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The Effect of Maize (*Zea mays* L.) / Soybean (*Glycine max* (L.) Merr.) Intercropping and Biofertilizer (Azotobacter) on Yield, Leaf Area Index and Land Equivalent Ratio

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ABSTRACT
This study was conducted to determine the effects of the different intercropping design of maize/soybean compared with sole cropping, in combination with Vitormone (biofertilizer; Azotobacter) during 2012 and 2013. There were four different intercropping designs were used in the experiment: *i*) sole maize and soybean, *ii*) 1 maize + 1 soybean in alternate rows, *iii*) 1 maize + 2 soybeans in alternate rows and *iv*) 1 maize + 2 soybeans in intra rows under control with the Vitormone application. The experiment was laid out in a randomized complete block design with two factors and three replications. The results showed that the seed/grain yields and the Leaf area index of sole soybean and sole maize outperformed the intercropping. The highest yields for both maize (8.0 t ha^{-1}) and soybean (3.26 t ha^{-1}) and land equivalent ratio (1.26) were recorded at 1 maize + 2 soybeans in alternate rows among the intercropping. The effect of Vitormone was significantly positive for all cropping system except 1 maize + 1 soybean in alternate rows. This suggests that 1 maize + 2 soybeans in alternate rows can intercrop and combine with the Vitormone. The intercropping of cereal/legume has the potential to improve the utilization of resources in monocropped lands.

Keywords: Alternate row and intra row, Intercropping, LAI (leaf area index), LER (land equivalent ratio), the Vitormone

1. Introduction

Intercropping can be defined as a multiple cropping system that two or more crops planted in a field during a growing season (Yong et al. 2015). Intercropping is a way to increase diversity in an agricultural ecosystem. The most benefit of intercropping is optimum utilization of plant resources such as nitrogen in Gramineae (or Poaceae)/Legumes (or Fabaceae) intercropping (Nasri et al. 2014). As known, Legume crop is used as soil N supply. When the crop with large canopy intercropped with the small crops, such as maize and soybean intercropping, soybean yield could decrease due to interspecific light competition (Liu et al. 2017). Thus, different intercropping designs such as alternate rows and intra rows were studied in many types of research (Ijoyah & Fanen 2012; Mandal et al. 2014). The optimum inter-row and intra-row distances are the most important to produce a high yield in maize/soybean intercropping (Kim et al. 2018). The LER has been recommended to evaluate the yield advantage of intercropping compared to monocropping (Mahallati et al. 2014). It was reported that the LER values above 1 determined in maize/soybean intercropping (Dolijanvic et al. 2009; Tsujimoto et al. 2015; Kamara et al. 2017).

The Büyük Menderes Basin has a typical Mediterranean climatic characteristic. Although monocropped cotton grown in many areas with saline and alkali soils, agricultural production is intensive, with winter wheat or forage crops–summer cotton or maize rotation. Yavas & Unay (2016 a, b) stated that poor nodulation of soybean and cowpea with maize intercropping occurred in the west of Büyük Menderes Basin. Nodulation failure is caused by soil compaction, flooding, and high pH levels. Vitormone is one of the foliar liquid bio-fertilizers containing *Azotobacter chroococcum* which species responsible for atmospheric nitrogen fixation (Mohiuddin et al. 2000; Ramteke et al. 2016). In many studies, it concluded that the Vitormone promoted the plant growth (Ghosh & Dayal 1998; Gaikwad et al. 2008).

There are few published reports on the poor nodulating of legume in grain/legume intercropping and interaction of bio-fertilizer (the Vitormone) and different intercropping design. Therefore, we conducted the present study to (a) determine the yield performance in maize and soybean intercropping under the Vitormone application; (b) explore the optimum intercropping design.

2. Material and Methods

2.1. Field layout and plant materials

This study was carried out at the field of research and application (37° 45' North, 27° 45' East) of the Field Crops Department of the Agricultural Faculty of Aydın Adnan Menderes University during the summer of the 2012 and 2013. The area is affected by a Mediterranean type of climate with hot and dry summer (Sensoy et al. 2007). The mean temperatures from April to September show that July and August exceed the long term mean temperatures (Figure 1) (provided by the Turkish State Meteorological Service).

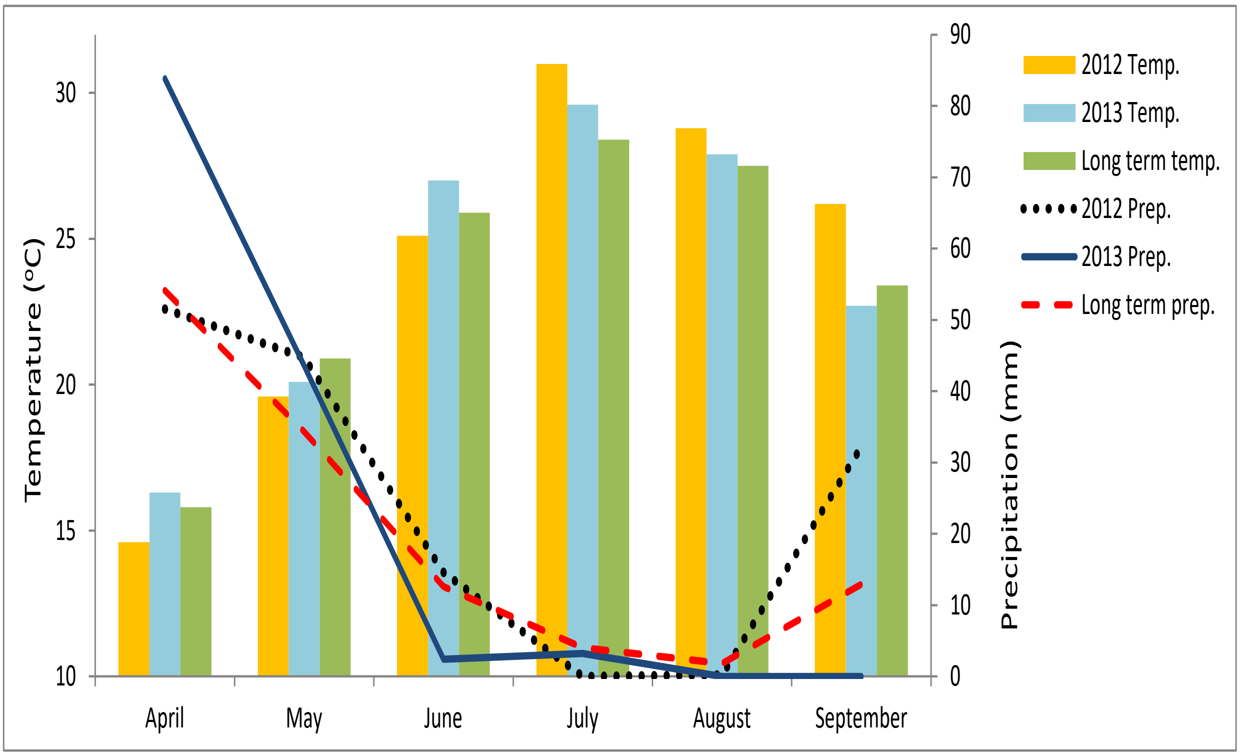

Figure 1- Precipitation and temperature during the cropping season in 2012 and 2013 with a long period

The experiment was sown on loamy sand soil having pH 7.60 (slight alkali), organic matter level is 1.20% (insufficient), total nitrogen 0.095% (medium), available P_2O_5 level is 2.08 mg kg^{-1} (insufficient) and available K_2O level is 1528 mg kg^{-1} (medium) (provided by Aegean University soil analysis laboratory).

P31G98 hybrid maize (*Zea mays* L.) and Umut soybean cultivar (*Glycine max* (L.) Merr.) were used as plant material. The soil characteristics where the experiment is carried out are alkali and poor in organic matter due to monoculture cotton growing. For this reason, it was difficult the nodulation of soybean. Therefore, Vitormone has been used as a factor in the application of biological fertilizer. The Vitormone provides the optimal environment for activating leaf surfaces by organisms and promotes a rich flora formation on the leaf surface (Golenberg & West 2013; Uddin et al. 2014).

In the study, “Randomized Complete Blocks Design with Two Factors" was used to simultaneously investigate the effects of intercropping and biofertilizer (the Vitormone) application (Cochran & Cox 1950). Sowings were carried out in 3 replications on May 1, 2012, and May 7, 2013. As a second factor, maize and soybean sowings were arranged as alternate rows, intra row and sole. The list of crops design in intercropping is given in Table 1 and schematized in Figure 2.

Sole maize (M_B) was planted at 0.70 x 0.15 m and soybean (S_B) planted at 0.70 x 0.05 m sowing standards. Each parcel consists of 12 rows (42 m^2) with a length of 5 m. The alternate rows were planted with 1 row of maize and 1 row of soybean and 35 cm rows and 15 cm and 5 cm rows respectively in the sowing areas (MS_A). There are approximately 90,000 plants ha^{-1} maize and 285,500 plants ha^{-1} soybean in the areas of plain seed and alternate seeding with soybean cultivation. These parcels consist of 24 rows with a length of 5 m (42 m^2). Another alternate sequence was 1 maize + 2 soybean cultivars (MS_D), and 1.05 m x 0.15 m sowing norm for maize and 0.35 m x 0.05 m sowing norm for soybeans were applied. In this regard, the plant densities were 63,490 plants ha^{-1} for maize and 380,940 plants ha^{-1} for soybean. The parcels consist of 24 rows of 5 m length (42 m^2). In the sowing on the same row (MS_C) parcels, two soybeans with 0.05 m space were 0.25 m between two maize. The plant densities were 57,140 plants ha^{-1} for maize and 114,280 plants ha^{-1} for soybean.

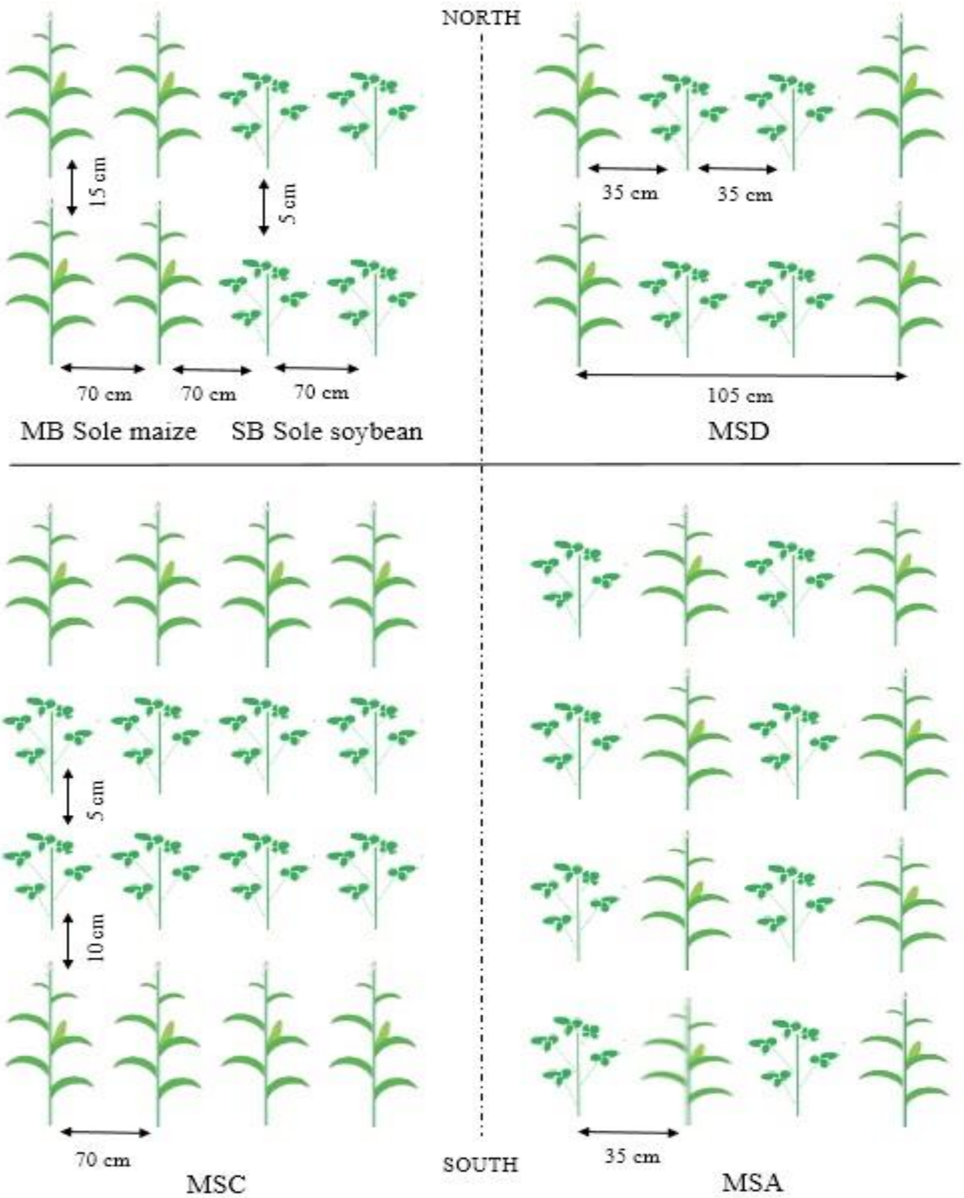

Figure 2- Diagrammatic sketch of different maize-soybean intercropping system

Table 1- The list of crops design in the cropping system

Crop	*Crop Design in intercropping*	*Symbol*
Maize	Sole	M_BV^-
Soybean	Sole	S_BV^-
Maize	Sole + Vitormone	M_BV^+
Soybean	Sole + Vitormone	S_BV^+
1 Maize + 1 Soybean	Alternate Rows	MS_AV^-
1 Maize + 1 Soybean	Alternate Rows + Vitormone	MS_AV^+
1 Maize + 2 Soybean	Alternate Rows	MS_DV^-
1 Maize + 2 Soybean	Alternate Rows + Vitormone	MS_DV^+
1 Maize + 2 Soybean	Intra row	MS_CV^-
1 Maize + 2 Soybean	Intra row + Vitormone	MS_CV^+

For all parcels, the recommended fertilizer rate of 180:80:80 of NPK was applied using a compound fertilizer (NPK 15:15:15) to supply 80 kg each of NPK ha^{-1} before sowing as basal application. Urea (46% N) to supply the remaining dose (100 kg ha^{-1}) of nitrogen was applied before first irrigation for all parcels except sole soybean. Three hoeings were applied for weeding. The Vitormone (Biological fertilizer) application, which is a factor in our study, prepared the Vitormone application (1 mL Vitormone + 1-L water which is the recommended the dose of Vitormone solution). It was applied twice with the mechanical back-sprayer at the flowering stage and 1 week after flowering in soybean.

2.2. Yield

For the yield of maize and soybean; 20 tagged plants were randomly selected from two rows of each plot leaving the outside rows as borders, and all obtained data were corrected to 14% grain moisture for both crops (Nyoki & Ndakidemi 2018).

2.3. Leaf area index (LAI)

For the LAI, data were collected from twenty plants at two the middle rows of each parcel at the stage of silking/tasseling for maize and peak flowering for soybean (Liu et al. 2017). The samples were separated into leaves. Leaf width and length measured in all leaves maize and multiplied to a coefficient factor of 0.75. Single leaf area for soybean was measured using an LI-3100 (LI-COR, Lincoln, NE) leaf area meter device. The LAI (m^2 m^{-2}) was determined by taking into account the number of plants in the unit area.

2.4. Land equivalent ratio (LER)

The LER is widely used to evaluate the land productivity of intercropping and was calculated according to the following formula (Ofori & Stern 1987);

LER= [Intercropping maize yield /Sole maize yield] + [Intercropping soybean yield /Sole soybean yield]

LER > 1 was evaluated as an effective intercropping system in terms of land utilization.

2.5. Statistical analysis

The data of yield and LAI were statistically analyzed using TARIST statistical Package Program (Acikgoz et al. 1994). The differences between the means were compared by the least significant difference (LSD) at the 5% level.

3. Results and Discussion

The results from the present study indicated that interactive effects of intercropping design and Vitormone application for maize yield were significant in the 2012 and 2013 cropping seasons. The high maize yields recorded in sole maize with the Vitormone (16.2 and 15.3 t ha^{-1}) and the non-Vitormone plots (14.9 and 14.3 t ha^{-1}) compared to intercropping plots (Table 2). In both years, MS_DV^+ (8.0 t ha^{-1}) and MS_CV^+ (7.0 and 7.6 t ha^{-1}) parcels produced the yields of the second group. In the parcels (MS_A) which alternate rows were planted with 1 row of maize and 1 row of soybean, maize yields of the non-Vitormone applications were higher than that of the Vitormone applications in both years.

Table 2- The results of variance analysis for the yield

Source of variation	*df*	*Yield*			
		Maize		*Soybean*	
		2012	*2013*	*2012*	*2013*
Block	2	14091.56	3826.54	66.14	332.65
Vitormone (A)	1	321.20	2795.04*	15595.80**	14113.50**
Crop Design (B)	3	1041140.43**	849598.15**	164448.33**	133185.98**
Ax B	3	80637.43**	11888.26**	3885.59**	682.18
Error	14	4540.51	515.83	126.93	620.48

*, **; significant at 5% and 1% probability level, respectively

The interaction of intercropping design and the Vitormone application was significant for soybean yield in 2012 whereas the differences of intercropping designs and the Vitormone applications were found significant in 2013. The yields of sole soybean parcels were higher than intercropping parcels in the Vitormone (4.8 t ha^{-1}) and the non-Vitormone (3.7 t ha^{-1}) parcels (Table 3). These values followed by MS_DV^+ (3.4 t ha^{-1}) and MS_DV^- (2.8 t ha^{-1}). The yields of MS_C and MS_A extensively decreased compared to sole cropping and MS_D. In 2013, sole soybean had the highest yield (4.3 t ha^{-1}) and was followed by MS_D (3.2 t ha^{-1}). Also, the yield of soybean in the Vitormone application recorded higher yield of 2.8 t ha^{-1} compared to the non-Vitormone (2.3 t ha^{-1}). Several studies (Ijoyah & Fanen 2012; Khan et al. 2012; Undie et al. 2012; Verdelli et al. 2012; Hirpa 2013; Ijoyah et al. 2013; Osang et al. 2014) suggested that the maize and soybean yield of intercropping over sole cropping decreased due to interspecific competition. In intercropping systems, yield advantage for maize and soybean could be found, which does not support findings of Li et al. (2001), Ngwira et al. (2012). Also, it was documented that the Vitormone application resulted with higher yield in the soybean (Tahir et al. 2009; Subowo et al. 2010; Koushal & Singh 2011; Ngalamu et al. 2013; Salih et al. 2014), faba bean (*Vicia faba* L.) (Osman et al. 2010), chickpea (*Cicer arietinum* L.) (Uddin et al. 2014). The additional Vitormone application could be enhanced the yields of maize and soybean in especially poor nodulating conditions.

Table 3- Mean yield of the intercropping system and Vitormone application in 2012 and 2013

	Maize (t ha^{-1})				*Soybean (t ha^{-1})*		
	2012		*2013*		*2012*		*2013*
	V$^+$	*V$^-$*	*V$^+$*	*V$^-$*	*V$^+$*	*V$^-$*	
A	6.2 e	9.6 c	6.2 f	7.3 e	0.8 e	0.9 e	1.3 c
B	16.2 a	14.9 b	15.3 a	14.3 b	4.8 a	3.7 b	4.3 a
C	7.0 de	6.4 e	7.6 d	7.2 e	1.3 d	0.9 e	1.2 c
D	8.0 d	6.4 e	8.0 c	7.5 de	3.4 b	2.8 c	3.2 b
LSD $_{(0.05)}$	1.18		0.39		0.20		0.30
V$^+$							2.8 a
V$^-$							2.3 b
LSD $_{(0.05)}$							0.2

A; 1 M + 1 S (Alternate Rows), B; Sole, C; 1 M + 2 S (intra row), D; 1 M + 2 S (Alternate Rows) , V; Vitormone. Within each column, values with the same letter do not significantly differ at 5%

The LAI is a positive indicator to improve the yield and to minimize the evaporation (Kubota et al. 2015). Also, it is probably the most important factor in the competition for light and critical to maintaining high yield in intercropping (Kamara et al. 2017). The effects of interaction between the intercropping system and Vitormone application on the LAI were found to be significant for the maize in the 2012 and 2013; for the soybean in 2013 (Table 4). The differences in the intercropping system and the Vitormone application for the soybean in 2012 were significant. The LAI values of sole maize with Vitormone (6.00 $m^2 m^{-2}$) or without the Vitormone (5.90 $m^2 m^{-2}$) were significantly higher than intercropping parcels in 2012 (Table 5). MS_A plots significantly exhibited the highest LAI values (4.41 $m^2 m^{-2}$ and 4.72 $m^2 m^{-2}$) compared to other intercropping parcels, MS_D and MS_C, respectively. The LAI values of 2013 growing season were similar to 2012 values for maize. In terms of the soybean's LAI, the sole soybean (7.28) had a significantly higher LAI value than that of intercropping systems followed by MS_D (6.40) in 2012. Similar ranging among the LAI values of intercropping systems in both V^+ and V^- parcels were recorded for soybean. However, it should be noted that the sum of maize and soybean LAI in intercropping was always superior to sole maize and soybean (Walker & Ogindo 2003; Kubota et al. 2015).

The LER provides an accurate evaluation of the competition in the intercropping system. Also, the LER of more than 1.0 reveals the yield advantage of intercropping. The LERs of different intercropping systems varied from 0.55 (MS_AV^+) to 1.32 (MS_DV^+). In contrast to many published literature, there was no strong the LER above 1.50 in maize/soybean intercropping (Rahman et al. 2017; Chen et al. 2018). The yield capacities of mentioned studies were 5.63-8.53 t ha^{-1} for the maize and 1.59-2.22 t ha^{-1} for soybean (Chen et al. 2018), 4.9-10.8 t ha^{-1} for the maize and 0.11-1.85 t ha^{-1} for soybean (Rahman et al. 2017), whereas yield values were 6.2-16.2 t ha^{-1} for the maize and 0.8-4.8 t ha^{-1} for the soybean in our study. It can be concluded that yield capacity should be considered when evaluating the LER, especially in the fertile areas. The MS_D, 1 Maize + 2 Soybean in alternate rows, produced greater than other intercropping systems (Table 6). The all LER values of Vitormone application parcels except MSA were found to be higher than the non-Vitormone application. In previous studies, it was documented that the highest LER value (1.26) was found in 1 maize + 2 soybeans in alternate rows (Addo-Quaye et al. 2011; Ijoyah & Fanen 2012; Mandal et al. 2014). Especially, the yield losses of MS_A and MS_C resulted from the continuous shading of the maize, and soybean yield decreased (Lv et al. 2014; Liu et al. 2017).

Table 4- The results of variance analysis for the LAI

		LAI			
Source of variation	*df*	*Maize*		*Soybean*	
		2012	*2013*	*2012*	*2013*
Block	2	0.01	0.00	0.02	0.00
Vitormone (A)	1	0.01	0.00	0.11*	0.11**
Crop Design (B)	3	8.15**	8.61**	14.39**	14.82**
A x B	3	0.05**	0.02**	0.04	0.02**
Error	14	0.00	0.00	0.01	0.00

*, **; significant at 5% and 1% probability level, respectively. LAI; leaf area index

Table 5- Mean LAI of the intercropping system and Vitormone application in 2012 and 2013

	Maize (m^2 m^{-2})				*Soybean (m^2 m^{-2})*		
	2012		*2013*		*2012*	*2013*	
	V^+	V^-	V^+	V^-		V^+	V^-
A	4.41 c	4.72 b	4.38 c	4.51 b	4.72 c	4.81 e	4.78 e
B	6.00 a	5.90 a	5.94 a	5.90 a	7.28 a	7.33 a	7.27 b
C	3.24 e	3.19 e	3.41 d	3.28 e	3.84 d	3.96 f	3.73 g
D	3.93 e	3.90 d	3.47 d	3.40 d	6.40 b	6.60 c	6.38 d
LSD $_{(0.05)}$	0.105		0.071		0.139	0.046	
V+					5.62 a		
V-					5.48 b		
LSD $_{(0.05)}$					0.098		

A; 1 M + 1 S (Alternate Rows), B; Sole, C; 1 M + 2 S (intra row), D; 1 M + 2 S (Alternate Rows) , V; Vitormone.
Within each column, values with the same letter do not significantly differ at 5%.

Table 6- The LER values for intercropping system and Vitormone application

	LER		
Intercropping System	*2012*	*2013*	*Mean*
MS_AV^-	0.88	0.76	0.82
MS_AV^+	0.55	0.70	0.63
MS_CV^-	0.67	0.72	0.69
MS_CV^+	0.70	0.85	0.78
MS_DV^-	1.19	1.21	1.20
MS_DV^+	1.20	1.32	1.26

LER; Land equivalence ratio

4. Conclusions

The interaction of the intercropping design and the Vitormone were significant for yield and the LAI in both years. The Sole maize and the sole soybean were shown to have a significantly higher yield and the LAI values than the intercropping system. The results of LER confirmed that the 1 maize + 2 soybeans in the alternate rows were preferable among the all intercropped yields. Finally, it can be concluded that the combination of intercropping and Vitormone was found suitable for the poor nodulating conditions in the monocropped agricultural system.

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