Integrated application of biochar and bio-fertilizer improves yield and yield components of Cowpea under water-deficient stress

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Abstract

A low amount of organic matter and insufficient irrigation are two main challenges facing successful crop production in arid and semiarid regions. Application of biochar as an organic amendment to soil not only can help increase organic matter in soil, but also may alleviate adverse effects of water deficit on plant growth and yield production. To test this hypothesis, a two-year field experiment was conducted to assess the effect of sugarcane biochar on yield and yield components of cowpea in water-deficient soil. Treatments consisted of two levels of seed treatment with nitroxin, three levels of biochar application (0, 4, and 8 ton/ha), and a three-level irrigation regime (60, 90, and 120 mm from evaporation pan class A), laid out in a split-factorial design. Results showed that the seed number per plant was significantly higher in cowpea when grown with biochar, possibly due to the relief of water-deficient stress and higher phosphorus and potassium content. Biomass production of cowpea declined under a severe water-deficit condition (ir_3) compared to normal irrigation (ir_1) in 2018 and 2019, decreasing by 39% and 42%, respectively. The maximum biomass obtained from application of 8 ton/ha biochar reached 617.43 and 664.92 g/m^2 in 2018 and 2019, respectively. Seeds treated with nitroxin exhibited 10% and 8% greater biomass production in 2018 and 2019 as compared with control treatments. Seed yield increased with the addition of biochar to soil under all irrigation regimes; however, the maximum seed yield of 266.46 and 275.36 g/m^2 was observed when there was no water-deficient stress condition and application of 8 ton/ha biochar in 2018 and 2019, respectively.

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Introduction

Cowpea (*Vigna unguiculata* L.) is a warm season crop that belongs to the Leguminosae family and that has a soil-enriching habit due to its capacity to fix atmospheric nitrogen (Chatterjee and Bandyopadhyay, 2017). This crop is widely cultivated, due to its high protein content and reasonable adaptation to harsh and arid environments. Due to an increase of drought conditions and low precipitation in arid and semiarid regions, cowpea is now considered to be a key crop in farming scenarios that take climate change into account (Carvalho *et al*., 2019). Higher temperatures coupled with lower rainfall decrease are projected in West Asia as well as the Middle East, where these changing conditions threaten agricultural production and food security in the region. Water deficiency is a serious challenge for successful crop production in arid and semiarid regions, particularly given the increasing periods of drought, and can drastically reduce crop yields (Toscano *et al*., 2016).

Irrigation represents a major part of the water used in the arid and semiarid regions of the world. Recently, the world resources of high quality water available for irrigation became significantly limited. Therefore, the water for irrigation in crop lands should be carefully used in an environmentally responsible manner (Connellan, 2002). In some cases the farmers apply excess amount of water to avoid yield loss. Irrigation scheduling is one of the main factors that affects successful crop production and farmers profit (Zhang and Oweis, 1999; Cai *et al*., 2001; Jones, 2004). It is very important to determine the right amount of water supplies required for crops during the growing season. In addition, it is essential to determine the most suitable irrigation regimes to obtain the optimum crop yield (Uçan *et al*., 2007; Comas *et al*., 2019).

Application of inorganic fertilizers has unfavorable impacts on the environment and human health. Therefore, proper fertilizer management in agroecosystems depends on finding safer and more environment-friendly methods to provide crop nutritional requirements (Adediran *et al*., 2005).

To maintain sustainable agricultural productivity, it is important to keep soil organic matter at reasonable levels to preserve healthy soil (Panwar *et al*., 2019). Chemical fertilizers and synthetic plant growth promoters can be replace by the natural derived amendments for more sustainability of crop production (Prakash and Prakash, 2000). Soil organic amendments such as vermicompost and bioslurry are great nutrient resources for crop during its growth and development (Murmu *et al*., 2013; Shahbaz *et al*., 2014).

In recent years, biochar has received attention worldwide due to its unique physical and biochemical properties (Saletnik *et al*., 2019). It is produced from organic biomasses through the process of pyrolysis. The physiochemical properties of biochar are mainly determined by the method of pyrolysis, including temperature levels and the chemical composition of the substrates used in biochar production (Song and Guo, 2012). As carbon-rich material, biochar consists of more than 50% carbon with a special porous structure and neutral or alkaline pH (Malińska, 2012). Biochar is not completely digested by soil microbial population and therefore, as a soil amendment, it could remain in soil as a permanent carbon-sequestration agent for many years (Weifu Lee, 2013).

It has been reported that biochar application to the soil results in higher potassium, phosphorus, nitrogen, and magnesium (Laird, 2008; Nelson *et al*., 2011). Biochar has great water-holding capacity and soil with biochar amendment has exhibited much higher water-holding capacity than biochar-free soil (Liu *et al*., 2016). Having a highly porous structure and nutrient-rich particles along with organic carbon molecules, biochar provides favourite growing media for microorganisms; as a consequence, it improves soil fertility (Cayuela *et al*., 2014). The effects of biochar on soil properties is largely depend on the method of biochar preparations, pyrolytic parameters and feedstock materials (Glaser *et al*., 2015; Pituello *et al*., 2015). Also, the type of soil is crucial factor in the effectiveness of biochar as a soil organic amendments (Ouyang *et al*., 2013). Soil water holding capacity is largely regulated by soil organic matter, particle size and aggregation (Verheijen *et al*., 2014). Reports has shown that biochar addition can improve crop growth but it differ greatly in different soil types (Keshavarz Afshar *et al*., 2016; Gaskin *et al*., 2010; Huang *et al*., 2019). In coarse-texture soils, organic particles of biochar improve the soil aggregation trough enhancement of the particle bindings of large particles. The positive effects of biochar in carbon depleted soil may grater rather than soils with a high organic carbon concentrations (Blanco-Canqui, 2017).

Bio-fertilizers are plant growth promoters mainly consisting of nitrogen-fixing and phosphate-solubilizing microorganisms providing nutritional needs for plants (Belimov *et al*., 1995; Goel *et al*., 1999). Bio-fertilizers improve soil biodiversity through an increase of the microbial biome. Azotobacter, Cyanobacteria, Rhizobium, Azospirillium, and Mycorrhizae are usually found in bio-fertilizer compounds (Hegde *et al*., 1999). Study revealed that addition of 15 ton/ha biochar to soil increase biological nitrogen fixation in common bean, considerably (Güereña *et al*., 2015). Despite of increasing research reports on the impact of biochar application in crop production, very few of these reports have been studied biofertilizers in legumes following application of biochar (Yusif *et al*., 2016; Egamberdieva *et al*., 2018).

However, organic amendment such as biofertilizer and biochar have reported to improve crop growth and yield production; there is not much information about the best use of them (sole or integrated) as well as the right amount of them under water stress conditions. Therefore, the present study seeks to provide evidence about cowpea yield production under water-deficient conditions when biochar and bio-fertilizers were used as organic soil amendments.

Materials and methods

Site and soil

A field experiment was conducted in the town of Ahwaz (31° 20'N, 48° 40'E, 12 m above sea level) in Khuzestan Province in Iran in the summer seasons of 2018 and 2019. The soil was a Silt clay with the following properties: pH 7.90, 0.01% organic matter, 0.06 mg kg^{-1} available N, 145 mg kg^{-1} available K, and 4 mg kg^{-1} available P. Samples taken from the 0-30 cm layer before the experiment was initiated early in the cowpea-growing season in 2018. Agro-climatically, Ahwaz falls under arid zone of Iran, which is characterized by very warm summers, moderate cold and experiences occasional rainfall during winters. Mean monthly meteorological data of Ahwaz during 2018 and 2019 were presented in Figure 1.

Experimental design and treatments

This research was conducted as a split-plot factorial in a randomized complete block design with three replications. Irrigation regimes were carried out as a main plot at three levels: $ir_{1(60)=}$ 60, $ir_{2(90)=}$90, and $ir_{3(120)=}$120 mm depth of water evaporated from a Class A evaporation pan, respectively (Roderick *et al*., 2009), biochar rates, *i.e*., $bio_{1(0)=}$ 0, $bio_{2(4)=}$ 4, and $bio_{3(8)=}$ 8 ton/ha and bio-fertilizer inoculated (nit^{+}) and not inoculated (nit^{-}) as a subplot.

Plants and treatments

The cowpea cultivar Omrani was used in the study. This cultivar has been widely cultivated by cowpea farmers in the southwest of Iran. Cowpea was seeded manually in rows spaced 60 cm apart with seeds spaced 15 cm apart. Seeds were planted at a depth of 3 cm. The plot sizes were 3 m by 4 m, and alleys of 0.5 m and 1 m, respectively, were left between the plots and the blocks. In order to keep the plot free from weeds, hand weeding was done 3 and 6 weeks after sowing.

Plots were irrigated using dead-end levelled furrow irrigation system. Irrigation regimes (ir_1, ir_2 and ir_3) in this experiment were based on the reference evapotranspiration, ETo (estimated from a Class A pan according to (Doorenbos *et al*., 1997; Neves *et al*., 2010).

The biochar was applied each year. The tested biochar (pH 7.5, 69.65% total C, 0.2% total N, 459 mg kg^{-1} total P, 2568 mg kg^{-1} total K, 1245 mg kg^{-1} total Fe, and 412 mg kg^{-1} total Mg) was produced from sugarcane bagasse by a pyrolysis reactor at 550°C for 3 h. In addition, Nitroxin bio-fertilizer was purchased from Mehr Asia Technology Company, Iran. Seeds were inoculated with Nitroxin. For Nitroxin treatments, 4 L of Nitroxin were added to 20 L of water and mixed gently. The seeds were soaked in prepared solution for about 10 min and then removed from the mixture; they were then spread in the shade and dried completely before being planted (Davod *et al*., 2011). Biochar was uniformly incorporated into the experimental plots at the beginning of the experiment (one day before cultivation).

Sampling and measurements

Ten plants were taken from two of the inner rows, about 50 cm from the beginning of the line. At physiological maturity, *i.e.*, when 95% of the pods had turned golden yellow, all the plants were harvested from the net plot excluding the border rows. Plants were hand-threshed, and filled pods were separated from unfilled pods. Total biomass was the summation of the dry weights of plant components: leaf, stem, and filled and unfilled pods. Dry weights were determined after oven drying at 70°C until constant weight. The number of seeds per pod, number of pods per m^2, 100- seed weight, and harvest index were calculated. Seed yield was determined from a 6 m^2 area in each subplot, and adjusted to a moisture content of 15%.

Statistical analysis

The data were analysed by three-way analysis of variance (ANOVA) by using Minitab ver. 16 statistical software. Homogeneity of error variances was tested using Bartlett's test. When the variance of traits error in two consecutive years of planting was homogeneous, the comparison of these traits was performed as a combined analysis. Mean comparison of data was made by using the least significant difference (LSD) test at 5% error probability. If the main treatments and interactions were significant, means comparison of interactions was merely described in results.

Results and discussion

Results of variance analysis showed that all mean effects had significant effects on all studied traits. The interaction between irrigation regimes ×biochar rates had no significant effect on the number of seed pods. Interaction between Irrigation and Nitroxin had no significant effects on biomass, number of pods, number of seeds per pod, or 100-seed weights. Biochar×Nitroxin had significant interaction effects on biomass only, whereas three-way interaction of irrigation regimes×biochar rate×Nitroxin had significant effects on 100-seed weight, biomass, and number of pods per plant, though only in 2018 (see Tables 1 and 2). The mean values for all of the traits across the main effects are presented in subsequent tables and figures. Biomass production of cowpea declined by –39.33% and –41.89% under the severe water-deficit condition (ir_3) as compared to normal irrigation (ir_1) in 2018 and 2019, respectively. Increase in biomass production was higher with the biochar treatments. The maximum biomass obtained from 8 ton/ha was 617.43 and 664.92 g/m^2 in 2018 and 2019, respectively. Seeds treated with Nitroxin exhibited +9.78% and +8.01% greater biomass production in 2018 and 2019 compared with control treatments. Thus, the three-way interaction effect of irrigation regimes × biochar × Nitroxin was promising. Biochar improves soil fertilizers, and if applied as organic amendment to the soil, it considerably improves nutrient absorption and plant uptake of nitrogen, phosphorus and potassium (Glaser *et al*., 2015; Huang *et al*., 2019; Zoghi *et al*., 2019). Application of 4 and 8 ton/ha biochar exhibited +27.56% and +54.57% increases in total biomass production during 2019. These results revealed that incorporation of biochar into water deficient soil ($ir_{2(90)}$*$bio_{3(8)}$) could significantly alleviate the damage to biomass production caused by drought stress. Zoghi *et al*. (2019) report that biochar application to soil not only increases the water-holding capacity of soil but also provides better conditions for crops to absorb nutrients more efficiently. They observed a 38% and 39% increase in photosynthesis and stomatal conductance of *Quercus castaneifolia* L. grown in biochar-treated soil compared with a control treatment consisting of a severely water-deficient condition. This result is in agreement with the findings from the present study.

The amounts of biomass produced by the ($ir_{2(90)}$*$bio_{3(8)}$) treatment in 2018 and 2019 were 717.67 and 725.67 g/m^2, respectively (Table 3). Under the severe water- deficient condition, biochar application ($ir_{3(120)}$*$bio_{3(8)}$) showed a +29.43% and a +28.18% increase of biomass in 2018 and 2019, respectively. Increase in the severity of drought stress reduced the beneficial effects of Nitroxin treatments in biomass production. The highest values of Nitroxin

Table 1. Analysis of variance for the effects of year (Y), replication (R), irrigation (i), biochar rates (b) and nitroxin (n) on grain yield and yield attributes in cowpea grown in 2018 to 2019.

SOV	df	Mean Square error						
		Biomass	Seed yield	HI	Nspod	Npod	100 seed weight	Seed Nitrogen
Y	1	16127.53**	531.46 ^{ns}	13.31^{ns}	105.55**	4.19^{ns}	15.51**	0.05**
R(Y)	4	967.41	316.70	5.69	17.07	29.32	4.85	0.029
ir	2	692641.58**	79341.28**	151.64**	93.28**	56.00**	313.717**	0.33**
Y*i	2	3897.22**	18.68 ^{ns}	4.05 ^{ns}	8.28**	0.28 ^{ns}	0.05^{ns}	0.001^{ns}
Y*i*R	8	685.34 ^{ns}	234.87 ^{ns}	10.60 ^{ns}	3.70^{ns}	9.13**	5.04**	0.007 ^{ns}
bio	2	426943.79**	35014.99**	76.13**	54.92**	76.59**	109.37**	1.49**
nit	1	56058.06**	8836.78**	6.73 ^{ns}	32.87**	43.58**	62.95**	0.72**
Ir*bio	4	36661.23**	1139.46**	95.57**	2.39^{ns}	2.03 ^{ns}	13.21**	0.07**
ir*nir	2	900.35^{ns}	3958.81**	114.59**	0.24 ^{ns}	1.29 ^{ns}	0.66 ^{ns}	0.01 ^{ns}
bio*nir	2	7100.73**	853.68**	4.76 ^{ns}	2.54 ^{ns}	2.48 ^{ns}	4.28 ^{ns}	0.07**
ir*bio*nit	4	3344.34**	92.01 ^{ns}	23.93 ^{ns}	2.06 ^{ns}	5.81**	10.82**	0.007 ^{ns}
Y*bio	2	3586.07**	108.00 ^{ns}	0.33 ^{ns}	8.69**	1.93^{ns}	0.05 ^{ns}	0.005 ^{ns}
Y*nit	1	296.73 ^{ns}	2.70 ^{ns}	0.12 ^{ns}	0.03 ^{ns}	1.20 ^{ns}	0.05 ^{ns}	0.007 ^{ns}
Y*ir*bio	4	3819.63**	32.88 ^{ns}	13.08 ^{ns}	2.31 ^{ns}	0.43 ^{ns}	0.04 ^{ns}	0.001 ^{ns}
Y*ir*nit	2	271.34 ^{ns}	15.75 ^{ns}	2.06 ^{ns}	1.56 ^{ns}	0.41 ^{ns}	0.05 ^{ns}	0.001 ^{ns}
Y*bio*nit	2	304.64 ^{ns}	208.22 ^{ns}	12.34 ^{ns}	1.72 ^{ns}	0.151 ^{ns}	0.05 ^{ns}	0.0054^{ns}
Y*ir*bio*nit	4	244.98 ^{ns}	16.78 ^{ns}	0.84 ^{ns}	0.46 ^{ns}	0.47 ^{ns}	0.04 ^{ns}	0.001 ^{ns}
Error	60	584.94	246.22	16.20	2.30	1.14	1.63	0.008 ^{ns}
CV(%)		4.51	7.93	10.73	14.77	10.95	5.97	2.58

*$P \leq 0.05$; **$P \leq 0.01$; ^{ns}, not significant.

application were obtained from ir_1 and ir_2 (Table 4). The contrasting results of ir_3 between 2018 and 2019 was due to the seasonal changes in climatic parameters during growing season (Figure 1). It was observed that incorporation of Nitroxin and biochar significantly increased the amount of dry biomass in both 2018 and 2019 (Table 5).

Overall, then, we believe that the increase in biomass and yield components of cowpea with biochar amendment is due to the soil's improved water-holding capacity as well as an improvement in the uptake of nutrient by plants. These findings are in agreement with de Melo Carvalho *et al*. (2014) and Tayyab *et al*. (2018). Also, previous researches revealed that biochar as an organic soil amendment improves soil fertility and facilities the biochemical cycling of nitrogen and phosphorus (Nelissen *et al*., 2012; Yao *et al*., 2012). It was shown that biochar provide inorganic nutrients to plants such as potassium, Magnesium and Calcium when incorporated with soil (Mukherjee and Zimmerman, 2013; Rajkovich *et al*., 2012). The increase of soil fertility in biochar amended plots facilitated plant growth and yield formation in cowpea.

Seed yield was decreased significantly under water-deficient

Table 2. Yield and yield attributes under different irrigation regimes, biochar rates and Nitroxin treatments during 2018 to 2019.

Year	ir	Biomass (g/m^2) LSD= 20.12	Seed yield (g/m^2) LSD= 11.78	HI LSD= 2.50	Npod/plant LSD= 2.32	Nspod/pod LSD= 1.47	100 seed weight (g) LSD= 1.72
2018	ir1 (60)	612.73	236.84	38.82	10.94	11.51	24.25
	ir2 (90)	587.29	204.15	35.29	9.44	8.96	20.59
	ir3 (120)	371.73	145.39	39.45	8.39	7.4	18.33
2019	ir1 (60)	661.2	242.93	37.37	11.18	12.39	24.92
	ir2 (90)	599.7	207.92	35.15	10.03	11.33	21.4
	ir3 (120)	384.17	148.84	38.94	8.75	10.08	19.13
	bio	Biomass (g/m^2) LSD= 16.12	Seed yield (g/m^2) LSD= 10.46	HI LSD= 2.68	Npod/plant LSD= 0.71	Nspod/pod LSD= 1.01	100 seed weight (g) LSD= 0.85
2018	bio 1 (0)	417.34	161.66	38.83	8.28	8.45	19.31
	bio 2 (4)	536.98	206.17	38.48	9.67	9.52	21.13
	bio 3 (8)	617.43	218.55	36.26	10.83	9.9	22.73
2019	bio 1 (0)	430.15	163.85	38.06	8.16	9.4	19.98
	bio 2 (4)	550.01	208.86	37.99	10.44	11.6	21.94
	bio 3 (8)	664.92	226.97	35.4	11.36	12.8	23.53
	nit	Biomass (g/m^2) LSD= 13.16	Seed yield (g/m^2) LSD= 8.54	HI LSD= 2.19	Npod/plant LSD= 0.58	Nspod/pod LSD= 0.82	100 seed weight (g) LSD= 0.69
2018	nit-	499.48	186.57	38.14	8.85	8.72	20.32
	nit+	548.36	204.35	37.57	10.33	9.86	21.8
2019	nit-	527.23	190.69	37.37	9.46	10.73	21.03
	nit+	569.48	209.1	36.94	10.52	11.8	22.6

Control: ir_1, bio_1, nit^-.

Table 3. Yield and yield attributes under different irrigation regimes and biochar rates during 2018 to 2019.

Year	ir	bio	Biomass (g/m^2) LSD= 27.93	Seed yield (g/m^2) LSD= 18.12	HI LSD= 4.64	Npod/plant LSD= 1.23	Nspod/pod LSD= 1.75	100 seed weight (g) LSD= 1.47
2018	ir1 (60)	bio 1 (0)	481.42	194.46	40.34	9.5	11.53	21.82
		bio 2 (4)	639.12	249.6	39.04	10.83	11.84	24.6
		bio 3 (8)	717.67	266.46	37.07	12.5	11.16	26.33
	ir2 (90)	bio 1 (0)	454.74	165.03	36.35	8.17	7.37	18.61
		bio 2 (4)	581.33	220.03	38.21	9.83	9.33	20.1
		bio 3 (8)	725.79	227.39	31.32	10.33	10.19	23.07
	ir3 (120)	bio 1 (0)	315.86	125.5	39.81	7.17	6.46	17.51
		bio 2 (4)	390.5	148.89	38.18	8.33	7.39	18.69
		bio 3 (8)	408.83	161.79	40.38	9.67	8.36	18.79
2019	ir1 (60)	bio 1 (0)	494.21	199.07	40.24	9.38	10.59	22.24
		bio 2 (4)	652.64	254.36	38.97	10.99	12.55	25.39
		bio 3 (8)	836.76	275.36	32.91	13.18	14.04	27.13
	ir2 (90)	bio 1 (0)	467.92	169.19	36.24	8.19	9.32	19.4
		bio 2 (4)	594.01	222.18	37.72	10.89	12.04	20.92
		bio 3 (8)	737.17	232.41	31.48	11.01	12.64	23.87
	ir3 (120)	bio 1 (0)	328.31	123.3	37.72	6.9	8.29	18.31
		bio 2 (4)	403.37	150.06	37.27	9.46	10.22	19.49
		bio 3 (8)	420.83	173.15	41.82	9.9	11.72	19.59

Control: ir_1, bio_1.

conditions in ir_2 compared to ir_1; the yield was decreased by –13.80% and –14.41% in 2018 and 2019, respectively. Biochar treatments produced higher seed yield than biochar-free treatments in both 2018 and 2019 (Table 2). The increase in seed yield in 2018 (+26.03%) and 2019 (+38.52%) was significant when comparing 8 ton/ha biochar to 0 ton/ha. Seed yield was increased by the addition of biochar to soil under all irrigation regimes; however, the maximum benefit of biochar was observed when there was no water-deficient stress condition. Seed yields of 266.46 and 275.36 g/m^2 were obtained from ($ir_{1(60)}$*$bio_{3(8)}$) in 2018 and 2019, respectively. In our experiment, the results showed that although cowpea yield is significantly decreased with $ir_{3(120)}$, cowpea is capable of providing yield under severe drought conditions. The amount of yield loss was not considerable at $ir_{2(120)}$ $bio_{3(8)}nit^+$ compared to that of crops under no water-deficit stress. This finding is in agreement with Fatokun *et al*. (2012) and Goufo *et al*. (2017), who suggested that cowpea is one of the most drought-tolerant crops in semi-arid regions. The amount of seed yield under ($ir_{2(120)}$*$bio_{3(8)}$) treatment did not exceed 180 g/m^2 during the two years of the experiment. Decrease in yield and yield components of many crops due to water-deficit stress has been well documented. Oktem *et al*. (2003) reported that corn yield was reduced when crops were exposed to water-deficit stress. Our results showed that the use of 8 ton/ha biochar in the soil could alleviate adverse effects of water deficit and provide stronger crops with higher seed yield. This benefit may arise from the way biochar increases the soil surface area and soil porosity, with these changes leading to higher water-holding capacity in biochar-amended soil (Agegnehu *et al*., 2017).

The treatments with a moderately water-deficient condition ($ir_{2(90)}$), when incorporated with biochar, produced seed yields without any significant decline compared to ($ir_{1(60)}$). Seed yields were only –14% and –15% lower in ($ir_{2(90)}$*$bio_{3(8)}$) in 2018 and 2019, as compared with the treatments with no water-deficient condition (Table 3). Results showed a significant decline in the harvest index under water-deficit stress. Incorporation of biochar

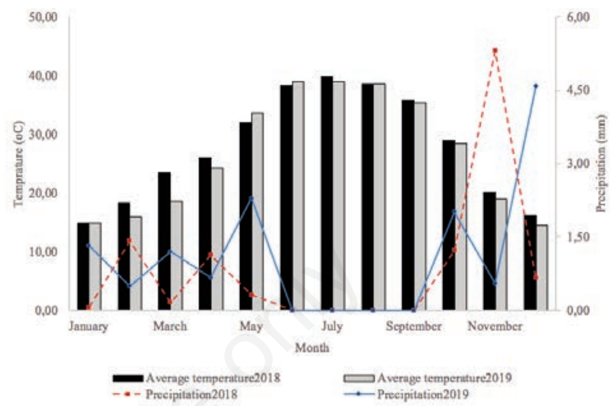

Figure 1. Meteorological data of Ahwaz (2018-2019) (www.irimo.ir; www.accuweather.com).

Table 4. Yield and yield attributes under different irrigation regimes and nitroxin treatments during 2018 to 2019.

Year	ir	nit	Biomass (g/m^2) LSD= 22.80	Seed yield (g/m^2) LSD= 14.79	HI LSD= 3.79	Npod/plant LSD= 1.01	Nspod/pod LSD=1.43	100 seed weight (g) LSD= 1.20
2018	ir1 (60)	nit-	581.65	216.92	37.41	10.11	11.1	23.7
		nit+	643.82	256.76	40.22	11.78	11.91	24.8
	ir2 (90)	nit-	562.01	197.65	35.89	8.78	8.07	19.8
		nit+	612.57	210.64	34.69	10.11	9.85	21.38
	ir3 (120)	nit-	354.78	145.15	41.11	7.67	6.98	17.44
		nit+	388.69	145.64	37.8	9.11	7.82	19.21
2019	ir1 (60)	nit-	639.76	222.14	35.45	10.31	11.7	24.24
		nit+	682.65	263.72	39.29	12.05	13.09	25.6
	ir2 (90)	nit-	574.92	200.16	35.62	9.71	10.95	20.6
		nit+	624.48	215.69	34.68	10.35	11.72	22.19
	ir3 (120)	nit-	367.02	149.79	41.03	8.35	9.56	18.25
		nit+	401.32	147.89	36.84	9.15	10.6	20.02

Control: ir_1nit^-.

Table 5. Yield and yield attributes under different biochar rates and nitroxin treatments during 2018 to 2019.

Year	bio	nit	Biomass (g/m^2) LSD= 22.80	Seed yield (g/m^2) LSD= 14.79	HI LSD= 3.79	Npod/plant LSD= 1.01	Nspod/pod LSD=1.43	100 seed weight (g) LSD= 1.20
2018	bio 1 (0)	nit-	404.81	155.23	38.48	7.67	7.81	18.99
		nit+	429.87	168.09	39.18	8.89	9.1	19.64
	bio 2 (4)	nit-	520.03	197.5	38.3	8.56	8.62	20.31
		nit+	553.93	214.84	38.65	10.78	10.41	21.96
	bio 3 (8)	nit-	573.59	206.99	37.63	10.33	9.73	21.65
		nit+	661.27	230.11	34.88	11.33	10.07	23.8
2019	bio 1 (0)	nit-	417.21	160.05	38.48	7.74	8.5	19.52
		nit+	443.08	167.65	37.65	8.57	10.3	20.44
	bio 2 (4)	nit-	533.39	202.48	38.25	9.68	11.24	21.11
		nit+	566.63	215.25	37.73	11.21	11.96	22.76
	bio 3 (8)	nit-	631.1	209.54	35.37	10.95	12.46	22.45
		nit+	698.74	244.4	35.44	11.78	13.14	24.6

Control: bio_1nit–

into the soil led to a +10.89 % increase in the harvest index in 2019 in a more severe water-deficient condition ($ir_{2(120)}$*$bio_{3(8)}$). Cowpea in this experiment showed good drought tolerance, especially when biochar was added to the soil. In their study, Goufo *et al*. (2017) investigated the drought- tolerance mechanism of cowpea. They found that changes in sugar content, amino acids, and proanthocyanidins of cowpea roots is the main regulatory network dealing with drought-stress conditions. They also suggested that proline, galactinol, and a quercetin are essential metabolites for cowpea to deal with drought conditions.

Under normal irrigation ($ir_{1(60)}$), the harvest index increased +7.5% and +10% with the application of Nitroxin bio-fertilizer in 2018 and 2019, respectively (Table 4). The differences revealed by comparing ir_1 to ir_3 for pods per m^2 was significant. The number of pods per plant decreased from 10.94 to 8.39 and 11.18 to 8.75, in 2018 and 2019, under ir_1 and ir_3, respectively. The adverse effects of water-deficit stress were significantly alleviated by the incorporation of biochar as a soil amendment. There is better root growth and biomass production in crops grown in biochar-treated soil. The increase in cowpea's yield components in biochar-treated soil may also arise from the fact that biochar is rich in phosphorus and potassium. These are two main requirements for healthy and productive crops.

The number of seeds per pod also declined under water-deficit treatments in 2018, while there was no significant reduction in the number of seeds per pod for ir_2 and ir_1 in 2019. Seed weights also declined with ir_3 as compared to ir_2 and ir_1. The maximum 100-seed weight was obtained at ir_1 while the lowest 100-seed weight was obtained at ir_3. Biochar treatment $bio_{3(8)}$ increased the number of pods per plant by +30% and +39% in 2018 and 2019, respectively.

The main goal of farming in arid and semiarid regions is to obtain the maximum grain yield from gradually decreasing water resources. When scheduling irrigation, it is crucial to avoid water-deficit stress; careful planning is required to advance the goal of yield production without any significant losses under limited irrigation regimes (Ertek, 2011). As expected, the maximum seed yield was obtained in $ir_{1(60)}$ regimes; this result was due to the adequate water supply in the soil during vegetative and grain-filling periods. Water scarcity in $ir_{3\ (120)}$ significantly decreased grain yield. In many legume crops, remobilization of assimilates from pod to grain is essential to have satisfactory grain filling and seed weight. Therefore, any defect in the current photosynthesis of cowpea may cause decreased seed weights and final seed yields (Bewley *et al*., 2012). When water is deficient in ir_2 and ir_3, the amount of assimilate production, dry matter production, and overall assimilate production is reduced, and this is the main reason for yield decline in the water-deficient treatments.

Application of Nitroxin increased the number of pods per plant, and this increase was more pronounced in 2018. Plants treated with Nitroxin exhibited 10.33 pods per plant, while non-treated plants produced only 8.85 pods per plant. The number of seeds per pod also decreased due to water deficiency. It was observed that Nitroxin-treated plants had more seeds per pod than non-treated plants. For instance, in 2019, the average number of seeds per pod was 10.73 in Nitroxin-free plants (nit^-), while nit^+ plants on average contained 11.8 seeds per pod (Table 2). Seed weight declined significantly under the water-deficit condition. However, the application of biochar and Nitroxin provided heavier seeds than the other treatments. The maximum 100-seed weight (26 g) was obtained from ($ir_{1(60)}$*$bio_{3(8)}$*nit^+), while the lowest 100-seed weight (17 g) was observed in the severe water-deficit condition without biochar or Nitroxin ($ir_{1(120)}$*$bio_{3(1)}$*nit^-).

Nitroxin has been documented as an effective bio-fertilizer for many agronomic crops, with Nabizadeh *et al*. (2012) reporting that the highest yield and yield attributes of *Pimpinella anisum* L. were obtained from Nitroxin-treated plants. The positive role of bio-fertilizers in increasing yield may arise from the characteristics of these fertilizers: they contain not only nitrogen-fixing microorganisms but also many biologically active compounds such as plant hormones and growth regulators, which improve root growth and development (Dey *et al*., 2004; Remans *et al*., 2008).

Figure 2 shows yield and biomass production of the cowpea; this spider plot summarizes the impact of the irrigation, biochar, and Nitroxin treatments. The chart shows that water-deficit stress decreases both seed yield and biomass production. However, seed yield and biomass production were promoted when the soil was mixed with biochar. The positive effect of biochar application on growth and yield was more pronounced with Nitroxin-treated plants. The maximum biomass production (783.05 g/m^3) was obtained from $ir_{1(60)}$*$bio_{3(8)}$*nit^+. Further, the data revealed that the highest seed yield (292.96 g/m^3) was produced from 8 ton/ha biochar and Nitroxin-treated plants when there was no water-deficient condition (Figure 2).

It has been well-documented that better root growth results in

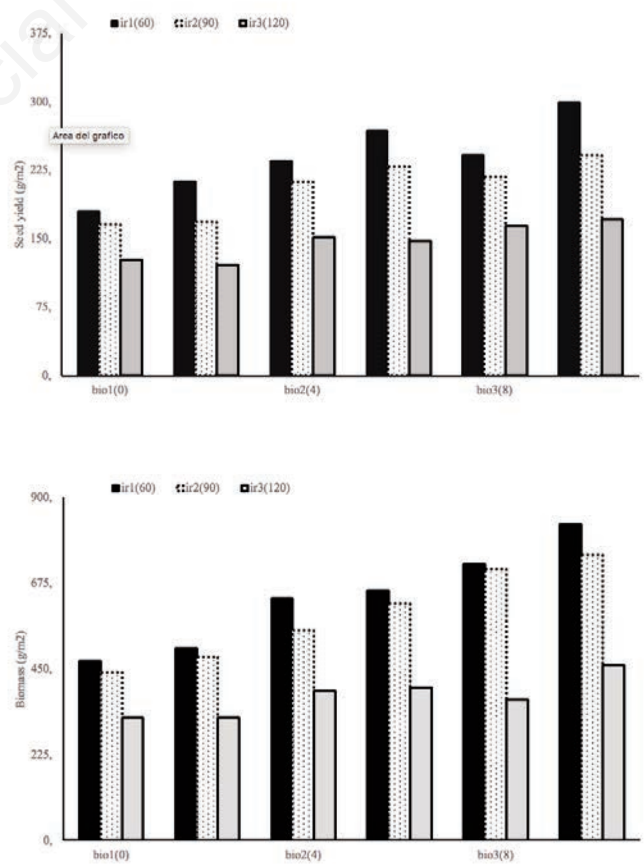

Figure 2. Yield (above) and biomass (down) of cowpea obtained from different biochar rates (0, 4, 8 ton/ha) and biofertilizer treatments (with nitroxin: nit^+, without nitroxin: nit^-) under various irrigation regimes (ir_1=line, ir_2=dot, ir_3=dash).

stronger crops, more efficient photosynthesis, and, ultimately, higher crop yield (Acharya and Sharma, 1994; Krishnakumar *et al*., 2013; Singh *et al*., 2005). Leithy *et al*. (2009) show that the increase in the number of pods per plant due to the application of bio-fertilizers is one of the main causes of higher yield in treated plants as compared with control crops. Our results reveal that cowpea likewise responds to bio-fertilizers very well. Cowpea plants treated with bio-fertilizer showed a significant increase in biomass production. Increase in the number of pods per plant and higher seed numbers per pod may have been due to the presence of biostimulants such as gibberellin and cytokinin in the Nitroxin fertilizer. In addition, the increase in the population of nitrogen-fixing bacteria sufficiently met the cowpea's needs for nitrogen fertilizer. Our study also showed that the application of bio-fertilizers has a more pronounced effect when they are incorporated with organic amendments. Similarly, Kamaei *et al*. (2019) reported that the highest root growth and biomass of sorghum were obtained when bio-fertilizers were mixed with organic compost.

Conclusions

It is concluded that, different irrigation regimes can influence the seed yield and yield components of cowpea. Among different biochar application rates, 8 ton/ha alleviated damaging effects of water deficit stress. This enhancement effect was more noticeable when integrated use of biochar at 8 ton/ha with Nitroxin bio-fertilizer applied in the reduced water irrigation (90 mm of class A pan evaporation) treatment. Based on the obtained results, in different cowpea irrigation regimes, the application of biochar as organic soil amendment increased biomass and yield components such 100 seed weight and number of pod per plant. Also, it should be evaluated the performance of other Agro-techniques combined with biochar application, especially those that increase number of seed per plant and crop productivity in cowpea production.

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