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Full Length Research Paper

Effects of vermicompost application and seed inoculation with biological nitrogen fertilizer under different plant densities in soybean [*Glycine max* (L.) cultivar, Williams]

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For studying the effects of vermicompost application, seeds inoculation with biological nitrogen fertilizer nitroxin and plant density management in soybean cultivar Williams, an experiment in factorial design with three factors based on randomized complete block design with 3 replication in the Astaneh Ashrafiyeh County (north of Iran) during 2010 was conducted. The factors of experiment consists off seed inoculation with biological nitrogen fertilizer nitroxin with two levels [N_1: control (without seed inoculation) and N_2: seed inoculation with nitroxin], application of vermicompost with three levels [V_1: control (without vermicompost application), V_2: 5 t/ha and V3: 10 t/ha] and plant density with three levels (D_1: 45 plants per m^2, D_2: 65 plants per m^2 and D_3: 85 plants per m^2). At maturity time seed yield, 100 seeds weight, plant height, number of pods per plant and number of seeds per plant were measured. Results of data analysis showed that, the effects of nitroxin biofertilizer inoculation, vermicompost application, plant density, interaction of nitroxin inoculation and density management and interaction of vermicompost application and density management on all studied traits was significant at 1% probability level. Interaction effect of nitroxin inoculation and vermicompost application on seed yield, 100 seeds weight, plant height and number of pods per plant at 1% and on number of seeds per plant at 5% was significant. Also, interaction effect of nitroxin inoculation, vermicompost application and plant density management on seed yield, 100 seeds weight and number of pods per plant showed significance differences at 1% probability level and on other traits was non significant. The highest seed yield was obtained from $N_2V_3D_2$ treatment (seeds inoculation with nitroxin, 10 t/ha vermicompost application and 65 plants per m^2) with 3831 kg/ha.

Key words: Iran, soybean, vermicompost, nitroxin biofertilizer, plant density, seed yield.

INTRODUCTION

Soybean (*Glycine max*) is one of the most important oil seed crop in the world. It contains 18 to 22% oil, highly desirable in diet and has 40 to 42% of good quality protein. Therefore, it is the best source of protein and oil and truly claims the title of the meat/oil on plants. Generally, it is used in the food industry for flour, oil, cookies, candy, milk, vegetable cheese, lecithin and many other products (Fatima et al., 2006). Organic fertilizers were common before of 1940˙s in Iran and many farmers use of chemical fertilizers know as unlawful and refrain its using. But many advertisements caused to use of chemical fertilizer by some farmers was extremist and forgot its effect on bioenvironmental pollution and in other words forgot organic and biological fertilizers (Malakoti, 1996). But in recent years due to unsuitable effect of chemical fertilizers on the soil, using of organic materials serves as a good and suitable source to supply soil food elements (Ntanos and Koutroubas, 2002). One of the best

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organic materials for increasing crops yield is vermicompost. Vermicomposting involves the bio-oxidation and stabilization of organic material by the joint action of earthworms and microorganisms. Although, it is the microorganisms that biochemically degrade the organic matter, earthworms are the crucial drivers of the process, as they aerate, condition and fragment the substrate, thereby drastically altering the microbial activity.

Earthworms act as mechanical blenders and by comminuting the organic matter they modify its physical and chemical status by gradually reducing the ratio of C:N and increasing the surface area exposed to micro-organisms – thus making it much more favourable for microbial activity and further decomposition (Domínguez et al., 1997). Vermicompost being a stable fine granular organic matter, when added to clay soil loosens the soil and improves the passage for the entry of air. The mucus associated with the cast being hydroscopic absorbs water and prevents water logging and improves water-holding capacity. The organic carbon in vermicompost releases the nutrients slowly and steadily into the system and enables the plant to absorb these nutrients. The soil enriched with vermicompost provides additional substances that are not found in chemical fertilizers (Kale, 1998). On the other hand, nitrogen is required by plants in comparatively larger amounts than other elements (Marschner, 1995). Excessive application of chemical nitrogen fertilizer can result in a high soil nitrate concentration after crop harvest (Jokela and Randall, 1989; Roth and Fox, 1992; Gordon et al., 1993). This situation can lead to an increase in the level of nitrate contamination of potable water, because nitrate remaining in the soil profile may leach to groundwater (Singh et al., 1995). A great way to solve these problems is usage of biological nitrogen fixation.

The utilization of biological nitrogen fixation method can decrease the use of chemical nitrogen fertilizer (urea), prevent the depletion of soil organic matter and reduce environmental pollution to a considerable extent (Choudhury and Kennedy, 2004). Several bacteria that are associated with the roots of crop plants can induce beneficial effects on their hosts and often are collectively referred to as PGPR (Plant Growth Promoting Rhizobacteria) (Vermeiren et al., 1999). The biological fixation of nitrogen produced by these organisms can constitute a significant and ecologically favorable contribution to soil fertility (Vlassak et al., 1992). Nitroxin is a biologic nitrogen fertilizer containing *Azospirillum* and *Azotobacter*. *Azospirillum* belongs to family *Spirilaceae*, heterotrophic and associative in nature. In addition to their nitrogen fixing ability of about 20 to 40 kg/ha, they also produce growth regulating substances (Arun, 2007). Although there are many species under this genus like, *Azospirillum amazonense, Azospirillum halopraeferens, Azospirillum brasilense*, but, worldwide distribution and benefits of inoculation have been proved mainly with the *Azospirillum lipoferum* and *A. brasilense. Azotobacter* belongs to family *Azotobacteriaceae*, aerobic, free living and heterotrophic in nature. Azotobacters are present in neutral or alkaline soils and *A. chroococcum* is the most commonly occurring species in arable soils. *Azotobacter vinelandii, Azotobacter beijerinckii, Azotobacter insignis* and *Azotobacter macrocytogenes* are other reported species. The number of *Azotobacter* rarely exceeds of 10^4 to 10^5 g^{-1} of soil due to lack of organic matter and presence of antagonistic microorganisms in soil (Subba, 2001). The bacterium produces anti-fungal antibiotics which inhibits the growth of several pathogenic fungi in the root region thereby preventing seedling mortality to a certain extent (Sheraz et al., 2010).

Bozorgi et al. (2011) with study effects of bio, mineral nitrogen fertilization and foliar zinc spraying on yield and yield components of faba bean were reported that the highest grain yield was obtained by 30 kg/ha pure nitrogen along with seeds inoculation with nitroxin. One of the other agricultural management practices for receiving to high yields is an optimum density designing. The plants largely depend on temperature, solar radiation, moisture and soil fertility for their growth and nutrition requirements. A dense population of crops may have limitations in the maximum availability of these factors. It is, therefore, necessary to determine the optimum density of plants population per area unit for obtaining maximum yields (Baloch et al., 2002). Optimum plant spacing ensures plants to grow properly both in their aerial and underground parts through different utilization of solar radiation and nutrients. The optimum plant density depends on different factors that most importance of this factors include: plant characteristics, growth period duration, planting time and methods, soil fertility, plant size, available moisture, sun shine, planting pattern and situation of weeds (Shirtliffe and Johnston, 2002).

The current study aim was to investigate the influence of vermicompost application and seed inoculation with biological nitrogen fertilizer (nitroxin) under plant densities management in soybean crop.

MATERIALS AND METHODS

In order to examine the effects of vermicompost application, seed inoculation with biological nitrogen fertilizer nitroxin and plant density management in soybean (*G. max* L. cultivar, Williams) an experiment in factorial format with three factors based on randomized complete block design with 3 replication under field conditions in the Astaneh Ashrafiyeh Township located in 37° 16′ N latitude and 49° 56′ E longitude in the Guilan province, north of Iran, in 2010 farming year was conducted. Soil analysis results show that (Table 1), the soil texture was Clay loam and pH 6.6. Factors of experiment were consisting of seed inoculation with biological nitrogen fertilizer nitroxin with two levels [N_1: control (without seed inoculation) and N2: seed inoculation with nitroxin], application of vermicompost with three levels [V_1: control (without vermicompost application), V_2: 5 t/ha vermicompost application and V3: 10 t/ha vermicompost application] and plant density with three levels (D_1: 45 plants per m^2, D_2: 65 plants per m^2 and D_3: 85 plants per m^2). Some chemical properties of used vermicompost in experiment is

Table 1. Soil physical and chemical properties of the experimental area.

Soil texture	Clay loam	EC (dsm^{-1})	0.511
Sand (%)	27.5	Organic carbon (%)	1.33
Silt (%)	40	Total nitrogen (%)	0.11
Clay (%)	35.5	Phosphorus (mg/kg)	92.07
pH	6.63	Potassium (mg/kg)	219.67

Table 2. Some chemical properties of used vermicompost.

Nitrogen (%)	Phosphorus (%)	Potassium (%)	Organic carbon (%)	pH	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)
1.5	1.8	1	20	7.2	800	400	120	180

Table 3. Results of variance analysis for effects of nitroxin biofertilizer inoculation, vermicompost application and plant density management on seed yield and other studied traits in soybean.

		MS				
Source of variance	**DF**	**Seed yield (kg/ha)**	**100 seed weight (g)**	**Plant height (cm)**	**Number of pods per plant**	**Number of seeds per plant**
Nitroxin (N)	1	707037.796**	9.217**	485.280**	137.920**	782.042**
Vermicompost (V)	2	6568239.389**	25.224**	716.810**	317.694**	1410.354**
N×V	2	68477.796**	0.638**	6.606**	5.567**	24.185*
Density (D)	2	14136622.722**	12.551**	1042.533**	430.634**	2122.042**
N×D	2	47322.463**	0.915**	57.187**	27.157**	158.641**
V×D	4	1235876.278**	1.381**	49.152**	39.852**	117.235**
N×V×D	4	76840.963**	0.565**	1.013ns	3.364**	11.100ns
Error	34	6238.408	0.033	0.768	0.442	6.662

ns,* and **: non significant, significant at the 5 and 1% level of probability respectively.

showed in Table 2. The operations of preparing land include first plough in winter and secondary plough along with giving nitrogen and potash before planting in spring was done.

According to soil analysis, 100 kg/ha urea fertilizer and 100 kg/ha potassium sulfate were implemented and due to presence of sufficient phosphorus in soil, do not use phosphorus fertilizer. Seeds of soybean were sown on the 5th of May, 2010. Each plot size was 2 m wide and 7 m long. Spacing between plots of each replication was 0.5 m and between replications were 1 m. The rows spacing was 50 cm and plant spacing on the rows was 10 cm. Irrigation practices was done once a week. During growth period, cultivate cares were done ordinarily. Measured traits were included seed yield, 100 seeds weight, plant height, number of pods per plant and number of seeds per plant. The data were analyzed by using MSTAT-C software. Also, the figures were draw by Excel 2003 software. The Duncan's multiple range tests was used to compare the means at 5% of significant.

RESULTS AND DISCUSSION

Effect of vermicompost application

With attention to variance analysis (Table 3), effect of vermicompost application showed significant differences in 1% probability level on all studied traits. Comparison of mean between vermicompost levels showed that with increasing of vermicompost rates up to 10 t/ha all measured traits was increased (Table 4). The highest seed yield with 2868 kg/ha, 100 seed weight with 16.25 g, plant height with 73.93 cm, number of pods per plant with 26.43 pods and number of seed per plant with 60.29 seeds was obtained from use of 10 t/ha vermicompost (V_3). On the other hand the lowest amounts of seed yield, 100 seed weight, plant height, number of pods per plant and number of seeds per plant respectively with 1661 kg/ha, 13.98 g, 62.07 cm, 18.42 pods and 42.96 seeds was recorded from V_1 treatment (without vermicompost application). Many researchers were reported that application of vermicompost because of supplying optimum nourishment condition cause to improve growth, yield and yield components in crops (Federico et al., 2007; Vijaya et al., 2008; Hernández et al., 2010).

Effect of seeds inoculation with biological nitrogen fertilizer (nitroxin)

Effect of seeds inoculation with nitroxin biological fertilizer

Table 4. Effect of nitroxin biofertilizer, vermicompost application and plant density management levels on yield and yield components of soybean.

Treatment	Seed yield (kg/ha)	100 seed weight (g)	Plant height (cm)	Number of pods per plant	Number of seeds per plant
Nitroxin					
N_1	2163.2[b]	14.9[b]	66.3[b]	21.6[b]	48.9[b]
N_2	2392[a]	15.7[a]	72.2[a]	24.8[a]	56.5[a]
Vermincompost					
V_1	1661[c]	13.98[c]	62.07[c]	18.42[c]	42.96[c]
V_2	2304[b]	15.71[b]	71.74[b]	24.62[b]	54.77[b]
V_3	2868[a]	16.25[a]	73.93[a]	26.43[a]	60.29[a]
Density					
D_1	1261[c]	14.40[c]	60.60[c]	17.64[c]	40.28[c]
D_2	2889[a]	16.04[a]	72.24[b]	24.86[b]	57.20[b]
D_3	2682[b]	15.50[b]	74.91[a]	26.97[a]	60.53[a]

Within each column, treatments that carry the same superscript letter are not significantly different at $P<0.05$.

had a significant difference ($p<0.01$) on all studied traits (Table 3). Mean comparison of data is presented in Table 4, seed inoculation with biological fertilizer nitroxin had a positive effect on all traits. The highest seed yield, 100 seed weight, plant height, number of pods per plant and number of seeds per plant respectively with 2392 kg/ha, 15.7 g, 72.2 cm, 24.8 pods and 56.5 seeds were found from N_2 treatment (seed inoculation with nitroxin). On the other hand, the lowest amounts of this traits was related to N_1 treatment (without nitroxin inoculation) respectively with 2163 kg/ha, 14.9 g, 66.3 cm, 21.6 pods and 48.9 seeds per plant. Bozorgi et al. (2011) with studying the effects of bio, mineral nitrogen fertilization and foliar zinc spraying on yield and yield components of faba bean were reported that the highest seed yield obtained by seeds inoculation with nitroxin. Similar results were reported by Molla et al. (2001) and Freeborn (2003).

Effect of plant density

Data presented in Table 3 showed that, the effect of plant density on all traits had significant differences in 1% probability level. With attention to Table 4, the highest seed yield with 2889 kg/ha and 100 seed weight with 16.04 g was found from 65 plants per square meter (D_2). Also, the lowest seed yield and 100 seed weight respectively with 1261 kg/ha and 14.40 g was recorded from 45 plants per square meter (D_1). Donovan et al. (1963) found that, with increase of plant density between rows and decrease between plants on rows, the seed yield was growth. Maximum amounts of plant height with 74.91 cm, number of pods per plant with 26.97 pods and number of seeds per plant with 60.53 seeds was obtained from 85 plants per square meter (D_3). Also, the lowest amounts of plant height, number of pods per plant and number of seeds per plants respectively with 60.60 cm, 17.64 pods and 40.28 seeds were recorded from 45 plants per square meter (D_1). Similar results were recorded by Larry et al. (2002), Khajouei-Nejad (2004) and Kashiri et al. (2006).

Interaction effects of nitrogen and vermicompost

Interaction effect of nitrogen and vermicompost on seed yield, 100 seed weight, plant height and number of pods per plant in 1% and on number of seeds per plant in 5% was significant (Table 3). Comparison of mean between interaction effect levels of nitrogen and vermicompost showed that, the highest seed yield, 100 seed weight, plant height, number of pods per plant and number of seeds per plant respectively with 2920 kg/ha, 16.76 g, 77.52 cm, 28.57 pods and 65.28 seeds were recorded from N_2V_3 (seed inoculation with nitroxin and 10 t/ha vermicompost application) treatment (Table 5). On the other hand, the lowest seed yield with 1486 kg/ha, 100 seed weight with 13.79 g, plant height with 59.70 cm, number of pods per plant with 17.39 pods and number of seeds per plant with 40.29 seeds were recorded from N_1V_1 treatment (without seed inoculation with nitroxin and no vermicompost application).

Similar results were recorded by Boquet (1990), Reddy and Reddy (1999), Hansen et al. (2001) and Arancon and Edwards (2005).

Interaction effects of nitrogen and plant density

Interaction effect of nitrogen and plant density on all

Table 5. Nitroxin inoculation x vermicompost application interactions for yield and yield components in soybean.

Nitroxin × Vermicompost					
Treatment	**Seed yield (kg/ha)**	**100 seed weight (g)**	**Plant height (cm)**	**Number of pods per plant**	**Number of seeds per plant**
N_1V_1	1486^{f}	13.79^{f}	59.70^{f}	17.39^{f}	40.29^{f}
N_1V_2	2187^{d}	15.18^{d}	68.70^{d}	22.99^{d}	51.01^{d}
N_1V_3	2817^{b}	15.73^{c}	70.35^{c}	24.29^{c}	55.30^{c}
N_2V_1	1836^{e}	14.18^{e}	64.44^{e}	19.44^{e}	45.63^{e}
N_2V_2	2421^{c}	16.24^{b}	74.78^{b}	26.24^{b}	58.52^{b}
N_2V_3	2920^{a}	16.76^{a}	77.52^{a}	28.57^{a}	65.28^{a}

Within each column, treatments that carry the same superscript letter are not significantly different at P<0.05.

Table 6. Nitroxin inoculation x plant density management levels interactions for yield and yield components in soybean.

Nitroxin × Density					
Teatment	**Seed yield (kg/ha)**	**100 seed weight (g)**	**Plant height (cm)**	**Number of pods per plant**	**Number of seeds per plant**
N_1D_1	1203^{f}	14.23^{f}	59.64^{f}	17.41^{e}	39.90^{e}
N_1D_2	2762^{c}	15.43^{c}	67.98^{d}	22.26^{d}	51.86^{d}
N_1D_3	2524^{d}	15.03^{d}	71.14^{c}	25^{c}	54.84^{c}
N_2D_1	1319^{e}	14.56^{e}	61.56^{e}	17.87^{e}	40.67^{e}
N_2D_2	3017^{a}	16.64^{a}	76.50^{b}	27.46^{b}	62.54^{b}
N_2D_3	2840^{b}	15.97^{b}	78.68^{a}	28.93^{a}	66.22^{a}

Within each column, treatments that carry the same superscript letter are not significantly different at P<0.05.

studied traits showed significant differences in 1% probability level (Table 3). Mean comparison of interaction effect levels of nitrogen and plant density is presented in Table 6. Use of seed inoculation by nitroxin biofertilizer and increase plant density had positive effect on yield and yield components of soybean. The N_2D_2 treatment (seed inoculation with nitroxin and 65 plants per square meter) with 3017 kg/ha seed yield and 16.64 g 100 seed weight was recorded maximum amounts of this traits. Also, the highest plant height, number of pods per plant and number of seeds per plant were recorded from N_2D_3 treatment (seed inoculation with nitroxin and 85 plants per square meter). On the other hand, the N_1D_1 treatment with 1203 kg/ha seed yield, 100 seed weight with 14.23 g, 59.64 cm plant height, 17.41 pods per plant and 39.90 seeds per plant was obtained the lowest values of this traits. Interaction effect treatment of n_2d_1 with 18.87 pods per plant and 40.67 seeds per plant was placed in same statistically level with N_1D_1 treatment. Similar results were reported by Federico et al. (2007), Jan et al. (2009) and Elhassan et al. (2010).

Interaction effects of vermicompost and plant density

With attention to variance analysis table (Table 3), interaction effect of vermicompost and plant density on all studied traits showed was significant (with 99% confidence coefficients). Comparison of mean between interaction effect levels of vermicompost and plant density is showed in Table 7. The highest seed yield with 3751 kg/ha and 100 seed weight with 17.16 g was obtained from V_3D_2 treatment (10 t/ha vermicompost application and 65 plants per square meter). Also, the maximum plant height with 80.25 cm, number of pods per plant with 31.10 pods and number of seeds per plant with 69.07 seeds were recorded from V_3D_3 treatment (10 t/ha vermicompost application and 85 plants per square meter). The interaction effect level of V_1D_1 (without vermicompost application and 45 plants per square meter) was recorded the lowest amounts of seed yield with 1145 kg/ha, 100 seed weight with 13.70 g, plant height with 57.21 cm, number of pods per plant with 16.32 pods and number of seeds per plant with 36.43 seeds. Similar results were reported by Khajouei-Nejad (2004), Arancon and Edwards (2005) and Hernández et al. (2010).

Interaction effects of nitroxin inoculation, vermicompost application and plant density management

Interaction effect nitroxin inoculation, vermicompost

Table 7. Vermicompost application x plant density management levels interactions for yield and yield components in soybean.

Vermincompost × Density					
Treatment	**Seed yield (kg/ha)**	**100 seed weight (g)**	**Plant height (cm)**	**Number of pods per plant**	**Number of seeds per plant**
V_1D_1	1145^{g}	13.70^{h}	57.21^{g}	16.32^{g}	36.43^{f}
V_1D_2	1882^{e}	14.31^{f}	63.32^{e}	18.50^{e}	44.48^{e}
V_1D_3	1956^{e}	13.94^{g}	65.69^{d}	20.43^{d}	47.97^{d}
V_2D_1	1289^{f}	14.53^{e}	61.03^{f}	17.65^{f}	39.07^{f}
V_2D_2	3036^{c}	16.64^{b}	75.41^{c}	26.83^{c}	60.67^{c}
V_2D_3	2587^{d}	15.96^{c}	78.78^{b}	29.37^{b}	64.57^{b}
V_3D_1	1350^{f}	14.97^{d}	63.55^{e}	18.95^{e}	45.35^{de}
V_3D_2	3751^{a}	17.16^{a}	78^{b}	29.23^{b}	66.45^{ab}
V_3D_3	3503^{b}	16.61^{b}	80.25^{a}	31.10^{a}	69.07^{a}

Within each column, treatments that carry the same superscript letter are not significantly different at $P<0.05$.

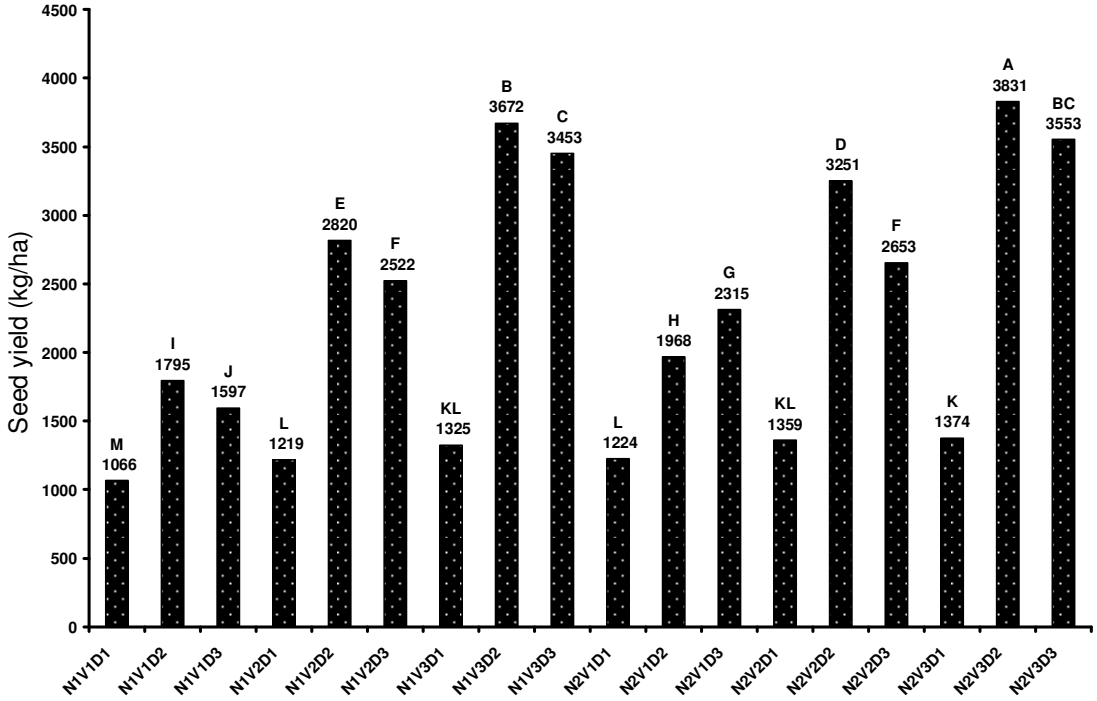

Figure 1. Interaction effect nitroxin inoculation, vermicompost application and plant density management on seed yield.

application and plant density management on seed yield, 100 seed weight and number of pods per plant in 1% probability level was significant. But on plant height and number of seeds per plant was non significant (Table 3). The highest seed yield with 3831 kg/ha and 100 seed weight with 18.01 g was recorded from $N_2V_3D_2$ (seed inoculation with nitroxin and 10 t/ha vermicompost and 65 plants per square meter). Also, the maximum number of pods per plant with 33.73 pods was recorded from $N_2V_3D_3$ (seed inoculation with nitroxin and 10 t/ha vermicompost and 85 plants per square meter). The $n_2v_3d_2$ treatment with 32.87 pods per plant was placed in same statistically level with $N_2V_3D_3$. The lowest seed yield, 100 seed weight and number of pods per plant was recorded from $N_1V_1D_1$ (without nitroxin inoculation and no vermicompost application and 45 plants per square meter) respectively with 1066 kg/ha, 13.36 g and 15.97 pods per plant (Figures 1, 2 and 3). Similar results were reported by Sorensen and Penas (1978), Ball et al. (2000) and Sailaja and Ushakumari (2002).

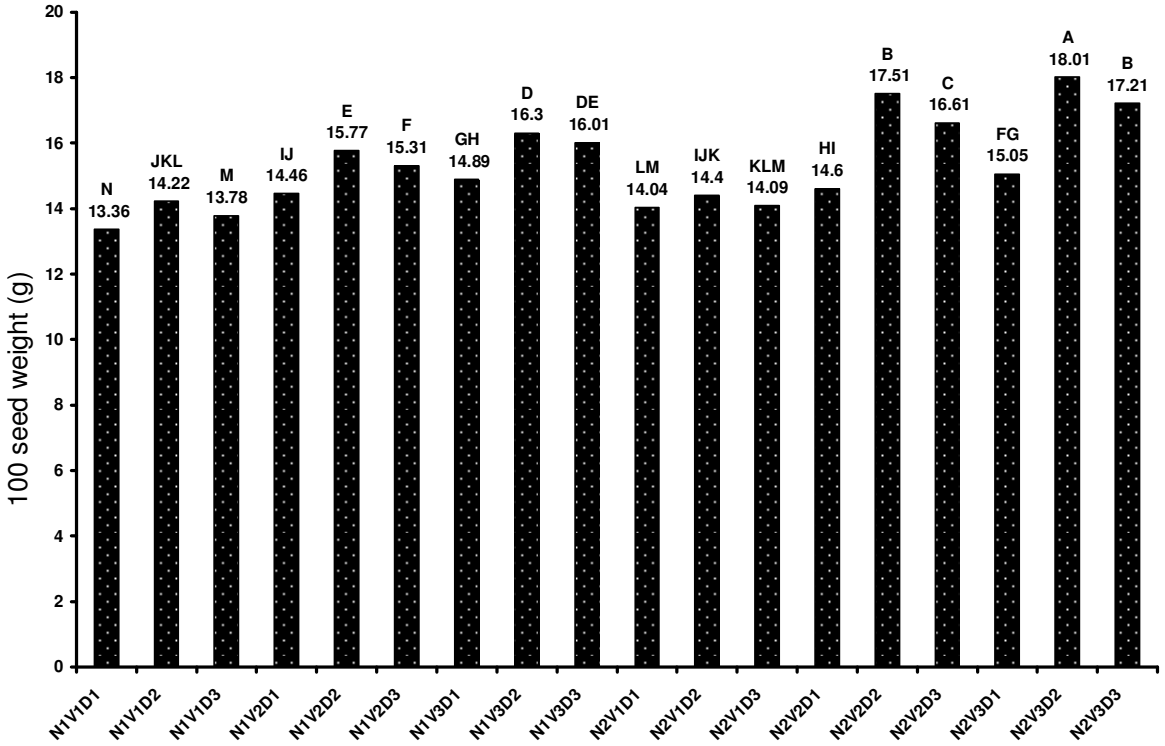

Figure 2. Interaction effect nitroxin inoculation, vermicompost application and plant density management on 100 seed weight.

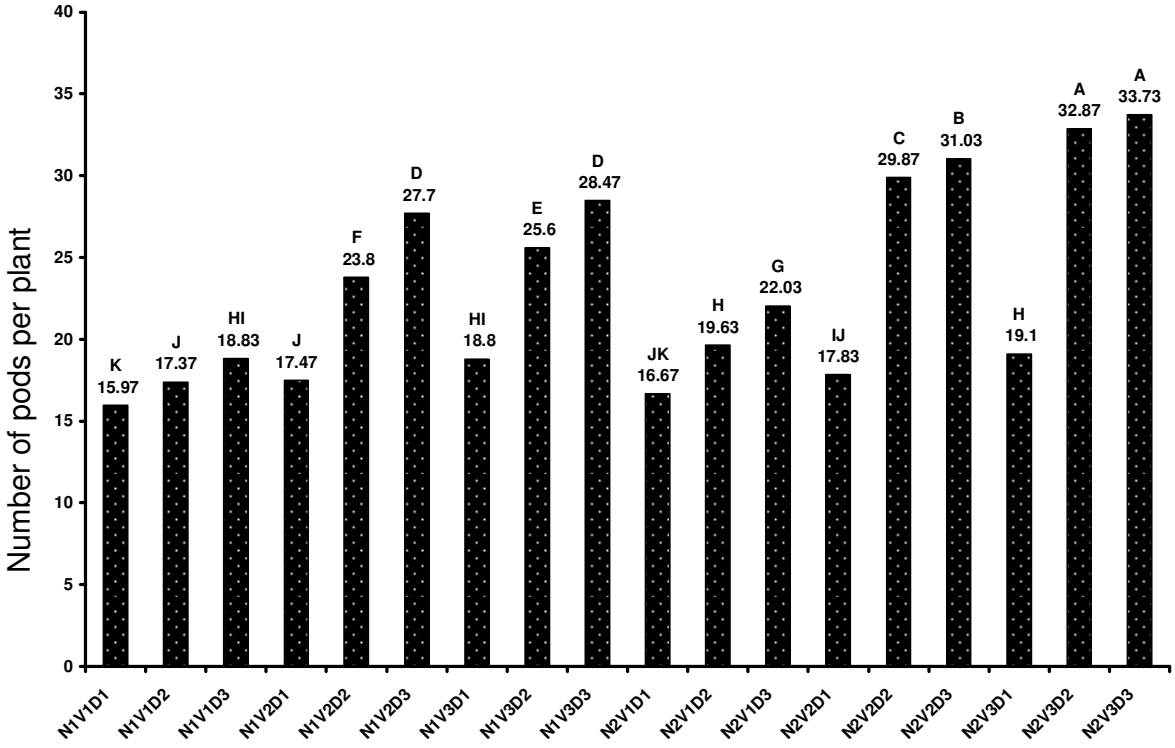

Figure 3. Interaction effect nitroxin inoculation, vermicompost application and plant density management on number of pods per plant.

Conclusion

Overall, the results clearly demonstrate that use of biological nitrogen fertilizer (nitroxin) had significant and positive effect on all measured traits. Application of nitroxin biofertilizer gave the greatest seed yield, 100 seeds weight, plant height, number of pods per plant and number of seeds per plant. On the other hand, vermicompost application significantly affect on all studied traits. When used of vermicompost at 10 t/ha, vermicompost increased seed yield, 100 seeds weight, plant height, number of pods per plant and number of seeds per plant in comparison with other vermicompost application treatments. Also, effect of plant density was significant on all studied traits. With increase plant density up to determined density (65 plants per m^2) the seed yield and 100 seed weight were increased. And on other traits, density of 85 plants per m^2 was superior. Based on the results of the present study, it can be suggested that vermicompost application at 10 t/ha, using a plant population density of 65 plants per m^2 and seed inoculation with biological nitrogen fertilizer would be suitable for growing soybean.

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