625**	28014352**	1	Irrigation(I)
3	26015	0.198	2	2	91	141	44044	2	Ei
0.738**	9954**	53**	67**	3870**	163333**	207375**	16154160**	1	Azospirillum(A)
4ns	72774ns	5ns	118^{ns}	1645^{ns}	1302^{ns}	792^{ns}	14770^{ns}	1	I×A
11	17637	0.029	0.213	0.332	169	240	89131	4	Ei
1**	11596693**	S	2**	551**	47048**	54417**	576230**	3	Nitrogen(N)
0.264ns	160939ns	s	3^{ns}	11^{ns}	920^{ns}	590^{ns}	58209^{ns}	3	I×N
0.005ns	361483ns	1ns	5^{ns}	35 ^{ns}	3993^{ns}	4451^{ns}	892136^{ns}	3	A×N
205**	514336**	10**	1**	61**	364**	813^{ns}	659577**	3	I×A×N
0.740	8062	0.175	0.555	1	243	227^{s}	659577	24	En
8.12	9.12	5.16	8.37	6.19	5.27	4.33	6.24	-	(%) CV

:**Significant difference at 1% level
:*Significant difference at 5% level
ns: non-significant difference

Table 2. The effect of irrigation levels, application of Azospirillum and nitrogen fertilizer on yield and yield components of rice.

Harvest index (%)	Biomass (kg ha^{-1})	1000-grains weight(g)	Unfilled grain (%)	Number of grains in panicle	Number of panicle/m^2	Number of tiller/m^2	Grain yield (kg ha^{-1})	Treatment
								Irrigation
47a	9103a	28.5a	7b	107a	347a	407a	4247a	I_1
42b	6442b	22.3b	11a	80b	245b	288b	2720b	I_2
								Azospirillum
46a	8883a	26.5a	2b	102a	354a	413a	4064a	A_1
44b	6602b	24.4b	5a	84b	237b	282b	2903b	A_2
								Nitrogen
39c	6860d	23.5c	11a	84d	225d	268d	2700c	N_1
42b	7681c	24.5b	7b	90c	280c	324c	3225b	N_2
45a	8516b	25.8a	6bc	97b	313b	370b	3800ab	N_3
45a	8813a	26.2a	5c	100a	373a	427a	3950a	N_4

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Table 3. Interactions of irrigation levels, application of Azospirillum and nitrogen fertilizer on yield and yield components of rice.

Harvest index(%)	Biomass (kg ha^{-1})	1000-grains weight(g)	Unfilled grain (%)	Number of grain in panicle	Number of panicle/m^2	Number of tiller/m^2	Grain yield (kg ha^{-1})	Treatment
42c	9281c	28.8bc	6.4gh	103d	317d	366e	3877b-d	I1A1N1
43bc	10830b	28.7bc	6.5gh	107c	367c	442cd	4652b	I1A1N2
45b	11800a	29.1b	6.2h	112b	408ab	483ab	5330a	I1A1N3
45b	12080a	29.9a	5.5i	116a	420a	510a	5381a	I1A1N4
43bc	7416g	27.2d	6.6gh	95e	242fg	292gh	3943bc	I1A2N1
44b	7595g	27.5d	6.9f	101d	258ef	315fg	3345c-g	I1A2N2
42c	8508e	28.1cd	7.1f	107c	317d	367e	3612b-e	I1A2N3
42c	9137f	28.5bc	7.4e	110b	358c	408d	3840b-d	I1A2N4
48a	6811d	21.7g	7.9de	79g	225fg	275h	3280c-g	I2A1N1
41d	7539h	23.2f	8.8de	92f	283de	333eh	3125c-g	I2A1N2
40de	8392g	24.7e	9.5d	102d	317d	367e	3387c-f	I2A1N3
39e	8629f	25.2e	9.7d	104d	408b	458bc	3331c-g	I2A1N4
48a	3931l	20.5h	16.7a	60j	170i	190j	1882g	I2A2N1
43bc	4763k	20.5h	14.5b	63i	187h	208i	2049fg	I2A2N2
41d	5366j	21.1gh	13.6bc	67h	208gh	267h	2187e-g	I2A2N3
39e	6102i	21.3gh	12.5c	70h	216gh	258h	2369d-g	I2A2N4

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The application of Azospirillum and the treatment without it produced 46 and 44% respectively (Table 2). N_4, N_3, N_2 and N_1 produced 45, 45, 42 and 39%, respectively (Table 2). $I_2A_1N_1$ and $I_2A_2N_1$ had maximum, while $I_2A_1N_4$ had minimum mean values with 48, 48 and 39%, respectively (Table 3). Drought stress had significant effects on harvest index, seed performance, straw performance, the number of fertile tiller/m^2, the number of full seeds, and seed emptiness rate (Sabetfar *et al*. 2013).

CONCLUSION

Irrigation interval of 11days compared with continuous submergence decreased grain yield to 56% because the number of tiller and panicle per square meter, number of grains in the panicle and the weight of 1000 grains decreased and unfilled grain percentage increased in the 11-days' irrigation interval. The use of biological fertilizer resulted in increased grain yield and it decreased the use of chemical fertilizers so that a reduced impact on the environment was achieved.

The application of different amounts of nitrogen showed positive effects on increasing grain yield and yield components. Also, the combination of applications of bacteria and different amounts of nitrogen improved these characteristics.

Therefore, it is concluded that the application of a balanced nitrogen fertilizer with Azospirillum could prevent the indiscriminate use of chemical fertilizers and reduce nitrate pollution, resulting in more sustainable agriculture in the north of Iran.

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کاهش آلودگی نیتروژن با استفاده از کود بیولوژیک در شالیزارهای برنج، جنوب حوضه دریای خزر، استان گیلان، ایران

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چکیده

استفاده از کودهای شیمیایی، به ویژه کود نیتروژن باعث آلودگی آب‌های زیرزمینی می‌شود. بنابراین یک راه برای کاهش آلودگی محیطی مصرف کود بیولوژیکی است. به منظور بررسی تأثیر رژیم آبیاری، آزوسپریلوم و سطوح کود نیتروژن بر عملکرد و اجزای عملکرد برنج آزمایشی در سال زراعی ۱۳۹۲ در شمال ایران انجام شد. آزمایش بصورت اسپلیت اسپلیت پلات (کرتهای دو بار خرد شده) و در قالب طرح بلوک‌های کامل تصادفی با سه تکرار انجام شد که در آن رژیم‌های آبیاری عامل اصلی و شامل: I1 = غرقاب دائم و I2 = 11 روز یکبار آبیاری، A1 = کاربرد و A2 = بدون کاربرد آزوسپریلوم ، عامل فرعی بود و سطوح کود نیتروژن شامل N1 = 0 ،N2 = 30 ،N3 = 60 و N4 = 90 کیلوگرم در هکتار به عنوان عامل فرعی فرعی بودند. در آبیاری غرقاب دائم و آبیاری ۱۱روزه عملکرد دانه به ترتیب به میزان ۴۲۴۷ و ۲۷۲۰ کیلوگرم در هکتار ، کاربرد آزوسپریلوم در مقایسه با عدم مصرف آن ۴۰۶۴و ۲۹۰۳ کیلوگرم در هکتار، در سطوح کود نیتروژن N4، N3، N2 و N1 به ترتیب به میزان ۳۹۵۰، ۳۸۰۰، ۳۲۲۵ و ۲۷۰۰ کیلوگرم در هکتار ودر I1A1N4 و I1A1N3 و I2A2N1 به ترتیب به میزان ۵۳۸۱، ۵۳۳۰ و ۱۸۸۲ کیلوگرم در هکتار بودند. نتایج حاصل از این آمایش نشان داد که مصرف متعادل کود نیتروژن همراه با آزوسپریلوم موجب کاهش بیش از ۳۰ درصد مصرف کود نیتروژن در شالیزار شده لذا آلودگی نیترات را کاهش داده و منجر به کشاورزی پایدار در شمال ایران می‌شود.

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