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Research Article

Inoculated Soybean Response to Starter Nitrogen in Conventional Cropping System in Moghan

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Abstract

To investigate the effects of inoculated seeds at different amounts of nitrogen on soybean growth and yield, we conducted a randomized complete block design with three replications on the Research Farm of the Moghan College of Agriculture and Natural Resources. Treatments were 0, 50, 100 and 150 kg ha⁻¹ urea; inoculation with Biosoy, 50 kg ha⁻¹ urea+inoculation with Biosoy, 100 kg ha⁻¹ urea+inoculation with Biosoy, 150 kg ha⁻¹ urea+inoculation with Biosoy. Increasing nitrogen fertilizer in non-inoculated plants reduced nodule dry weight but enhanced plant height, stems dry weight, the pods dry weight, biological yield and grains per plant. When seeds inoculated, non-application and application of 100 kg ha⁻¹ urea produced more stems dry weight, pods dry weight, biological yield, grain numbers per plant and grain yield which were as much as application of 150 kg ha⁻¹ urea for non-inoculated plants. Therefore, based on this investigation results we suggest applying of diammonium phosphate as a P fertilizer at the rate of 200 kg ha⁻¹ +non application or 50 kg ha⁻¹ urea application plus inoculation of soybean seeds by Biosoy in Moghan region. As a result, we can save all or part of the urea fertilizer.

Key words: Biosoy, nitrogen, nodule, soybean, yield

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The global population continues to rise by about eighty million people per year. There is, therefore, an urgent need for improved varieties of crops, such as soybean that can sustain high yields even on poor soils (Park *et al.*, 2011). Soybean is the most important grain legume crop in the world and among the top five of all food crops grown (Sheaffer *et al.*, 2009). Soybeans can suffer from nitrogen deficiency under field conditions, particularly at flowering when the nodules start to senescence or when seeds are either planted without inoculation of soils with proper symbiotic bacteria, particularly in areas where soybean has not been grown before, or on acid soils that prevent successful nodulation (Mengel and Diaz, 2012). Nitrogen is a major limiting factor in plant growth and development (Albareda *et al.*, 2009). Osborne and Riede (2006) reported that application of nitrogen at 0, 8, 16 and 24 kg N ha⁻¹ increased significantly plant biomass, growth and grain yield. Yet, excess use of N-fertilizer wastes non-renewable resources and often results in environmental pollution. In contrast, biological nitrogen fixation uses photosynthetically produced energy and is environmentally cleaner (Albareda *et al.*, 2009). Biological Nitrogen Fixation (BNF) is an important part of sustainable agriculture (Sessitsch *et al.*, 2002) and growers apply rhizobial inoculum often as bio-fertilizers. Inoculation of seeds with *Rhizobium* increases nodule number (Salih *et al.*, 2015; Tahir *et al.*, 2009) and its dry matter (Zuffo *et al.*, 2015; Mohamed and Hassan, 2015). Nodules are tissues that capture N₂ from air and give it to plant as ammonia. According to Hungria *et al.* (2006), the amounts of nitrogen fixed by soybean through BNF was up to 300 kg N ha⁻¹, supplying up to 94% of crop needs. The fixed nitrogen leads to increase relative growth rate (Salih *et al.*, 2015). Several studies reported significant increase in soybean growth parameters and grain yield due to inoculation of bradyrhizobial isolates (Purcino *et al.*, 2000; Pant and Prasad, 2004; Soe *et al.*, 2010; Jalaluddin, 2005; Kala *et al.*, 2011). Salih *et al.* (2015) reported that relative growth rate and grain yield was much higher in combined of *Bradyrhizobium* and 100 kg ha⁻¹ diammonium phosphate. Plant height and shoot dry matter subjected to application of *Bradyrhizobium japonicum* in soybean were higher (Zuffo *et al.*, 2015). Also, Mohamed and Hassan (2015) reported that inoculated plants produced higher nodule dry weight, grain yield, number of filled pods, seed numbers compared to un-inoculated plants.

The purpose of this experiment was to test reducing the use of urea fertilizer in conventional cropping systems using Biosoy bio-fertilizer in Moghan.

MATERIALS AND METHODS

To investigate the effects of nitrogen fixing bio-fertilizer at different levels of chemical fertilizer, urea, we conducted a field experiment on the Research Farm of the Moghan College of Agriculture and Natural Resources, University of Mohaghegh Ardabili (39°42'N Latitude 47°10'E Longitude with altitude of 45 m) in a clay loam soil in 2014. Some characteristics of the soil of the experimental land have shown in Table 1. We arranged treatments on a randomized complete block design with three replications. Treatments were 0, 50, 100 and 150 kg ha⁻¹ urea; 0 kg ha⁻¹ urea+inoculation with Biosoy, 50 kg ha⁻¹ urea+inoculation with Biosoy, 100 kg ha⁻¹ urea+inoculation with Biosoy, 150 kg ha⁻¹ urea+inoculation with Biosoy. Recommended rates of nitrogen and phosphorous fertilizer calculated based on application of nitrogen and phosphorous fertilizers in conventional cropping system in Moghan region (150 kg ha⁻¹ urea+200 kg ha⁻¹ diammonium phosphate). According to positive effect of phosphorous fertilizer on nodule forming the amount of diammonium phosphate for all plots considered 200 kg ha⁻¹ and different levels of urea fertilizer considered as treatments.

Soybean inoculant Biosoy, including effective *Bradyrhizobium japonicum* populations obtained from the Mehr Asia Biotechnology Company (MABCo.) the bio-fertilizer had 10⁸ bacteria per gram. Seeds of soybean (cv. Williams) divided into two sub-samples, one of which was kept as non-inoculated. The other sub-samples inoculated with Biosoy. Each plot consisted of five rows with three meter length, spaced 50 centimeter apart. The optimum plant density (50 plants per m²) obtained by extra seed sowing and hand thinning. Chemical fertilizers applied as strip method after emerging. Weeds controlled by hand. At physiological maturity, 10 plants were randomly harvested from center part of each plot and growth traits, yield and yield components determined.

Table 1: Some characteristics of the soil of the experimental land

Soil characteristics	Values
Zn (ppm)	3.62
Fe (ppm)	4.58
Cu (ppm)	3.80
Mn (ppm)	9.67
EC (pH)	7.45
N (%)	0.09
P (ppm)	1.5
K (ppm)	627
Texture	Loamy clay

RESULTS AND DISCUSSION

Nodule dry weight: In the plants from seeds inoculated with Biosoy, control and application of 50 kg ha⁻¹ urea produced the highest nodule dry weight, that was not statistically significant compared to 100 and 150 kg ha⁻¹ urea. In the plants from seeds not inoculated with bacteria increasing the use of urea decreased nodule dry weight so that the largest weight of nodules was in the control and the highest amount of urea produced the lowest amount. However, decrease in nodule dry weight was not significant and treatments were statistically same. Inoculated plants with Biosoy in use 0, 50 and 100 kg ha⁻¹ urea had statistically significant superiority compared to the non-inoculated plants but at 150 kg ha⁻¹ urea there was no significant advantage (Table 2). Nodule growth and dry weight was higher in inoculated relative to the non-inoculated control (Hungria *et al.*, 2015) and nitrogen fixation activities significantly increased in the soybean plants grown on the soil which nodule bacteria on biochar had been applied (Iijima *et al.*, 2015). Most researchers have reported that the nodule forming and nitrogen fixation prevented in large amounts of nitrogen fertilizer (Solaiman and Rabbani, 2003; Solaiman and Hossain, 2006). The most nodule dry weight of soybean obtained at inoculation with Rhizobium+25 kg N+90 kg P ha⁻¹ (Tahir *et al.*, 2009). But, Mishra *et al.* (2010) reported that most nodules dry weight produced in integrated application of all nitrogen, phosphorus and potassium chemical fertilizers along with seed inoculation with Rhizobium+PSB+Plant growth promoting rhizobacteria.

Plant height: In plants from inoculated seeds with Biosoy the highest height produced at 50 kg ha⁻¹ urea fertilizer, which is of a significant advantage compared to 0, 100 and 150 kg ha⁻¹ fertilizer. With increasing values of urea fertilizer to 100 and 150 kg ha⁻¹ plant height reduced and the lowest amount was gained at 150 kg ha⁻¹ urea. Increased uses of fertilizer in non inoculated plants increased plant height. However, the difference between 0, 50 and 100 kg ha⁻¹ was insignificant. But, 150 kg ha⁻¹ urea produced significantly higher height

than the 0 and 50 kg ha⁻¹ urea fertilizer. Plants from inoculated seeds had significantly higher height than non inoculated plants at 0 and 50 kg ha⁻¹ urea but at 100 and 150 kg ha⁻¹ inoculated and non inoculated plants height were similar. Soybean plants of all genotypes inoculated with Rhizobium sp. BARIRGm901 produced greater plant height than non-inoculated plants (Alam *et al.*, 2015). Tahir *et al.* (2009) reported that the highest amount of plant height produced in the inoculation of seeds with Rhizobium+25 kg N ha⁻¹ +90 kg P ha⁻¹. But Mishra *et al.* (2010) reported that integrated application of all nitrogen, phosphorus and potassium chemical fertilizers along with seed inoculation with Rhizobium+PSB+Plant growth promoting rhizobacteria produced the greatest height. Argaw (2012) showed significant effect of nitrogen fertilizer on soybean but noted that adding nitrogen to the 46 kg ha⁻¹ compared to control had not significant effect on plant height. Tahir *et al.* (2009) resulted that the increase of nitrogen in soybean was significantly increased plant height.

Stem dry weight: In plants from seeds inoculation with *Bradyrhizobium japonicum* the higher stem dry weight was for 50 kg ha⁻¹ urea. Yet, differences among 0, 50 and 100 kg ha⁻¹ urea were insignificant. In these plants, the use of urea in quantities greater than 50 kg ha⁻¹ reduced plant height and the highest amount of urea produced the lowest plant height. Increased use of urea fertilizer for non-inoculated plants led to increase stem dry weight, so that the highest and lowest shoot dry weight produced at 150 and 0 kg ha⁻¹ urea fertilizer, respectively. The differences between 0 and 50 kg ha⁻¹ of urea and as well as the differences between 100 and 150 kg ha⁻¹ urea was not significant. The plants were inoculated with bacteria only in use 0 and 50 kg ha⁻¹ of urea were significantly higher than non-inoculated plants. Mishra *et al.* (2009) reported a significant increase in peas and lentils shoot biomass when they inoculated by Rhizobium. Similar result stated in soybean (Alam *et al.*, 2015). Elkoca *et al.* (2007) also reported that inoculation with nitrogen fixing bacteria significantly increased pea plant

Table 2: Analysis of variance (ANOVA) for growth and yield traits of soybeans

MS									
SOV	df	Nodule dry weight/plant	Plant height	Stem dry weight/plant	Pods dry weight/plant	Biological yield	Grains/plant	Grain yield	Harvest index
Block	2	0.30	30.71	1.25	6.58	8.68	0.16	4103.6	0.0015
Treatment	7	0.60*	212/87**	4.38**	14.35**	23.44**	0.07**	6533.5**	0.025*
Error	14	0.16	105.9	0.29	0.67	1.46	0.01	587.0	0.00067
CV (%)		33.75	5.87	12.41	10.88	10.15	5.3	13.8	8.7

NS: Non significant, *,** Significant at 1 and 5% probability level, respectively, Df: Degree of freedom, SOV: Source of variation, CV: Coefficient of variance

height and its stem biomass compared to control treatments and this increase was equal to or greater than nitrogen, phosphorus and nitrogen+phosphorus treatments. Tahir *et al.* (2009) noted that the increase in soybean fertilization significantly increased plant biomass. But testing diammonium phosphate and triple superphosphate at the amount of 60 kg ha⁻¹ showed no significant effect on bean stalk biomass (Zafar *et al.*, 2011).

Pod dry weight: Application of 50 kg ha⁻¹ urea fertilizer compared to non application of urea increased the dry weight of pods per plant in the plants from seeds inoculated with *Biosoy* but more use of urea in quantities of 100 and 150 kg ha⁻¹ decreased dry weight of pods per plant, so that the least amount obtained at 150 kg ha⁻¹ urea. Also, the difference among 0, 100 and 150 kg ha⁻¹ urea was not significant. In plants from non inoculated seeds, increased use of urea fertilizer, improved the dry weight of the pods. Also difference between 0 and 50 kg ha⁻¹ was non-significant. The difference between 100 and 150 kg ha⁻¹ was also non-significant. However, application of 150 kg ha⁻¹ urea produced the highest dry weight of pods per plant which was equal to the application of 0 and 50 kg ha⁻¹ urea in inoculated plants with bacteria *B. japonicum*. Also, it is noteworthy that 0 and 50 kg ha⁻¹ of urea produced significantly higher pod dry weight in the plants from seed inoculation with bacteria than from non-inoculated plants. Alam *et al.* (2015) noted that inoculation of soybean with *Rhizobium* sp. resulted in higher pod yields, compared with yields of non-inoculated plants, in both 2010 and 2011. El-Shaarawi *et al.* (2011) reported that the integrated application of *B. japonicum* at smaller amounts of nitrogen in contrast to nitrogen alone application improved the growth and yield of soybean and *B. japonicum* beneficial effect on pods dry weight at low levels of nitrogen fertilizer improved and the shortest amount of fertilizer had maximum effectiveness. Mishra *et al.* (2010) have suggested that wet weight of peanut pods per plant increased significantly as a result of the use of biological nitrogen fixing in comparison with control. El-Shaarawi *et al.* (2011) also reported that increasing nitrogen fertilizer resulted in increased biomass of leaves, stems and pods. Increase pea pods weight per plant with application of more fertilizer has also been reported by Negi *et al.* (2007).

Biological yield: In plants from seeds inoculated with *Biosoy* application of 50 kg ha⁻¹ urea fertilizer non-significantly increased biological yield compared to non-use of the fertilizer or control. But, more use of urea in quantities of 100 and 150 kg ha⁻¹ decreased biological yield compared with

50 kg ha⁻¹ urea, so that 150 kg ha⁻¹ of urea produced the lowest amount of biological yield. Differences among 0, 100 and 150 kg ha⁻¹ urea was insignificant. In plants were not inoculated with *Biosoy*, increase in chemical fertilizer enhanced the biological yield so that no application of urea fertilizer and the 150 kg ha⁻¹ urea produced the lowest and the highest values. For the non-application of urea (0) and application of 50 kg ha⁻¹ urea, plants inoculated with bacteria were much superior to non inoculated plants. But, at 150 kg ha⁻¹ of urea plants not inoculated had non-significantly higher biological yield than inoculated plants. Biomass of leaves, stems, pods and biological yield increased with enhance in nitrogen fertilizer (El-Shaarawi *et al.*, 2011). Tahir *et al.* (2009) also showed that significantly increased dry matter yield of soybeans have as a result of increased nitrogen fertilizer. Also, Zafar *et al.* (2011) noted that application of triple super phosphate and diammonium phosphate at amount of 60 kg ha⁻¹ increase dry matter of beans. Increase in biological yield with inoculation seeds of soybean by *Rhizobium* was stated by Alam *et al.* (2015). El-Shaarawi *et al.* (2011) stated that the beneficial effect of *B. japonicum* on total dry weight was seen in the shortest amount of the fertilizers.

Grains per plant: Grains per plant in plants from non inoculated seeds significantly were higher for plots received fertilizer compared with plots not received fertilizer. Use 100 kg ha⁻¹ urea fertilizer led to non-significant increase in the number of grains per plant compared to 50 kg ha⁻¹ urea fertilizer application but 150 kg ha⁻¹ fertilizer significantly increased the number of seeds per plant. The plants from seeds inoculated with *Biosoy* had largest number of grains per plant at 50 kg ha⁻¹ urea were similar to non fertilizer application. Increase in fertilizer amounts (100 and 150 kg ha⁻¹ urea) reduced the number of grains and the highest amount of fertilizer produced the lowest grains. A significant increase in the number of seeds per soybean plants with increasing nitrogen fertilizer by El-Shaarawi *et al.* (2011) and Argaw (2012) have been reported. Increase in the number of seeds per pea plants with *Rhizobium* application was reported by Erman *et al.* (2011). Mrkovacki *et al.* (2008) noted that maximum number of seeds created in the seed inoculation along with the use of nitrogen fertilizer.

Grain yield: Grain yield significantly affected by treatments (Table 2). Means comparison (Table 3) showed that in plants were not inoculated with *Biosoy*, the use of 50 and 100 kg ha⁻¹ urea relative to the non-application of fertilizer non-significantly improved the yield but the use of 150 kg ha⁻¹

Table 3: Mean comparisons for growth and yield traits of soybeans

Treatments	Traits							
	Nodule dry weight/plant	Plant height	Stem dry weight/plant	Pods dry weight/plant	Biological yield	Grains/plant	Grain yield	Harvest index
Non inoculated								
0	0.83 ^{bc}	45.83 ^d	2.32 ^e	3.55 ^d	5.86 ^d	20.00 ^e	1.99 ^d	34 ^a
50	0.81 ^{bc}	46.5 ^d	3.15 ^{de}	5.46 ^{cd}	8.61 ^{cd}	29.25 ^d	2.9 ^{cd}	33 ^a
100	0.75 ^c	50.16 ^{cd}	3.92 ^{cd}	7.54 ^{bc}	11.47 ^{bc}	33.08 ^{cd}	3.06 ^{bcd}	26 ^b
150	0.72 ^c	58.44 ^{bc}	5.17 ^{abc}	9.1 ^{ab}	14.27 ^{ab}	42.05 ^{ab}	4.29 ^{ab}	30 ^{ab}
Inoculated by Biosoy								
0	1.73 ^a	60.83 ^b	5.37 ^{ab}	8.77 ^{ab}	14.15 ^{ab}	40.00 ^{abc}	3.91 ^{abc}	27 ^b
50	1.71 ^a	71.29 ^a	5.92 ^a	10.53 ^a	16.45 ^a	47.00 ^a	5.02 ^a	30 ^{ab}
100	1.55 ^{ab}	57.0 ^c	4.87 ^{abc}	8.07 ^b	12.95 ^b	37.66 ^{bcd}	3.71 ^{bc}	28 ^b
150	1.36 ^{abc}	53.3 ^{bcd}	4.18 ^{bcd}	7.42 ^{bc}	11.61 ^{bc}	35.00 ^{bcd}	3.17 ^{bcd}	27 ^b

Same letters in columns are non-significant differences by the Duncan's test at 5% level (0, 50, 100 and 150 are different nitrogen levels, Biosoy is a bio fertilizer)

urea led to significant increase in grain yield compared to non-application of fertilizer. There were no significant differences between the values of 50 and 100 kg ha⁻¹ urea and 100 and 150 kg ha⁻¹ urea. When soybean seeds were inoculated with Biosoy, application of 50 kg ha⁻¹ urea produced the highest grain yield which is statistically similar to the non-application of urea in inoculated plants and urea application of 150 kg ha⁻¹ in the non-inoculated plants. More use of urea for non inoculated plants reduced grain yield and the lowest yield was achieved in 150 kg ha⁻¹ urea. In the non-application of fertilizers and application of 50 kg of urea fertilizer, inoculated plants produced higher yield compared with non-inoculated plants. Increase in Soybean yield with increased use of nitrogen fertilizer has been reported by El-Shaarawi *et al.* (2011). But, Argaw (2012) reported that the addition of nitrogen fertilizer at a rate of 46 kg ha⁻¹ urea compared with the control could not significantly increase the seed yield of soybean. Kumar and Rao (1991) also reported similar results. A positive correlation between grain yield and total biomass or leaf area of soybean seed by Frederick *et al.* (1991) have been reported. Bhat *et al.* (2010), Hungria *et al.* (2015) and Mmbaga *et al.* (2015) stated that seeds inoculation with Rhizobium increased seed yield relative to control. Similar results have been reported in canola (Yasari and Patwardhan, 2007; Alam *et al.*, 2015). Despite this, Javaid *et al.* (2002) reported that *B. japonicum* strains effect on seed yield was not significant. El-Shaarawi *et al.* (2011) reported that *B. japonicum* had greatest effect on grain yield in the least amount of chemical fertilizer. Also, Hungria *et al.* (2015) demonstrated that co-inoculation promotes yield increases without adding any chemical N fertilizers even in soils where established populations of soybean bradyrhizobial exist.

Harvest index: Harvest index was significantly affected by treatments (Table 2). Means comparison (Table 3) showed

that harvest index of the non-inoculated plants at 0, 50 and 150 kg ha⁻¹ urea, were similar and higher than 150 kg ha⁻¹ urea. In plants from inoculated seeds, despite of insignificant differences between the application of 0, 50, 100 and 150 kg ha⁻¹ of urea the application of 50 kg urea had a higher harvest index than 0, 100 and 150 kg of nitrogen. Non-inoculated plants in the non-application of urea fertilizer had more harvest index than plants from seed inoculation and at 50, 100 and 150 kg of urea. There was no difference between the inoculated and non-inoculated plants. Imran *et al.* (2015) reported that Inoculation either with *O. ciceri* alone or its co-inoculation with *M. ciceri* produced on average higher nodules (42%), biomass (31%), grains yield (64%) and harvest index (72%) in both chickpea genotypes over non-inoculated controls in both soils.

CONCLUSION

Experimental treatments significantly influenced. Nodule dry weight and harvest index at 5% of probability level and the others at 1% of probability level. Increasing nitrogen fertilizer in non-inoculated plants insignificantly reduced nodule dry weight but enhanced plant height, stems dry weight, the pods dry weight, biological yield, grains per plant and grain yield. Application of 0 and 50 kg ha⁻¹ urea for inoculated plants produced as much as application of 150 kg ha⁻¹ urea for non-inoculated plants stems dry weight, pods dry weight, biological yield, grains per plant and grain yield. Therefore, based on this investigation result to economic production, we suggest application of diammonium phosphate as a P fertilizer at the rate of 200 kg ha⁻¹ + non application or 50 kg ha⁻¹ urea application plus inoculation of soybean seeds by Biosoy in Moghan region. As a result, in this way we can save all or part of the urea fertilizer.

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