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# Changes of seed yield, seed protein and seed oil in rapeseed (*Brassica napus* L.) under application of different bio fertilizers

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## ABSTRACT

This experiment was lay out in order to evaluate the effects of different biofertilizers on seed yield, protein and oil of rapeseed in Iran. The experiment was a factorial design with three replications. Treatments were four nitrogen biofrtilizers (control ($N_1$), Nitroksin ($N_2$), Azotobacter ($N_3$) and Supernitroplat ($N_4$)) and three phosphate biofrtilizers ( control($P_1$), Biosfer phosphate ($P_2$) and Phosphate barvar2 ($P_3$)).In this study seed yield, seed protein, seed oil, protein yield and oil yield were determined. Results showed that that there were significant differences in the response of rapeseed to the effect of treatments on seed yield seed protein, seed oil, protein yield and oil yield. However, seed yield, seed protein, seed oil, protein yield and oil yield significantly higher in application of Azotobacter treatments. The highest among the phosphate biofertilizers, seed yield, seed protein, seed oil, protein yield and oil yield were belonged at application of Phosphat barvar2. Interaction between N×P shows that $N_3P_3$ treatment has the highest seed yield, protein yield and oil yield and the $N_1P_1$ treatment has the lowest seed yield, protein yield and oil yield and the differences were significant. In final results of this study reviled that application nitrogen and phosphate biofertilizers specially Azotobacter and Phosphat barvar2 increased seed yield, seed protein, seed oil, protein yield and oil yield under Iran environmental condition.

**Key words:** Bio fertilizer, Oil, Protein and rapeseed

## INTRODUCTION

Biofertilizer is a material containing microorganism(s) added to a soil to directly or indirectly make certain essential elements available to plants for their nutrition. Various sources of biofertilizers include nitrogen fixers, phytostimulators, phosphate solubilizing bacteria, plant growth promoting rhizobacteria, etc… (Shekh, 2006). Application of biofertilizers became of great necessity to get a yield of high quality and to avoid the environmental pollution (Shevananda, 2008). Bio-fertilizer usually contains microorganisms having specific function such as Azospirillum to fix N2 and P solubilizing bacteria to solubilize P from the soil and fertilizer to be available to the plants (Saraswati & Sumarno, 2008). Several

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researchers had conducted the experiments to evaluate the responses of various plants such as young Robusta coffee (Junaedi et al., 1999), soybean (Noor, 2003; Totok & Rahayu, 2007), and turfgrass (Guntoro et al., 2007) to the biofertilizer application, but the results were still inconsistent In a way, microorganisms serve as biofertilizers (El-kholy ., 2005). An example is the fungus Penicillium bilaii, which allows plants to absorb phosphates from the soil. It does this by producing anorganic acid which dissolves soil phosphates into a form which plants may use. In field experiments in Argentina, corn inoculated with Azospirillum lipoferum showed double the seeds per ear, an increase in seed dry weight by 59 % , and a significant stimulation in root development at harvest time (Fulchieri and Frioni, 1994). Another example is the bacterium Rhizobium. (Shekh, 2006). Use of these microorganisms as environment friendly biofertilizer helps to reduce the much expensive phosphatic fertilizers. Phosphorus biofertilizers could help to increase the availability of accumulated phosphate (by solubilization), efficiency of biological nitrogen fixation and increase the availability of Fe, Zn etc., through production of plant growth promoting substances (Kucey ., 1989). Increased root, shoot weight with dual inoculation in maize have been reported by (Chabot et al ., 1993), while grain yields of the different maize genotypes treated with Azospirillum spp. Seed inoculation with Rhizobium , phosphorus solubilizing bacteria, and organic amendment increased seed production of the crop(Panwar et al ., 2006). For give to highest seed yield in agriculture addition to both nitrogen and phosphate fertilizer is very important (Shaban, 2013a,b For give the highest seed yield and protein yield in barley (Azimi et al, 2013) and maize (Beyranvand et al, 2013) should apply both nitrogen and phosphate biofertilizers. Rapeseed (Brassica napus L.) was cultivated by ancient civilization in Asia and Middle East. Rapeseed meal has always been recognised as having the potential to be an alternative protein source for human consumption (Uppstrom, 1995). The most recent reviews on the subject have concluded that the presence of antinutritional factors such as glucosinolates, phytic acid, and phenolics, including sinapine and tannins remains a major drawback to their utilisation for food manufacture (Aider and Barbana, 2011; Tan et al., 2011). Proteins are the major constituent in oilseed meal and the growing demand for rapeseed oil world wide implies that more meal will be produced. Therefore, this alternative protein source needs further investigation. Oilseed protein isolates are normally prepared by direct alkaline extraction (DIR) in an environment of high pH followed by acid precipitation, presumably due to the high nitrogen yield obtained in the isolate (Pedroche et al., 2004). Apart from poor solubility issues, there is little information about the level of antinutritional factors in the isolates (Yoshie-Stark et al., 2008). however, not grown as an oilseed due to the low oil content, relatively high erucic acid and high glucosinolate content (Raney and Rakov, 2007). While the oil-free meal of S. alba possesses a relatively high protein content (45-48%) and the amino acid composition of the meal is fairly well balanced, its protein extracts also show useful technological functionalities (Aluko et al., 2005). In this study, therefore, we extracted the protein fractions from S. alba and B. napus oilseed meals by using both DIR and OSB methods to study the protein extractability and the contents of major antinutritional components of each fraction namely total phenolics, sinapine, and glucosinolate contents.Therefore this study was planned to examine effect of different biofertilizers on protein and oil of rapeseed at Iran.

## MATERIALS AND METHODS

This study was conducted in the Faculty of agronomy and plant breeding, Islamic Azad University, Boroujerd Branch, Boroujerd, Iran during the growing seasons 2012-2013. The experiment was lay out in order to evaluate the effects of nitrogen and phosphate biofertilizers on protein and oil of rapeseed (*Brassica napus* L.). The experimental region has a continental semi-arid climate with annual precipitation of 369 mm. The Okapi rapeseed cultivar was supply from station of agricultural research center, Hamedan provience, Iran. Soil of field was loam (pH= 7.9) with organic matter content 1.43%, 8.2

ppm of P and N 0.15%. The experiment was a factorial design with three replications. Treatments were four nitrogen biofrtilizers (control ($N_1$), Nitroksin ($N_2$), Azotobacter ($N_3$) and Supernitroplat ($N_4$)) and three phosphate biofrtilizers ( control($P_1$), Biosfer phosphate ($P_2$) and Phosphate barvar2 ($P_3$)).The Okapi rapeseed cultivar seeds were inoculate with biofertilizers before planting and seeds was planted in a 6m long, 10-row plot. Row to row and plant - plant distance was maintained at 30cm and, 4cm respectively. Plant samples for detrmined os seed yield were taken with 10 plants from each plot. To determine grain yieldwe removed and cleaned all the seeds produced within two central rows in the field. Then grain yield and biomass yield recorded on a dry weight basis. Yield was defined in terms of grams per square meter and quintals per hectare.

Seeds oil was determined by Nuclear Magnetic Resonance method in NMR system.Then oil yield was determined by following formula:

Oil yield= % oil × seed yield

Seed nitrogen determined by NMR system and total protein obtained by following formula:

Protein= %nitrogen×0.54

Protein yield was determined by following formula:

Protein yield= % protein × seed yield

The statistical analyses to determine the individual and interactive effects of time cultivation and weeds control methods were conducted using MSTAT-C and SPSS programs. Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$. Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis.

## RESULTS

### Seed yield

The effect of all treatments on grain yield was significant (Table 1). The comparison of the mean values of the grain yield showed that among the nitrogen biofertilizers, Azotobacter treatment has the highest (2816kg/ha) grain yield and spernitroplat treatment has the lowest grain yield (2442kh/ha) and the differences were significant (Table 2). Among the phosphate biofertilizers treatments, the highest grain yield (2679kg/ha) was belonged at application of Phosphat barvar2 and the lowest grain yield (2309kg/ha) was belonged at control (Table 2). Interaction between N×P (Table 3) shows that $N_3P_3$ treatment has the highest (3087kg/ha) grain yield and the $N_1P_1$ treatment has the lowest grain yield (1868kg/ha) and the differences were significant.

**Table1.** Analysis of variance (mean squares) for effects of different bio fertilizers on seed yield, protein and oil of rapeseed

| source | df | Seed yield | Seed protein | Seed oil | Protein yield | oil yield |
|---|---|---|---|---|---|---|
| R | 2 | 142237 | 2.44 | 2.12 | 2241 | 22838 |
| Nitrogen biofertilizer(N) | 3 | 620555** | 8** | 18.28** | 55101** | 195907** |
| Phosphate biofertilizer(P) | 2 | 41655** | 4.35** | 7.51** | 34321** | 120281** |
| N*P | 6 | 30652** | 0.76 | 0.01 | 2553** | 7520** |
| E | 22 | 1960.4 | 0.061 | 0.4 | 77.8 | 970.82 |
| CV | | 2.7 | 2.2 | 2.5 | 2.6 | 3.9 |

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

**Seed protein**

The effect of all treatments on seed protein was significant, excluding interaction between N×P (Table 1). The comparison of the mean values of the seed protein showed that among the nitrogen biofertilizers, Azotobacter treatment has the highest (22%) seed protein and control treatment has the lowest seed protein (20%) and the differences were significant (Table 2). Among the phosphate biofertilizers treatments, the highest seed protein (21.8%) was belonged at application of Phosphat barvar2 and the lowest seed protein (20%) was belonged at control (Table 2).

**Seed oil**

The effect of all treatments on seed oil was significant, excluding interaction between N×P (Table 1). The comparison of the mean values of the seed oil showed that among the nitrogen biofertilizers, Azotobacter treatment has the highest (43.48%) seed oil and Supernitroplat treatment has the lowest seed oil (40.9%) and the differences were significant (Table 2). Among the phosphate biofertilizers treatments, the highest seed oil (42.3%) was belonged at application of Phosphat barvar2 and the lowest seed oil (40.76) was belonged at control (Table 2).

**Protein yield**

The effect of all treatments on protein yield was significant (Table 1). The comparison of the mean values of the protein yield showed that among the nitrogen biofertilizers, Azotobacter treatment has the highest (628kg/ha) protein yield and control treatment has the lowest protein yield (441kg/ha) and the differences were significant (Table 2). Among the phosphate biofertilizers treatments, the highest protein yield (585kg/ha) was belonged at application of Phosphat barvar2 and the lowest protein yield (479kg/ha) was belonged at control (Table 2). Interaction between N×P (Table 3) shows that $N_3P_3$ treatment has the highest (701kg/ha) protein yield and the $N_1P_1$ treatment has the lowest protein yield (341kg/ha) and the differences were significant.

**Table 2.** Mean comparisons for effects of different bio fertilizers on seed yield, protein and oil of rapeseed

| treatments | Seed yield (kg/ha) | Seed protein (%) | Seed oil(%) | Protein yield(kg/ha) | oil yield(kg/ha) |
|---|---|---|---|---|---|
| Nitrogen biofertilizer(N) | | | | | |
| control ($N_1$) | 2190d | 20d | 40.17d | 441d | 882d |
| Nitroksin($N_2$) | 2591b | 21b | 41.94b | 560b | 1086b |
| Azotobacter($N_3$) | 2816a | 22a | 43.48a | 628a | 1226a |
| Supernitroplat($N_4$) | 2442c | 21b | 40.9c | 518c | 979c |
| Phosphate biofertilizer(N) | | | | | |
| control ($P_1$) | 2309c | 20c | 40.76c | 479c | 937c |
| Biospher phosphate($P_2$) | 2541b | 21.4ab | 41.8b | 545b | 1057b |
| Phosphat barvar2 ($P_3$) | 2679a | 21.8a | 42.30a | 585a | 1136a |

Means by the uncommon letter in each column are significantly different ($p<0.05$)

## Oil yield

The effect of all treatments on oil yield was significant (Table 1). The comparison of the mean values of the oil yield showed that among the nitrogen biofertilizers, Azotobacter treatment has the highest (1226kg/ha) oil yield and control treatment has the lowest oil yield (882kg/ha) and the differences were significant (Table 2). Among the phosphate biofertilizers treatments, the highest oil yield (1136kg/ha) was belonged at application of Phosphat barvar2 and the lowest protein yield (937kg/ha) was belonged at control (Table 2). Interaction between N×P (Table 3) shows that $N_3P_3$ treatment has the highest (1372kg/ha) oil yield and the $N_1P_1$ treatment has the lowest oil yield (721kg/ha) and the differences were significant.

**Table 3.** Interaction effect of treats for effects of different bio fertilizers on seed yield, protein and oil of rapeseed

| treatments | Seed yield(kg/ha) | Protein yield(kg/ha) | Oil yield(kg/ha) |
|---|---|---|---|
| $N_1P_1$ | 1868h | 346h | 721i |
| $N_1P_2$ | 2310f | 477g | 944gh |
| $N_1P_3$ | 2393g | 500f | 981fg |
| $N_2P_1$ | 25.2ef | 532e | 1031ef |
| $N_2P_2$ | 2593d | 561d | 1088cd |
| $N_2P_3$ | 2676c | 586c | 1139c |
| $N_3P_1$ | 2595d | 566d | 1100cd |
| $N_3P_2$ | 2777b | 517b | 1206b |
| $N_3P_3$ | 3087a | 701a | 1372a |
| $N_4P_1$ | 2276f | 475g | 897h |
| $N_4P_2$ | 2486f | 525e | 990fg |
| $N_4P_3$ | 2576de | 554d | 1051de |

Means by the uncommon letter in each column are significantly different ($p<0.05$)

Three nitrogen biofertilizers (control (N1), Nitroksin (N2), Azotobacter (N3) and Supernitroplat (N4)) and three phosphate biofrtilizers ( control(P1), Biosfer phosphate (P2) and Phosphate barvar2 (P3)).

## DISCUSSION

Table 1. indicates that there were significant differences in the response of rapeseed to the effect of treatments on seed protein and seed oil. According to the data of table 2, the effect of nitrogen and phosphate biofertilizers were evaluated positively, there were an increase in seed yield, seed protein, seed oil, protein yield and oil yield. In this study protein determined by NMR system, but there are many study that extract ant determined proteins by OSB fractions and DIR fractions and various extraction methods reported previously (Gillberg and Tornell, 1976; Xu and Diosady, 1994). According to Gillberg and Tornell (1976), rapeseed has a very complex protein composition, with widely different isoelectric points. This has apparently caused a considerably large amount of proteins which solubilised. Therefore, it may be concluded that photosynthetic capacity of plants treated with phosphors-solving microorganisms increases due to increased supply of phosphors nutrition. Seed weight also increases due to better transfer of photosynthetic substances. The content of rapeseed seeds in terms of conservation of plant materials is a function of numbers of endosperm and starch granules generated 10 to 14 days after pollination (Hay and Gilbert, 2001). In this present study in nitrogen biofertilizers treatments seed yield, seed protein, seed oil, protein yield and oil yield were highest in application of Azotobacter (table2). However, among the phosphate biofertilizers treatment Phosphat barvar2 has the positive effect on seed yield, seed protein, seed oil, protein yield and oil yield, compared to other P fertilizers. Therefore, reduced production of photosynthetic substances due to a smaller green surface area, decreased the conservation content of photosynthetic substances due to having short internodes or high levels of absysic acid during the above–mentioned critical period, restrict the seed yield variance analysis, the effects biological fertilizers. Results were similar to previous research(Shekh, 2006, El-kholy et al ., 2005 and Sarig et al ., 1990). Seed yield increase may under the effect of the phosphorus biofertilizer which induced the uptake ability of the roots to nutrients and positive increase in the yield parameters because of improving the root system as a source-sink relationship to the reproductive part (shoot), that agree with (Mohammed et al ., 2001 ), (Ozturk et al ., 2003) and (Panwar et al ., 2006). There were indications to shoot increase too under the effect of biofertilizer because there were general modifications in growth performance. Grain yield increasing was reported with the biofertilizer application which account important benefit to the rapeseed producers and rapeseed production, causing decreasing in the inputs of production because of economizing much money to chemical fertilizers and increasing in seed yield, which positively influenced the rapeseed photosynthesis and dry matter accumulation more actively that agree with (Lin et al ., 1983, Salmone and Dobereiner, 2004, Shevananda, 2008, and Darzi et al ., 2009). Long term field studies showed a significant contribution of biofertilizers for the yield increase of the field crops, which vary in range from 8–30% of control value depending on crop and soil fertility. The rhizosphere competence of native bacteria for C sources was major determinant for the success of inoculants (Gyaneshwar et al., 2002). Mixed microbial cultures allow their components to interact with each other synergistically, thus, stimulating each other through physical or biochemical activities (Vassilev et al., 2001). The interaction of N2-fixing bacteria with other bacteria could also inhibit their diazotrophic activity (Rojas et al., 2001). As well as increasing the availability of phosphorus for a plant microorganisms may release growth-increasing compounds such as oxin, gibberellin, and cytokines that are effective in increasing root and plant growth (Sattar and Gaur, 1987). Research by Ortas *et al.,* (1996) showed that these microorganisms increase absorption of food elements and yield by lowering the pH level in the soil. Results of this research clearly demonstrated the useful effect of integrating microbial fertilizer to increase seed yield, seed protein, seed oil, protein yield and oil yield under Iran condition. The research of various other studies has demonstrated that

mixed treatments increase plant vegetative growth, resulting in increased yield in crops and legumes under farm conditions (Hoflich *et al*., 1994). In final results of this study reviled that application nitrogen and phosphate biofertilizers specially Azotobacter and Phosphat barvar2 increased seed yield, seed protein, seed oil, protein yield and oil yield under Iran environmental condition.

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