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Fertilizers and Inoculants Effects on Soybean Yield and Yield Components

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Abstract

Soybean (Glycine max L.) has been a significant source of plant origin proteins for both the livestock feed and humans industries for many years. Soybean yield is low in smallholder farms in Afghanistan. The objective of this study was to examine the impact of DAP, nitrogen fertilizer and Rhizobia on soybean yield and yield components performance. To achieve this objective, field experiments were conducted, using a randomized complete block design, with three replications, different levels of fertilizers, inoculant and control as well. Soybean responded remarkably to the added nitrogen, phosphorus sources fertilizers likewise bio fertilizer. The crop characters were significantly influenced by these treatments. Grain yields varied from 465 kg ha^{-1} to 2532.8 kg ha^{-1} in control treatment to 595 kg ha^{-1}. Analysis of variance was applied to find the effects of different levels of DAP, N and Rhizobia in yield and yield components of soybean. The results revealed that there was no significant differences in pod length, pods per plant, seeds per pod and 100 grain-weight at 5% level. The result of yield showed a significant difference at 1% level. Based on the results a positive correlation (0.15366) was between 100 grains weight and grain yield. There was a significant difference in days to flowering and plant height among treatments, the longest days to flowering was detected in Rhizobia treatments as well as the shortest days to flowering in DAP treatments. The amount of chlorophyll concentration was measured using SPAD 502, there was no a significant difference among treatments. It was ranged from 2.67 to 3 mg in all treatments. Cluster analysis using UPGAM was applied to determine the effects of different types of fertilizers on soybean agronomic relationship. Based on this analysis all treatments were classified in to three groups. This study's novelty is using rhizobia in Afghanistan.

Key words*: Fertilizers, Inoculation, SPAD – 502, Yield, Yield Components, Chlorophyll Concentration, Soybean*

Introduction

Soybean (*Glycine max*) as well known as a 'miracle crop' with over 40% protein and 20% oil, originated in China. As early as in 2853 BC, the Emperor Sheng-Nung of China named it as one of the five sacred seeds. Thus, soybean has been cultivated in China for more than 4,000 years. It is believed that with the development of sea and land trades, soybean moved out of China to nearby countries such as Burma (Myanmar), Japan, India, Indonesia, Malaysia, Nepal, the Philippines, Thailand and Vietnam between the first century AD and 1100 AD. Soybean is grown in more than 50 countries and is the leading oilseed crop produced and consumed worldwide (Wilcox, 2004). Soybean has now become the largest source of vegetable oil and protein in the world and its large-scale cultivation is concentrated in a few countries such as Argentina, Brazil, Canada, China, India, Paraguay and USA which together produce about 96 per cent of the world's, 189 million tons annual soybean production.

Several studies on N in soybean-based systems reported that N removal at harvest may exceed biological fixation (Harper et al., 1989; Peoples & Craswell, 1992; Vanotti & Bundy, 1995). The combination of high rates of N removal with a potential reduction of biological N fixation due to soil compaction may make it necessary to balance N , either through N fertilizer application or by enhancing nodulation and N fixation. Most studies on the effect of fertilizer-N on soybean growth and N fixation by rhizobium conducted elsewhere concluded that fertilization reduces N fixation through a reduction in the number, weight and activity of nodules (Starling et al., 1998; Chen et al., 1992). Coating soybean seeds with rhizobial inoculants has been suggested as a way to improve N fixation but the success of inoculation was found to be highly variable (Peoples & Craswell, 1992). Starter N increased caused N accumulation in plants but seed yields usually remained unchanged (Salvagiotti et al., 2008). Poor nodulation and variable response to inoculation is mainly attributed to intrinsic characteristics of the host plant (Michiels et al., 1998). As a result, application of high amounts of inorganic-nitrogen fertilizers is becoming a common practice which has detrimental environmental consequences (Salton et al., 2008). Biological nitrogen fixation reduces costs of production.

The use of inoculants as alternatives to N fertilizer avoids problems of contamination of water resources from leaching and runoff of excess fertilizer. Utilizing biological nitrogen fixation (BNF) is part of responsible natural resource

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management. Legume inoculants do not require high levels of energy for their production or distribution. Application on the seed is simple compared to spreading fertilizer on the field. Inoculants increase legume crop yields in many areas. Biological Nitrogen Fixation often improves the quality of dietary protein of legume seed even when yield increases are not detected. Through practices such as green manuring, crop rotations and alley cropping, N fixing legumes can increase soil fertility, permeability and organic matter to benefit non legume crops. Using BNF is part of the wise management of agricultural systems. The economic, environmental and agronomic advantages of BNF make it a cornerstone of sustainable agricultural systems. Grain yields varied from 1.0 t ha^{-1} to 1.7 t ha^{-1} in unamended control plots to 4.8 t ha^{-1} for common bean and 3.8 t ha^{-1} for soybean in inoculated plots with both P and manure addition (Rurangwa et al., 2018).Legumes comprise the most important plant families in agriculture. Many soils do not have sufficient numbers of appropriate rhizobia for maximum BNF. Rhizobial inoculants and legume crops must be properly matched. There are several methods of inoculating legumes. Inoculants require some special care to maintain their Viability Although BNF is a natural process, many soils do not have sufficient numbers of appropriate rhizobia for effective symbiosis (Denwar et al., 2012). Inoculating legume crops with compatible rhizobia ensures maximal BNF. Inoculation is especially important when introducing new legumes to an area (Dobbelaere et al., 2003; Zhang et al., 1997).
The main objective of this research was to compare different levels of Urea and DAP including Rhizobia treatments and their effects on soybean yield and yield components.

Materials and Methods

Variety of (Stine3400 - 2) was used as plant material

Different levels of chemical fertilizers (Urea fertilizer and DAP), and biological fertilizer (Rhizobia) were used to evaluate the effects of these fertilizers on yield and yield components of soybean. Inoculant and DAP were used at cultivation time, and N fertilizer was applied at different stages of plant growth.

Experimental site

Field trials were established in the experimental farm of Agriculture Faculty of Kabul University (34 `N 69`E) in 2016 and 2017. The soil physical and chemical properties were tested. Randomized complete block design was used with three replications and 36 plots. The area for each plot was prepared 3 x 2 m (6 m^2), the space between rows and plants considered 40 cm and 20 cm, respectively.
Germination test were applied to find germination capacity of soybean seeds, the result of germination test of this cultivar was more than 80%). The seeds were directly planted in the field.
Vernier clipper instrument was used to measure the length of pod and seed. SPAD (Soil Plant Analysis Development) used to measure the amount of N in the leaves.
Protein and oil analysis
DK Series Kjeldahl Apparatus Made in Italy (DKL 8 (8-position with 250 ml tubes, Ø 42 mm) was used to analyze the percentage of protein and oil of soybean (Figure 1). Statistical Tool for Agricultural Research (STAR), Version 2.0.1, January 2014 was used to analyse the data.

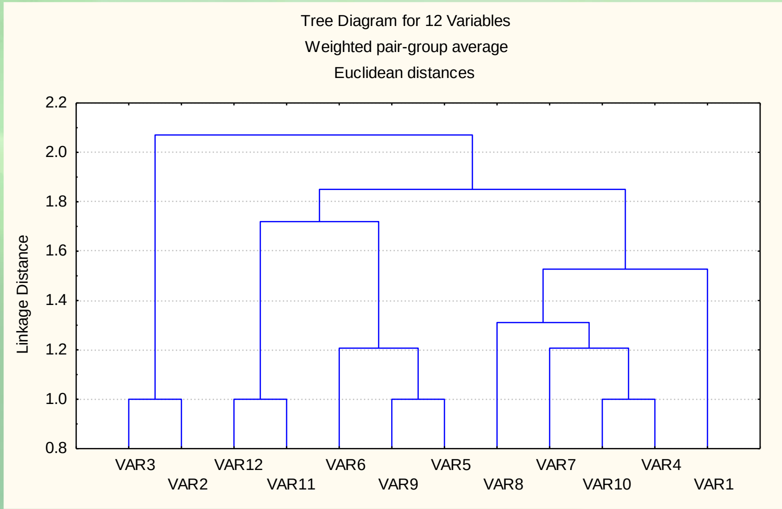

Figure 1. Tree diagram of different levels of DAP, N and Rhizobia and their effects on soybean yield, yield components and agronomic traits.

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Results and Discussion

Yield and yield components evaluation

Seed is the starting point of agriculture. Hence, the seeds were tested for percentage of germination and the result of germination was more than 80%. Yield is one of the main objectives for researchers and farmers. All efforts are accomplished for quantity and quality of products. Therefore, yield and yield components were measured and analyzed in the present research. Pod length plays a key role in number of seeds per pod, as much as longer pod produce much more seeds than the shorter one. In using different levels of inoculants, exhibited longer pod length (more than 42 mm) (Table 1).

Number of pod per plant is also one of the important yield components. It means large number of pods per plant performs higher yield than less number of pods per plant. In this research large number of seeds per plot detected in DAP levels and 125 kg ha^{-1} N as well. Hence, we can conclude that P is an important element in somatic growth of crops. Number of seeds per plot were 72.87, 74.33 and 127.40 seeds per pod in 50 kg ha^{-1} and 100 kg ha^{-1} DAP, and 125 kg ha^{-1} N respectively (Table 1).

Seed number per pod, seed appearance, seeds color, and seed quality play a major role in yield per unit area. Thus, we decided to measure and analyze this useful parameter. Seeds per pod showed the same response to DAP, N and Rhizobia (Table 1).

South Dokota Agriculture Station reported 4.97 t ha^{-1} yield (Kleinjan et al., 2018), but in this research the highest yield obtained from DAP application, which is 2.5 t ha^{-1}. As was observed with early growth, no significant effect of either soybean cultivar or N treatment was present at EVS91 or WGS91, with an average yield of 34.9 and 31.4 bu/acre, respectively (wood & Weaver, 1993). In this study the highest yield using N fertilizer was 0.9 t ha^{-1} that has a coincidence with the result of the above mentioned authors (34.9 Bu acre^{-1} is equal to 0.494 t ha^{-1}). Ntambo et al (2017) reported 0.5 t ha^{-1} of soybean yield using inoculant. When inoculated with Rhizobium sp. BARIRGm901, three of the four soybean genotypes tested (BARI soybean6 + R, MTD10 + R, and BGM02026 + R) showed significant increases ($p \leq 0.05$) in seed yields (3.60, 3.08, and 3.22 t ha−1, respectively) compared with plants of other treatments genotypes (Alma et al., 2015; Albareda et al., 2015). But in this study using different rates of inoculant, we obtained 1.008 t ha^{-1} of soybean yield that is two times higher than has been reported by Ntambo et al (2017).

100 grain-weight shows the healthiness of seeds. Because, wrinkled seeds are softer and smoother seeds are heavier. The smooth seeds are healthy and their yield performance is also

There is a strong coincidence between 100 grains weight and grain yield in this study. The different levels of DAP performed heavier grain weight as well as high yield than levels of N and Rhizobia. The yield of soybean in 125, 50, 75 and 100 kg ha^{-1} levels of DAP was 2532.8, 2061.8, 1567.2 and 1238.3 kg ha^{-1} respectively. The lowest yield was recognized in 120 kg h^{-1} level of N per hectare (Table 1).

Table 1. Evaluation of DAP, Nitrogen and Rhizobia treatments effects on yield and yield components of soybean.

Treatment	PL (mm)	PPP	SPP	100 GW (g)	GY kg ha^{-1}
125 DAP	39.16	66.93	3.00	11.23	2532.8 a
50 DAP	42.47	72.87	3.00	12.37	2061.1 b
75 DAP	40.58	55.47	2.67	12.27	1567.2 c
100 DAP	39.91	74.33	3.00	11.90	1238.3 d
125 N	41.18	127.40	3.00	12.37	900.6 ef
120 N	40.66	55.67	3.00	11.80	465 g
130 N	39.35	52.73	3.33	10.93	712.8 fg
135 N	39.11	53.07	3.00	10.90	1021.7 de
0 Control	41.91	41.27	3.33	12.03	595 g
0.2635 R (1X)	38.85	37.27	3.00	10.90	953.9 ef
0.527 R (2X)	42.09	54.87	3.00	11.33	903.3 ef
0.7905 R (3X)	42.37	58.60	3.33	10.63	1008.3 de
Mean	40.64	62.54	3.06	11.56	1163.3
F-test	ns	ns	ns	ns	**
SEM	1.11	17.19	0.23	0.62	94.9
SED	1.57	24.31	0.33	0.87	134.2
LSD (p= 0.05)	3.27	50.42	0.69	1.81	278.2
C.V	4.75	47.61	13.26	9.25	14.1

Pod Length (PL), Pod per Plant (PPP), Seed per Pod (SPP), 100 Grain Weight (100 GW), grain yield (GY), Means in a column with the same letter are not significantly different *, ** at P< 0.05 and P< 0.001, respectively, ns = Not significant, Di-Ammonium phosphate (DAP, Nitrogen (N), Rhizobia (R).

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Analysis of variance was applied to find the effects of different levels of DAP, N and Rhizobia among yield and yield components of soybean. The results revealed that there was no significant differences in pod length, pod per plant, seed per pod and 100 grain weight at 5% level. The result of yield showed a significant difference at 1% level (Table 1). Based on the results a positive correlation (0.15366) was between 100 grains weight and grain yield. Least significant difference test was applied to compare different treatments and there was similarity and differences among treatments (Table 1). Based on LSD 125, 50 and 75 kg ha^{-1} levels of DAP were different from the others. Coefficient of variation in pod per plant is larger but in the other parameters is lower than 20% and it shows the preciseness of the research (Table 1). Control exhibited the lowest yield (596 kg ha^{-1}) than DAP, N and Rhizobia treatments (Table 1).

Agronomic characters measurement and analysis

Agronomic characters play a useful role in increasing of crops yield. Days to flowering, nitrogen amount, plant height and branch per plant were measured and analyzed in this research. Based on the results, days to flowering were earlier in DAP treatments than N treatment and in N treatments earlier than Rhizobia treatments, respectively. Days to flowering in DAP different levels ranged from 65.33 – 70 days, in nitrogen different levels ranged from 67.33 – 70.67 days and in Rhizobia ranged from 70 – 73.33 days. Analysis of variance (ANOVA) was also applied to statistically compare the differences; the results of ANOVA were shown that there is a significant difference among treatments at 1% level. According to least significant difference test (LSD), Rhizobia treatments exhibited later flowering performance than DAP and N treatments (Table 2). We can conclude that it is the effect of N, which has been fixed by inoculants. Days to flowering adjustment are one of the important factors in breeding program as well as diseases and drought escape factors.

SPAD 502 chlorophyll meter made in Japan was used to measure chlorophyll concentration, in soybean plant. Application of this instrument helped the group to record chlorophyll concentration in different treatments. The results showed that the high level of chlorophyll recorded in DAP and Rhizobia treatments than N treatments. These results might be physiological expression between soil and atmospheric N. while the amount of N is low in the soil, chlorophyll concentration increases. Result of ANOVA revealed that there is no significant differences among treatments in chlorophyll concentration of different treatments. (Table 2).

Plant height is one of the key traits for obtaining high yield. Plant height has also been focused for measurement and analysis. The data were regularly collected and ANOVA applied to determine the differences among different levels of nutrients, which are important for plant growth. Outcome of ANOVA indicated that there is a significant difference among DAP, N and Rhizobia levels at 1% level. Least significant difference test LSD has also been applied and the results exhibited that the treatments were classified in different categories via this method (Table 2).

Desirable branching performs high yield, which is the main objective of researchers, specialists and farmers as well. The branching of soybean was measured in this study. Apparently, we can see some difference, but statistically there is no significant difference among treatments in branching of soybean plant (Table 2).

Table 2. Analysis the effects of DAP, N and Rhizobia on agronomic traits of soybean.

Treatments (Kg ha^{-1})	DTF	Chlorophyll concentration (mg)	PHT (cm)	BPP
125 DAP	68 bcd	39.00	58 a	3.80
50 DAP	65.33 a	41.00	65 a	5.27
75 DAP	66 ab	40.00	62 b	4.47
100 DAP	70 de	40.00	60 c	4.67
125 N	67.33abc	38.00	66 a	5.73
120 N	69.33 cde	38.00	47.3 g	3.33
130 N	70.67 e	38.00	53 e	3.47
135 N	68 bcd	39.47	50 f	4.07
0 Control	68 bcd	39.20	50 f	3.27
0.2635 R (1X)	73.67 f	40.00	51 f	4.13
0.527 R (2X)	73.33 f	41.00	57 d	4.40
0.7905 R (3X)	70.33 e	40.00	61 bc	4.87
Mean	69.17	39.47	0.68	4.29
F-test	**	ns	**	ns
SEM	0.75	0.74	56.69	0.72
SED	1.06	1.04	0.96	1.02
LSD (p= 0.05)	2.20	2.16	1.99	2.11
C.V	1.88	3.23	2.07	29.01

Days to Flowering (DTF),SPAD and plant height (PHT),Pod per Plant (PPP) , Means in a column with the same letter are not significantly different *, ** at P< 0.05 and P< 0.001, respectively; NS= Not significant, Di-Ammonium phosphate (DAP, Nitrogen (N), Rhizobia (R) .

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Cluster analysis

The term *cluster analysis* (first used by Tryon, 1939) encompasses a number of different algorithms and methods for grouping objects of similar kind into respective categories. The *EM* algorithm for clustering is described in detail in Witten and Frank (2001).

Cluster analysis using UPGAM was applied to determine the effects of different types of fertilizers on soybean agronomic relationship. Tree Diagram for 12 Variables Weighted pair-group average Euclidean distances was used to detect the relationship among 12 treatments, which were applied in this research. Based on the cluster results these treatments were classified into three main groups and each main group divided into some sub groups. Main group (I) was consisted of 50 and 75 kg ha^{-1} DAP, these variables exhibited similar effect on soybean different parameters. Main group (II) was consisted of 0.7905 and 0.5270 kg/ha of inoculant, 120 and 125 kg/ha N, and 0 levels (control), these variables had also the same effect on soybean growth. Main group (III) was consisted of 130 and 135 kg/ha N, 0.2635 kg/ha inoculant and 100 and 125 kg/ha DAP, these variables, classified in the same class, so performed the same expression on soybean life from different points of view (Figure 2). According to this cluster group, we can conclude that the treatments under the certain group had similar effects on yield, yield components and agronomic characters.

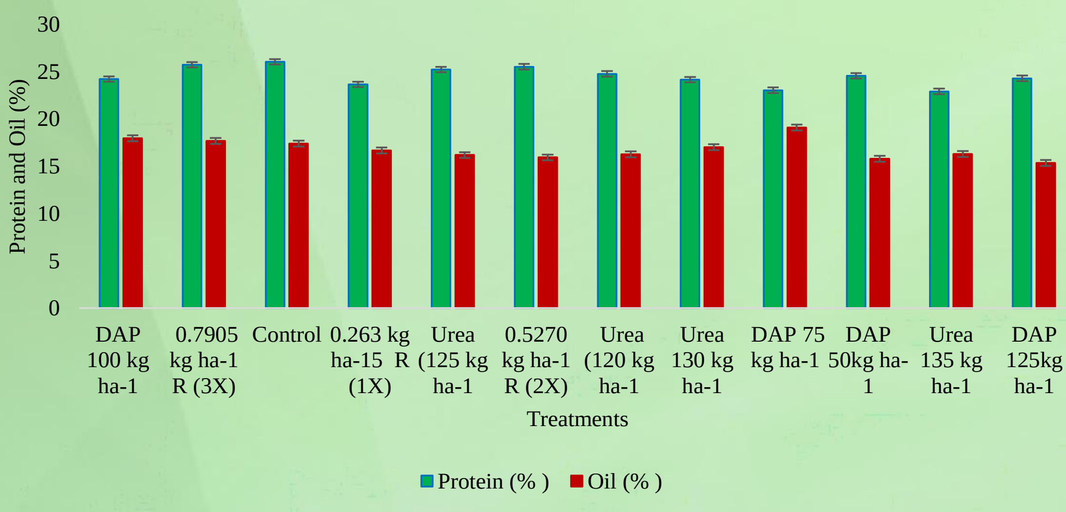

Figure 2. Effects of different levels of chemical and biological fertilizers on soybean Protein and Oil percentage.

Protein and Oil percentage analysis

Based on laboratory results using DKL 8 (8-position with 250 ml tubes, Ø 42 mm) instrument, the highest level of protein was detected in control and 3X Rhizobia treatments (26.03 & 25.71% respectively) and the lowest level of protein has been detected in 135 kg ha^{-1} Urea treatment (22.9%). The oil percentage has also been analyzed and based on the results, the highest level of oil was recognized in 75 kg ha^{-1} DAP treatment (19.08%) and the lowest level was found in 125 kg ha^{-1} DAP treatment (15.35%). Mainly there was no big differences of protein and Oil among treatments using the chemical and biological fertilizers.

Economic Return

Higher cost of cultivations are under inoculant rhizobium treatments (US $. 3504, 2450 & 1396 ha^{-1}) which it was because of the highest price of inoculant rhizobium (US $. 4 g^{-1}).

Maximum net return was obtained from 125 and 50 kg-DAP ha^{-1} in the value of (US $.1377.07 – 1243.33 ha^{-1}). As medium NR was obtained from treatments 75,100 DAP ha^{-1} and 100 Urea ha^{-1} ranging from (US $. 973.60, 812.40, 716.93 ha^{-1}) and the lowest NR ranging from (US $. 528.40, 474.53, 440.13, 276.93 ha^{-1}) was obtained from treatments 250 Urea, control, 200 Urea and 150 Urea ha^{-1} respectively (Table 3).

The economic analysis revealed that the highest net benefit of (US $.1377.07 ha^{-1}) was obtained from the application of 125 kg ha^{-1} DAP fertilizer whereas the rhizobium treatments gave the negative net returns (US $. -2406.67, -1453.73, -431.47 ha^{-1}) (Table 3) .

Therefore, with economic basis, the application of 125kg DAP ha^{-1} , 50 kg DAP ha^{-1} and in the case of Urea 100 kg ha^{-1} on soybean would be recommended in Kabul province of Afghanistan.

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Table 3. Cost benefit analysis of chemical and biological fertilizers application.

Treatments	Cost of Cultivation (US $/ha)	Gross Return (US $/ha)	Net returns (US $/ha)	Net returns per US $ invested
T1	425.33	1802.40	1377.07	3.24
T2	375.33	1618.67	1243.33	3.31
T3	392.00	1365.60	973.60	2.48
T4	408.67	1221.07	812.40	1.99
T5	382.00	1098.93	716.93	1.88
T6	402.00	678.93	276.93	0.69
T7	422.00	862.13	440.13	1.04
T8	442.00	970.40	528.40	1.20
T9	342.00	816.53	474.53	1.39
T10	1396.00	964.53	-431.47	-0.31
T11	2450.00	996.27	-1453.73	-0.59
T12	3504.00	1097.33	-2406.67	-0.69

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