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Pinto bean and black mustard responses to bio-fertilizers under intercropping system

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Abstract: In order to evaluate the response of pinto bean and black mustard intercropping to application of biological and chemical nitrogen fertilizers, a factorial set of treatments was arranged within randomized complete block design (RCBD) with three replications. In this experiment, fertilizer treatments were non-fertilizer, bio-fertilizers, bio-fertilizers + 50% chemical urea fertilizer (125 kg/ha) and bio-fertilizers + 100% chemical fertilizer (250 kg/ha). The cropping patterns comprised pure stands of bean and black mustard, additive intercropping with a ratio of 50% black mustard + optimum density of pinto bean mono cultures and an additive intercropping with optimum density of two species in mono cultures. Application of bio-fertilizers and chemical fertilizer increased most of the agronomic traits in pinto bean and black mustard plants. The bio-fertilizers + 100% of urea followed by bio-fertilizers + 50% of urea were the superior treatments, compared with other fertilizers. Evaluation of intercropping patterns with using land equivalent ratio (LER), relative yield total (RYT), relative value total (RVT) and relative crowding coefficient (RCC) indices showed that the highest LER and RYT were recorded for bio-fertilizer + 100% chemical fertilizer treatment. The highest RVT and RCC were obtained from control treatment (non-fertilization) in inter-cropping (optimum density of two species). Based on the LER, RVT, RYT and RCC indices, it was evident that intercropping of pinto bean and black mustard was more beneficial than mono cultures. Therefore, it was generally concluded that intercropping pattern was better than monocultures of two species at different levels of fertilizers and also bio-fertilizers application could increase efficiency of chemical fertilizer. Thus, bio-fertilizers + 100% chemical fertilizer and intercropping of pinto bean and black mustard was the better treatment.

1. Introduction

To increase the efficacy of crop production, improve soil fertility and environmental protection, an alternative cropping system could be needed (Kiminami *et al.*, 2010). Intercropping is a method for moving towards sustainable agriculture and environmental protection (Habimana *et al.*, 2019; Moghbeli *et al.*, 2019). One of the farming practices is concurrent cultivation of two or more crops in the same field which is experienced in many regions of the world (Tüzel and Öztekin, 2017). Some reasons have been identified for farmers engaging in intercropping which are still valid today. First, it leads to increase in the utilization of environmental factors. This has both space and time dimension.

Plants are different in rooting habitat and have different nutrient requirements. Thus, the intercropping of plants can increase the utilization of nutrients, water and light. Also, intercropping can lead to reduction of adverse conditions in the agroecosystem (Lithourgidis *et al*., 2011). Intercropping may also lead to better soil management because of the fact that may crops overlap in terms of the time they are in the soil. Other economic reasons such as dependability of returns and increased returns from the same piece of land may make farmers adopt intercropping (Alabi and Esobhawan, 2006). Watikai *et al*. (1993) and Willy (1990) confirmed that increasing the yield of biomass in intercropping is due to the more absorption of light. The highest performance is achieved when intercropping canopy is composed of two layers: (1) tall plants with narrow leaves and high photosynthetic capacity; (2) dwarf plants with lying leaves and low photosynthetic capacity. In general, the productivity in intercropping is more than sole cropping (Raei *et al*., 2015).

Among nutrient elements, nitrogen is an important nutrient and has vital functions in plant growth and development. Nitrogen deficiency imposes most limits on crop production compared to other nutrients. With large areas of the arable land in Iran being located in arid and semiarid regions, most of them face low organic matter content as well as nitrogen deficiency and also, to achieve an economically sound production, nitrogen plays a significant role in these regions (Joorabi *et al*., 2015). On the contrary, slow-release nitrogen fertilizers are effective and inexpensive alternative to soluble N (Jiao *et al*., 2005). The yield of pea in intercropping of pea and wheat increased by application of slow release nitrogen fertilizer (Abbady *et al*., 2016). In all around of the globe, for achieved high yield of plants, the chemical fertilizers are extensively being used. However, this type of fertilizers has devastating effects on the health of the soil animals. A better alternative of these chemicals might be to exploit the microbial capabilities to be served as bio-fertilizer (Tomer *et al*., 2016). Bio-fertilizers colonize at the rhizosphere and improve nutrient accessibility of plants and increase the growth of plants. Microorganisms residing in rhizosphere immensely facilitate trace element's uptake. They may act as biocontrol agent, by means of antagonistic activity against phytopathogenic microorganisms, interfering in the bacterial quorum sensing systems, etc. However, bio-fertilizers perform more than one mechanism for accomplishing plant growth enhancement (Kumar *et al*., 2014; Dutta and Patel, 2016).

Black mustard is an important oilseed crop. It is often grown as an intercrop or mixed crop either with pulses or cereals crops, but its productivity is very low due to improper combination (Kumar *et al*., 2014). Bean is also one of the most important food supplements for human, and its protein content is rich (Arija *et al*., 2007). It is also tolerant to shadow and can be planted in intercropping system and grows well. It can increase the soil nitrogen by nitrogen fixation (Kowal and Kassam, 1978). Intercropping of legumes with non-legumes increases yield per unit area, because they use different nitrogen sources and have low competition for nitrogen (Haugard-Nielsan *et al*., 2001). The importance of this pulse crop is based on its good nutritive composition and its high market value, which mainly depends on the consumption quality of the product (nutritional and culinary quality of either the seed or the pod). Thus, the present investigation was carried out to study pinto bean and black mustard responses to bio-fertilizers and chemical nitrogen fertilizer, intercropping system and interaction of intercropping system × nitrogen fertilizer.

2. Materials and Methods

Field conditions

The experiment was conducted in 2016 at the Research Farm of the Faculty of Agriculture, University of Tabriz, Iran (Latitude 38°05' N, Longitude 46°17' E, Altitude 1360 m above sea level with the mean annual rainfall of 285 mm). Some physical and chemical properties of soil in experimental area and averages of maximum and minimum temperatures and rainfall during the work in 2016 were shown in Table 1.

Experimental design and treatments

A factorial set of treatments was arranged with three replications. In this experiment, fertilizer treatments were control (non-fertilizer), bio-fertilizers (azotobarvar 1 and barvar 2), bio-fertilizers + 50% the recommended chemical urea fertilizer (125 kg/ha) and bio-fertilizers + 100% chemical fertilizer (250

Table 1 - Some physical and chemical properties of experimental soil and averages of maximum and minimum temperatures and rainfall during the work in 2016

Physical and chemical properties of experimental soil												
Depth (cm)	EC (ds/m)	PH	Organic Carbon (%)	N (%)	P (mg/kg)	K (mg/kg)	Fe (mg/kg)	Ca (mg/g)	Sand (%)	Silt (%)	Clay (%)	Soil type
0-35	2.77	7.75	0.37	0.04	4.90	255	2.60	780	74	14	12	Sandy loam

Months	Averages of maximum and minimum temperatures and rainfall	
	Temperature (°C)	Rainfall (mm)
April	9.4	78.2
May	16.9	13.5
June	22	14.8
July	28	0
August	29.4	15

kg/ha). Azotobarvar 1 contains the azoto bacter-vinelandii (strain O4) and barvar 2contains the pantoea agglomerans (strain P5) and pseudomonas putida (strain P13). The cropping patterns comprised pure stands of bean and black mustard, additive intercropping with a ratio of 50% black mustard + optimum density of pinto bean mono cultures and an additive intercropping with optimum density of two species in mono cultures.

Measurements

Yield and yield components. At maturity and when the moisture content of seeds decreased by about 18%, 10 plants were harvested from each plot and 100 grains weight of pinto bean and black mustard were recorded. Also to determine of grain and biological yields, an area equal to 1 m^2 was harvested from middle part of each plot considering marginal effect and dried in an oven at 75°C for 48 hours. Subsequently, biological and grain yields per unit area were determined. Harvest index was calculated by the following equation:

$$\text{Harvest index} = (\text{Grain yield}/\text{Biological yield}) \times 100$$

Evaluative indices of intercropping

Land equivalent ratio (LER), as an agronomic index, indicates the efficiency of intercropping for using the resources of the environment compared with mono cultures (Mead and Willey, 1980). The value of unity is the critical value. When the LER is greater than one, the intercropping improves the growth and yield of the cultivars. In contrast, when LER is lower than one the intercropping negatively affects the growth and yield of the plants grown in mixtures (Caballero *et al*., 1995). The LER was calculated as:

$$LER = \frac{Y_{pb}}{Y_p} + \frac{Y_{bp}}{Y_b}$$

where Y_p and Y_b are the yields of pinto bean and black mustard, respectively, as sole crops and Y_{pb} and Y_{bp} are the yields of pinto bean and black mustard, respectively, as intercrops.

Relative value total (RVT) as an economic index proposed by Schultz *et al.* (1982).

This index is widely used now and has been used by many researchers. The RVT was calculated as:

$$RVT = \frac{aP_1 + bP_2}{aM_i}$$

where, P_1 and P_2 are the yields of two different crops in intercropping and M_1 and M_2 are the yields of those of these crops in monocultures ($M_1 > M_2$). Also, a and b are the market prices of crop 1 and 2 respectively.

If the RVT >1, the mixture crop has the advantage and if the RVT <1, pure stand will have an economic advantage. If RVT =1, then these two methods are not economically advantageous to each other.

Relative yield is the ratio of the species response in the mixture to the species response when grown in monoculture. Relative yield total (RYT) is the total RY of the two associated species, as shown in below:

$$RYT = RYa + RYb$$
$$RY_a = \text{Ya in mixture}/\text{Ya in monoculture}$$
$$RY_b = \text{Yb in mixture}/\text{Yb in monoculture}$$

A RYT of 1 indicates that species A and B are making demands on the same resources. If RYT is <1, this shows antagonism between species A and B. If the RYT is >1, the yield of the mixture is greater than that of the single and is preferred.

The Relative Crowding Coefficient (RCC) is a measure of the relative dominance of one species over the other in a mixture (De Wit, 1960). The RCC was calculated as:

$$RCC = (Y_{pb}/Y_p)/(Y_{bp}/Y_b)$$

where Y_p and Y_b are the yields of pinto bean and black mustard, respectively, as sole crops and Y_{pb} and Y_{bp} are the yields of pinto bean and black mustard, respectively, as intercrops.

If RCC= 1, the amount of crop in the mixture will be equal to monocropping. Also, if RCC <1 indicates that the amount of the product in the mixture has decreased relative to solecrop and if RCC >1, the yield of the mixture is higher than that of pure stand of crops and the mixing is beneficial.

Statistical analysis

Analyses of variance for data based on theexperimental design and comparison of means (Duncan multiple range test) at $p \leq 0.05$ were carried out, using MSTATC software. Excel software 2013 was used to draw figures.

3. Results

Analyses of variance showed significant effects of cropping pattern and fertilizers on 100 grains weight, biological and grain yields per unit area of pinto bean and also biological and grain yields per unit area of black mustard. 100 grains weight of black mustard was significantly affected by fertilizer treatments and interaction of cropping pattern × fertilizers (Table 2).

The highest 100 grains weight, biological and grain yields per unit area of pinto bean and grain yield of black mustard were achieved in pure stands of bean and black mustard and also inbio-fertilizers + 100% chemical fertilizer (urea). Maximum biological yield of black mustard was achieved inpure stands of black mustard culture, but there were no significant differences with additive intercropping with optimum density of two species in mono cultures treatment. Also, maximum of this trait was achieved in bio-fertilizers + 100% chemical fertilizer (urea) but, there were no significant differences with bio-fertilizer + 50% chemical fertilizer (Table 3).

Significantly, maximum 100 grains weight of black mustard in different cropping patterns was observed in intercropping with a ratio of 50% black mustard + optimum density of pinto bean mono cultures and bio-fertilizers + 100% chemical fertilizer (urea). Generally, in other cropping patterns there were no considerable differences between fertilizer treatments (Fig. 1).

Evaluation of intercropping efficiency of treatments indicated that land equivalent ratio (LER) is >1 in all intercropping and fertilizer treatments and this showing the superiority of intercropping compared to single cropping. Maximum of LER and relative yield total (RYT) were attended in optimum density of two species and bio-fertilizers + 100% chemical fertilizer (urea). Maximum relative value total (RVT) is related to optimum density of two species with non-fertilizer. Maximum of relative crowding coefficient (RCC) was related to non-fertilizer treatment in 50% of optimum density of two species in pinto bean and optimum density of two species in black mustard (Table 4).

Table 2 - Analysis of variance of the agronomic traits in pinto bean and black mustard under different cropping patterns and fertilizer treatments

Source	df	Mean Square							
		Pinto bean (*Phaseolus vulgaris* L.)				Black mustard (*Brassica nigra* L.)			
		100 grains weight	Biological yield	Grain yield	Harvest index	100 grains weight	Biological yield	Grain yield	Harvest index
Replication	2	72.94	3680597	1131685	2.03	0.01	1786035	157796	2.60
Cropping pattern	2	79.10 **	40310058 **	9924157 **	0.70 NS	0.01 NS	122550980 **	1961700 **	5.77 NS
Fertilizer (F)	3	140.98 **	37336605 **	9970014 **	17.55 NS	0.14 *	24340924 **	832223 **	2.44 NS
C × F	6	4.78 NS	989636 NS	236278 NS	6.49 NS	0.17 **	8957965 NS	11308 NS	6.06 NS
Error	22	2.75	559597	133108	10.29	0.04	5045637	21412	3.77
Cv %	-	5.23	16.85	16.27	6.40	4.46	17.12	8.53	14.59

Ns, * and **: non-significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 3 - Means of the agronomic traits in pinto bean and black mustard under different cropping patterns and fertilizer treatments

Treatment	Pinto bean (*Phaseolus vulgaris* L.)			Black mustard (*Brassica nigra* L.)	
	100 grains weight (g)	Biological yield (kg/ha)	Grain yield (kg/ha)	Biological yield (kg/ha)	Grain yield (kg/ha)
Cropping pattern					
C1	34.08± 5.83 a	6505.70± 80.65 a	3269.90± 57.18 a	15181.70± 123.21 a	2066.60± 45.45 a
C2	32.19± 5.67 b	3793.90± 61.59 b	1911.80± 43.72 b	9437.90± 97.14 b	1272.60± 35.67 c
C3	29.00±5.38 b	3013.80± 54.89 c	1543.10± 39.28 c	14737.50±121.39 a	1802.10± 42.45 b
Fertilizer treatments					
F1	26.54±5.15 d	2117.40± 46.01 d	1048.90± 32.38 d	11022.00± 104.98 c	1372.80± 37.05 c
F2	31.23± 5.58 c	3537.50± 59.47 c	1771.20± 42.08 c	12606.00± 112.27	1542.00±39.26 b
F3	33.37± 5.77 b	5138.70± 71.68 b	2688.20± 51.84 b	14266.00± 119.44 a	1959.50± 44.26 a
F4	35.87± 5.98 a	6777.60± 82.32 a	3458.10± 58.80 a	14581.00± 120.75 a	1980.70± 44.50 a

Different letters in each column indicate significant difference at P≤0.05. Means are average values of three replicates ± standard errors.
C1, C2, C3= pure stands of bean and black mustard, additive intercropping with a ratio of 50% black mustard + optimum density of pinto bean mono cultures and additive intercropping with optimum density of two species in mono cultures, respectively.
F1, F2, F3, F4= control (non-fertilizer), bio-fertilizers (azotobarvar 1 and barvar 2), bio-fertilizer + 50% chemical fertilizer (urea) and bio-fertilizers + 100% chemical fertilizer (urea), respectively.

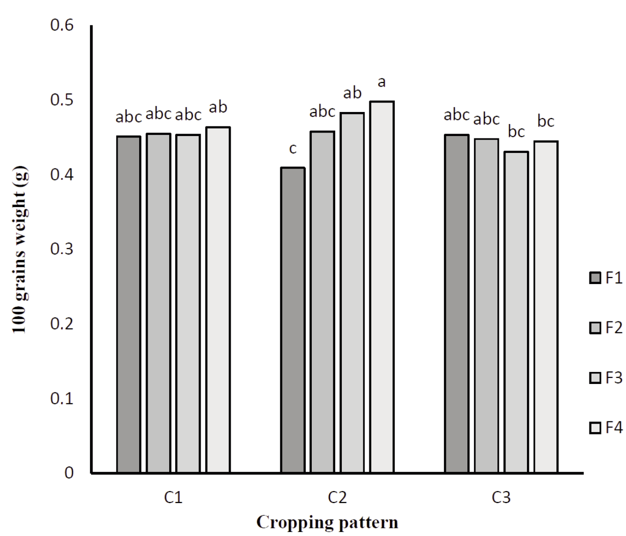

Fig. 1 - Mean 100 grain weight of black mustard for interaction of cropping pattern × fertilizers. Different letters indicate significant difference at p ≤ 0.05 (Duncan test). C1, C2, C3= pure stands of bean and black mustard,additive intercropping with a ratio of 50% black mustard + optimum density of pinto bean mono cultures and additive intercropping with optimum density of two species in mono cultures, respectively. F1, F2, F3, F4= control (non-fertilizer), bio-fertilizers (azotobarvar 1 and barvar 2), bio-fertilizer + 50% chemical fertilizer urea and bio-fertilizers + 100% chemical fertilizer (urea), respectively.

4. Discussion and Conclusions

According to the results, bio-fertilizers + 100% chemical fertilizer (urea) was the best fertilizer treatment in pinto bean and black mustard as, it significantly increased the field performance of these plants (Table 3, Fig. 1), followed by bio-fertilizers + 50% chemical fertilizer. However, biological and grain yields for black mustard was affected as similar to bio-fertilizer + 50% chemical fertilizer (urea) with bio-fertilizers + 100% chemical fertilizer (urea). Therefore, bio-fertilizer application resulted in decreasing 50% of chemical fertilizing. Chemical fertilizer has various negative environmental effects such as soil, water and air pollution, which increase environmental production cost (Moradi *et al*., 2011). Bio-fertilizer as essential components of organic farming, play a vital role in maintaining long term fertility and sustainability of soil. Bio-fertilizers have the ability to access a major part of nutrients for growing plant along with growth promoting factors (Cordovilla *et al*., 1999).

Significant reduction of grain and biological yields in intercropping (Table 3) was attributed to interspecific competition between two crops (Bybee-Finley and Matthew, 2018). Pilbeam *et al*. (1994) has noted that grain yield of maize in sole culture was greater than intercropping with bean. Competition for nutrient uptake and deficiency of nitrogen transport are responsible for the reduction of maize yield in intercropping with legumes (Tomar *et al*., 1988). However, there were not significant differences between sole cropping and optimum density of two species in intercropping system. Therefore, the presence of pinto bean plants hasn't considerable interspecific competition on black mustard plants. Always grain yield of plants did not reduce in intercropping.

As an illustration, Long *et al*. (2001) showed that the grain yield of wheat increased 28 to 30% in intercropping with soybean compared to monoculture.

The land equivalent ratio (LER) of the all intercropping treatments was more than 1, which indicated an advantage of intercropping in comparison with monocultures of pinto bean and black mustard (Table 4). This can be attributed to increasing plant density/m^2 and more use efficiency of environmental resources (Nasrollahzadeh Asl *et al*., 2009). Bio-fertilizers improved LER at all plant population as were applied alone or along with chemical fertilizer. In intercropping system, root interaction could increase the root activity and microbial quantity in the rhizosphere (Zhang, 2013). Interspecific interaction between species in the rhizosphere can also affect nutrient availability and uptake in intercropping (Haugard-Nielsan, 2001). Dua *et al*. (2005) found that intercropping potato and French bean in all intercropping treatments enhanced yield compared to sole cropping and the amount of LER was more than one. Specific competition usually includes competition for soil water, available nutrients, and solar radiation (Buxton and Fales, 1993). Competition can also have a significant impact on the growth rate of the presented species in intercropping.

Relative value total (RVT) of intercropping treatments was higher than 1 which showed the economic advantage of intercropping compared to monocultures. The highest RVT were observed in the non-fertilizerwith optimum densities of two species. RVT was improved as plant density increased. On these biases RVT values of optimum densities for two species were higher than 50% optimum density at the same fertilizer treatments (Table 4). It was attributed to more improvement intercropping yields compared to monocultures (Javanmard *et al*., 2018). Several indices such as LER, RVT, relative yield total (RYT), relative crowding coefficient (RCC) (Table 4), competitive ratio, aggressively, actual yield loss, monetary advantage, and intercropping advantage have been developed to describe competition and economic advantage in intercropping (Ghosh, 2004; Midya *et al*., 2005).

RCC is ability of a species to use limited resource in intercropping with its ability to gain the same resource in intercropping system by using yield comparing and shows the competitive advantage of intercropping components (Snaydon, 1991). RCC of black mustard in most treatment was higher than RCC of pinto been. Its maximum value was observed in treatment non-fertilizer and optimum density of two species about 2.803. The highest value of RCC of pinto been in treatment non-fertilizer and 50% of optimum density of two species. Fertilizer application result in decreasing RCC of pinto bean and increasing

Table 4 - Evaluation of intercropping efficiency of treatme

Fertilizer treatments	Land equivalent ratio (LER)		Relative value total (RVT)		Relative yield total (RYT)		Relative crowding coefficient (RCC) of Pinto bean		Relative crowding coefficient (RCC) of Black mustard	
	Inter-cropping (50% of optimum density of two species)	Inter-cropping (optimum density of two species)	Inter-cropping (50% of optimum density of two species)	Inter-cropping (optimum density of two species)	Inter-cropping (50% of optimum density of two species)	Inter-cropping (optimum density of two species)	Inter-cropping (50% of optimum density of two species)	Inter-cropping (optimum density of two species)	Inter-cropping (50% of optimum density of two species)	Inter-cropping (optimum density of two species)
Control (non-fertilizer)	1.003	1.102	3.181	4.758	1.003	1.102	1.073	0.356	0.931	2.803
Bio-fertilizers (azotobarvar 1 and barvar 2)	1.107	1.349	2.717	3.874	1.107	1.349	1.007	0.559	0.992	1.787
Bio-fertilizer + 50% chemical fertilizer (urea)	1.253	1.381	2.661	3.229	1.253	1.381	0.879	0.515	1.136	1.940
Bio-fertilizers + 100% chemical fertilizer (urea)	1.304	1.418	2.401	2.817	1.304	1.418	0.804	0.602	1.242	1.658

RCC of black mustard in 50% of optimum density of two species. Also, with increasing black mustard density in intercropping, RCC of black mustard was higher than bean at all fertilizer treatments. Generally, fertilizer application, change the superiority of bean toblack mustard (Table 4).

Fertilizer treatments, particularly bio-fertilizers + 100% chemical fertilizer (urea) improved grain yields of pinto bean and black mustard via higher 100 grains weight and biological yield per unit area. Resource use efficiency was increased in intercropping systems. Intercropping diversify agroecosystem, and resulted in sustainable production and increase economic income, in addition, can be effective the use of agricultural land considerably. Finally, it was concluded that intercropping pattern was better than monocultures of two species at different levels of fertilizers and also bio-fertilizers application could increase efficiency of chemical fertilizer and it can reduce the environmental risk and increase field performance of pinto bean and black mustard.

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