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Effect of seed inoculation with nitroxin and different urea chemical fertilizer levels on yield, yield components and oil yield of canola (*Brassica napus*L.)

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ABSTRACT
To evaluate the effect of nitroxin and urea chemical fertilizer on yield, yield components and oil yield of canola, an experiment was carried out at the Research Farm of the University of Tabriz, Iran in 2012. A factorial experiment based on randomized complete blocks design with three replications was conducted in this study. The first factor was the amounts of nitroxinbio-fertilizer in two levels (a_1: control and a_2: inoculation with nitroxin) and the second factor was the urea chemical fertilizer treatments (b_1, b_2, b_3, b_4 and b_5: utilization of 0, 80, 160, 240 and 320 Kg/ha urea chemical fertilizer, respectively). Siliques per plant, grain per siliques, grain and biological yield and grain oil yield were recorded.Results showed that urea fertilizer treatment had a significant effect on yield, yield components and grain oil yield of canola. Significant increase was observed in all characters with applying nitroxin bio-fertilizer. Interaction of effect of nitrogen and nitroxin treatments on grain yield and oil yield showed when nitrogen fertilizer was used, the highest grain and oil yield were obtained from the combined use of nitroxin and 320 Kg/ha urea chemical fertilizer, but difference between 240 Kg/ha and 320 Kg/ha urea was not significant.According to the results obtained, nitroxin bio-fertilizer and application of 240 Kg/ha urea is the best combination for canola grain and oil production in this research.

Key Words: Canola, Grain yield, Nitroxin, Oil yield, Urea.

INTRODUCTION

Oilseeds are the second important source of human energy requirements after cereals. Optimal use of agricultural inputs, especially fertilizer is one of the objections of soil fertility improvement in order to achieve high performances. Canola (*Brassica napus*L.) is considered as one of the most important oilseeds in recent decades. Canola with more than 40% oil and about 40% protein is one of the world's major oilseeds (Grant&Bailey 1993). Its oil also has potentially developed in the bio-diesel market. In addition to oil production, the leaves and stems of canola provide high quality fodder. Canola is one of the main oil crops in many countries especially in Iran.Plant nutrition is one of the most important factors that increase plant production. The use of chemical fertilizers has been increased worldwide for plant production (Abril*et al.* 2007) due to availability of inexpensive fertilizers (Graham & Vance 2000). The continued use of chemical fertilizers causes health and

environmental hazards such as ground and surface water pollution by nitrate leaching (Pimentel 1996). Intensive use of chemical fertilizers and other chemicals has produced environmental problems and increased production costs. There centesimo crisis and environmental problems has raised interest in environmental friendly sustainable agricultural practices, which can reduce input costs (Salantur*et al.* 2005). Canola is nitrogen demanding crop plant. Nitrogen plays vital role in its healthy growth and is one of the main precursors of protein which absorbs in the form of mineral, ammonium or nitrate by canola plant (Hopkins & Hunter 2004). The seed yield, total dry matter and harvest index in some genotypes of *Brassica napus*has been found to improve with higher rates of N (Kopsell*et al.* 2004). N2-fixing maybe important for plant nutrition by increasing N uptake by the plants and playing significant role as plant growth promoting rhizobacteria (PGPR) in the bio fertilization of crops.Nitroxin biological fertilizer contains the most effective nitrogen fixation bacteria of *Azotobacter* and *Azospirillium*, which stabilizes the nitrogen, balance absorption of micronutrient and macronutrient rate needed by plant, as it causes growth and development of root and shoots of plant by synthesis and excretion of stimulants of plant growth such as types of regulating hormones such as Oxine, and also production of different amino acids and types of antibiotics, Cyanide hydrogen, Siderophore, etc, and causes increase of quality and quantity of product by protecting root such as terrestrial pathogenic agents.*Azotobacter* is a nitrogen fixation bacteria of atmosphere, and the nitrogen fixation rate via this bacteria varies from 20-40 Kg/ha depending on bacteria strain, soil and climatic conditions of the region as it has been reported at 7-12% and maximum up to 39% in case of positive answer of product (Cardoso &Kuyper2006). Bio-fertilizer improves the chemical, physical and biological characteristics of soils and increases the yield and quality of crops. Thus, this study was designed to evaluate the effects of nitroxin bio-fertilizer on yield, yield components and oil production of canola under different urea chemical fertilizer treatments.

MATERIALS ANDMETHODS

Site description and experimental design

The field experiment was conducted in 2012 at the Research Farm of the University of Tabriz, Iran (latitude 38°05_N, longitude 46°17_E, altitude 1360 m above sea level). The climate of research area is characterized by mean annual precipitation of 285 mm, mean annual temperature of 10°C, mean annual maximum temperature of 16.6°C and mean annual minimum temperature of 4.2°C. Experiment was conducted in factorial within a randomized complete block design with three replications. The first factor was the amounts of nitroxinbio-fertilizer in two levels (a_1: control and a_2: inoculation with nitroxin bio-fertilizer) and the second factor was the urea chemical fertilizer treatments (b_1, b_2, b_3, b_4 and b_5: utilization of 0, 80, 160, 240 and 320 Kg/ha urea chemical fertilizer, respectively). All plots were irrigated immediately after sowing and all fertilizers treatments were applied before sowing. Hand weeding of the experimental area was performed as required.

Measurement of traits

To specify siliques per plant and grain per siliques ten plants were selected from the middle of the plots and then, they were measured. Also at maturity, to determine of grain and biological yield an area equal to 1 m^2 was harvested from middle part of each plot considering border effect. Harvested plants were dried in 25°^{C} and under shadow and air flow, and then grains were separated from the remains by threshing. Seed oil content was determined with using soxhlet apparatus and diethyl ether as a solvent. Oil yield were calculated via multiply oil percentage × grain yield.

Statistical analysis

Statistical analysis of the data based on factorial design was performed using MSTAT-C software. Duncan multiple range test was applied to compare means of each trait at the 5% probability level.

RESULTS AND DISCUSSION

Inoculation with nitroxin and application of urea chemical fertilizer had a significant effect onsiliques per plant of canola (Table 1). Maximum siliques per plant belonged to no inoculation treatment with application of 320 Kg/ha urea fertilizer, but difference with 240 Kg/ha and inoculation with nitroxin was not significant (227.5) (Fig. 1). The lowest of siliques per plant was observed in not inoculation (118.6) and control chemical fertilizer (Fig. 1). Studies of Moradi*et al.* (2011) on wheat showed that the using effective microorganisms as a bio-fertilizer increased all studied vegetative growth characters.

Effect of bio-fertilizer treatment, urea chemical application and interaction of urea ×nitroxin on grain per siliques was significant (Table 1). Bio-fertilizer caused to increasing grain number per siliques. Maximum number of grain per silique (37.6) was obtained by nitroxin bio-fertilizer and 240 Kg/ha urea chemical fertilizer (Fig. 2). Gan*et al.* (2004) revealed the positive effect of bio-fertilizer on the grain per siliques of mustard.Soleimanzadeh(2011) showed the positive effect of bio-fertilizers on the most plant growth parameters of sunflower.

Nitrogen and bio-fertilizer had significant effect on 1000 grain weight (Table 1). The highest

Table 1: Analysis of variance yield and yield components of canola affected by bio and chemical fertilizer treatments.

S.O.V	df	Mean Square							
		Siliques per plant	Grain per siliques	1000 grain weigh t	Biological yield	Grain yield	Harvest t index	Grain oil percentage	Grain oil yield
Replication	2	10.53	0.154	0.002	69269.4	1345.6	0.43	0.609	530.1
Bio-fertilizer	1	12346.4 **	158.74 **	2.144 **	37820395.2 **	899947.2 **	14.519 **	5.95 *	224354.6 **
Chemical fertilizer	4	6262.3 **	140.13 **	1.312 **	15094996.05 **	1159849.4 **	1.294	3.04 *	142579.5 **
Interaction	4	1888.3 **	21.139 *	0.262 *	2800772.6 **	206903.6 **	5.244 **	0.159	36369.2 **
Error	18	199.43	6.207	0.065	316155.5	8990.8	0.835	0.748	2584.5

* and **: Significant at 1 and 5% probability levels, respectively.

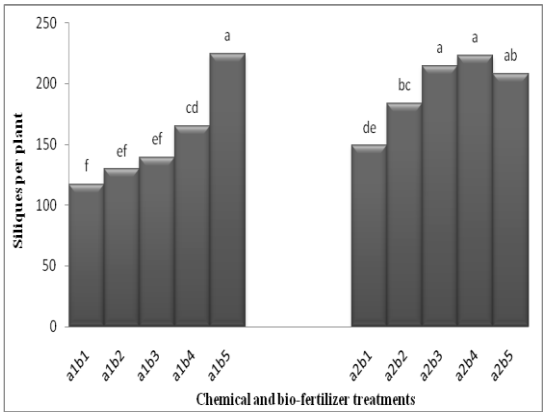

Fig. 1: Effect of different urea fertilizer levels (b_1, b_2, b_3, b_4 and b_5: utilization of 0, 80, 160, 240 and 320 Kg/ha urea chemical fertilizer, respectively) and bio-fertilizer (a_1: control and a_2: nitroxin) on siliques per plant of canola (Different letters indicate significant difference at $p \leq 0.05$).

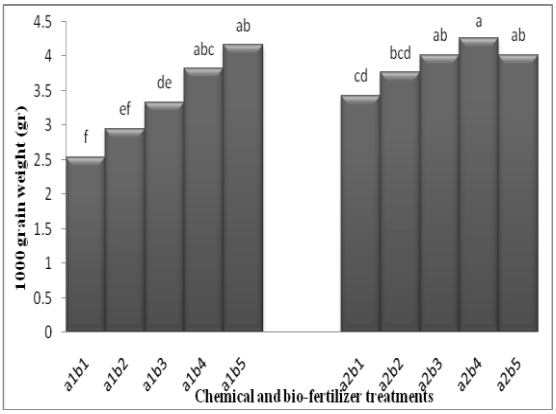

Fig. 3: Effect of different urea fertilizer levels (b1, b2, b3, b4 and b5: utilization of 0, 80, 160, 240 and 320 kg/ha urea chemical fertilizer, respectively) and bio-fertilizer (a1: control and a2: nitroxin) on 1000 grain weight of canola (Different letters indicate significant difference at $p \leq 0.05$).

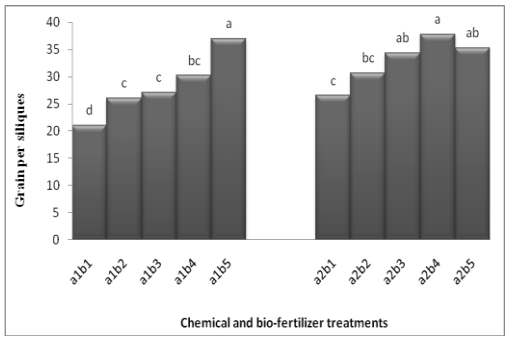

Fig. 2: Effect of different urea fertilizer levels (b_1, b_2, b_3, b_4 and b_5: utilization of 0, 80, 160, 240 and 320 Kg/ha urea chemical fertilizer, respectively) and bio-fertilizer (a_1: control and a_2: nitroxin) on grain per siliques of canola (Different letters indicate significant difference at $p \leq 0.05$).

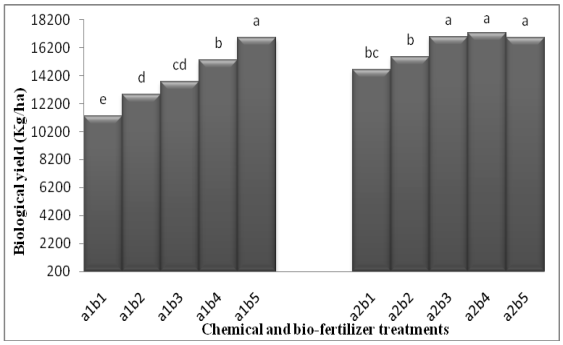

Fig. 4: Effect of different urea fertilizer levels (b_1, b_2, b_3, b_4 and b_5: utilization of 0, 80, 160, 240 and 320 Kg/ha urea chemical fertilizer, respectively) and bio-fertilizer (a_1: control and a_2: nitroxin) on biological yield of canola (Different letters indicate significant difference at $p \leq 0.05$).

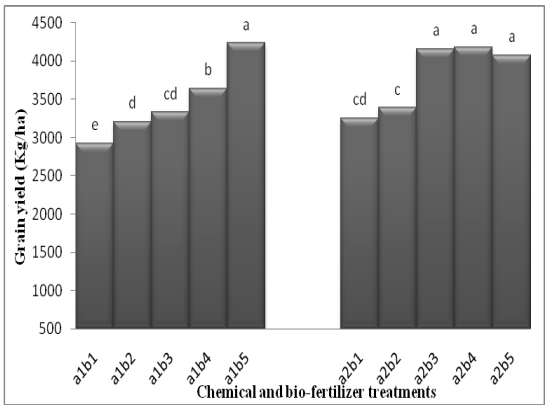

Fig. 5: Effect of different urea fertilizer levels (b_1, b_2, b_3, b_4 and b_5: utilization of 0, 80, 160, 240 and 320 kg/ha urea chemical fertilizer, respectively) and bio-fertilizer (a_1: control and a_2: nitroxin) on grain yield of canola (Different letters indicate significant difference at $p\leq 0.05$).

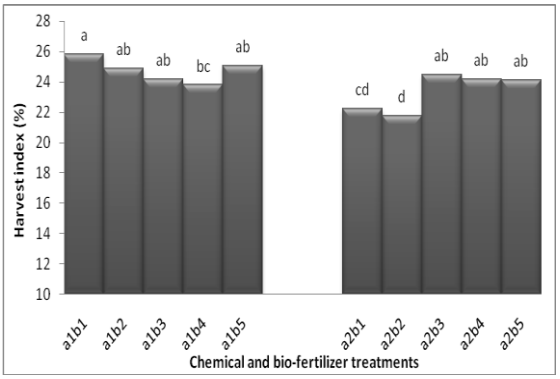

Fig. 6: Effect of different urea fertilizer levels (b_1, b_2, b_3, b_4 and b_5: utilization of 0, 80, 160, 240 and 320 Kg/ha urea chemical fertilizer, respectively) and bio-fertilizer (a_1: control and a_2: nitroxin) on harvest index of canola (Different letters indicate significant difference at $p\leq 0.05$).

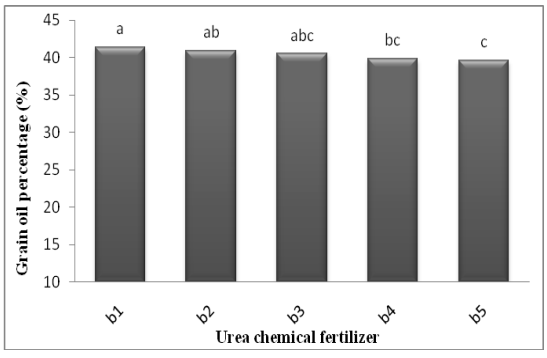

Fig. 7: Effect of different urea fertilizer levels (b_1, b_2, b_3, b_4 and b_5: utilization of 0, 80, 160, 240 and 320 kg/ha urea chemical fertilizer, respectively) on grain oil percentage of canola (Different letters indicate significant difference at $p\leq 0.05$).

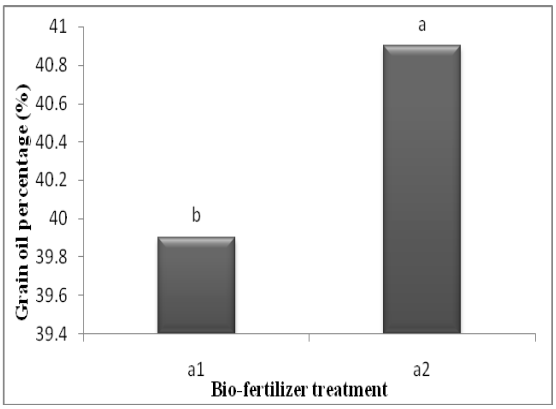

Fig. 8: Effect of bio-fertilizer (a_1: control and a_2: nitroxin) on grain oil percentage of canola (Different letters indicate significant difference at $p\leq 0.05$).

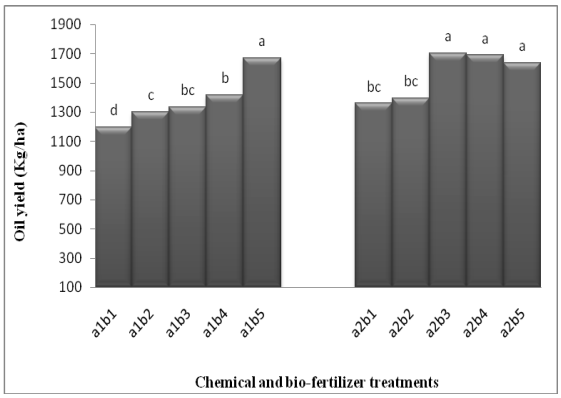

Fig. 9: Effect of different urea fertilizer levels (b_1, b_2, b_3, b_4 and b_5: utilization of 0, 80, 160, 240 and 320 kg/ha urea chemical fertilizer, respectively) and bio-fertilizer (a_1: control and a_2: nitroxin) on oil yield of canola (Different letters indicate significant difference at **$p\leq 0.05$**).

1000 grain weight was obtained from 240 kg/haN treatment. The trait of 1000 grain weight also increased due to inoculating of the grains with the bacteria under study in comparison with the case of no inoculation (Fig. 3). Bio-fertilizers may improve photosynthesis by increasing water and nutrients absorption leading to produce more assimilate and improve plant growth and thus, 1000 grain weight increased in comparison with no inoculation treatment. Hamidi*et al.* (2007) reported that 1000 grain weight of corn increased by inoculation of grain with PGPR bacteria compared to no inoculation.

Urea chemical fertilizer and inoculation with nitroxin have significant effects on the biological yield of canola (Table 1). Means comparison indicated that the maximum biological yield (17723 Kg/ha) produced by b_4(240 Kg/ha urea chemical fertilizer) and inoculation with nitroxin bio-fertilizer (Fig. 4), but difference

between b_2 and b_4 treatments was not significant. The minimum biological yield (11312 Kg/ha) caused by b_1 (no application of urea chemical fertilizer) and no inoculation of grains (Fig. 4). The positive effects of inoculation on plant growth were confirmed by various studies (Ibiene*et al.* 2012). Vijayan*et al.* (2007) demonstrated the beneficial effect of inoculation of *Azotobacterchroococcum*on biological yield.

The effect of bio-fertilizer application and urea fertilizer on grain yield of canola was significant (Table 1). The highest grain yield (4281 Kg/ha) was obtained at 320 Kg/ha urea chemical fertilizer and no inculcation of grains and the lowest (2910 Kg/ha) was obtained in no inoculation with bio-fertilizer and no application of urea, respectively (Fig. 5). N fertilizer in this study had a significant effect on canola grain yield. Another study on the effect of N fertilization on growth and yield components showed increase in canola grain yield (Ahmadi&Bahrani 2009). This finding was supported by Lin *et al.* (1983) who reported that application of *Azotobacter*and *Azospirillum*increased corn yield.

The obtained results showed that the interaction of nitrogen and bio-fertilizer was significant for the harvest index (Table 1). Maximum harvest index (25.8 %) was obtained from control urea treatment with no inoculation of nitroxin (Fig. 6). Similarly,Daneshvar*et al.* (2008) reported that N fertilizer increased harvest index of canola.

Our results showed that nitroxin and N fertilizer application had significant effect on grain oil percentage (Table 1). The highest grain oil percentage was obtained at control fertilizer treatment and inoculation of grains with nitroxin (Figs. 7 and 8).Analysis of variance indicated that interaction of N fertilizer and nitroxinsignificantly affected grain oil yield (Table 1).An increase in grain oil yield was observed underinoculation of grains with nitroxin (Fig. 9). Our results showed that grain oil yieldgradually increased ($p<0.01$) as N fertilizer levels increased from b1 to b_5, however grain oil percentage significantlyincreased in this condition.It seems that increase of grain oil yield from b_1 to b_5 treatments were due to increasing of grain yield under application of urea fertilizer. There are various studies on the effects of N fertilizerand bio-fertilizers on production of oil in different crops. Zaman Khan*et al.* (2004) employed different fertilizers and they found yield changes of canola in the first year but no changes of pepperyield in the second year. Sharief&Keshta(2002) foundincreasing in the oil yield of canola using bio and chemical fertilizers.

CONCLUSION

In this experiment, inoculation of canola seed with nitroxin showed significant effect on grain yield, oil yield and other studied traits. The highest oil and grain yield was obtained from inoculation of canola with nitroxin and application of 240 Kg/ha urea chemical fertilizer and difference with 320 Kg/ha urea was not significant for oil and grain production. It seems that can be saving to fertilizer by application of bio-fertilizer combined chemical fertilizer. Totally, the obtained results revealed that using bio-fertilizer combined with urea significantly improved the growth characters and grain and oil yield compared to control.

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