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# Changes in oil and protein of soybean under application of biological fertilizers

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**ABSTRACT**: A field experiment was lay out in order to evaluate the effects of different biofertilizers on protein and oil of soybean in Iran. The experiment was a factorial design with three replications. Treatments were four nitrogen biofrtilizers (control (N1), Nitroksin (N2), Azotobacter (N3) and Supernitroplat (N4)) and three phosphate biofrtilizers ( control(P1), Biosfer phosphate (P2) and Phosphate barvar2 (P3)). In this study seed protein and seed oil were determined. Results showed that that there were significant differences in the response of soybean to the effect of treatments on seed protein and oil. However, seed protein and oil significantly higher in application of Azotobacter treatments. The highest among the phosphate biofertilizers, seed protein and oil were belonged at application of Phosphate barvar2. In final results of this study reviled that application nitrogen and phosphate biofertilizers specially Azotobacter and Phosphat barvar2 increased seed protein and oil.

**Key words**: Bio fertilizer, oil, protein and soybean

## INTRODUCTION

Soybean (*Glycine max* L.) is one of the most widely adopted grain legumes. Seeds for human consumption is the principal product of the soybean plant. Soybean is also used in the production of oil and protein. Rainfed crops grown in semiarid regions experience unpredictable water deficits during their life cycle. Lack of available water is the main factor limiting chickpea yields in the semiarid regions (Arya et al., 1998). Soil water and nutrients are the two key factors limiting agricultural productivity in the arid to semiarid areas (Ogola et al., 2002). However, recent studies have shown that crop yields are not necessarily decreased with a moderate level of water deficit under irrigation conditions (Zhang et al., 1998).

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 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).
Therefore this study was planned to examine effect of different biofertilizers on protein and oil of soybean 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 soybean. The experimental region has a continental semi-arid climate with annual precipitation of 369 mm. 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 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.

Seeds oil was determined by Nuclear Magnetic Resonance method in NMR system.
Seed nitrogen determined by NMR system and total protein obtained by following formula:

Protein= %nitrogen×0.54

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 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 (27%) seed protein and control treatment has the lowest seed protein (25%) and the differences were significant (Table 2). Among the phosphate biofertilizers treatments, the highest seed protein (26.8%) was belonged at application of Phosphat barvar2 and the lowest seed protein (25%) was belonged at control (Table 2).

Table1. Analysis of variance (mean squares) for effects of different bio fertilizers on seed protein and oil of soybean

| source | df | Seed protein | Seed oil |
|---|---|---|---|
| R | 2 | 2.69 | 3.11 |
| Nitrogen biofertilizer (N) | 3 | 9.2** | 15.33** |
| Phosphate biofertilizer (P | 2 | 7.66** | 6.99** |
| N*P | 6 | 0.623 | 0.05 |
| E | 22 | 0.0658 | 0.53 |
| CV | | 4.1 | 3.1 |

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

### 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 (38.48%) seed oil and Supernitroplat treatment has the lowest seed oil (35.9%) and the differences were significant (Table 2). Among the phosphate biofertilizers treatments, the highest seed oil (37.3%) was belonged at application of Phosphat barvar2 and the lowest seed oil (35.7%) was belonged at control (Table 2).

## DISCUSSION

There were significant differences in the response of rapeseed to the effect of treatments on seed protein and seed oil (table1). According to the data of table 2, the effect of nitrogen and phosphate biofertilizers were evaluated positively, there were an increase in seed protein and oil.

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), oil seeds has a very complex protein composition, with widely different isoelectric points. This has apparently caused a considerably large amount of proteins which solubilised.

Table2. Mean comparisons for effects of different bio fertilizers on seed protein and oil of soybean

| treatments | Seed protein (%) | Seed oil(%) |
|---|---|---|
| Nitrogen biofertilizer(N) | | |
| control ($N_1$) | $25^{d}$ | $35.17^{d}$ |
| Nitroksin($N_2$) | $26^{b}$ | $36.9^{b}$ |
| Azotobacter($N_3$) | $27^{a}$ | $38.4^{a}$ |
| Supernitroplat($N_4$) | $26^{b}$ | $35.9^{c}$ |
| Phosphate biofertilizer(N) | | |
| control ($P_1$) | $25^{c}$ | $35.7^{c}$ |
| Biosphere phosphate($P_2$) | $26.4^{ab}$ | $36.8^{b}$ |
| Phosphate barvar2 ($P_3$) | $26.8^{a}$ | $37.3^{a}$ |

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

Therefore, it may be concluded that photosynthetic capacity of plants treated with phosphors-solving microorganism's increases due to increased supply of phosphors nutrition. In this present study in nitrogen biofertilizers treatments seed protein and oil were highest in application of Azotobacter (table2). However, among the phosphate biofertilizers treatment Phosphate barvar2 has the positive effect on seed protein and oil compared to other P fertilizers. 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 protein and oil under Iran condition. In final results of this study reviled that application nitrogen and phosphate biofertilizers specially Azotobacter and Phosphat barvar2 increased seed protein and oil.

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