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Effects of Vermicompost and Chemical Fertilizers on Phonological and Phytochemical Traits of Soybean (*Glycine max* L.)

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ABSTRACT

One of the basic principles of sustainable agriculture is the use of organic fertilizers in agricultural ecosystems with the purpose of eliminating or substantially reducing the use of chemical inputs. To evaluate the effect of different amounts of vermicompost on some phonological and phytochemical traits of soybean, an experiment was conducted at Agriculture and Natural Resources Research Center of Shahrekord in 2013. The experiment was conducted in a randomized complete block design including vermicompost treatments (10, 20, 30, 40 and 50 ton.ha^{-1}), chemical fertilizer based on soil testing analysis in the amount of 300 (kg.ha^{-1}) (N.P.K: 20.20.20), and control (no fertilizer). The results showed that 40 ton.ha^{-1} vermicompost had a positive and significant effect on the soybean phonological stages, the content of chlorophyll a and b and carotenoid. Significant reduction of the required GDD from planting to germination, flowering, and pod stage, but required GDD was observed from increased the pod stage to full maturity was significantly increased. In addition, the chlorophylls a and b content and carotenoid were increased in comparison with control treatment. It appears that due to the positive effects of organic fertilizers on soil physical and chemical properties and gradual releasing of the soil's required elements along with the high capacity of the humidity perseverance, they can be appropriate alternatives for chemical fertilizers.

Keywords: Soybean, Vermicompost, Photochemistry, Phonological, GDD, Chlorophyll a and b, Carotenoid

INTRODUCTION

Soybean (*Glycine max* L.) is an annual diploid plant (2n=40) from the species of *legume* (Leguminosae) grown in the form of a strong and relatively full foliage shrub. Its dry seeds content 18% to 25% proteins and serve as one of the most important oilseed crops. It contains digestible nutritional ingredients such as calcium, iron, and is rich in vitamins with great value for human nutrition (Khajepur, 2007).

In recent decades, use of chemical inputs in farmlands has led to several environmental problems including water pollution, the slump of the agriculture products quality and the reduction of soils fertility. It is why there is a more tendency to use non-chemical fertilizers for supplying the plants' needs (Sharma, 2002).

In addition to their nutritional roles, organic fertilizers have a significant effect on improving the products quality and the soil physical traits and its biological activity. One of the organic fertilizers is vermin-compost made by earthworms using organic waste including manure and plants' residue. It has a high porosity, a high power in absorption and preservation of the minerals, an appropriate ventilation and drain, a high capacity for water preservation and is free of odor and pathogens, and nowadays, its usage in sustainable agriculture is common in order to improve the growth and quality of the garden and agriculture products (Arankon *et al.*, 2004). Doing a research on red clover, Sayons *et al*., 1998, stated that using the earthworms' waste as organic fertilizers can improve its flowering conditions and growth indices led to increasing the density of the nutritional elements of organic fertilizers.

The amount of temperature needed for a plant from the time of cultivation to each phonological stage and, finally, complete ripe is named growing degree days of GDD. In addition, Radmehr 1998 stated that increasing the temperature leads to increasing the plants' physiological development. As a result, regardless of temperature, for completion of each stage of growth, a stable and specific amount of GDD is needed.

The leaves' chlorophyll content is one the key factors in determining the photosynthesis speed and producing the dry matter (Ghush *et al*., 2004). In the report of Ma *et al.* (1995), there was a positive correlation between the soybean photosynthesis rate, chlorophyll amount and the leaves proteins. In fact, the chlorophyll amount indicates the rate of germination.Due to harmful effects of chemical fertilizers, this study was conducted to examine the organic fertilizer effects of vermicompost on some soybean physiological and agronomic traits as an alternative to chemical fertilizers.

MATERIALS AND METHODS

This study was conducted in 2013 at Agriculture and Natural Resources Research Station of Shahrekord (50°, 56´ East longitude and 32°, 18 ´north latitude and altitude of 2037 m above sea level. The annual rainfall average is 330 mm and the glacial average is 115 days early. The experiment was arranged in a randomized complete block design with three replications. The treatments included vermicompost in 5 levels (10, 20, 30, 40 and 50 $ton.ha^{-1}$), 300 $kg.h^{-1}$ chemical fertilizers, N.P.K: 20.20.20 based on soil analysis and control (without fertilizer).

The vermicompost and chemical fertilizers treatments were applied before planting. Soil and vermicompost characteristics are shown in Tables 1 and 2, respectively. Soybean seeds (CV M9) were planted in high density in 21 karts (8×5.2 m). Experimental plots were consisted 4 rows 50 cm. Seeds were thinned to 5 cm distances at 2 leaf stage. Irrigation was done every 5-7 days depending on the temperature. Weed control was done by hand in two stages.

By using the daily temperature data in growing season, the required GDD for each stage of soybean development was calculated as follows:

$GDD = \sum (T_{min} + T_{max}) /2 - (T_b)$

In which T_{min}=the minimum temperature, T_{max}=the maximum temperature, and T_b=the base temperature (for soybean it is 10°C)

The chlorophyll measurement was done by Arnon technique (1949) in a way that 0.5 g sampled at pollination stage leaf was poured in Chinese oven, smashed by liquid nitrogen and squashed, then 20 ml acetone 80% was added to it. After that, it was put in centrifuges at the speed of 6000 rpm for 10 minutes, and separated extract was transferred into the balloon glasses. An amount of extract was poured in a spectrophotometer. The absorption amount by spectrophotometer was conducted for chlorophyll a in wavelength of 663 and 470 nm, for chlorophylls a, b and carotenoid, respectively. Finally, by using the below formula, the amount of carotenoid and chlorophyll a and b were calculated based on mg/ml.

Chlorophyll a = 12.25(A663) – 2.79(A645)

Chlorophyll b = 21.21(A645) – 5.1(A663)

Carotenoides = (1000(A470) – 1.8(chl.a) – 58.02(chl.b))/198.

The required data were analyzed using Minitab software of ver: 16. Means were compared using Duncan's multiple range tests at 5% and 1% probability levels.

Table 1. Results site soil analyse

Depth Cm	E.C. $ds.m^{-1}$	pH. of past	O.C %	P ava. $mg.kg^{-1}$	K ava. $mg.kg^{-1}$	N %	Zn. $mg.kg^{-1}$	Mn. $mg.kg^{-1}$	Fe. $mg.kg^{-1}$	Cu $mg.kg^{-1}$
0 – 30	0.664	8.44	0.443	18.8	239	0.054	0.55	8.26	4.87	0.92

Table 2. Material analyses of vermicompost

pH	EC $ds.m^{-1}$	N %	P_2O_5 %	K_2O %	Na %	Ca $mg.kg^{-1}$	Mg $mg.kg^{-1}$	Zn $mg.kg^{-1}$	Fe $mg.kg^{-1}$	Mn $mg.kg^{-1}$	Cu $mg.kg^{-1}$	moisture %	O.C %
7.75	3.428	1.620	0.421	1.441	0.63	1.91	0.72	106.11	4581.23	247.23	19.71	11.28	16.11

RESULTS AND DISCUSSION

GDD from planting to germination

The variance analysis results (Table 3) indicated the significant effect of treatments on this growth stage. It seems that because of using the vermicompost, the soil structure has improved due to increasing of porosity and reduction of soil bulk density and therefore less resistance against germination and faster emergence of the seedlings from the soil. In a study of Sajadinik and Yadavi (2013), the GDD from planting to germination stage in sesame was reduced because of the effect vermicompost. Vermicompost has growth enzymes and hormones that increase the activity of the germination enzymes and causes a faster emergence of Seedlings from the soil by making an appropriate bed (Cherniad *et al*., 2003). Lebaschi *et al*. (2003) stated that the amount of GDD in germination stage was reduced in sesame and Hypericum because of using vermicompost.

GDD from germination to flowering

The fertilizer treatments had significant effect on GDD from germination to flowering (Table 3). The highest GDD for this period was seen in control and 10 ton/ha^{-1}vermin-compost treatments. In addition, the least GDD was found for the vermin-compost treatment 40
ton/ha^{-1}. It seems that the presence of organic fertilizers leads to a reduction of grown in comparison to chemical fertilizers. Consequently, the plant reaches to the flowering stage with less GDD. On the other hand, because of earlier germination of the seeds in vermin-compost treatments, the flowering stage will begin sooner. Pandi (2005) showed that using of vermicompost increases the occurrence speed of development stages via improving the useful vital reactions in soil and absorption of water and nutrition. Ataie *et al*. (2013), in examining the procedure of changes in soil nitrogen and phosphorus by using organic and chemical materials, concluded that using organic materials, reduces the nitrogen sources in soil and make the flowering stage of chamomile faster.

GDD from complete flowering to pot stage

The fertilizing treatments had a significant effect on GDD from germination to flowering (Table. 3). According to the results there was no significant differences among the control (without fertilizer), chemical fertilizer and low amount of compost (10 and 20 ton/ha^{-1}) treatments. On the other hand, treatments of 30, 40 and 60 ton/ha^{-1} of vermicompost had the least GDD rate for this period. It seems that by faster the seed germination, the flowering and pot stages will occur sooner. The significant and positive correlations between the these stages are an evidence for this above reasoning.

Sajadinik and Yadavi (2013) and Atai *et al*. (2013) found similar results stating that using vermicompost causes a good growing conditions and accessibility of nutrition for growth and leafe development because of the more solubility of micronutrients in soil and as a result amending of the soil physical, chemical and biological traits.

GDD from complete pot stage to ripening

The results of variance analysis (Table 3) indicated that GDD, from complete pot stage to ripening is significantly under the effect of fertilizer treatments. That the maximum and minimum GDD were found in 40 ton.ha^{-1} vermin-compost and control treatments, respectively. It seems that faster growing period and reduce of nitrogen sources in the early stages increases the required GDD for this stage and therefore seed. Filling will be increased. The negative and significant correlations (Table. 4) of this stage with germination to flowering stage (r=-0.887) confirms this reasoning. Sajadinik and Yadavi (2013) stated that faster ripening of sesame was under the effect temperature in final growth stages. Kuchaki and Nasiri Mahallati (1993) stated that one of the most important merits of determining the phonology of the plant is optimized by using of ecological factors for increasing its functionality. Wilix *et al.* (1987) stated that for growth of soybean cultivars, the temperature is more important than day-length. In addition, they stated that there was a significant correlation between the soybean growth stages and the received GDD from flowering period to complete ripening.

Carotenoid

According to the results of variance analysis (Table 3), the soybean carotenoid contact was affected by fertilizer treatments. The highest amount of leaf carotenoid was related to of 30 and 4o ton.ha^{-1} vermicompost treatments (Figure 1) and the least amount was obtained control (without fertilizer) and chemical fertilizer treatments. Furthermore, there was a negative and significant correlation between the leaf carotenoid and GDD from flowering to pot stage (Table 4). It seems that increasing the soil microbial activity and gradual releasing of the mineral compounds and elements such as nitrogen and phosphor, some appropriate conditions have occur studied the for growing and producing the carotenoid pigments. Ataie Kachuie *et al*. (2012) effect of mineral and organic fertilizers on marigold’s photochemical traits is dand showed that the carotenoid amount was increased significantly by adding organic fertilizers. Pant *et al*. (2011) and Garcia Martins (2002) found that decomposition of vermicompost under aerial conditions leads to the production of some compounds similar to the chemical structure of auxin and cytokinin in the methanol extract of different kind of vermicompost which an important factor in stimulating the production and storage of carotenoid.

Table 3. The variance analysis of different traits

S.O.V	d.f	MS				Chla	Chlb	Carotenoid
		planting to germination	germination to flowering	flowering to pod stage	pod stage to ripening			
Rep	2	5.7 ^{ns}	3.18 ^{ns}	20.63 ^{ns}	6.05 ^{ns}	0.108 ^{ns}	0.^{01ns}	0.11 ^{ns}
Treat	6	2672.5**	394.24**	432.76**	142.63**	47.32**	14.480**	13.70**
Error	12	19.1	42308	13.56	12236	0.142	0.058	0.062

ns,** and * : non significant and significant probability levels.

Table 4. Means comparing of different to phonological traits soybeen based on GDD

Adjective treatments	(planting to germination)	(germination to flowering)	(flowering to pod stage)	(pod stage to ripening)
Control	142.37±3.51^{b}	637±2.00 ^{b}	208.02±3.61 ^{a}	414.33±4.04 ^{c}
Mineral fertilizer	169.27±4.03 ^{a}	647.67±2.52 ^{a}	210.27±2.05 ^{a}	423±2.65 ^{b}
Vermi 10Ton/ha	130.7±4.03 ^{b}	634±4a ^{bc}	215.67±5.13 ^{a}	418.33±1.15 ^{bc}
Vermi 20Ton/ha	113.15±3.42 ^{c}	636.23±3.33 ^{b}	214.33±4.73 ^{a}	418.33±2.08 ^{bc}
Vermi 30Ton/ha	94.73±4.61 ^{d}	624.33±2.52 ^{c}	189.78±4.53 ^{b}	421.33±2.08 ^{b}
Vermi40Ton/ha	94.67±5.51 ^{d}	614±3.61 ^{d}	190.78±3.02 ^{b}	436.67±2.08 ^{a}
Vermi 50Ton/ha	88.67±3.51 ^{d}	631.67±2.89 ^{bc}	189.75±4.52 ^{b}	422.33±3.51 ^{b}

For each traits means with at least one common letter are not significantly different based on duncans test at 5% probability levels

Table 5. Correlation coefficient between traits

	planting to germination	germination to flowering	flowering to pod stage	pod stage to ripening	Chla	Chlb	Carotenoid
planting to germination	1						
germination to flowering	0.42^{ns}	1					
to pod stage flowering	0.708**	0.651**	1				
pod stage to ripening	-0.347^{ns}	-0.887**	-0.519^{ns}	1			
Chla	-0.61*	-0.61*	-0.49^{ns}	0.687**	1		
Chlb	-0.5^{ns}	-0.783**	-0.398**	0.821**	0.828**	1	
Carotenoid	-0.662*	-0.549^{ns}	-0.65*	0.47^{ns}	0.4^{ns}	0.536^{ns}	1

ns,** and * : non significant and significant probability levels.

Chlorophylls a and b

As it is indicated in Table 3 except in treatment of 40 ton/ha of vermicompost which had the highest amount of Chlorophylls a and b (54.18 and 9.8 respectively mg/ml), there was low amount of chlorophyll in other fertilizer treatments, although the differences significant (Figure 2). The least amounts (8.6 and 65.1mg/ml) for Chlorophyll a and b, respectively were found in control treatment which was decreased to about 63% and 81%. It seems that high nutritional elements content of vermin-compost and gradual releasing of some elements such as nitrogen and phosphor reduced leaching and provide adequate amount caused of the elements for growth and were found, as the result, the content of leaves chlorophyll increased.

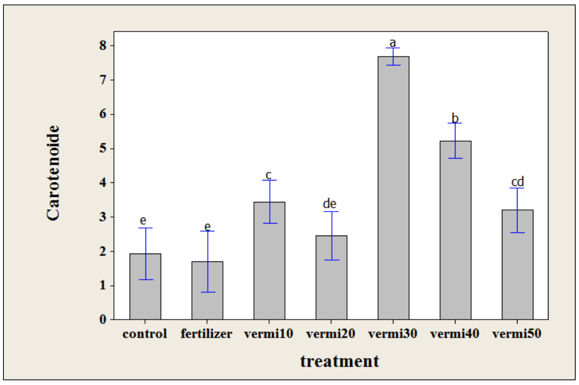

Figure1. Comparing fertilizer treatments effects on carcinoid

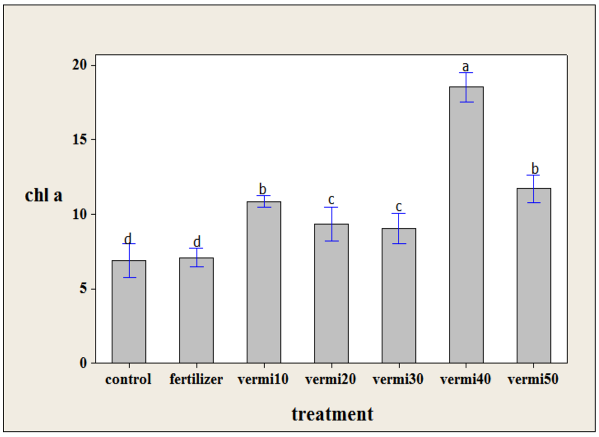

Figure 2. Comparing fertilizer treatments effects on chlorophyll- a

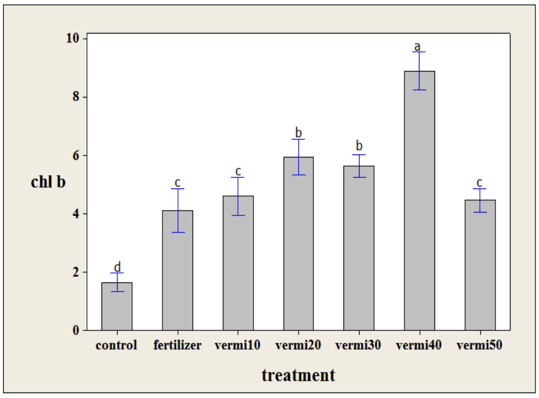

Figure 3. Comparing fertilizer treatments effects on chlorophyll - b

Joigbe's (2007) studied about the effects of mineral and organic fertilizers on function rate and chlorophyll content of maize and sorghum and indicated that mutual use of these fertilizers significantly increased the rate of chlorophylls a and b. Rajan *et al*. (2000) studeid the effects of vermicompost on some soybean agronomic traits and indicated that the use of organic fertilizers had a significant effect on chlorophyll content. Increasing of chlorophyll will increase the photosynthesis naturally leading to increasing the production of elaborated sap, seed filling rate and period and, leaf surface resistance. Wilekekas *et al*. (1987) stated that there is a high correlation between soybean growth rate and GDD from flowering stage to complete ripening. Also Mojab Ghasrodashti *et al*. (2011), Abdolsabur and Abolsaud (1996) found that the chlorophyll content will be increased because of using organic fertilizer.

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