Irrigation, Phosphorus Fertilizer and Phosphorus Solubilizing Microorganism Effects on Yield and Forage Quality of Turnip (*Brassica rapa* L.) in an Arid Region of Iran

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Abstract Turnip has a high potential to produce abundant nutritious and valuable forage when availability of perennial warm and cool-season species is limited. Water deficit and fertility affect not only growth and yield of crops but also their quality and nutritional value. To evaluate the effect of different phosphorous fertilizers on forage yield and quality of turnip under deficit irrigation regimes, a field experiment was conducted during 2009. The experimental treatments arranged as split plots were five levels of irrigation, no irrigation (IR_0), irrigation at sowing time (IR_1), irrigation at sowing time + commencement of tuber formation (IR_2), (irrigation at sowing time + commencement of tuber formation + commencement of stem elongation (IR_3) and normal irrigation (IR_N), assigned to main plots and four levels of fertilizers, no fertilizer (F_{Co}), 100 % F_{Ch} (100 % chemical fertilizer), seed inoculation by *Pseudomonas putida* (F_{Bi}), 50 % F_{Ch} + F_{Bi} , assigned to subplots. The maximum forage yield was obtained at IR_N , which followed a decreasing trend as the number of irrigations decreased. The best phosphorous fertilizer treatments affecting forage yield were 100 % F_{Ch} and 50 % F_{Ch} + F_{Bi} in sequence. As the water stress increased, in vitro dry matter digestibility (IVDMD), metabolizable energy, and protein yield followed a decreasing trend while in the same situation the crude protein, water soluble carbohydrates, acid detergent fiber, and ash content followed an increasing trend.

Keywords Forage yield · Forage quality · IVDMD · Crude protein · Biological fertilizer

Introduction

Forage turnip is an annual crop that has been long known for its use as a reliable forage source in many regions of the world. This crop has a high potential to produce abundant nutritious and valuable forage when availability of perennial warm and cool-season species is limited. In addition, turnip is relatively higher yielding and also more nutritious than other forage crops like cereals [29].

Environmental factors like soil water and fertility affect not only growth and yield of crops but also their quality and nutritional value as well. Among all factors challenging plant growth and yield, including biotic and abiotic stresses, water stress is the most important factor that limits plant productivity especially in arid and semi-arid regions [30]. Water deficit or limited irrigation regime is an advantageous strategy that can be conducted in these areas to improve water use productivity by less water consumption. Water deficit regime refers to providing required water for plants only in critical growth stages and inducing water stress in non-critical growth stages [39]. The yield of crops will be negatively affected by this strategy and severity of this effect is dependent on the time of inducing water stress [25]. As the water deficit affects both quantitative and qualitative characteristics of the product, determining the critical growth stages as well as magnitude effects of water stress happened in each stage, sounds important in different crops like turnip.

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Phosphorus (P) is an essential element playing a key role in plant growth and metabolism and is the major nutrient after nitrogen that limits plant growth and production [12]. Also, it is well understood that P could increase the plant resistance to drought stress and has a tendency to moderate the adverse effect of drought stress in plants [16]. It is known that under drought stress conditions, plants lack of sufficient P uptake from soils is a major issue. Phosphorus can moderate the severity of the drought stress through different mechanisms including enhancement of the growth of the plant's root system [9, 16]. However, between 75 and 90 % of P applied as chemical fertilizer in agro-ecosystems remains unavailable to the plant due to iron, aluminum, and calcium complexes [34]. Indeed, many soils are defined as having high P-fixation capacity [38]. Efforts have been made to solubilize insoluble P and therefore increase its availability through biologically mediated processes by phosphate solubilizing microorganisms (PSM) [18]. For supplying required P to crops, the safe and feasible strategy for sustainable agriculture is to apply fertilizer P in conjunction with phosphorus solubilizing microorganisms (PSM), which assist in dissolving a wide range of insoluble P compounds in soils leading to higher crop yields while reducing P leaching and surface runoff to water bodies causing eutrophication [5]. This has been mainly attributed to bacterial production of extracellular enzymes and exudation of organic acids, which stimulates plants root growth [31].

The objectives of the present field study were to evaluate the individual and combined effects of irrigation regimes, fertilizer P and phosphorus solubilizing microorganism on the yield and forage quality of turnip (*Brassica rapa*) in an arid region of Iran.

Materials and Methods

Site Description

This research was conducted at the Research Farm of the College of Agriculture and Natural Resources, University of Tehran, in Karaj, Iran (N35°56", E50°58"), in 2009. The climate type of this site is considered arid to semi-arid with annual average air temperature, soil temperature, and rainfall of 13.5°C, 14.5 °C, and 262 mm, respectively. Total monthly rainfall of the experimental site during the study period and for the long term is shown in Table 1.

The soil texture of the experimental field is clay loam (33 % sand, 36 % silt, and 31 % clay) with pH = 8.2 and $E_c = 3.41$ ds/m. The organic carbon content of the surface layer (0–15 cm) was 1.02 %. The soil had no salinity and drainage problem. Some physical and chemical properties of the soil are presented in Table 2.

Table 1 Total monthly rainfall of the experimental site during the study period (2009) and for the long term

Month	Precipitation (mm)	
	2009	Long term
March	12.6	47.7
April	4.5	34.7
May	3.1	20.8
June	0.0	2.3

Experimental Details

The design of the experiment was split plots with five levels of irrigation regimes and four levels of P fertilizers. A randomized complete block design with four replications was employed for data analyses. The experimental treatments were as follows:

Irrigation regimes comprised of: control (no irrigation) (IR_0), irrigation at sowing time (IR_1), irrigation at sowing time + commencement of tuber formation (IR_2), irrigation at sowing time + commencement of tuber formation + commencement of stem elongation (IR_3), and normal irrigation or no drought stress (IR_N). The time of irrigation in the normal irrigation treatment was scheduled based on the common practice in the area, which consists of irrigating at 7 to 8-day intervals. Subplots consisted of control (no P fertilizer) (F_{CO}), sole chemical fertilizer according to soil test (100 % F_{Ch}), sole biological fertilizer (seed inoculation by *Pseudomonas putida* bacteria strains 41 and 168) (F_{Bi}), integrated fertilizer (50 % chemical P fertilizer + seed inoculation by *pseudomonas putida* strains 41 and 168) (50 % F_{Ch} + F_{Bi}). The chemical P was provided by Triple Super Phosphate (200 kg/ha according to soil test), which was applied in strips 5 cm apart from the seed. After field preparations, recommended amounts of urea and potassium sulphate (75 and 100 kg/ha, respectively) were added to the soil surface layer and mixed to 20 cm of the soil with disk. After fertilizer application, the experimental site was divided into 80 plots. The plot size for each treatment was 5 × 3 m. A border of 2 m between adjacent plots in each replication and 5 m between replications were considered to avoid drainage water from entering the other plots. Another 75 kg/ha of urea was also applied in the commencement of stem elongation.

Biological P Fertilizer Characterization and Seed Treatments

Biological P fertilizer comprised of two strains of *Pseudomonas putida* (41 and 168) isolated from the rhizosphere of wheat by the Soil Biology Laboratory in Soil and Water

Table 2 Chemical properties of the soil at the experimental site

Available (mg/kg) %							Soluble (meq/l)		
Cu	Mn	Zn	Fe	K	P	N	Mg ⁺⁺	Ca ⁺⁺	Na ⁺
1.58	12.7	1.25	6.43	237	14	0.09	8.2	20	7.1

Research Institute of Iran. The turnip seed (a small tuber indigenous ecotype) was immediately planted after inoculation by bacteria in the rate of 10^8 colony-forming units (CFU) on March 3, 2009. Plant-to-plant spacing was 10 cm and plant rows were 50 cm apart. The depth of sowing was 2 cm. The crop was harvested on June 26, 2009, and samples were collected from 1 m² quadrat per plot after elimination of border effects. Fresh weight of turnip forage was measured on site. The dry weight was measured only after the forage was dried in an oven (45 °C) for 7 days.

Forage Quality Characteristics

The qualitative properties including in vitro dry matter digestibility (IVDMD), crude protein (CP), water soluble carbohydrate (WSC), acid detergent fiber (ADF), and ash content of three replications of all treatments were analyzed by the following methods: According to the AOAC [4], each dried forage sample used for quantitative analysis was ground through a 1-mm sieve. IVDMD was measured according to the two-stage fermentation technique of Tilley and Terry [33]. CP was determined according to the Kjeldahl method [4]. ADF was determined using the procedure by Goering and Van Soest [13]. WSC was determined by the phenol–sulfuric acid method according to Dubois et al. [11]. Ash was determined by igniting the samples in a muffle furnace at 600 °C for over 4 h [4]. Metabolizable energy (ME) was determined by Eq. (1)[8].

$$ME = 0.17IVDMD\% - 2 \quad (1)$$

Statistical Analysis

A randomized complete block design with four replications was employed for data analyses. All statistical analyses were performed using GLM procedure of SAS (SAS, 1996). An ANOVA technique for split plot design was carried out. Mean comparison was done using Duncan's multiple range test at the 95 % level of probability. All differences reported are significant at $p \leq 0.05$ unless otherwise stated.

Results

Forage Yield

Deficit irrigation regimes and different P fertilizers significantly affected the shoot forage yield of turnip ($p < 0.01$)

(Table 3). The highest forage yield of 2,709 kg/ha was observed in IR_N while the lowest yield of 461.8 kg/ha was obtained from IR₀ (Table 3). With only one irrigation at sowing time (IR₁), the shoot forage yield reached 1,600 kg/ha (almost a fourfold increment compared to IR₀). This is while the extra irrigation at tuber formation (IR₂) increased only 20 % in shoot production and extra irrigation at stem formation (IR₃) provided no significant increase in shoot production compared to previous treatments (Table 3).

The highest forage yield of 2,008 kg/ha was obtained from 100 % F_{Ch} treatment, which was 25 % more than control (F_{Co}). The integrated fertilizer, 50 % F_{Ch} + F_{Bi}, also significantly over produced the control treatment (F_{Co}) by 12 % (Table 3).

In severe water stress conditions (IR₀), integrated fertilizer (50 % F_{Ch} + F_{Bi}) produced the highest forage yield. In other irrigation systems of IR₁ to IR_N, always 100 % F_{Ch} out-produced shoot biomass compared to other P fertilizers (Fig. 1). The highest forage yield of turnip was obtained in IR_N*100 % F_{Ch} treatment while the lowest was obtained in control (no irrigation and no fertilizer treatment) (Fig. 1).

Forage Quality

IVDMD

As the water stress severity increased, digestibility of dry matter decreased. The lowest IVDMD (520 g/kg dry matter) was obtained at IR₀ and the highest (622 g/kg) was achieved at IR_N (Table 3).

Application of P fertilizers (in all fertilizer treatments) significantly increased IVDMD compared to the control (Table 3).

In severe water stress conditions, IR₀ and IR₁, F_{Bi} and 50 % F_{Ch} + F_{Bi} treatments provided the highest IVDMD (higher than chemical P fertilizer and control), respectively. However, by increasing the number of irrigations, the positive effect of chemical P fertilizer on IVDMD was better pronounced (Fig. 2). The table of correlation coefficient showed a positive correlation between turnip shoot yield and IVDMD ($r = 0.782$) (Table 4).

CP Content and Protein Yield

According to Tables 3 and 4, the effect of irrigation regimes and P fertilizers on CP and protein yield of turnip

Table 3 The mean of forage quantitative (kg/ha) and qualitative characteristics (g/kg dry matter) of turnip as affected by deficit irrigation regimes and phosphorous fertilizers

Irrigation regimes	Forage yield (kg/ha)	IVDMD (g/kg)	CP (g/kg)	Protein yield (kg/ha)	WSC (g/kg)	ADF (g/kg)	ASH (g/kg)	ME MJ/D
I ₀	461.8 (±37.4) ^d	529 (±11.3) ^b	234 (±4.6) ^a	111.5 (±12.0) ⁰	209 (±5.0) ^a	340 (±8.1) ^a	85.2 (±1.2) ^a	7.0 (±0.2) ^d
I ₁	1600.3 (±44.4) ^c	548 (±6.4) ^b	221 (±5.9) ^{ab}	350.3 (±9.6) ^c	195 (±5.2) ^b	320 (±7.0) ^b	84.1 (±1.6) ^a	7.3 (±0.1) ^c
I ₂	1890.7 (±84.9) ^b	603 (±2.7) ^a	210 (±5.2) ^{bc}	398.3 (±23.5) ^b	196 (±3.2) ^b	272 (±6.7) ^c	83.7 (±0.6) ^a	8.2 (±0.1) ^b
I ₃	1935.3 (±82.0) ^b	621 (±9.2) ^a	199 (±3.7) ^c	387.2 (±22.0) ^b	195 (±5.1) ^b	275 (±6.4) ^c	79.5 (±1.3) ^b	8.6 (±0.2) ^a
I ₄	2709.8 (±54.2) ^a	622 (±7.4) ^a	182 (±3.1) ^d	490.3 (±14.6) ^a	180 (±3.3) ^c	240 (±5.0) ^d	79.2 (±1.3) ^b	8.6 (±0.1) ^a
P fertilizer								
P ₀	1558.4 (±167.5) ^c	571 (±13.5) ^b	199 (±4.0) ^c	300.1 (±35.0) ^b	185 (±2.9) ^b	303 (±11.0) ^a	81.5 (±0.9) ^a	7.7 (±0.2) ^b
P ₂₀₀	2008.2 (±191.9) ^a	591 (±15.4) ^a	210 (±7.6) ^b	402.1 (±38.8) ^a	191 (±3.0) ^b	299 (±12.0) ^a	83.3 (±1.7) ^a	8.0 (±0.3) ^a
P ₁₀₀ + PSM	1767.1 (±161.4) ^b	589 (±1.0) ^a	222 (±5.6) ^a	379.3 (±34.3) ^a	212 (±4.7) ^a	285 (±11.0) ^b	81.7 (±0.6) ^a	8.0 (±0.2) ^a
PSM	1544 (±161.5) ^c	587 (±8.4) ^a	206 (±5.7) ^{bc}	308.6 (±32.6) ^b	192 (±4.5) ^b	271 (±8.4) ^c	83.0 (±1.6) ^a	8.0 (±0.1) ^a
ANOVA								
I	**	**	**	*	**	**	**	**
P	**	**	**	*	**	**	ns	**
I*P	**	**	*	ns	ns	ns	*	**

Within columns, means followed by the different letter are significantly different at ($p < 0.05$) (for each factor separately). Values in parentheses represent standard error

* $p < 0.05$; *** $p < 0.001$

ns no significance

Treatments abbreviations: I₀ no irrigation, I₁ irrigation at sowing time, I₂ irrigation at sowing time + commencement of tuber formation, I₃ irrigation at sowing time + commencement of tuber formation + commencement of stem elongation, I₄ normal irrigation. P₀ no phosphorous fertilizer, P₁₀₀ 100 % chemical fertilizer, P₅₀ + PSM 50 % chemical fertilizer + seed inoculation by *Pseudomonas putida* strains 41 and 168, PSM seed inoculation by *Pseudomonas putida* bacteria strains 41 and 168

Fig. 1 Interaction effects of deficit irrigation and different P fertilizers on shoot forage of turnip. Error bars represent standard error. Different small letters above columns indicate significant differences ($p < 0.05$) between treatments. Treatment abbreviations: IR₀ no irrigation, IR₁ irrigation at sowing time, IR₂ irrigation at sowing time + commencement of tuber formation, IR₃ irrigation at

sowing time + commencement of tuber formation + commencement of stem elongation, IR_N normal irrigation. F_{co} No phosphorous fertilizer, 100 % F_{ch} 100 % chemical fertilizer, 50 % F_{ch} + F_{bi} 50 % chemical fertilizer + seed inoculation by *Pseudomonas putida* strains 41 and 168, F_{bi} seed inoculation by *Pseudomonas putida* bacteria strains 41 and 168

were significant ($p < 0.01$). By increasing the water stress severity, the CP content increased while the protein yield followed a decreasing trend (Table 3). The highest CP was observed in IR₀ (234 g/kg dry matter) while in IR_N by more than 21 % decrement, it reached 182 g/kg dry matter. In contrast, the highest protein yield (490 kg/ha) was obtained by IR_N while the lowest yield (111.4 kg/ha) was obtained from IR₀ (nearly fourfold decrement). By one

irrigation at sowing time (IR₁) the protein yield achieved a threefold increment compared to the control (from 111 to 350 kg/ha) (Table 3). However, the extra irrigation at tuber formation (IR₂) and third irrigation at stem elongation stage (IR₃) had a lower efficiency in increasing the protein yield of forage turnip.

Among different P fertilizers, integrated fertilizer (50 % F_{ch} + F_{bi}) treatment produced the highest crude protein

Fig. 2 Interaction effects of deficit irrigation and different P fertilizers on IVDMD of turnip shoot forage. *Different small letters above columns indicate significant differences ($p < 0.05$) between treatments. Error bars represent standard error. Treatment abbreviations: IR_0 no irrigation, IR_1 irrigation at sowing time, IR_2 irrigation at sowing time + commencement of tuber formation, IR_3 irrigation at*

sowing time + commencement of tuber formation + commencement of stem elongation, IR_N normal irrigation. F_{CO} No phosphorous fertilizer, 100 % F_{Ch} 100 % chemical fertilizer, 50 % $F_{Ch} + F_{Bi}$ 50 % chemical fertilizer + seed inoculation by *Pseudomonas putida* strains 41 and 168, F_{Bi} seed inoculation by *Pseudomonas putida* bacteria strains 41 and 168

Table 4 Pearson correlation between some quantitative and qualitative parameters of the turnip shoot

	Shoot yield	IVDMD	CP	WSC	ADF	Ash	ME	CP yield
Shoot yield	1							
IVDMD	0.782(**)	1						
CP	-0.745(**)	-0.654(**)	1					
WSC	-0.523(*)	-0.194	0.690(**)	1				
ADF	-0.793(**)	-0.879(**)	0.692(**)	0.335	1			
Ash	-0.395	-0.542(*)	0.529(*)	0.144	0.434	1		
ME	0.782(**)	1.000(**)	-0.655(**)	-0.194	-0.879(**)	-0.542(*)	1	
CP yield	0.980(**)	0.759(**)	-0.618(**)	-0.421	-0.751(**)	-0.332	0.759(**)	1

** Correlation is significant at the 0.01 level (two-tailed)

* Correlation is significant at the 0.05 level (two-tailed)

Fig. 3 Interaction effects of deficit irrigation and different P fertilizers on CP content of turnip forage. *Different small letters above columns indicate significant differences ($p < 0.05$) between treatments. Error bars represent standard error. Treatment abbreviations: IR_0 no irrigation, IR_1 irrigation at sowing time, IR_2 irrigation at sowing time + commencement of tuber formation, IR_3 irrigation at*

sowing time + commencement of tuber formation + commencement of stem elongation, IR_N normal irrigation. F_{CO} No phosphorous fertilizer, 100 % F_{Ch} 100 % chemical fertilizer, 50 % $F_{Ch} + F_{Bi}$ 50 % chemical fertilizer + seed inoculation by *Pseudomonas putida* strains 41 and 168, F_{Bi} seed inoculation by *Pseudomonas putida* bacteria strains 41 and 168

(11 % more than the control) (Table 3), but the highest protein yield was obtained by 100 % F_{Ch} and 50 % $F_{Ch} + F_{Bi}$ fertilizers, 34 % and 26 % more than the control, respectively.

The interaction effect of irrigation regimes and different P fertilizers showed that in IR_0 the highest CP was produced by 100 % F_{Ch} while in other irrigation treatments, 50 % $F_{Ch} + F_{Bi}$ produced the highest CP (Fig. 3). There

was a negative correlation between forage yield and CP content ($r = -0.745$) as well as between CP and IVDMD ($r = -0.654$) (Table 4).

Water-Soluble Carbohydrate

The highest WSC (209 g/kg dry matter) was obtained in IR_0 , which followed an increasing trend as the severity of water stress decreased (Table 3).

Application of chemical P fertilizer and biological fertilizer had no positive effect on WSC compared to the control. This is while the highest WSC was obtained by 50 % $F_{Ch} + F_{Bi}$ application indicating the superiority of integrated fertilizer over the sole chemical fertilizer or sole bio-fertilizer (Table 3). There was a negative correlation between turnip forage yield and WSC content ($r = -0.523$).

Acid Detergent Fiber

The highest ADF was observed in IR_0 (340 g/kg dry matter) and the lowest (240 g/kg dry matter) by 29 % reduction was observed in IR_N (Table 3).

Application of PSM as a sole bio-fertilizer or as part of integrated fertilizer decreased ADF in turnip while application of chemical fertilizer provided with no significant response in this trait.

Ash

By increasing in water stress severity, the ash content of turnip forage followed an increasing trend (Table 3). The highest ash content of 90 g/kg dry matter was obtained in IR_0 * 100 % F_{Ch} (Fig. 4). A negative correlation ($r = -0.542$) between

IVDMD and ash was observed while the correlation between ash and CP was positive ($r = 0.692$) (Table 4).

Metabolizable Energy

As the water stress severity increased, ME followed a decreasing trend. The ME in IR_0 was 6.9 MJ/kg dry matter while in IR_N it was 8.6 MJ/kg (Table 3).

Application of chemical P fertilizer, integrated fertilizer and sole bio fertilizer equally increased turnip ME compared to control (Table 3).

At IR_0 irrigation treatment only, application of bio-fertilizer (F_{Bi}) increased ME and in IR_1 integrated application of chemical P fertilizer and bio-fertilizer produced the highest value of ME among different fertilizer treatments. As the soil water content increased in IR_3 and IR_N , chemical P fertilizer had a better influence on ME compared to other fertilizers (Fig. 5).

Discussion

Forage Yield

The lack of water during vegetative growth stage not only reduces the plant leaf area, resulting in declined plant ability to photosynthesis, but will also reduce the persistence of plant leaves [14, 26]. This is happened because of less turgor pressure and cell elongation which ultimately cause less plant growth under water stress [1]. Turnip is very sensitive to soil moisture at seed germination and establishment stages [23]. In this experiment with only one irrigation at sowing time (IR_1 treatment), probably the germination percentage and germination rate increased

Fig. 4 Interaction effects of deficit irrigation and different P fertilizers on ash content of turnip shoot forage. Different small letters above columns indicate significant differences ($p < 0.05$) between treatments. Error bars represent standard error. Treatment abbreviations: IR_0 no irrigation, IR_1 irrigation at sowing tune, IR_2 irrigation at sowing time + commencement of ruber formation, IR_3

irrigation at sowing time + commencement of tuber formation + commencement of stem elongation, IR_N normal irrigation. F_{CO} No phosphorous fertilizer, 100 % F_{Ch} 100 % chemical fertilizer, 50 % $F_{Ch} + F_{Bi}$ 50 % chemical fertilizer + seed inoculation by *Pseudomonas putida* strains 41 and 168, F_{Bi} seed inoculation by *Pseudomonas putida* bacteria strains 41 and 168

Fig. 5 Interaction effects of deficit irrigation and different P fertilizers on ME content of turnip shoot forage. Error bars represent standard error. Different small letters above columns indicate significant differences ($p < 0.05$) between treatments. Treatment abbreviations: IR₀ no irrigation, IR₁ irrigation at sowing time, IR₂ irrigation at sowing time + commencement of tuber formation, IR₃

irrigation at sowing time + commencement of tuber formation + commencement of stem elongation, IR_N normal irrigation. F_{co} No phosphorous fertilizer, 100 % F_{ch} 100 % chemical fertilizer, 100 % F_{ch} + F_{bi} 50 % chemical fertilizer + seed inoculation by *Pseudomonas putida* strains 41 and 168, F_{bi} seed inoculation by *Pseudomonasputida* bacteria strains 41 and 168

leading to a better seedling establishment. This situation supported a better access of the roots to moisture and nutrients in lower layers of the soil. In a water stress condition, the nutrients are less accessible by the crop root. Also, the ability of the root to uptake nutrients is decreased because of the lower plant transpiration. In this case, the absorption of the major contributing nutrients to the plant growth like N and P is reduced, causing a major reduction in plant growth [22]. The relative success in plant to survive and grow well at water deficit regime is dependent on the stage of growth in which the water stress happens [25]. This could probably explain the threefold biomass production in IR₁ compared to the control (IR₀).

The positive effect of P fertilizer on turnip forage yield found in this study supports the findings by Türk et al. [35]. Also, the advantages of integrated application of chemical and biological P fertilizer have been reported in different crops such as cotton [28] and soybean [36]. The higher availability of P nutrient in 100 % F_{ch} along with the suitable environmental conditions provided by no water stress treatment (IR_N) could explain the better adsorption of P and other necessary nutrients in the soil, which leads to significantly higher forage yield by turnip in this treatment.

Interaction effects of water deficit regimes and P fertilizers showed that in severe water stress conditions (IR₀), adverse effects of water stress were modified by the application of 50 % F_{ch} + F_{bi} treatment. It has been shown that integrated application of chemical and biological P fertilizer could produce the highest yield in lentil [2] and mustard [21] and this treatment was more effective than sole chemical or sole biological P fertilizer. In other irrigation regimes, sole chemical P fertilizer out-produced forage yield, indicating the positive effect of P on growth and yield of turnip, which supports the findings of Türk et al. [35].

Forage Quality and Chemical Composition

There are different research results on the effect of water stress on forage quality characteristics, especially on IV-DMD. The results of some experiments showed that water stress could increase the amount of structural carbohydrates and fibers leading to less IVDMD [17]. These results are similar to our findings in the present study. However, some other research works showed that the digestibility of dry matter was not affected by water stress [19] or even increased by water stress [20, 24, 37]. The decrease in IVDMD under water deficit regimes in this experiment could be explained by increment in fiber content of turnip. The existing negative correlation between IVDMD and fiber content of plants such as ADF and NDF has been well understood (for more details, see [15, 20]). In our study, the same outcome was obtained and a strong negative correlation between ADF and IVDMD ($r = -0.879$) was perceived (Table 4). The higher ADF value observed in IR₀ treatment indicated that water stress could increase ADF. Increasing ADF as a result of water stress could justify lower IVDMD in this condition. The increment in insoluble fibers in cell walls is one of the physiological responses of plants towards water stress to prevent moisture loss as a means to persist under water stress conditions. These results are supported by findings of Wilson [43] on tropical pasture species and Haung and Duncan [17] on turf grass. These results could explain why by increasing water stress severity, ME, a key factor for determining forage nutritional value, followed a decreasing trend. In our study, the favorable moisture condition in the soil positively affected ME content. Haung and Duncan [17] reported that drought stress could stimulate forage structural carbohydrates content (fiber), which can cause a reduction in forage ME.

Positive response of IVDMD and ME to application of different P fertilizers observed in this study (Table 3) indicates that P element could be considered as an important means to improve nutritional value of turnip forage. Our results in regard to IVDMD are supported by Türk et al. [35]. However, they reported that by application of chemical P fertilizer, turnip fiber (ADF and NDF) decreased, which lead to higher IVDMD. Nevertheless, our results showed that chemical P fertilizer had no significant effect on decreasing ADF while Bi and integrated fertilizer (50 % F_{Ch} + F_{Bi}) could decrease ADF (Table 3). Reducing ADF and increasing IVDMD as a result of biological P fertilizer application has been reported earlier [11]. No significant difference between P fertilizers observed in this study convinced us that the biological P fertilizer could be considered as a promising substitute for chemical fertilizer in this regard.

Evaluating the interaction effect of irrigation regimes and P fertilizers demonstrated that application of biological P fertilizer at severe water stress condition (IR_0) increased IVDMD. However, in moderate and no-water stress conditions, as the availability of water increased, the role of chemical fertilizer was more pronounced in producing forage with higher IVDMD. These results indicate that in water deficit conditions, the application of biological P fertilizer could increase turnip forage IVDMD, which causes an increase in the quality of produced forage.

Increment in CP as a result of water stress could be explained by rising nitrogen concentration in plant tissues [27]. The same findings about increasing CP content in perennial rye grass and tall fescue under water deficit stress has been well documented [3, 19]. These results could be explained by a higher nitrogen concentration and availability in a dry soil [20]. Buxton et al. [6] indicated that the content of crude protein in forage crops is strongly correlated with the availability of nitrogen in the soil. Collins [7] reported an increasing tendency in crude protein content due to the higher soil nitrogen concentration causing by water stress. The existing negative correlation between IVDMD and CP would be proper evidence to support these results. A negative correlation between these two characteristics in annual forage crops has been reported earlier by Ward et al. [40].

An increase in CP and protein yield of turnip forage by application of 100 % F_{Ch} and 50 % F_{Ch} + F_{Bi} convinced us that providing required P for turnip is a crucial means to improve the produced crude protein and protein yield per hectare. The positive effect of P element on CP content of turnip and artichoke has been reported by Türk et al. [35] and Salamah [32], respectively. Protein yield per hectare is calculated by multiplying CP by the forage yield. In our study, a noticeable positive correlation between shoot yield and protein yield was observed ($r = 0.98$), while protein yield and CP were negatively correlated ($r = -0.618$) (Table 4). The reason for this result could be explained by a significant

decrement in CP content in plants grown at no-water stress conditions. The correlation between forage yield and protein yield was stronger than the correlation between forage yield and CP content (Table 4). By increasing the forage yield, the protein yield was also increased.

Turnip responded to water stress, as in some other crops, with an increase in WSC content [41, 42]. Weinberg et al. [41] showed that in plants under water stress, more water-soluble carbohydrate is produced, which in turn increases the plant ability to produce a higher silage quality. They also reported a negative correlation between complementary irrigation and WSC.

In plants grown in more moist soils, the concentration of elements in plant tissues will decrease, resulting in a significant decrement in ash content. The results of this experiment showed that under severe water stress conditions, the concentration of mineral elements in the plant tissues increased. This increment was more pronounced when chemical P fertilizer was applied. The higher concentration of elements in plant sap and cellular vacuoles tends to increase the osmotic pressure of plant water, which in turn leads to a reduction in the transpiration rate from leaf area.

Conclusions

Turnip forage yield and quality was substantially affected by water stress. The results indicated that turnip is very sensitive to water stress at germination, establishment and early growth stages. Application of integrated fertilizer (50 % F_{Ch} + F_{Bi}) reduced turnip forage yield by 12 % compared to full chemical P fertilizer, but moderated the adverse effects of water stress on turnip forage yield, increased forage quality and saved 50 % in chemical P fertilizer. Long-term environmental and economic analysis is required to make the final judgment on superiority of the best fertilizer for turnip forage production under deficit irrigation regimes.

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References

1. Araus JL, Bort J, Steduto P, Villegase D, Royo C (2003) Breeding cereals for Mediterranean conditions: ecophysiological clues for biotechnology application. *Ann Appl Biol* 142:129–141
2. Arpana N, Kumar SD, Prasad TN (2002) Effect of seed inoculation, fertility and irrigation on uptake of major nutrients and soil fertility status after harvest of late sown lentil. *J Appl Biol* 12:23–26

3. Asay KH, Jensen KB, Waldron BL, Han G, Johnson DA, Monaco TA (2002) Forage quality of tall fescue across an irrigation gradient. *Agron J* 94:1337–1343
4. Association of Official Analytical Chemists (1999) Official methods of analysis, 16th ed. (930.15) AOAC, Washington, DC
5. Aulakh MS, Garg AK, Kabba BS (2007) Phosphorus accumulation and leaching, and residual effects on crop yields from long-term applications in subtropics. *Soil Use Manag* 23:417–427
6. Buxton DR, Mertens V, Fisher DS (1996) Forage quality and ruminant utilization. In: Moser LE, Buxton DR, Casler MD (eds) Cool-season forage grasses. American Society of Agronomy Monograph, Series 34, Madison, pp 229–266
7. Collins M (1991) Nitrogen effects on yield and forage quality of perennial ryegrass and tall fescue. *Agron J* 83:588–595
8. CSIRO (1990) Feeding standards for Australian ruminants, standing committee on agriculture and resource management, Ruminants subcommittee, Melbourne
9. Day AD, Ludeke KL (1992) Plant nutrients in desert environment. Springer, Berlin Heidelberg New York, 117 p
10. Dubois M, Gilles KA, Hamilton JK, Rebes PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350–356
11. Fateh S (2008) Effects of organic and chemical fertilizers on forage yield and quality of Globe artichoke (*Cynara scolymus*). PhD thesis, University of Tehran, 250 p
12. Fernandez LA, Zalba P, Gomez MA, Sagardoy MA (2007) Phosphate-solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. *Biol Fertil Soils* 43:805–809
13. Goering HK, Van Soest PJ (1970) Forage fiber analysis: apparatus reagents, procedures, and some applications. Agriculture Handbook 379. U.S. Government Printing Office, Washington, DC
14. Guinta FR, Motzo R, Diedo M (1995) Effect of drought on leaf area development, biomass production and nitrogen uptake of durum wheat grown in a Mediterranean environment. *Aust J Agri Res* 44:99–111
15. Hacker JB (1982) Selecting and breeding better quality grasses. In: Hacker JB (ed) Nutritional limits to animal production from pasture, Proceedings of an International Symposium, Queensland, August 1981, pp 305–326
16. Hardie K, Leyton L (1981) The influence of vesicular-arbuscular mycorrhiza on growth and water relations of red clover. I. In: phosphate deficient soil. *New Phytologist* 89:599–608
17. Haug R, Duncan R (1997) Drought resistance mechanisms of seven warm season turf grass-soil drying. *Crop Sci* 37:1663–1858
18. Illmer P, Schinner F (1995) Solubilization of inorganic calcium phosphates-solubilization mechanisms soil. *Soil Biol Biochem* 27:257–263
19. Jensen KB, Asay KH, Waldron BL, Johnson DA, Monaco TA (2003) Forage quality traits of orchard grass and perennial ryegrass at five irrigation levels. *Agron J* 95:668–675
20. Jensen KB, Waldron BL, Peel MD, Robins JG, Monaco TA (2007) Forage quality of irrigated pasture species as affected by irrigation rate. Proceedings of the XXVIIth Eucarpia symposium on improvement of fodder crops and amenity grasses. 19–23 Aug, Copenhagen
21. Kantwa SR, Meena NL (2002) Effect of irrigation, phosphorus and PSB on growth and yield of mustard. *Ann Agric Res* 23: 456–460
22. Karner JK, Boyer JS (1995) Water relations of plant and soils. Academic, Waltham
23. Keshavarz Afshar R, Keykhah M, Chaichi MR, Ansari Jovini M (2012) Effect of different levels of salinity and drought stresses on seed germination characteristics and seedling growth of forage turnip (*Brassica rapa* L.). *Iran J Field Crop Sci*. (Accepted)
24. Kipni T, Krivat G, Dvash L, Granoth I, Jonathan R (1994) Yield and quality of forage sorghum as affected by water supply. In: Proceedings of the 15th general meeting of the European Grassland Federation. Grassland and Society, Wageningen, 6–9 Jun, pp 173–176
25. Kirda C (2000) Deficit irrigation scheduling based on plant growth stages showing water stress tolerance. Publishing and Multimedia service, Information Division, FAO, Rome
26. Nagarajan S, Rane J, Maheswari M, Gambhir P (1999) Effect of post anthesis water stress on accumulation of dry matter, carbon and nitrogen and their partitioning of dry matter, carbon and nitrogen and their partitioning in wheat varieties differing in drought tolerance. *J Agro Crop Sci* 183:129–136
27. Pessarakli MM, Morgan PV, Gilbert J (2005) Dry matter yield, protein synthesis, starch and fiber content of barley and wheat plants under two irrigation regimes. *J Plant Nutr* 28:1227–1241
28. Poberejskaya SI, Egamberdiyeva D (2003) Improvement of the productivity of cotton by phosphate solubilizing bacteria inoculants. *Plant Nutrition-Food Security and sustainability of Agro ecosystems*, 670–671
29. Rao SC, Dao TH (1987) Soil water effects on low-temperature seedling emergence of five *Brassica* cultivars. *Agron J* 79: 517–519
30. Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. *J Plant Physiol* 161:1189–1202
31. Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv* 17:319–339
32. Salamah FS (1997) Effect of some agriculture treatments on productivity of globe artichoke under Ismailia conditions. MSc thesis, Suez Canal University, Ismailia
33. Tilley JM, Terry RA (1963) A two-stage technique for the in vitro digestion of forage crops. *J British Grassland* 18:104–111
34. Turan M, Ataoglu N, Sahin F (2006) Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. *J Sustain Agric* 28:99–108
35. Türk M, Albayrak S, Balabanli C, Yüksel O (2009) Effects of fertilization on root and leaf yields and quality of forage turnip (*Brassica rapa* L.). *J Food Agric Environ* 7:339–342
36. Umale SM, Thosar VR, Chorey AB, Chimote AN (2002) Growth responses of soybean to phosphorus solubilizing bacteria and phosphorus levels. *J Soils Crops* 12:258–261
37. Van Soest PJ, Mertens DR, Deinum B (1978) Pre harvest factors influencing quality of conserved forage. *J Animal Sci* 47:712–720
38. Vessey JK (2003) Plant growth promoting rhizobacteria as bio-fertilizers. *Plant Soil* 255:571–586
39. Wang H, Zhang L, Dawes WR, Liu C (2001) Improving water use efficiency of irrigated crops in North China plain-measurements and modeling. *Agric Water Manag* 48:151–167
40. Ward JD, Redfearn DD, McCormick ME, Cuomo GJ (2001) Chemical composition, ensiling characteristics, and apparent digestibility of summer annual forages in a subtropical double-cropping system with annual ryegrass. *Dairy Sci J* 84:177–182
41. Weinberg ZG, Landau SY, Bar-Tal A, Chen Y, Gamburg M, Brener S, Dvash L (2005) Ensiling safflower (*Carthamus tinctorius*) as an alternative winter forage crop in Israel. In: Park RS, Stronge MD (ed) Proceedings of the 15th International Silage Conference. Belfast, 3–6 July. Wageningen Academic, Wageningen, p 169
42. Weinberg ZG, Bar-Tal A, Chen Y, Gamburg M, Brener S, Dvash L, Markovitz T, Landau S (2007) The effects of irrigation and nitrogen fertilization on the ensiling of safflower (*Carthamus tinctorius*). *Animal Feed Sci Technol* 134:152–161
43. Wilson J (1981) Effects of water stress on herbage quality. Proceedings of the 15th international grassland conference, Lexington