

Effect of nanosilver application on agronomic traits of soybean in relation to different fertilizers and weed density in field conditions

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Abstract

A field experiment was conducted during the vegetation season of 2012 to evaluate the effect of nanosilver application and weed density in an integrated fertilization system on agronomic traits of soybean. The experimental design was arranged in split-split plots based on a randomized complete block design. Main plots consisted of six fertilizer resources including farmyard manure (F1); compost (F2); chemical fertilizer (F3); farmyard manure + compost (F4), farmyard manure + compost + chemical fertilizer (F5); and a control (F6). Subplots with three weed densities were 0 (D1), 10 (D2) and 20 (D3) pigweed plants m⁻². Sub-subplots were seed treated with nanosilver (N1) and control (N2, without nanosilver). The results showed that coapplication of organic and chemical fertilizers (F5) increased leaf chlorophyll significantly. The highest grain nitrogen was obtained from F5D1 and D1N1 treatments. The highest grain P and K concentration was obtained from F5 treatment. Coapplication of compost, farmyard manure and chemical fertilizer produced a higher amount of pods per plant, seed number per pod and 100-seed mass. Since the highest amounts of grain yield components were obtained from F5 and D1 treatments, F5D1 produced the highest grain yield.

Key words: farmyard manure, competition, compost, nanosilver, nutrients.

Abbreviations: FYM, farmyard manure.

Introduction

Nanotechnology with materials having unique properties has promised applications in various fields. It has provided new solutions to problems in plants and food science (post-harvest products) and offers novel approaches to the rational selection of raw materials, the processing of such towards applying disease control molecules, slow-release pesticides and developing diagnostic tools. Nanosilver, a new class of material with remarkably different physicochemical and biological characteristics from convenient silver-containing substances, has been shown to have antibacterial, antifungal and antiviral effects and it can reduce damage and losses caused by diseases (Choi et al. 2009; Eo, Lee 2009). Recently, silver nanoparticles have been used in agricultural systems. For example, treatment with nanosilver can extend the postharvest storage period of asparagus spears by up to 20 or 25 days (An et al. 2008). Use of silver nanoparticles instead of silver nitrate increased seed yield in borage (*Borago officinalis* L.) plants (Seif Sahandi et al. 2011). Moreover, unfavorable effects of flooding stress on growth of saffron plants can be diminished by foliar application of nanosilver (Sorooshzadeh et al. 2012).

The soybean [*Glycine max* (L.) Merr.] or soya bean is a species of legume native to Asia, widely grown for its

edible bean which has numerous uses. Weeds can be one of the biggest challenges a soybean grower will face. Weeds compete with soybeans for light, moisture, and nutrients, with early-season competition being most critical. Most of the yield reduction occurs during the first six weeks after planting (Dielman et al. 1995). Soybean is especially sensitive to weed competition during the first four to nine weeks after planting (Blackshaw 1991). Weed interference can reduce soybean yield by 83% depending on the weed species, density, and duration of the competition (Aguyoh, Masiunas 2003). In general, a high competitive ability with weeds is associated with traits that allow a crop to establish ground cover faster and absorb more available resources than the competing weeds. Competition between a crop and weeds for growth resources is a major cause of reduced crop yield. Weed density also has an important impact on weed-crop competition. Redroot pigweed (*Amaranthus retroflexus* L.) is a common weed in soybean fields. Rapid growth and tall plant traits make redroot pigweed extremely competitive with crops.

The ability of redroot pigweed to cause serious yield losses is documented for some crops such as cotton, soybean and snap beans (Bensch et al. 2003; Culpepper et al. 2006). However, information related to the density effects of redroot pigweed in different fertilization systems

are lacking. The degree of weed competition is influenced by several other factors such as nutrient availability, mostly affected by fertilization. Chemical fertilizers are the dominant fertilizers in Iranian soybean farms and application of organic fertilizers are not common. Incorporation of organic matter either in the form of crop residues or farmyard manure has been shown to improve soil structure and water retention capacity, increase infiltration rate, decrease bulk density and increase biological nitrogen fixation by soybean (Mohammadi et al. 2012). This can lead to greater uptake of nitrogen by soybean in competition with redroot pigweed. This study was conducted to evaluate soybean response to different pigweed density and nanosilver in an integrated fertilization system.

Materials and methods

The trial was conducted in the Botanical Garden of Hamedan, the northwest region of Iran in the vegetation season of 2012. The soil type is sandy loam (100 g kg⁻¹ clay, 460 g kg⁻¹ silt, and 440 g kg⁻¹ sand) with a water holding capacity of 272 g kg⁻¹. The experimental design was a split-split plot based on a randomized complete block design with three replications. The main plots were six methods of fertilization including 5 t ha⁻¹ farmyard manure (FYM; F1); 5 t ha⁻¹ compost (F2); 50 kg ha⁻¹ triple superphosphate + 30 kg ha⁻¹ urea (F3); 2.5 t ha⁻¹ FYM + 2.5 t ha⁻¹ compost (F4); 2.5 t ha⁻¹ FYM + 2.5 t ha⁻¹ compost + 25 kg ha⁻¹ triple super phosphate + 15 kg ha⁻¹ urea (F5), and a control (without fertilizer; F6). Subplots were three pigweed densities including 0 (D1), 10 (D2) and 20 (D3) plants m⁻². Sub-sub plots were seed treated with nanosilver (N1) and a control (without nanosilver, N2). Redroot pigweed seed was purchased from a commercial supplier and sown in a 1-cm-deep furrow; seeds were sown by hand, covered with soil, and firmly packed. Nanosilver particles were provided by Pars Nano Nasb Company, Tehran, Iran.

FYM and compost were analyzed for chemical properties (Table 1). FYM, compost and chemical fertilizer were added to respective plots before sowing of soybean. Soybean and pigweed seeds were planted on April 10, 2012. Main plot size was 5 × 10 m and spaces between main plots were 2 m. All weeds other than redroot pigweed were removed by hand, interrow cultivation, and through selective herbicides throughout the growing season.

Chlorophyll measurements were performed with a hand-held dual wavelength chlorophyll meter (SPAD 502, Minolta Camera Co., Japan) at the flowering stage. After

soybean harvesting, seeds were collected to determine soybean seed yield and yield components. Area harvested was 2 × 10 m for each subplot. Seed yield of soybean was adjusted to 9% moisture content. The nitrogen content of mature seeds was determined by microkjeldhal method (Jakson 1973). The phosphorus content of mature seeds was determined by vanado molybdate phosphoric acid yellow colour method (AOAC 1990). The potassium content was determined by a flame photometer (EEL; Parsad 1998). For oil extraction, the seeds were oven-dried at 40 °C for 4 h, by using a ventilated vacuum oven, up to a moisture content of about 5%; then they were ground with a Waring Blender. The meal was extracted with petroleum ether (for 6 h) in a Soxhlet type extractor according to Pomeranz and Clifton (1994). The oil extract was evaporated by distillation at reduced pressure (15 Torr) in a rotary evaporator (Heildof) at 35 °C until the solvent was totally removed.

The data collected in this study was subjected to analysis of variance (ANOVA). GLM was used for the analysis of variance and to test differences between treatments. Means comparison was done through a LSD test by using the SAS statistical package (SAS Institute, 2002).

Results

Soybean seed yield was affected by fertilization, pigweed density and nanosilver (Fig. 1). Two-way interaction of fertilization × pigweed density had significant effect on seed yield (Table 2). Since the highest amount of seed yield components was obtained from F5 and D1 treatments, F5D1N1 produced the highest seed yield. All fertilization treatments had a lower seed yield in D3 plots compared to other pigweed densities (Fig. 1A). Nanosilver application caused increased seed yield as compared to the control.

Fertilization, pigweed density and nanosilver had significant effect on pod number per plant. Fertilization and pigweed density had significant effects on seed number per pod and 100-seed mass. Also, fertilization × pigweed density interaction had a significant effect on 100-seed mass (Table 2). The highest pod number per plant, seed number per pod and 100-seed mass were observed in coapplication of organic and chemical fertilizer (Table 3). Simultaneous application of FYM, compost and chemical fertilizer significantly increased pod number per plant, seed number per pod and 100-seed mass. Despite the increase in 100 seed mass in F5 compared to F3, there was no significant difference between them. However, combined application of compost and FYM in comparison with individual

Table 1. Chemical characteristics of farmyard manure and compost used in the present study

Treatment	pH	N	P (%)	K	Ca	Mg	Zn	Cu
						(mg kg ⁻¹)		
Farmyard manure	7.42	0.44	0.42	0.30	766	1210	11	23
Compost	7.23	0.68	1.11	0.45	1687	1786	81	288

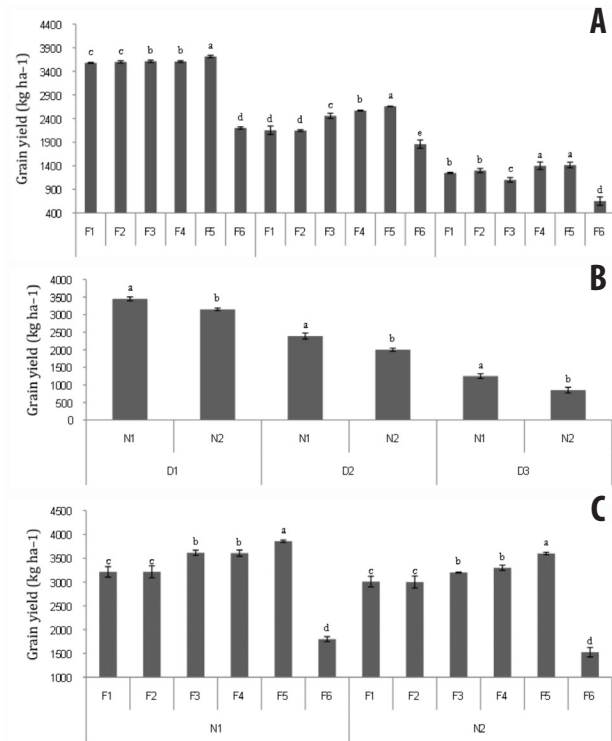


Fig. 1. Interactive effects of fertilization × pigweed density (A), pigweed density × nanosilver (B) and fertilization × nanosilver (C) on soybean seed yield. F1, farmyard manure; F2, compost; F3, chemical fertilizer; F4, farmyard manure + compost; F5, farmyard manure + compost + chemical fertilizers; F6, control; D1, weed-free; D2, 10 plants m⁻²; D3, 20 plants m⁻². Mean values with the same letter in each section are not significantly different (LSD tests at 5% probability). Bars indicate SE.

application of them increased 100 seed mass (Table 3).

Seed nitrogen concentration was affected by fertilization, pigweed density and nanosilver. There was a significant interaction effect between fertilization × pigweed density

and pigweed density × nanosilver application (Table 2). The highest seed nitrogen was obtained from F5D1 and D1N1 treatments (Fig. 2). It was also found that in high pigweed density with FYM and compost application (F4D3), soybean plants had more seed nitrogen than in treatment with chemical fertilizer (F3D3) (Fig. 2A). The nanosilver effect on nitrogen in weed free treatments was not significant. However, in the presence of pigweeds, nanosilver increased nitrogen uptake by soybean (Fig. 2B).

Fertilization and pigweed density had a significant effect on seed phosphorus and potassium concentrations. Nanosilver had no significant effect on phosphorus and potassium concentration (Table 2). The highest seed P and K concentration was obtained in the N5 treatment (Table 3). Triple super phosphate fertilizer (F3) significantly increased seed P concentration in comparison with compost and FYM. The maximum seed P and K of soybean was reached when no redroot pigweed was present. Not surprisingly, the lowest seed P and K for soybean was recorded in the plots with highest weed density. At the highest densities of redroot pigweed, seed P and K of soybean was reduced by 111 and 49% in comparison with the control, respectively (Table 3).

The effect of fertilization, pigweed density and nanosilver on leaf chlorophyll is presented in Table 2. Data analysis showed that pigweed density and nanosilver did not significantly affect leaf chlorophyll concentration. Comparison of basal fertilizer level showed that the highest chlorophyll concentration was obtained on the F5 treatment, and the least was in the F3 treatment (Fig. 3).

Fertilization had significant effect on oil yield, while no significant differences were found between pigweed density and nanosilver levels. There was a significant interaction ($P < 0.01$) between fertilization × nanosilver treatments. The highest percentage of seed oil was obtained from F1 and F2 treatments (Fig. 4) and the highest oil yield was from plants

Table 2. Analysis of variance (means square) of soybean traits as affected by fertilization, pigweed density and nanosilver. ns, not significant; *, significant at $p < 0.05$; **, significant at $p < 0.01$

Treatment	df	Seed oil (%)	Leaf chlorophyll	Seed potassium	Seed phosphorus	Seed nitrogen	Mass of 100 seeds	Number of seeds per pod	Number of pods per plant	Seed yield
Block	2	17.9 **	22.6 *	65182.2 **	1112.2 **	15701.2 **	85.2 **	1.2 ns	18.6 *	358520.3 **
Fertilization (F)	5	19.5 **	35.2 **	98128.1 **	1058.2 **	85365.2 **	92.5 **	11.9 **	65.2 **	7552013.5 **
Error a	10	2.2	4.1	1526.2	51.2	910.1	5.5	0.8	4.2	37084.1
Pigweed density (D)	2	10.2 ns	12.9 ns	41256.2 **	910.2 **	95695.2 **	66.1 **	9.1 **	58.1 **	5623564.2 **
F × D	10	9.2 ns	11.2 ns	4158.2 ns	111.2 ns	22365.2 **	45.1 **	1.6 ns	3.1 ns	3528316.2 **
Error b	24	4.1	7.2	2100.1	61.5	1985.2	7.1	0.9	5.5	90010.1
Nanosilver (N)	1	11.2 ns	6.2 ns	3959.1 ns	69.2 ns	21958.5 **	34.1 ns	5.2 ns	54.2 **	5695258.2 **
F × N	5	45.1 *	11.2 ns	5005.3 ns	61.1 ns	1256.2 ns	14.5 ns	2.1 ns	2.1 ns	25665.2 ns
D × N	2	9.2 ns	8.1 ns	1589.6 ns	44.6 ns	19546.3 **	19.4 ns	0.9 ns	0.5 ns	56985.5 ns
F × D × N	10	8.2 ns	8.9 ns	890.1 ns	10.2 ns	256.2 ns	10.2 ns	0.2 ns	1.1 ns	1256.2 ns
Error c	36	5.1	8.2	996.5	63.5	2123.3	9.9	2.4	6.6	25695.2

Table 3. The main effects of fertilization, pigweed density and nanosilver on some agronomic traits of soybean. Mean values in each column with the same letter are not significantly different (LSD at 5% probability)

Treatment	Mass of 100 seeds (g)	Number of seeds per pod	Number of pods per plant	Seed potassium (mg 100 g ⁻¹)	Seed phosphorus (mg 100 g ⁻¹)
F1 (FYM)	13.9 b	2.0 c	19.8 c	1001.5 b	222.8 b
F2 (compost)	13.7 b	2.3 bc	17.7 c	954.1 c	224.1 b
F3 (chemical fertilizer)	14.8 a	2.5 b	22.7 b	879.5 d	228.2 b
F4 (FYM + compost)	13.8 b	2.5 b	22.6 b	1198.6 a	229.6 b
F5 (FYM + compost + chemical fertilizer)	15.0 a	2.7 a	26.2 a	1201.4 a	241.2 a
F6 (control)	13.2 b	1.6 d	6.9 d	444.7 d	178.1 d
D1 (weed-free)	14.1 a	2.9 a	24.5 a	1121.4 a	298.5 a
D2 (10 plants m ⁻²)	14.1 a	2.2 b	21.2 b	900.1 b	210.8 b
D3 (20 plants m ⁻²)	14.0 a	1.6 b	12.3 c	818.2 c	152.6 c
N1 (with nanosilver)	14.1 a	2.3 a	22.1 a	951.2 a	221.1 a
N2 (without nanosilver)	14.1 a	2.2 a	16.5 b	942.2 a	219.8 a

in the F5 treatment (data not shown). In this study, high negative correlation ($r^2 = -0.60$) was observed between seed N and oil concentration (Fig. 5). The interactive effect of fertilization × nanosilver showed that the highest seed oil concentration was obtained in F2N1 treatment.

Discussion

Application of silver ions can displace copper ions from certain receptor proteins. Consequently, silver application blocks ethylene perception, since copper ions have a critical role in ethylene binding with the receptors (Hedden, Thomas 2006). Therefore, some results in this study can be attributed to the effect of silver in preventing ethylene action. Also, with application of chemical fertilizer, most of the rhizosphere nitrogen is absorbed by the weed, but in organic manure application, the gradual release of nitrogen occurred and soybean was more successful in competition. Even the high density of pigweed in organic manures may cause dominance of soybean in competition. Hatch et al. (2007) reported that incorporation of farmyard manure in the soil had beneficial effects of increasing biological nitrogen fixation, dry matter, and N yields in red clover.

Sufficient moisture level, availability of nutrients, and lack of pathogens are the most important factors for plant fertility and seed production. Rudresh et al. (2005) showed

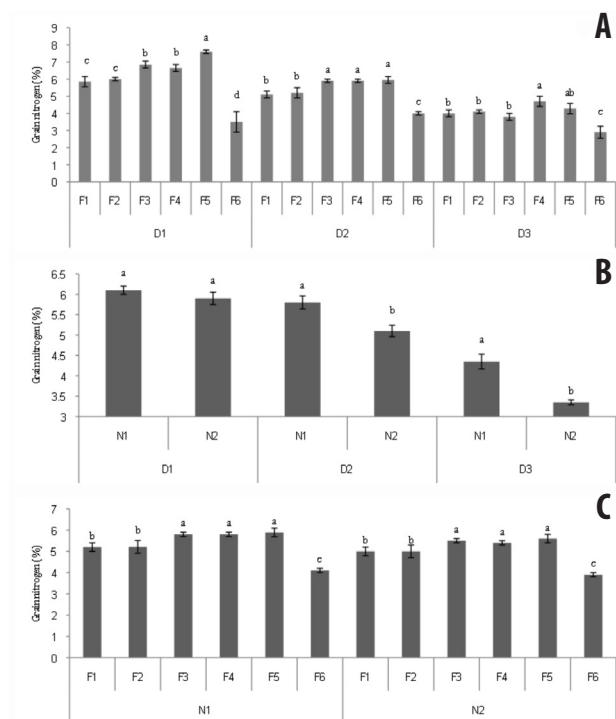


Fig. 2. Interactive effects of fertilization × pigweed density (A), pigweed density × nanosilver (B) and fertilization × nanosilver (C) on soybean seed nitrogen. F1, farmyard manure; F2, compost; F3, chemical fertilizer; F4, farmyard manure + compost; F5, farmyard manure + compost + chemical fertilizers; F6, control; D1, weed-free; D2, 10 plants m⁻²; D3, 20 plants m⁻². Mean values with the same letter in each section are not significantly different (LSD tests at 5% probability). Bars indicate SE.

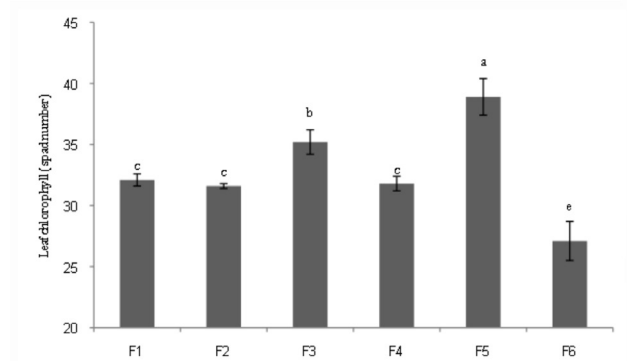


Fig. 3. Effect of fertilization on leaf chlorophyll concentration. F1, farmyard manure; F2, compost; F3, chemical fertilizer; F4, farmyard manure + compost; F5, farmyard manure + compost + chemical fertilizers; F6, control. Mean values with the same letter in each section are not significantly different (LSD tests at 5% probability). Bars indicate SE.

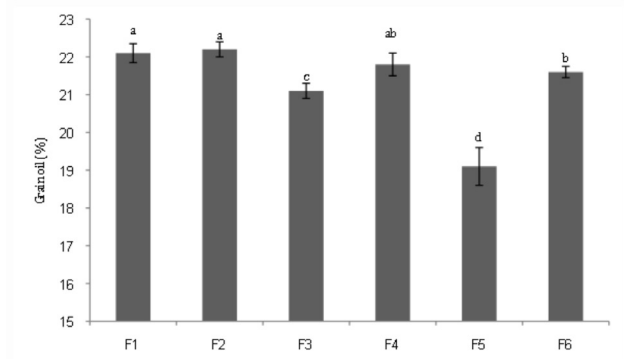


Fig. 4. Effect of fertilization on soybean oil concentration. F1, farmyard manure; F2, compost; F3, chemical fertilizer; F4, farmyard manure + compost; F5, farmyard manure + compost + chemical fertilizers; F6, control. Mean values with the same letter in each section are not significantly different (LSD tests at 5% probability). Bars indicate SE.

that nutrient availability plays an important role in increasing seed number per pod. As mentioned, F5D1N1 produced the highest seed yield. Perhaps, along with meeting plant need for phosphorus, adding compost and farmyard manure to soil can provide microelements for plant in weed-free conditions. Increasing densities of redroot pigweed resulted in more soybean yield losses. Compost applied in the current study has been shown to contain elevated concentrations of microelements including zinc, magnesium and calcium. Moreover, it seems that organic manure improves soil structure and optimizes root growth conditions by providing organic matter and nutrients.

As mentioned, silver can prevent ethylene action, and the study of Wilmowicz et al. (2008) showed that no flower bud formation was observed when seedlings were treated with ethylene, which was thought to be a strong inhibitor of flowering. Therefore, increased number of pods in nanosilver treated plants in this study may be due to the effect of silver in preventing undesirable ethylene action on flowering.

Combined application of compost and FYM increases soil enzymatic activity such as phosphatase and, as a result, P availability for plants increases (Mohammadi et al. 2011). Also, application of organic manure increased the ability of *Bacillus* sp. to produce organic acids such as gluconic, citric and fumaric acids under P-limiting conditions, in consequence increasing the solubility of poorly soluble phosphorus compounds (Veneklaas et al. 2003). It has been reported that nitrogen availability is the most limiting factor for crop yield in organic farming, because of insufficient mineralization rate of added organic matter or immobilization of nitrogen by soil microorganisms (Clark et al. 1999).

In the present study, the comparison of leaf chlorophyll concentration between treatments (Fig. 2) indicated that severe competition for nitrogen occurred between soybean and pigweeds, and that this competition was alleviated by

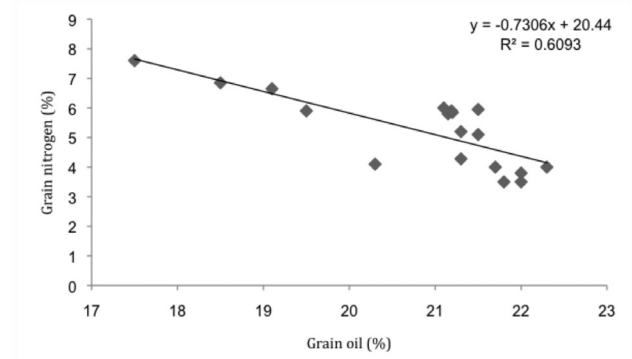


Fig. 5. Correlation between oil and nitrogen concentration of soybean seed.

application of organic manure. Comparison of fertilization levels showed that the highest chlorophyll concentration was obtained from integrated fertilizer treatment (farmyard manure plus chemical fertilizer). Regarding the key role of nitrogen in chlorophyll structure, it seems that supply of this element by combined application of organic and chemical fertilizers is the main reason for the observed increased leaf chlorophyll concentration. Considering existence of the highest nitrogen level in this treatment, achieving high rates of chlorophyll was not unexpected. Positive correlations between leaf nitrogen and chlorophyll concentration have been reported in previous studies (Dordas, Sioulas 2008). Increased leaf chlorophyll concentration in these treatments is related to more mineral elements, such as iron, magnesium, and manganese, provided by simultaneous application of compost and farm manure.

The application of chemical fertilizer alongside with organic fertilizer in the F5 treatment increased nitrogen uptake by soybean and caused the lowest percentage of oil obtained from this treatment. Similarly, previous studies have shown decreased seed oil with increasing nitrogen concentration (Mohammadi, Rokhzadi 2012). High application of nitrogen fertilizer can cause decrease in carbohydrate availability for oil synthesis, but increased protein synthesis (Rathke et al. 2005). The physiological reason for the negative correlation is related to the competition for carbon skeletons during carbohydrate metabolism. The synthesis of both fatty acids and amino acids requires carbon compounds from the decomposition of carbohydrates. Since the carbohydrate content of proteins is lower than that of oils, increased nitrogen supply intensifies the synthesis of proteins at the expense of fatty acid synthesis and thus, reducing the oil concentration of the soybean seed. In contrast, the study of Hao et al. (2004) showed that application of FYM led to increase in seed oil concentration.

Also, applying organic manure in high pigweed infested plots (F4D3) increased soil organic matter, and release of nutrients occurred gradually. This can lead to greater uptake of nitrogen by soybean in competition with redroot pigweed. Moreover, organic fertilizers with improved soil

physical properties provided suitable conditions for root development and biological nitrogen fixation (Ouedraogo et al. 2001). Labraba and Araus (1991) reported that spraying silver nitrate caused improvement of seed yield in wheat. Seed abscission is one of the main factors causing reduced seed yield in plants (Seifshahandi et al. 2011). It has been shown that one of the reasons for plant organ abscission is imbalance between phytohormones. Ethylene plays an important role in this process. Thus, the observed increase in seed yield was a result of reduced seed abscission due to the inhibitory effect of silver on ethylene action.

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