Bulgarian Journal of Agricultural Science, 19 (No 6) 2013, 1268-1274
Agricultural Academy

EFFECT OF WATER STRESS, BIO-FERTILIZER AND MANURE ON SEED AND ESSENTIAL OIL YIELD AND SOME MORPHOLOGICAL TRAITS OF CUMIN

M. SEGHATOLESLAMI
Islamic Azad University, Faculty of Agriculture, Birjand Branch, Birjand, Iran

Abstract

SEGHATOLESLAMI, M., 2013. Effect of water stress, bio-fertilizer and manure on seed and essential oil yield and some morphological traits of cumin. *Bulg. J. Agric. Sci.,* 19: 1268-1274

Cumin is a drought resistant medicinal plant adapted to arid and semi arid regions. Primary productivity in these regions is generally low. In order to study the effect of water stress, manure and bio-fertilizer on cumin a split plot design based on randomized complete block with 3 replications was conducted in two successive growth seasons (2009-2010 and 2010-2011) in Birjand, Iran. Two irrigation treatments (rainfall+3 irrigations as water stress and rainfall+6 irrigations as control) were in main plots and 7 fertilization methods (control- no fertilizer, 10 t/ha manure- 10M, 5t/ha manure- 5M, 50 kg/ha nitrogen as urea- 50N, 5M+25N and seed inoculation with *Pseudomonas putida* and *P. fluorescence*) were in sub plots. Results demonstrated that seed and essential oil yield, plant height and umbel number per plant significantly declined in water stress conditions, but branch number per plant and essential oil and seed germination percentage were not significantly affected by water stress. In water stress conditions, the highest seed yield related to 5M+25N treatment. The effect of fertilizer treatments on plant height, branch number per plant and seed essential oil and germination percentage were not significant. In conclusion, results showed that manure could be used effectively to modify the impact of water shortage and to stimulate an increase in cumin seed and essential oil yields probably through improving the water holding capacity of the soil. Nitrogen application with manure improved the effect of manure application. Seed inoculation with *Pseudomonas sp.* demonstrated no positive effect on plant growth and yield.

Key words: cumin, manure, bio fertilizer, essential oil, germination, morphology

Introduction

Crop production in the arid and semi arid environment of Birjand, Iran relies on irrigation. Many growers in this area sow local medicinal plants along with their commercial crops in the belief that in dry years when harvest predictions may not be fully realized from their commercial crops, local plants with more tolerance to drought can provide a supplementary source of income (Khazaie et al., 2008).

Water deficit is considered as a major environmental factor affecting many aspects of plant physiology and biochemistry (Charles et al., 1994). Because morphological and physiological mechanisms are involved in a plant's response to drought stress, these traits are frequently studied in some research. Baher et al. (2002) demonstrated that water stress decreased plant height of *Satureja hortensis*. Colom and Vazzana (2002) showed that stem number per plant was negatively affected by water stress in *Eragrostis curvula*.

Drought may cause changes in some metabolites yield (Petropoulos et al., 2008). In medicinal plants, essential oil production depends upon the metabolic state of source tissues as well as stress factors (Sangwan et al., 2001). Drought stress increases the essential oil percentage of more medicinal and aromatic plants, because in this condition, more metabolites are produced to prevent oxidization in the cells (Aliabadi Farahani et al., 2009).

Arid and semi-arid regions of the world are generally noted for their low primary productivity. Fertilization increases the availability of limited nutrients, stimulates plant growth and improves the nutrient use efficiency in an infertile and dry environment (Dang et al., 2006). Addition of organic material affects some properties of the soil. Organic matter

E-mail: mjseghat@yahoo.com

improves soil structure and aggregate stability, as well as its moisture retention capacity by increasing the total number of storage pores (Bhattacharyya et al., 2008).

Organic agriculture relies heavily on boosting the level of organic matter in the soil; this is typically achieved by replacing inorganic fertilizers with animal manure as the fertility source of choice (Evanylo et al., 2008). Manure is typically applied to the soil at rates designed to supply a crop's nitrogen requirement (Evanylo et al., 2008). Nitrogen applications generally increase oil yield in aromatic plants by enhancing the amount of seed or biomass yield per unit land area (Sangwan et al., 2001).

In addition to the supply of sufficient nutrients, N application can lead to environmental contamination resulting from excessive nitrate leaching (Dong et al., 2005). More recently there has been a revival of interest in environmentally friendly, sustainable and organic agricultural practices (Esitken et al., 2005). The use of bio-fertilizers is known to improve plant growth through the supply of plant nutrients and may help to sustain environmental health and soil productivity (O'Connell, 1992).

Research has demonstrated that using bio-fertilizer could reduce the need for chemical fertilizer and decrease its adverse effects on the environment (Karlidag et al., 2007). Plant growth and development both improve in response to seed or root inoculation with microbial inoculants producing plant growth regulators (Zahir et al., 2004). This experiment was conducted to evaluate responses of cumin to manure, nitrogen and bio-fertilizer under water stress.

Materials and Methods

This experiment was conducted in the 2009-2010 and 2010-2011 growing seasons at the Agricultural Research center of Islamic Azad University, Birjand branch, Birjand, Iran. Longitude, latitude and altitude of Birjand are 59°13', 32°53' and 1480 m, respectively. Birjand has a dry and warm climate and rainfall mainly occurs between Novembers to April. Annual average rainfall is 167 mm. Table 1 shows maximum and minimum temperature averages and precipitation levels for both years of this experiment. Electrical conductivity (EC) of the irrigation water was 2.6 dS.m^{-1}. Soil characteristics are shown in Table 2.

A split-plot design was used based on a randomized complete block with three replications. Two irrigation treatments (rainfall+3 irrigations as water stress and rainfall+6 irrigations as control) were as main plots and 7 fertilization methods (control- no fertilizer, 10 t/ha manure- 10M, 5t/ha manure- 5M, 50 kg/ha nitrogen as urea- 50N, 5M+25N and seed inoculation with *Pseudomonas putida* and *P. fluorescence*) were as sub plot. Each sub-plot was 4 m long and 3 m wide. Manure was used only in the first year of the experiment. Nitrogen (as urea) was used at two stages (two weeks after emergence and at the start of flowering) in both years of the study. Seed inoculation with bacteria was conducted before planting.

Base fertilizer (100 kg. ha^{-1} triple phosphate and 150 kg. ha^{-1} potassium sulfate) was applied before sowing in both years of the experiment. To evaluate the effect of different

Table 2
Properties of the soil (0-30 cm) and manure used in experiment

Soil		Manure	
Parameters	Values	Parameters	Values
pH	8.2	pH	7.4
Texture	Sandy	Ash (%)	82
Organic C (%)	0.33	C (%)	6.8
N (%)	0.033	N (%)	0.72
P (ppm)	6.8	P (%)	0.2
K (ppm)	133	K (%)	0.5
Fe (ppm)	4.08	Fe (ppm)	3670
Mn (ppm)	4.34	Mn (ppm)	287
Zn (ppm)	1.82	Zn (ppm)	17
Cu (ppm)	0.88	Cu (ppm)	19

Table 1
Rainfall and average monthly temperatures in 2 experiment years in Birjand

Month	Rainfall, mm		T_{min}, °C		T_{max}, °C	
	2009-2010	2010-2011	2009-2010	2010-2011	2009-2010	2010-2011
December	37.1	0	0.8	-4.1	12.7	17.9
January	22	8.2	-0.8	-5.3	15.1	11.9
February	18	30.6	0.3	0	14.3	12
March	58.3	17.3	6.5	2.7	21.6	16.6
April	8.3	6.8	9.4	7.2	24.4	23.6
May	18.1	5.1	14.1	10.1	29.6	32.4

treatments, especially manure and bacteria on plant growth over a longer period of time, plots and treatment positions were not changed in the second year of the experiment.

Sowing was done manually on 6th and 11th December, respectively in 2009-2010 and 2010-2011 growing season. Seeding quantity was 40 kg. ha^{-1}. Weed control was done by hand as and when required.

Seed yield was determined by harvesting 2 m^2 on 15th May, with regard to border effects. Morphological traits were measured randomly in 10 plants from each sub-plot. Seed essential oil percentage was determined using a Clevenger type apparatus. Data analysis was conducted by mstat-c software and means were compared by Duncan's multiple range tests at 5% probability level.

Results

Morphological traits

Plant height at the second year of the experiment was significantly affected by irrigation treatments (Table 3) and it declined in water stress conditions (Table 4).

There was a non significant effect of irrigation on branch number per plant (Table 3), but water stress significantly reduced the umbel number per plant in both years of the experiment (Table 4).

The effect of fertilizer treatments on plant height and branch number per plant was not significant, while umbel number per plant was significantly affected by the treatment (Table 3). Table 5 shows that umbel number per plant was in the lower statistical group in N_0 and 50N treatments and at higher in treatments with manure (10M, 5M and 5M+25N).

The interaction among treatments was not significant on any of the measured morphological traits (Table 3).

Seed yield

Seed yield was significantly affected by irrigation treatments (Table 3) and declined in water stress conditions at 46.9% and 62.9% in the first and second years of the experiment respectively (Table 6).

The effect of fertilizer treatment and its interaction with irrigation on seed yield was significant (Table 3). In water stress conditions, higher seed yield was related to 5M+25N treatment (Table 6) and that was not significantly different with other treatments of 5M and 10M. In this condition non-fertilizer, B1 and B2 treatments had lower seed yields. In the non-stress condition, the highest seed yield (Table 6) was related to 50N (in 2009-2010) and 5M+25N (in 2010-2011) treatments.

Seed qualitative traits

The effect of irrigation on the seed's essential oil content and germination percentage was not significant, but seed essential oil yield was significantly affected by the treatment (Table 7).

Seed essential oil percentage was not significantly different in response to fertilizer treatments, but its yield was sig-

Table 3
Analysis of variance for cumin yield and its morphological traits as affected by irrigation and fertilizing methods

Sourec of variation	df	Mean squares							
		Seed yield		Plant height		Branch number per plant		Umbel number per plant	
		2009-2010	2010-2011	2009-2010	2010-2011	2009-2010	2010-2011	2009-2010	2010-2011
Replication	2	20.4	47.6	17.44	3.1	3.81	0.018	2.07	0.003
Irrigation (I)	1	8038 **	10218 **	44.85 ns	145.6 **	0.75 ns	0.004 ns	2.83 **	8.24 *
Error 1	2	14.2	16.7	19.66	1.42	0.21	0.018	0.03	0.354
Fertilizing (F)	6	246.7 **	253.7 **	3.75 ns	4.73 ns	0.08 ns	0.088 ns	1.04 **	1.90 *
I×F	6	76.5 **	55.5 **	6.31 ns	14.1 ns	0.55 ns	0.081 ns	0.43 ns	0.895 ns
Error 2	24	10.2	10.8	4.85	6.7	0.28	0.181	0.25	0.573

Ns: Non significant and * and ** significant at 5 and 1% probability level, respectively.

Table 4
Effect of irrigation on some morphological traits of cumin

Treatments	Plant height		Branch number per plant		Umbel number per plant	
	2009-2010	2010-2011	2009-2010	2010-2011	2009-2010	2010-2011
Stress (Supplemental irrigation)	15.0 a	12.7 b	2.44 a	2.12 a	5.17 b	4.73 b
Complete irrigation	17.1 a	16.4 a	2.17 a	2.14 a	5.69 a	5.61 a

-Means followed by the same letter within each column are not significantly different according to Duncan multiple test (P=0.05) nificantly affected by these treatments (Table 9). Treatment of 5M+25N, with the highest seed yield, also had the highest essential oil yield. Essential oil yield increased in all treatments from any type of fertilizer and results were not significantly different (Table 9).

Interaction of irrigation and fertilizer treatments on essential oil yield and percentage was not significant (Table 8). Simple and interaction effects of irrigation and fertilizer treatments on germination percentage of seeds were not significant (Table 7).

Table 5
Effect of fertilization method on some morphological traits of cumin

Treatments	Plant height		Branch number per plant		Umbel number per plant	
	2009-2010	2010-2011	2009-2010	2010-2011	2009-2010	2010-2011
Control	16.1 a	15.1 a	2.40 a	2.30 a	4.72 c	4.88 abc
10 M	15.5 a	14.5 a	2.33 a	2.03 a	5.77 ab	5.40 ab
5 M	16.0 a	14.0 a	2.33 a	2.03 a	5.60 ab	5.72 a
50 N	17.8 a	14.2 a	2.23 a	2.15 a	5.12 bc	4.42 c
5 M + 25 N	15.9 a	14.9 a	2.47 a	2.08 a	5.97 a	5.68 a
B 1	15.6 a	16.0 a	2.13 a	2.30 a	5.37 ab	5.60 a
B 2	15.6 a	13.2 a	2.23 a	2.03 a	5.48 ab	4.50 bc

-10M: 10 t/ha manure- 5M: 5t/ha manure- 50N: 50 kg/ha nitrogen- and B1 and B2 seed inoculation with *Pseudomonas putida* and *P. fluorescence*, respectively;
-Means followed by the same letter within each column are not significantly different according to Duncan multiple test (P=0.05)

Table 6
Effect of irrigation and fertilization method on cumin seed yield (g.m^{-2})

Fertilization treatment	Irrigation treatments					
	2009-2010			2010-2011		
	Stress (Supplemental irrigation)	Complete irrigation	Means	Stress (Supplemental irrigation)	Complete irrigation	Means
Control	25.83 b	50.37 c	38.10 b	16.17 bc	38.50 d	27.33 d
10 M	38.27 a	60.23 b	49.25 a	22.30 ab	61.07 a	41.68 a
5 M	37.30 a	61.24 b	49.27 a	19.20 abc	50.00 b	34.60 b
50 N	30.10 b	73.20 a	51.65 a	18.10 abc	45.97 bc	32.03 bc
5 M + 25 N	38.20 a	62.90 b	50.55 a	24.17 a	63.63 a	43.90 a
B 1	24.50 b	53.37 c	38.93 b	13.90 c	44.27 bcd	29.08 cd
B 2	24.57 b	51.15 c	37.86 b	14.73 c	43.50 cd	29.12 cd
Means	31.25 b	58.92 a	45.09	18.37 b	49.56 a	33.97

-Means followed by the same letter within each column are not significantly different according to Duncan multiple test (P=0.05)

Table 7
Analysis of variance for some seed quality traits of cumin as affected by irrigation and fertilizing methods

Sourec of variation	df	Mean squares					
		Essential oil percentage		Essential oil yield		Germination percentage	
		2009-2010	2010-2011	2009-2010	2010-2011	2009-2010	2010-2011
Replication	2	0.001	0.012	0.64	1.81	413.4	5.21
Irrigation (I)	1	0.153 ns	0.027 ns	470.6 **	497.8 **	2002 ns	180.2 ns
Error 1	2	0.035	0.006	0.196	0.843	379.2	11.2
Fertilizing (F)	6	0.137 ns	0.033 ns	8.53 **	15.2 **	122.8 ns	27.3 ns
I×F	6	0.088 ns	0.034 ns	2.79 ns	3.12 ns	251.7 ns	54.6 ns
Error 2	24	0.056	0.093	2.03	1.47	166.6	156.5

Ns: Non significant and * and ** significant at 5 and 1% probability level, respectively

Table 8
Effect of irrigation on some qualitative traits of seed cumin

Treatments	Essential oil percentage		Essential oil yield, kg.ha^{-1}		Germination percentage	
	2009-2010	2010-2011	2009-2010	2010-2011	2009-2010	2010-2011
Stress (Supplemental irrigation)	2.179 a	2.284 a	6.81 b	4.20 b	55.9 a	55.4 a
Complete irrigation	2.300 a	2.233 a	13.5 a	11.09 a	42.1 a	51.3 a

-Means followed by the same letter within each column are not significantly different according to Duncan multiple test (P=0.05)

Table 9
Effect of fertilization method on some qualitative traits of seed cumin

Treatments	Essential oil percentage		Essential oil yield, kg.ha^{-1}		Germination percentage	
	2009-2010	2010-2011	2009-2010	2010-2011	2009-2010	2010-2011
Control	2.495 a	2.201 a	9.35 bc	6.09 c	55.3 a	52.5 a
10 M	2.231 a	2.278 a	11.01 ab	9.59 a	49.2 a	54.8 a
5 M	2.131 a	2.259 a	10.79 ab	7.76 b	45.7 a	55.2 a
50 N	2.043 a	2.143 a	10.58 abc	6.74 bc	48.5 a	51.5 a
5 M + 25 N	2.318 a	2.361 a	11.81 a	10.10 a	46.3 a	56.5 a
B 1	2.142 a	2.239 a	8.80 c	6.57 bc	43.3 a	52.2 a
B 2	2.315 a	2.327 a	8.75 c	6.66 bc	54.7 a	50.8 a

-10M: 10 t/ha manure- 5M: 5t/ha manure- 50N: 50 kg/ha nitrogen- and B1 and B2 seed inoculation with *Pseudomonas putida* and *P. fluorescence*, respectively.
-Means followed by the same letter within each column are not significantly different according to Duncan multiple test (P=0.05)

Discussion

There was probably too much reduction of precipitation in the year 2010-2011 (especially in a sandy loam textured soil condition making the soil water holding capacity was too low) this could be a reason for different responses of plant height to water stress at each year of the experiment. Baher et al. (2002), Razmjoo et al. (2008) and Bettaieb et al. (2009) have also reported plant height reduction under water stress in *Satureja hortensis*, *Matricaria chamomile* and *Salvia officinalis*, respectively.

There was a non significant effect of irrigation on branch number per plant (Table 3) probably as a result of soil infertility in the area where the experiment was conducted and as a consequence plant growth was low and the formation of branches was inhibited. These results are conflicting with those obtained by Colom and Vazzanz (2002) and Ahmadian et al. (2011b) in *Eragrostis curvula* and Chamomile, respectively that reported a reduction of branch number per plant under drought.

The reduction of cumin umbel number per plant in water stress condition also has reported by Ahmadian et al. (2011a). In that experiment, the highest number of umbels per plant resulted from 3 applications of irrigation with an application of manure.

There was a non significant effect of nitrogen application (N_0) on umbel number per plant and therefore seed yield and the positive effect on plants treated with manure indicated that in addition to making nutrients available to plants, manure caused an improvement in the soil's physical properties, especially its water holding capacity, which affected plant growth and yield. Also Bhattacharyya et al. (2008) in a study on sustainability under combined application of mineral and organic fertilizers indicated that an addition of manure with NPK fertilizer increased soil organic carbon content and total nitrogen compared to the treatment of NPK alone.

Seed yield reductions under water stress conditions have been reported in Coriandrum by Aliabadi Farahani et al. (2008). However, in another research Tatari (2004) indicated that increasing irrigation times in cumin improved biomass yield but decreased seed yield.

Water stress reduces yield of medicinal and aromatic plants by three mechanisms (Aliabadi Farahani et al. 2009): 1- Reduction in PAR absorption as affected by limitation of leaf area expansion, temporary leaf wilting or rolling during periods of water stress, or by early leaf senescence. 2- Reduction in radiation use efficiency and 3- Reduction in harvest index. In this experiment, because HI was not significantly affected by water stress (data are not showed) it seems that reduction in PAR absorption and radiation use efficiency caused a decline in cumin growth and yield. This reduced yield could also be attributed to preferential allocation of assimilates to roots under water stress.

There are many reasons showing that cumin has adapted to drought stress (Kizil et al. 2003; Tavoosi, 2001), but the substantial reduction in seed yield in this experiment may be as a result of the low water holding capacity of the soil (the soil had a sandy texture). Generally seed yield in the second year of the experiment was lower than in the first year (Table 6). This was as a result of lower rainfall and an unfavorable pattern of rainfall in the second year of the experiment (Table 1).

Organic amendments improved soil structure and aggregate stability in the soil, as well as its moisture retention capacity by increasing the total number of storage pores (Bhattacharyya et al. 2008). In a rain-fed soybean-wheat system, Bhattacharyya et al. (2008) showed that addition of farmyard manure with NPK fertilizer treatments compared to NPK alone, increased seed yield through increasing the soil's organic carbon content and total nitrogen. In addition, Mallanagouda (1995) reported that using manure with mineral fertilizer in coriander resulted in a higher increase in yield than single applications of each nutrient.

In the non-stress condition, the highest seed yield (Table 6) was related to 50N (in 2009-2010) and 5M+25N (in 2010-2011) treatments. This may have been due to the increasing the soil's water holding capacity as a result of manure application in the second year of the experiment when the rate of precipitation was very low (especially because the soil had a sandy texture with low water holding capacity).

Ahmadian et al. (2011b) reported that, in a non-stress condition, mineral fertilizer treatments resulted in higher yields of chamomile flower. In another study on cumin, Ahmadian et al. (2011a) indicated that an application of organic fertilizer caused an increase to the soil's water holding capacity.

There was not any significant difference between seed yield of the control treatment (no fertilizer) with bio-fertilizer treatments (B_1 and B_2) in both years of the experiment (Table 6). These results are contrary to those of Shaalan (2005) demonstrating that bio-fertilizer application such as *Pseudomonas sp.* increased growth parameters in *Nigella sativum*. This might be due to an unfavorable soil condition (pH, EC and texture) for activating bacteria, making the bacteria unsuitable for the plant species.

The complete irrigation treatment produced better seed yield thus more essential oil yield (Table 8). Other research has demonstrated an increasing of seed oil and essential oil percentages respectively in *Calendula officinalis* (Rahmani et al. 2008) and *Ocimum sp.* (Khalid 2006) and a decreasing essential oil yield of *Pelargonium graveolens* (Putievsky et al. 1990) in drought stress conditions, while Khazaie et al. (2008) found no significant difference in the essential oil percentages of *Thymus vulgaris* and *Hyssopus officinalis* using irrigation intervals of 7, 14 and 21 days in a 2 year experiment.

It has been reported that nitrogen application generally increases essential oil yield in aromatic plants by increasing the biomass yield per unit land area (Sangwan et al., 2001; Rao, 2001).

Low production potential and thus low growth and yield in the location of this experiment may be one of the reasons for no change to seed germination under different treatments in this study.

Conclusions

In conclusion, these results show that manure application could be used to modify the impact of water shortage and stimulate an increase in cumin seed and essential oil yield by improving a soil's water holding capacity. Nitrogen application with manure had the effect of improving the soil probably due to increasing its organic matter, mineralization and mineral absorption. As seed inoculation with *Pseudomonas sp.* in this study had no positive effect on plant growth and yield, then it can be maintained that climate, soil condition and plant species are the most important factors to determine suitable bacteria species for application as bio fertilizer.

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Received November, 13, 2012; accepted for printing June, 2, 2013.