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Original Article

Black cumin *(Nigella sativa* L.) Yield Affected by Irrigation and Plant Growth Promoting Bacteria

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Abstract

This study was conducted to assess the effects of irrigation and plant growth promoting bactera on seed yield and qualitative characteristics of Black cumin (*Nigella sativa* L.) at the Experimental Station of the Research Institute of Forest and Rangeland, Damavan region, Iran. A split-plot experimental design, based on Randomized Complete Block Design (RCBD) with three replications, was used. Irrigation treatment at three levels (normal irrigation (control), irrigation terminated at the start of budding and irrigation terminated at the start of flowering) allocated in main plots and plant growth promoting bacteria treatment at four levels (non-inoculated, seed inoculated, spraying on the plant base at stem elongation stage and seed inoculated + spraying on the plant base at stem elongation stage) were arranged in sub-plots. Measured traits were consisted of harvest index, seed yield, essential oil, carvone and thymoquinon content. Results showed that the highest harvest index (28.20%) and seed yield (722.85 kg/ha) were obtained by normal irrigation. But, irrigation terminated at the start of budding caused the maximum essential oil (0.45%), carvone (0.018 mM) and thymoquinon content (0.021mM). Applying spraying plant growth promoting bacteria on the plant base at stem elongation stage and seed inoculated + spraying on the plant base at stem elongation stage caused highest seed yield (611.59 kg/ha) and thymoquinon content (0.019 mM). Maximum essential oil (0.45%) and carvone content (0.020 mM) was obtained by seed inoculation with plant growth promoting bacteria.

Keywords: Irrigation termination, Black cumin, Essential oil, Carvone, Thymoquinone

Introduction

Nigella sativa L. (black cumin) is an annual flowering plant, native to southwest Asia and used widely in traditional and industrial pharmacology [1,2]. According to the literature, Black cumin seeds and their extracts contain anti-abetic, antihistaminic, anti-hypertensive, anti inflammatory, anti-microbial, antitumor, galactagogue and insect repellent properties [3].
One of the most important constituents of volatile oil of the *Nigella sativa* seeds are thymoquinone [4]. Thymoquinone belongs to class of compounds known as terpenoids [5]. In vivo, it inhibits for stomach and fibro sarcoma tumor incidence and multiplicity in mice [6]. Pharmacological investigations explored the effectiveness thymoquinone and carvone against various maladies like oxidative stress, cancer, immune dysfunction and diabetic complications [7].
Some studies shown that the Black cumin is able to tolerate moderate levels of water stress [5, 8]. Some researchers have focused on response of Black cumin to different irrigation intervals [8] and

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irrigation scheduling based on developmental stage [5].

Inoculation of plants with *Azospirillum* can result in a significant change in various plant growth parameters. Positive effects of inoculation have been demonstrated on various root parameters, including increase in root length, particularly of the root elongation zone [9,10]. *Azospirillum* as N_2 – fixing bacteria could be a beneficial source to enhance plant growth and produce considerable amounts of biologically active substances that can promote growth of reproductive organs and increase the plants productivity. *Azospirillum* play an important role in yield- attributed characters owing to the production of siderophores which regulate the availability of nutrients to the crop [11]. The best performances of *Azospirillum* under field conditions are usually associated with non-optimal conditions for plant growth (limited fertilization and water) and they occur mainly in semi-arid agriculture [12].

Drought stress is one of the most important environmental stresses affecting agricultural productivity around the world and may result in considerable yield reductions [13,14]. Drought occurs when soil moisture level and relative humidity in air is low while temperature is also high [15]. According to the United Nations Environment Program, Iran will be one of 100 countries around the world with a low rate of renewable fresh water per capita [16].

Almost every plant process is affected directly or indirectly by water supply [17]. Certainly, most land plants are exposed to short or long term water stress at some times in their life cycle and have tended to develop some adaptive mechanisms for adapting to changing environmental conditions [15].

Crop yields are restricted by water shortages in many parts of the world [18]. On a global basis, about one-third of potential arable land suffers from inadequate water supply, and the yields of much of the remainder are periodically reduced by drought. Moreover, water deficits may occur during a plant's life cycle outside of arid and semi-arid regions [19,20]. It is well known that water stress not only affects morphological appearance but also changes biomass ratio. Bradford and Hsiao [21] reported that water stress drastically decreases root elongation and leaf area expansion but that these two processes are not equally affected. Timpa *et al*. [22] and Akinci and Lösel [23,24] reported that the water stress caused major reductions in height, leaf number, leaf area index, fresh and dry weight of cotton plants.

Water stress resulted in significant reduction of fresh and dry matter, nutrient content, and essential oil yield of Japanese mint plants [25]. Fresh and dry weights of *Ocimum basilicum* L. were decreased as plant water deficit increased [26]. Ghamarnia and Jalili [16] showed that with increasing water stress seed yield, oil yield and water use efficiency significantly decreased. Water stress reduced fresh and dry weights of *Satureja hortensis* L. (Savory) plants. Severe water stress increased essential oil content more than moderate water stress [27].

Solinas and Deiana [28] reported that secondary products of plants can be altered by environmental factors and water stress is a major factor affecting the synthesis of natural products. Changes in essential oils extracted from aromatic plants and their composition were observed with water stress [29]. The essential oil yield of basil was increased by subjecting plants to water stress just before harvesting [30].

The results were indicated that water deficit during the vegetative growth of mint can result in reduction in plants height and smaller leaf areas in mint [31], yarrow [32] and chicory [33]; reduction in plant biomass of calendula [34] and reduction in dry matter of balm [13]. The numerous studies were indicated that grain yield can be significantly reduced as a result of water deficit during the reproductive period of coriander [35], Mexican marigold [36] and grapevine [37].

Singh-Sangwan *et al*. [38] indicated that the level of essential oils was maintained or enhanced under drought condition in two lemon grasses (*Cymbopogon nardus* (L.) Rendle and *Cymbopogon pendulus* (Nees ex Steud.) W.Watson). Other studies have shown that water stress has negative effects on yield and yield components of some medicinal plants [39-48]. Also, water stress during budding, flowering and anthesis stages of plants, will cause most reduction in seed number and seed yield [49].

The potential of medicinal and aromatic plants for growing under limited water conditions make them suitable alternative crops in such agro- ecosystems [50,51]. Some research has been carried out on responses of Black cumin to different irrigation intervals [5,8,52] but irrigation scheduling based on developmental stage has not been studied well.

And also, there are a few studies on *Azospirillum* inoculation in black cumin. So, the present research was carried out to evaluate the effects of *Azospirillum* and irrigation treatments on seed yield and active substances of Black cumin seeds.

Materials and Methods

Field Experiment

This experiment was carried out during the growing season of 2011 in order to investigate the effects of different irrigation treatments and biofertilizer on yield of Black cumin plants at the Experimental Station of the Research Institute of Forest and Rangeland, Damavand region, Iran (Latitude: 35° 39´ N; Longitude: 52° 05´ E; Elevation: 1800m). Soil was sampled random at the top 0–30 cm soil-layer of experimental site. According to the results of soil analysis (Table 1), soil was a loamy-clay with pH 7.1, containing total N (0.08 %), total P (40 mg/kg), and total K (550 mg/kg) with an EC of 0.70 ds/m. Data on rainfall and mean temperature during growing stages of Black cumin are presented at Table 2.

A split-plot experimental design, based on Randomized Complete Block Design (RCBD) with three replications, was used to assess the effects of treatments on Black cumin. Irrigation treatment (W1 = normal irrigation from emergence to harvest (control), W2 = irrigation terminated at the start of budding and W3 = irrigation terminated at the start of flowering) allocated in main plots and *Azospirillum* treatment (A1=non-inoculated, A2=seed inoculated and A3= spraying on the plant base at stem elongation stage, A4= seed inoculated + spraying on the plant base at stem elongation stage) were arranged in sub-plots.

Growth stages of plants were monitored in two middles rows of each plot. The developmental stages were determined when one plant in each plot indicated that stage. The developmental stages were: budding, when at least one folded flower bud was observed per plant and flowering, when at least one unfolded flower was observed per plant.

Azospirillum lipoferum inoculation was carried out by dipping the black cumin seeds in the cells suspension of 10^8 CFU/ml for 15 min [53]. Seeds of *Nigella sativa* were obtained from Medicinal Plants Research Station of Isfahan, Iran. Each experimental plot was 3 m long and 2 m. Black cumin seeds were directly sown by hand, 0.5 cm depth and in rows with 25 cm apart on 9 April 2011. Three weeks after sowing, the seedlings were thinned up to 180-plant m−2. Irrigation furrows with uniform slopes were constructed in each experimental plot. A one-time irrigation was applied immediately after sowing for uniform emergence. Soil water content was measured for each development stage weekly across all treatments and was determined on a dry mass basis and converted to a volumetric basis using 1.35 g cm^{-3} as soil bulk density. In order to determine the soil water content, 24 h after irrigation 5 soil samples were taken by sampling drill, then samples were weighed by electrical scale and placed under 105°C in electrical oven for 48 h [54, 55]. Based on field capacity (26.5% θ) and wilting point (11% θ) of the study site, 50% of available soil water was considered as the water stress threshold (17.2% θ). There was no incidence of pest or disease on Black cumin during the experiment and weeding was done manually. Weeds were controlled manually. All necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation.

Table1 Soil analysis of experiment location

Texture	pH	Cu	Zn	Mn	Fe	K	P	N	O.C	EC
		mg/kg						%	%	ds/m
Loamy-Clay	7.1	0.46	1.2	8.8	7.7	550	40	0.08	0.8	0.7

Table2 Mean temperature and rainfall during various developmental stages of Black cumin

Developmental stage	Mean temperature (°C)	Rainfall (mm)
Planting–Seed emergence	23.6	0
Seed emergence–Budding stage	26.5	5.7
Budding stage–Flowering stage	24.1	2.1
Flowering stage–Maturity stage	24.8	0.2

Measurements

Final seed yield measured from 1m^2 of each plot. To determine the amount of essential oil, a sample of 10 g of seeds was mixed with 50 ml of tap water in a flask and the water was distilled for 3 h using a rotavapor instrument and flask put over the heat produced by electro-thermal instrument for 3 hours (for providing direct heat condition). Distillated were put in the centrifuge (at 1000 rpm for 30 min) and supernatants were separated. UV monitoring of the eluted solutes was carried out [4] by using spectrophotometer UV-visible (model: Cary 100 Boi, VARIAN). Quantities of thymoquinone and carvone were determined by measuring the peak area.

Statistical Analysis

All data were subjected to one-way analysis of variance using a general lineal models procedure (PROC GLM) in the Statistical Analysis System [56] to test for significant differences between treatments (P<0.05). Means of comparisons were performed by Duncan's Multiple Range Test (DMRT). Data were transformed when necessary before analysis to satisfy the assumptions of normality. However, any values mentioned in this section refer to the original data of present experiment.

Harvest Index

Analysis of variance showed that harvest index affected by irrigation treatments and *Azospirillum* inoculation, significantly (Table 3). Mean comparison, also showed significant differences between various levels of irrigation treatments (Table 4). The highest harvest index (28.20%) was recorded under the W1 treatment. but, there were not significant differences between W1 and W3 treatments. Irrigation termination at start of budding stage caused 9.4% reduction in harvest index compared to the control (W1).

Results

The present results have indicated that all measured traits were significantly affected by irrigation and *Azospirillum* treatments. Only seed yield affected by interaction between treatments, significantly (Table 3).

Table 3 Effects of irrigation treatments and Azospirillum inoculation on measured traits of Black cumin (*Nigella sativa* L.)

S.O.V	DF	Harvest Index	Seed Yield	Essential Oil Content	Carvone Content	Thymoquinon Content
rep	2	4918.6219	5058.3333**	0.0052	0.0004211	0.0018571
Irrigation	2	47329.5574**	359363.5574**	3.4817*	0.1549303*	0.3927190**
rep×a	4	44.6590	66.6667	0.0041	0.0034297	0.0001849
b	3	37219.7774**	57049.7774**	4.7931*	0.2364501**	0.2840194**
a×b	6	6193.3162	642.1245**	0.0162	0.0943166	0.0056171
error	18	10.3333	8.3333	0.0011	0.0002960	0.0011573
CV %		10.39	7.53	3.15	4.52	3.76

a: Irrigation treatments; b: *Azospirillum lipoferum* inoculation.

*, ** are significant at P≤ 0.05 and P≤ 0.01 levels of probability, respectively.

Table 4 Mean comparison of the some characteristics of black cumin at various levels of irrigation treatment and *Azospirillum* inoculation

Treatments	Harvest index (%)	Seed yield (kg/ha)	Essential Oil (%)	Carvone (mM)*	Thymoquinone (mM)*
Irrigation treatment					
W_1	28.20a	722.853a	0.38b	0.011953b	0.012684b
W_2	25.54b	377.319c	0.45a	0.018199a	0.021964a
W_3	28.18a	532.883b	0.42ab	0.018196a	0.015004b
Azospirillum lipoferum					
A_1	24.98b	430.719d	0.40b	0.020348a	0.018250b
A_2	27.68a	583.453b	0.45a	0.020348a	0.019159a
A_3	28.60a	551.636c	0.41b	0.018524b	0.018747b
A_4	28.47a	611.590a	0.44a	0.019845a	0.019381a

*based on millimolar per 10 g seed weight.

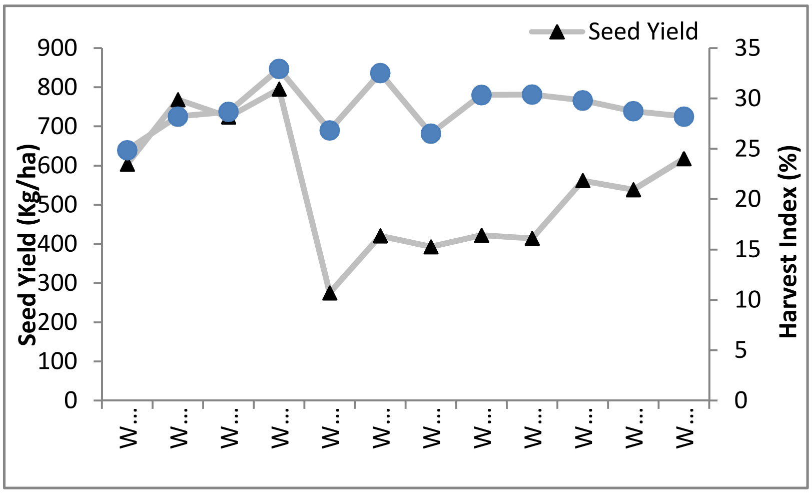

Fig. 1 Effects of interaction between treatments on Black cumin Seed Yield and Harvest Index(W_1=normal irrigation from emergence to harvest (control), W_2=irrigation terminated at the start of budding and W_3=irrigation terminated at the start of flowering; A_1=non-inoculated, A_2=seed inoculated, A_3=spraying on the plant base at stem elongation stage and A_4= seed inoculated+spraying on the plant base at stem elongation stage).

Mean comparison showed significant differences between various levels of *Azospirillum* inoculation. A1 caused the plant to reach the lowest harvest index (24.98%). As a general, *Azospirillum* could increase harvest index significantly in comparison to A1 (control). But, there were not significant changes between A2, A3 and A4 treatment (Table 4).

Seed Yield

Results showed that irrigation treatments and *Azospirillum* inoculation had significant effects on the seed yield (Table 3). Mean Comparison showed that seed yield varied between 722.85 and 372.32 kg/ha (Table 4), which was obtained from control (W1) and W2, respectively.
Seed yield in response to irrigation cessation at the start of budding (W2) showed the highest decrease compared to controls. In combination between various levels of irrigation treatment and *Azospirillum* inoculation, W1A4 and W1A2 resulted in the highest seed yield (Fig 1). There were significant differences in seed yield between the plants inoculated with various levels of *Azospirillum lipoferum*. A4 caused the highest seed yield in black cumin (Table 4).

Essential Oil Content

The essential oil content of seeds is affected by irrigation and biofertilizer. But Interactions between treatments could not cause significant differences (Table 3). The highest essential oil content (0.45%) was obtained at irrigation terminated at the start of budding stage. But, there were not significant differences between W1 and W3 treatment for essential oil content (Table 4). *Azospirillum* could increase essential oil content significantly as compared to the control (A1). The maximum of essential oil content (0.44%) was obtained at *Azospirillum* inoculation with seeds (A2). Although, according to the Table 4, there is no significant difference between A2 and A4 treatments.

Thymoquinone Content

Irrigation treatments and *Azospirillum* had significant effects on thymoquinone content. But, it was not affected by interactions between various levels of treatments, significantly (Table 3). Maximum thymoquinone content (0.021964 mM/10 g seed) was obtained when irrigation terminated at the start of budding stage. But, there were not significant differences between W1 and W3 treatment for thymoquinone content (Table 4). *Azospirillum* could increase thymoquinone content significantly as compared to the control (A1). The highest amount of thymoquinone (0.020159 mM) was obtained at A2. Although, according to the Table 4, there is no significant difference between A2 and A4 treatments.

Carvone Content

The carvone content in the seeds is affected by irrigation termination levels and *Azospirillum lipoferum* inoculation. Interactions between various levels of treatments did not cause significant differences (Table 3). Maximum carvone content (0.018199 mM/10 g seed) was obtained when irrigation terminated at the start of flowering stage. But, there were not significant differences between W1 and W2 treatment for carvone content (Table 4). *Azospirillum* could increase carvone content significantly as compared to the control (A1). The highest amount of carvone (0.020348mM) was obtained at A2. Although, according to the Table 4, there is no significant difference between A1, A2 and A4 treatments. The minimum carvone content was obtained when *Azospirillum* sprayed at the base of plants (A3).

Discussions

According to the results of this study, irrigation treatments had significant effects on all measured traits. At W2, number of follicle per plant and seed yield and harvest index was at least. While, the maximum essential oil, thymoquinone and carvone content were obtaind at W2.

The harvest index from W2 was 9.43% and 9.36% lower in comparison with W1 (control) and W3, respectively. Water stress conditions and improper irrigation management, will result in lower assimilation production in the plants. Also, allocation of assimilates among seeds and vegetative organs will change. Reduction of harvest index under water stress conditions is because of sooner leaves senescence, reduction in seed filling period and lower seed weight [57]. Similar result was obtained by Paknejad *et al*. [58].

Azospirrilum inoculation could enhance growth and production of black cumin in comparison with control. In A2 treatment (seed inoculated), the highest harvest index was obtained. Although, there were not significant differences between inoculations seeds with *Azospirillum* or spraying at plant base at stem elongation stage.

The numerous studies were indicated that seed yield can be significantly reduced as a result of water deficit during the reproductive period of coriander [35], Mexican marigold [36] and grapevine [37].

Other studies have shown that water stress has negative effects on yield and yield components of some medicinal plants such as *Nigella sativa* L, [39, 40], *Plantago ovata* Forssk. [41], *Matricaria chamomilla* L. [42], *Cuminum cyminum* L. [43], *Mentha piperita* L. [44], *Melissa officinalis* L. (Lemon balm) [45], *Nepeta racemosa* Lam. (Lemon catmint) [46], *Origanum vulgare* L. [47] and *Salvia officinalis* L. [48].

Total extraction of soil moisture by Azospirillum-inoculated plants is greater and water extracts from deeper layers in the soil profile [10]. Therefore, seed yield increase in inoculated plants was attributed primarily to improved utilization of soil moisture. These effects could result in more water uptake, especially in arid and semi-arid regions.

Water stress resulted in significant reduction of essential oil yield of Japanese mint plants [25]. The essential oil content of basil was increased by subjecting plants to water stress just before harvesting [30]. Singh-Sangwan *et al*. [38] indicated that the level of essential oils was maintained or enhanced under drought condition in two lemon grasses [*Cymbopogon nardus* (L.) Rendle and *Cymbopogon pendulus* (Nees ex Steud.) W.Watson].

According to the results, the highest thymoquinone content obtained when irrigation terminated at the start of budding stage. In the other hand, water stress increased active compounds of black cumin. Other studies have shown that water stress could enhance active substance and quality of some medicinal plants such as menthol of peppermint [44], plumbagin of Chitrak [59], essential oil of balm [45], essential oil of cumin [43] and essential oil of basil [27].

It must be taken into consideration that a concentration increase of active compounds induced by moderate drought stress in general is associated with a reduction of biomass production. Consequently, it has to be clarified the putative gain in quality by increasing the secondary plant product concentration by applying deliberately drought stress would be compensated by decreasing yields in biomass. Thus, a corresponding decision must strongly be based on the question related to the nature of the desired product.

It is obvious that in the case of medicinal plants which are used as pharmaceuticals, the quality and thus the concentration of active compounds is much more relevant than the total yield, whereas in all cases, where the desired compounds will be extracted, the overall yield has to be very high. A successful and effective application of deliberate drought stress for quality improvement, e.g. by applying special watering regimes is an encouraging new tool for the production of medicinal and pharmaceutical relevant plants [60].

For thymoquinone content in the seeds, *Azospirillum* had positive effects and could increase differences between control (A1) and other levels of bacteria application (A2-A4). *Azospirillum* inoculation with seeds could improve seed quality.

Conclusions

Our findings revealed that the start of budding stage of Black cumin was the most sensitive to irrigation termination. Irrigation termination at budding stage had negative influence on seed yield, while by at W2 (irrigation termination at budding stage), essential oil, carvone and thymoquinone content of seeds were maximum.

According to the advantages of *Azospirillum* inoculation, which it has mentioned previously; it seems using *Azospirillum lipoferum* in agro-ecosystems could increase seed yield and quality of seeds and, of course, help farmers to save water in arid and semi-arid regions.

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