Evaluating the Effect of Bio Fertilizers and Nitrogen Fertilizer on Rate of Biological Nitrogen Fixation (BNF) and Nodulation of Cowpea Varieties in Ahwaz, Iran.

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ABSTRACT

In order to examine the effect of phosphate bio-fertilizer containing symbiotic and non-symbiotic bacteria and nitrogen fertilizer on indigenous and non-indigenous varieties of cowpea a factorial experiment in the form of randomized complete block design was carried out in Ahvaz in 2013. Biological fertilizers with trade name of phosphate (fertile 2) for soybean containing Brady rhizobium Japonicum and Nano biological fertilizer of Biozer containing Rhizobium Phaseolii, nitrogen fertilizer, and control treatment and the indigenous cultivars including Kamran, Baghdadi, Farokhshahi, and Texas Sabili (Mustache) and the non-indigenous cultivar of dwarf Italian were examined in three replications. The ANOVA results showed that there was a significant difference between the effect of fertilizer levels, varieties, and their interactive effect in terms of the number of nodes, active nodes, root dry weight, and the rate of biological nitrogen fixation. The highest rate in the mentioned factors belonged to the treatment with biological phosphate (fertile 2) for soybean and then Nano biological fertilizer of Biozer and the treatment with cultivar of Texas Sabili (mustache) and F3V4 and the lowest rate belonged to the treatment with nitrogen fertilizer and F1V1. In general, the treatment inoculated with biological fertilizers and indigenous varieties showed better biological fixation ability; therefore, the use of native cultivars and fertilizers containing symbiotic bacteria for biological nitrogen fixation in terms of economy and development of sustainable and organic agriculture can improve the cowpea production in the current conditions.

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INTRODUCTION

Providing sufficient nutrients is one of the most important constraints in the realization of potential yield of crops which has been removed in conventional farming by using chemical fertilizers. Nevertheless, environmental problems caused by excessive use of chemical fertilizers, energy and production costs and their adverse effects on biological cycles and self-sustainability of ecological farming systems on the one hand, and the problem of providing sufficient and quality food for the growing population of the world on the other hand, have made it necessary to revise the methods of increasing crops production in order to reduce the consumption of chemical fertilizers. To produce crops while reducing the use of chemical fertilizers in ecological farming systems in future, the development of methods for increasing the rotation of nutrients is very important [21].

Nowadays, what encourages developed countries to produce and use bio-fertilizers is their real attention to the negative environmental effects resulting from the unbalanced and excessive use of chemical fertilizers. Considering the annual consumption of over 850000 tons of chemical fertilizers in lands under the cultivation of legumes in Iran it is essential to provide for the farmers the effective and efficient inoculums of major legumes of the country such as cowpea which is one of the most important crops for human consumption through appropriate planning [16].

Cowpea (Vigna unguiculata L.) is an annual legume with quick growth and its growth period is reported to be 90 to 120 days [7]. The area under cultivation of beans in Iran and in Khuzestan Province in 2009-2010 was...
about 90844 and 14290 hectares respectively and the production rate was 254111 and 39915 tons. In terms of cultivation area and production rate Khuzestan is ranked as the second province after Lorestan [1].

In Iran few studies have been done on biological nitrogen fixation but in recent years special attention has been paid to this matter. A handful of studies have been done on beans particularly the cowpeas in Iran in relation to investigation of various legumes' germplasms in terms of adaptation with coexistent strains [23] such as the report made by Taherkhani [21].

In many parts of the country where the cowpea is cultivated, considerable amounts of nitrogen fertilizers are used which makes the effective native rhizobium's inactive and consequently the nodulation decreases. This not only imposes an expensive cost to the farmers but also causes environmental pollution. The present study has been done to select the best combination of cowpea cultivar and Brady rhizobium Japonicum and Rhizobium Phaseoli in Ahvaz. Mohammad Ali Khalaj et al. (2013) investigated the potential of biological nitrogen fixation by rhizobium strains in areas under the cultivation of beans in Ghazvin at two stages (50% flowering and harvest stage) to measure the desired traits (dry weight of shoot, number and weight of tubers, nitrogen uptake, and grain yield) for selecting the superior strain and reported that the results of two years of field experiments and the measurement of the desired traits showed that inoculation of bean seeds with effective strains of rhizobium increased the plant growth and grain yield and had a significant effect on reduction of nitrogen fertilizer consumption [15]. Mehrpooyan et al. (2009) examined the effect of several inoculants containing phaseoli bacteria on nutrients uptake in three varieties of bean during 2006 and 2007 in Khoramdarehin Zanjan Province and reported that in general, inoculation of different bean cultivars with bacteria strains increased the quantitative and qualitative traits compared with the treatment without inoculation and the consumption of 100 kg nitrogen fertilizer, in some cases. Rhizobium bacteria in addition to their very important role in nitrogen fixation can increase the growth and yield of crops by influencing better absorption of essential elements [18].

Mahdavi et al. (2006) investigated the effect of root zone temperature on nodulation and nitrogen fixation of three ecotypes of Zanjan, Ardebil, and Shahrekord in Lathyrus and showed that all three ecotypes at 25°C had the highest nodulation and nitrogen fixation and the temperatures of 5 and 10°C decreased nitrogen fixation and nodulation in the studied ecotypes [17].

Yadegari et al. (2004) examined the effects of inoculation of one cultivar and one line of soybean with different strains of symbiotic bacteria in order to determine the best combination of bacteria and cultivar in greenhouse conditions. They reported that acetic strains had better efficiency than other strains in nodulation and nitrogen fixation and soybean line was superior to Williams cultivar due to better coexistence and adaptation in the region. The highest amounts associated with the number of nodes per plant (13.2), dry weight of node (0.18 g per plant), plant dry weight at pod emergence stage (33.16 g) and the weight ratio of shoots to root (6.6) under greenhouse conditions belonged to the combination of acetic bacteria and line 11. Generally, this combination was selected as the best combination for soybean in Karaj [23].

Dadivar et al. (2005) evaluated the efficiency of rhizobium inoculants in major areas of bean cultivation in Markazi Province and reported that the use of inoculants increased the yield of bean compared to the treatment with consumption of nitrogen fertilizer, so that the increase rate compared to the fertilizer was 11% in Arak, 1.6% in Khomein, and 8.6% in Shazand. The increase of yield was significant in other cities except in Khomein where due to high consumption of nitrogen fertilizer and more cultivation experience the yield in treatment with rhizobium inoculation was not significantly different from the fertilizer treatment. Nevertheless, the consumption of rhizobium inoculants has positive effects on environment protection and the increase of yield and decrease of costs. As a result, the consumption of rhizobium bio-fertilizer is recommended in bean cultivating areas in Markazi Province [4].

Fernando et al. (2008) found that strains separated from fast growing species compared with strains separated from slow growing species are more effective in the formation of tuber and biological nitrogen fixation [8]. Christian et al. (2002) found that legumes such as beans under the shortage of nitrogen would have an important role in nitrogen fixation and its increase in soil and thus in some countries beans are planted as the soil enhancer [3]. In 1992, Vasilas and Nelson studied the effect of different rhizobium strains on beans in International Center for Tropical Agriculture (CIAT). In this experiment, 19 various and effective strains of rhizobium and two varieties of bean were examined in Latin American countries, England, and Canada. The highest rate of nitrogen fixation by 121 kg/ha belonged to Aurora variety. In this experiment it was identified that bean can provide about 50% of its nitrogen requirements. Various studies have been done in this regard in different regions and countries [22]. Evaluation of the best combination of fertilizer and cowpea varieties in order to optimum nodulation and highest amount of nitrogen fixation in Ahwaz, Iran, was purpose of this study.

MATERIALS AND METHODS

This experiment was carried out in 2013 in Shahid Mansour ZargaranKhozani Greenhouse in an area of 15 m² at latitude 31°18’55.5”N and longitude 48°41’16.9”E. in Ahwaz as the pot. In order to conduct the
experiment the factorial method was used in the form of randomized complete block design with three replications and 5 treatments of cowpea varieties and 4 fertilizer treatments based on the objectives which were considered for the project. The treatments contain different cultivars of cowpea including Kamran, Baghdadi, Texas-Sabili, Farokhshahr, and dwarf Italian cultivar and fertilizer treatments including Nanobiological fertilizer of Biozer, biological phosphate (fertile 2) for soybean, granular urea fertilizer, and control treatment without fertilizer. The soil of pots was prepared from a piece of land in Albaji Village in Ahvaz 15 km away from Ahvaz-Andimeshk road and it was carried to the laboratory in plastic bags for doing the necessary experiments. The experiments included the measurement of soil texture via hydrometric method according to Stock’s Law (the effect of mass and time on the collapse of soil particles) [9]. In order to measure the soil acidity, the saturated soil was prepared and after 24 hours by pH meter, the soil acidity was measured. Then, the extract of saturated soil was prepared by the suction apparatus and was used in the subsequent experiments [19].

The saturated mud was prepared from the soil sample and it was extracted. Then, the electrical conductivity of the soil was measured by EC meter [19].

In order to measure the total nitrogen the Kjeldahl method was used. At first, the soil was digested due to the heat and sulfuric acid and catalyst and then it was heated by soda in Kjeldahl distillation flask. Afterwards, the condensed liquid was titrated with sulfuric acid and finally, total nitrogen percentage was calculated [20].

According to the investigations and the information obtained from Ahvaz Agriculture Research Station, no legume has been cultivated in the selected part of the experimental field in recent years; therefore, the desired soil is relatively isolated in terms of the presence of cowpea legume symbionts. First of all, the base plan was designed in dimensions of 4×1.5 m and the pots were placed as the batches of 20 each with three replicates. The soil prepared from the field got upside down and leveled well and then the pots were filled with the soil as much as their capacity so that the soil was not compact. It was tried to provide appropriate drainage at the bottom of each pot to prevent the water logging in pots. In order to carry out the experiment, 60 pots (20 treatments with 3 replications) were prepared which were placed next to each other in categories of 4×5.

RESULTS AND DISCUSSION

Number of Nodes:

The ANOVA results showed that the number of nodes was significantly different at different levels of cowpea cultivars and fertilizer, i.e. different treatments of cowpea cultivars and different kinds of fertilizer had a significant effect on the number of nodes. Daneshian (1995) studied the interactive effect of Brady rhizobium strains and different cultivars of soybean on the number of nodes and referred to the significant effect of cultivar [5]. Hungeria (2000) studied soybean cultivars and Brady rhizobium bacteria and stated that the interactive effect of cultivar and bacteria on the trait was significant [12].

The mean of the number of nodes in cowpea cultivars of Baghdadi (10.72), Farokhshahri (10.83), and dwarf Italian (10.60) are placed in the same sub-group which indicates lack of difference among them. The number of nodes in cultivars of Kamran (13.83) and Texas-Sabili (14.61) are placed in the same sub-group and they are not significantly different from each other. The highest mean of the number of nodes is related to Texas-Sabili cultivar (diagram 1, Table 1).

Hafeez et al. did an experiment on the rate of nodulation of different cultivars of lentils with strains of leguminous bacteria and showed that strains and cultivars had a significant effect on the number of nodes [10]. Also, Dashti and Khodabande (2008) studied the symbiotic effect of Sino rhizobium meliloti strain on three alfalfa cultivars and referred to the significant effect of bacteria and cultivar on the number of nodes [6].

Diagram 1: Mean comparison of the number of nodes in different cultivars of cowpea
Comparing the number of nodes at different levels of fertilizer through Duncan's test showed that the highest mean of the number of nodes belonged to the treatments with bio-fertilizers containing Rhizobium Japonicum (20.42) and biological Nano fertilizer containing Rhizobium Phaseoli (18.09) which were categorized in the same sub-group and nitrogen fertilizer (0.778) and control (9.19) were in the same sub-group and were significantly different from biological fertilizers containing Rhizobium Japonicum and biological Nano fertilizer containing Rhizobium Phaseoli. Therefore, it is concluded that the use of biological fertilizers has an increasing effect on the number of nodes (Diagram 2 and Table 1). Comparison of the means showed that the interactive effect of different cultivars of cowpea and different fertilizers on the number of nodes was significant, so that the highest number of nodes by 25.557 belonged to F_3V_4 treatment and the lowest number by 0.233 belonged to F_1V_1 treatment. Studying the effects of inoculation of pea and faba bean with the strain of Azospirillum Brasilense in different conditions showed that the number of nodes significantly increased in crops treated with this strain under greenhouse conditions in comparison to the control treatment which used just the native bacteria [13]. The results were consistent with the findings of Karimi who studied the symbiotic effect of meliloti strains on three cultivars of alfalfa [14].

**Number of Active Nodes:**

The ANOVA results showed that the effect of different levels of cowpea cultivars, different levels of fertilizer and their interactive effect on the number of active nodes were significant.

Comparison of the means through Duncan’s method showed that the means of the number of active nodes in Baghdad (5.45) and Farokhshahri (5.27) were placed in the same sub-group which means they were not different from each other. The cultivars of Kamran (6.84) and Texas-Sabili (7.54) were placed in two sub-groups and there was a significant difference between them. Dwarf Italian cultivar (6.10) shares with this sub-group (Diagram 3, Table 1).

**Diagram 2:** Mean comparison of the number of nodes at different levels of fertilizer

**Diagram 3:** Mean comparison of the number of active nodes in different cultivars of cowpea
The mean of the number of active nodes is significantly different at different levels of fertilizer and each one is placed in one sub-group. Comparison of the means indicates that the use of biological fertilizers containing Rhizobium japonicum (15.21) and biological Nano fertilizer containing Rhizobium Phaseoli (8.61) increases the number of active nodes more effectively (Diagram 4, Table 1).

Mean comparison results show that the interactive effect of different cowpea cultivars and different fertilizer on the number of active node is significant (Table 1). Accordingly, the highest number of active node belongs to F_{3}V_{4} treatment (19) and the lowest number belongs to F_{1}V_{1} treatment (0.000). Vasilas and Nelson (1992) studied the effect of different strains of rhizobium on beans in International Center for Tropical Agriculture (CIAT). In this experiment, 19 various and effective strains of rhizobium and two varieties of bean (Kentwood, Aurara) were examined in Latin American countries, England, and Canada. The highest rate of nitrogen fixation by 121 kg/ha belonged to Aurara variety. In this experiment it was identified that bean can provide about 50% of its nitrogen requirements [22].

**Root Dry Weight:**

The ANOVA results showed that the root dry weight was significantly different at different levels of cowpea cultivars, different levels of fertilizer, and their interactive effect.

Comparison of the means through Duncan’s method showed that the means of the root dry weight in Farokhshahri (0.76) and dwarf Italian (0.77) were placed in the same sub-group which means they were not different from each other. The cultivars of Kamran (0.62), Baghdadi (0.57) and Texas-Sabili (0.98) were placed in different sub-groups and there was a significant difference between them. As it is observed, the mean of root dry weight is more in non-native cultivars (Diagram 5, Table 1).
Comparing the root dry weight at different levels of fertilizer through Duncan's test showed that the mean of root dry weight was significantly different at all levels of fertilizer and each one was placed in one sub-group. Comparison of the means show that the highest mean (0.944) belongs to the treatment with consumption of biological fertilizers containing Rhizobium Japonicum, and the treatment with biological Nano fertilizer containing Rhizobium Phaseoli by 0.708 has an increasing effect on the root dry weight (Diagram 6, Table 1).

Mean comparison results show that the interactive effect of different cowpea cultivars and different fertilizer on the root dry weight is significant. Accordingly, the highest dry weight of root belongs to F$_2$V$_4$ treatment (1.953) and the lowest dry weight of root belongs to F$_1$V$_1$ treatment (0.333) (Table 1). In this regard, Bailay (1988) in an experiment reported that the effect of different strains of Brady rhizobium Japonicum on the root weight of soybean cultivars was significant [2]. Hungeria et al. (2000) on the soybean [12], Hafeez et al. (2000) on different cultivars of lentil [10] Dashti and Khoda Bande (2008) on three varieties of alfalfa [6] got similar results.

**Root Nitrogen:**

The ANOVA results showed that the root nitrogen was significantly different just at different levels of fertilizer and the effect of different levels of cowpea cultivars and their interactive effect on the root nitrogen were not significantly different.

Comparing the root nitrogen at different levels of fertilizer through Duncan’s test showed that the mean of root nitrogen was significantly different at all levels of fertilizer and each one was placed in one sub-group. Comparison of the means show that consumption of biological fertilizers containing Rhizobium Japonicum by 0.762 and biological Nano fertilizer containing Rhizobium Phaseoli by 0.648 have the highest effect on the root nitrogen (Diagram 6, Table 1). In different experiments the rate of nitrogen fixation by rhizobium's has been estimated to be about 50% of the total nitrogen needed by plant. Due to insufficient rate of nitrogen fixation for bean, along inoculation the consumption of a little amount of nitrogen fertilizer as the starter can be effective [11].
The obtained results indicate the superiority of treatments which were fed with bio-fertilizers of Biozer and phosphate fertile 2 for soybean and the treatments which were fed with nitrogen fertilizer didn’t have any significant economic productivity. Thus, in order to improve biological nitrogen fixation in cowpea in Ahvaz and in order to economize the production and use of bio-fertilizers particularly phosphate fertile 2 for soybean the following points are suggested:

1. If possible, the native Brady rhizobium bacterium in the region should be identified and purified and used in the inoculants.
2. In order to protect the bacteria in inoculants against the environmental stresses the protective coating materials such as oil should be used.
3. For better verification of this investigation it is suggested to repeat the project with other cultivars and bio-fertilizers and even the inoculants of different strains of Brady rhizobium Japonicum, so that the best treatment for Khuzestan Province could be selected through the obtained results.

REFERENCES


