

Effect of Melatonin, Salicylic Acid, and Mycorrhizal Fungi Application on Agronomic and Grain Quality Traits in Wheat Grown under Different Water Irrigation Levels

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Abstract: Water stress is considered one of the most critical environmental factors affecting wheat growth and productivity worldwide. So, a field experiment was conducted at the experimental farm of Shandaweel Agricultural Research Station, Sohag governorate, Egypt, during the 2017/2018 and 2018/2019 growing seasons to study the effect of the application of melatonin (30 ppm), salicylic acid (200 ppm), and mycorrhizal fungi inoculation on wheat cultivar Shandaweel 1 under three irrigation levels i.e., 5476 (I₁₀₀), 4380 (I₈₀) and 3285 (I₆₀) m³ ha⁻¹. Results showed that decreasing irrigation water amount from 5476 to 3285 m³ ha⁻¹ caused a significant decrease in days to heading, days to maturity, plant height, leaf area index at 75 and days after sowing, leaf chlorophyll content, spike length, number of spikes m⁻², number of spikelets spike⁻¹, number of kernels spike⁻¹, thousand kernel weight, grain yield, biological yield, and grain carbohydrate content, while it caused a significant increase in grain protein content. Application of melatonin, salicylic acid, and mycorrhizal fungi treatments separately or in combination (salicylic acid + mycorrhizal fungi) or (melatonin + mycorrhizal fungi) reduced the negative effects of water stress in wheat as compared to the control treatment. The greatest reduction for the negative effect of water stress and the highest mean values of all studied traits were obtained with the combined application of (salicylic acid + mycorrhizal fungi) or (melatonin + mycorrhizal fungi) treatments and these combinations can play an important role in the reclamation of drought-affected land in arid and semi-arid regions.

Keywords: wheat, water stress, melatonin, salicylic acid, mycorrhizal fungi, grain yield.

1. Introduction

Wheat (*Triticum aestivum* L. family Poaceae) is considered a major crop among cereals all over the globe and it represents the principal crop for human food. [1,2]. In the meantime, drought stress or water scarcity as well as a global warming is one of the most limited factors to growth and productivity of several plant species which cause a serious threat to food security in many regions worldwide [3,4]. In this context, the predictions for the year 2050, drought stress will cause serious problems for more than 50% of arable lands [5]. The cultivated land area of wheat in Egypt reached 1.39 million hectares in 2019/2020 growing season, with an average yield of 6.4 ton/hectare, and the total production was about 8.9 million tons [6]. Currently, wheat production is not enough for local consumption as a result of the steady increase in the population of Egypt. Therefore, increasing wheat production is an essential national target to fill the gap between production and consumption. Water shortage is common in many countries including Egypt and it is one of the serious environmental threats for plant growth, development, and yield. Water stress during vegetative growth caused a reduction in leaf area index of wheat [7]. Nikolaeva et al. [8] noted a decline in chlorophyll content from 13% to 15% in water stressed wheat compared with the well-watered plants in three varieties of wheat. Water stress reduces grain yield of wheat through negative affecting

the yield components, i.e., number of plants per unit area, number of spikes and grains per plant, or unit area and single grain weight, which are determined at different stages of plant development [9,10]. Wheat yield and its components decreased with decreasing irrigation water amounts [11]. Water stress stimulates senescence and enhances the remobilization of pre-anthesis stored carbohydrates from leaves to grains [12]. Decreasing irrigation from 100% to 50% significantly decreased yield and yield attributes and grain carbohydrate, while grain protein increased [13,14]. Recently, exogenous application of plant growth regulators has been considered as an efficient way to enhance plant drought tolerance in crop production [15]. Consequently, finding new plant growth regulators that improve crop drought tolerance is an effective approach to improve crop production. In this context, exogenous application of melatonin has also been found to improve plant drought tolerance. Melatonin (N-acetyl-5-methoxytryptamine) has widely existed in living organisms [16]. It is also widely found in a wide range of concentrations in plants [17, 18, 19]. Many studies reported that melatonin increases the drought tolerance and plays multiple roles in plant including delaying leaf senescence, regulating water balance, promoting lateral root formation and seed germination, maintaining the integrity of leaf and chloroplast, modulating nitro-oxidative homeostasis and proline

metabolism [20, 21, 22, 23, 24]. Meng[25] suggested that wheat seed priming with melatonin could enhance the biomass and yield once plants suffered from water stress during filling stage. Foliar application of melatonin can be a useful strategy to help plant combat adverse conditions for enhancing yield of wheat plants [26]. Another growth regulator of salicylic acid (SA) enhances plant defense against various biotic and abiotic stresses through morphological, biochemical and physiological mechanisms [27]. Salicylic Acid (SA) is a signal molecule for modulating plant responses to environmental stresses [3, 28]. Salicylic acid can significantly affect plant water relations, photosynthesis, growth, and stomatal regulation under abiotic stress conditions. Application of Salicylic acid (SA) at 400 mg L⁻¹ caused an increase in yield and its components, protein and carbohydrate content in wheat [14]. Growth traits of wheat were improved as a result of SA spray on wheat plants [29]. Soil microorganisms are a very important component in the plant/soil system [30, 31, 32]. Arbuscular mycorrhizal fungi (AMF) is a root obligate symbiotic that exchange mutual benefits with about 90% of terrestrial plants and represents a key link between plants and soil mineral nutrients. The symbiosis of arbuscular mycorrhiza (AM) fungi with plant roots has been shown to be helpful to tolerate and overcome water stress in different plant species including wheat [33]. Extra radical mycelia of mycorrhizal fungi transfer water to their host plants under low soil moisture conditions and the AM fungi improve plant growth, development, and yield [34]. Wheat plants inoculated with mycorrhizal fungi showed a marked effect on the seed yield per plant and its components in comparison to the untreated plants [35]. Therefore, the aim of this research was to investigate the effect of melatonin, salicylic acid and mycorrhizal fungi and their interaction on growth, productivity and grain quality traits, chlorophyll, protein and carbohydrate contents under different irrigation levels.

2. Materials and Methods

A field experiment was carried out at the experimental farm of Shandaweel Agricultural Research Station, Sohag Governorate, Egypt (31°42'E, 26°33'N and 61 m altitude) during the two growing seasons 2017/2018 and 2018/2019 to study the effect of melatonin, salicylic acid and mycorrhizal fungi and their interaction application on agronomic and grain quality traits on wheat cultivar Shandaweel 1 under three different irrigation levels. The average annual rainfall and temperature are 1mm and 23.5°C. The soil texture is a clay loam and pH is 7.4. Soil available N, P, K contents of the cultivated layer (0-30 cm) are 54, 15, 310 ppm, respectively. The experiment was laid out in strip plot design with three replications. The sowing of wheat grains for the two seasons was carried out on November 25th. The plot size was 8.4 m² (12 rows, 20 cm apart × 3.5 m long). The three-water irrigation amounts 5476, 4380 and 3285 m³ ha⁻¹ (referred as I₁₀₀, I₈₀, I₆₀) were allotted to horizontal plots and the six applied treatments i.e., control (water), melatonin (30 ppm), salicylic acid (200 ppm), mycorrhizal fungi, salicylic acid (200 ppm) + mycorrhizal fungi and melatonin (30 ppm) + mycorrhizal fungi (referred as T1, T2, T3, T4, T5 and T6) to vertical plots.

Melatonin (30 ppm) and Salicylic acid (200 ppm) treatments were foliar applied twice at 45 and 65 days after

sowing. Inoculation with VA- mycorrhizae fungi at the rate of 250 spores added with grains at sowing time. Local strain of *Glomus macrocarpum* was kindly obtained from plant production Dept., Fac. of Agric. (Saba Basha). The recommended agricultural practices were applied from sowing to harvest. The studied traits were; days to heading (day), days to maturity (day), plant height (cm), Leaf area index at 75 and 105 day after sowing (DAS), Total chlorophyll content, spike length (cm), number of spikes m⁻², number of spikelets spike⁻¹, number of kernels spike⁻¹, thousand kernel weight (g), grain yield (ton/ha), biological yield (ton/ha), grain protein content (%) according to Lowry et al., [36] and grain carbohydrate content (%) by the anthrone-sulphuric acid method content according to Fales [37], Schlegel [38] and Badour [39], respectively.

2.1 Statistical analysis

Results of the two growing seasons were subjected to appropriate statistical analysis using MSTATC computer program in strip plot design. The least significant difference test (L.S.D) at 0.05 level of probability was used to test the significant differences among the means of each treatment according to Steel and Torrie [40].

3. Results

3.1. Phonological and growth traits

3.1.1. Irrigation levels effect

Data presented in Table (1) showed that the irrigation levels had significant effects on days to heading, days to maturity, plant height, leaf area index at 75 and 105 DAS and leaf chlorophyll content in both seasons. For the different irrigation levels, Phonological and growth traits showed the same order I₁₀₀ > I₈₀ > I₆₀ in both seasons. Decreasing irrigation level from 100% to 80% significantly reduced all studied phonological and growth traits under both seasons except days to heading in the first season, while Decreasing irrigation levels from 100% to 60% significantly decreased all studied phonological and growth traits under both seasons. As compared to effect I₁₀₀ treatment (5476 m³ ha⁻¹), the effect I₈₀ treatment (4380 m³ ha⁻¹) reduced days to heading by 0.57 and 1.17 %, days to maturity by 1.06 and 0.99%, plant height by 3.14 and 3.20%, leaf area index at 75 DAS by 6.92 and 8.75% , leaf area index at 105 DAS by 7.85 and 8.22 and total chlorophyll content by 6.69 and 3.29% (Fig. 1) in the first and second seasons, respectively. While the I₆₀ treatment (3285 m³ ha⁻¹) caused a decrease of (1.97 and 2.05%), (2.55 and 2.16%), (6.75 and 8.13 %), (11.38 and 13.62), (13.18 and 13.71) and (9.18 and 5.56) compared to the I₁₀₀ treatment (Fig. 1) for the above-mentioned traits in the first and second seasons, respectively.

3.1.2. Melatonin, salicylic acid and mycorrhizal fungi treatments effect.

Data presented in Table (1) revealed that melatonin, salicylic acid and mycorrhizal fungi treatments differed significantly in phonological and growth traits in the two growing seasons. Also, all treatments of melatonin (ME), salicylic acid (SA) and mycorrhizal fungi (MF) and their combination significantly increased all phonological and

Table 1. Means of phonological and some growth traits as affected by melatonin (ME), salicylic acid (SA) and mycorrhizal fungi (MF) treatments under three water irrigation levels in 2017/2018 and 2018/2019 seasons.

Trait	Days to heading (day)		Days to maturity (day)		Plant height (cm)		Leaf area index (75 DAS)		Leaf area index (105 DAS)		Leaf Chlorophyll content (SPAD)	
	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19
Irrigation levels												
I ₁ (100%)	94.75	95.54	142.13	142.58	115.63	122.46	4.48	5.14	7.13	7.66	46.07	46.57
I ₂ (80%)	94.21	94.42	140.63	141.17	112.00	118.54	4.17	4.69	6.57	7.03	42.99	45.04
I ₃ (60%)	92.88	93.58	138.50	139.50	107.83	112.50	3.97	4.44	6.19	6.61	41.84	43.98
F test	**	**	**	**	**	**	**	**	**	**	**	**
LSD 0.05	0.96	0.36	1.0	0.44	2.0	2.0	0.07	0.07	0.08	0.07	0.71	1.30
Melatonin (ME), salicylic acid (SA) and mycorrhizal fungi (MF) treatments												
Control	91.08	92.75	137.58	138.92	105.25	111.08	3.81	4.27	6.05	6.40	41.08	42.71
ME	92.92	93.75	138.25	139.92	110.00	115.58	3.94	4.43	6.24	6.63	42.36	44.79
SA	94.33	94.08	140.08	140.17	110.75	116.25	4.28	4.78	6.65	7.15	43.88	45.38
MF	94.08	94.67	140.58	141.08	111.92	119.25	4.16	4.67	6.41	6.88	43.72	44.88
SA+MF	95.83	96.00	143.08	143.25	116.58	123.25	4.63	5.29	7.40	7.95	45.88	46.92
ME+MF	95.42	95.83	142.92	143.17	116.42	121.58	4.42	5.10	7.02	7.59	44.90	46.49
F test	**	**	**	**	**	**	**	**	**	**	**	**
LSD 0.05	0.67	0.77	1.6	1.25	3.2	2.8	0.16	0.16	0.16	0.15	1.14	1.11

** refer to significant at 0.01 level of probability

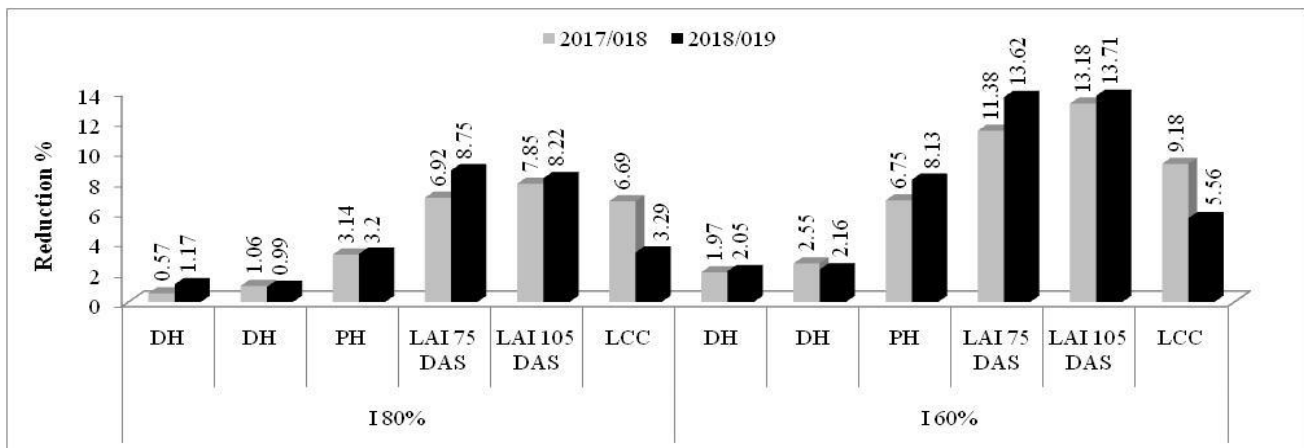


Fig. 1. Reduction of days to heading (DH), days to maturity (DM), plant height (PH), leaf area index at 75 day after sowing (LAI 75 DAS), leaf area index at 105 day after sowing (LAI 105 DAS) and total chlorophyll content (TCC)(Unit) under I_{80%} and I_{60%} as compared to I_{100%} irrigation level in 2017/018 and 2018/019 seasons.

growth studied traits compared to control treatment except days to maturity in both seasons as well as leaf area index at 75 DAS in the first season under ME treatment. The highest mean values of phonological and growth traits were obtained from plants which were treated by SA + MF treatment in both seasons. However, SA + MF treatment and ME + MF were statistically similar for all phonological and growth traits except leaf area index at 75 and 105 DAS in both seasons. While, the lowest mean values of phonological and growth traits were recorded with the control treatment in the two growing seasons. On the other hand, insignificant differences between the SA and MF treatments for all phonological and growth traits in both seasons except plant height in the second season and leaf area index at 105 DAS in the first season.

3.1.3. Interaction effect

The interaction effect between the three irrigation levels and melatonin, salicylic acid as well as mycorrhizal fungi inoculation (Table 2) was significant for plant height and leaf area index at 75 and 105 DAS, while it was insignificant for heading, days to maturity and total chlorophyll contents in both seasons. The highest mean values of plant height (120 and 126.75 cm), leaf area index at 75 DAS (5.00 and 5.85) and leaf area index at 105 DAS (8.00 and 8.60) were recorded under the combination of I₁₀₀ and SA + MF treatment in the first and second season, respectively. In contrast, the lowest mean values for the traits mentioned above were obtained under I₆₀ and control treatment in both seasons.

Table 2. Effect of the interaction between irrigation levels and melatonin (ME), salicylic acid (SA) and mycorrhizal fungi (MF) treatments for phonological and some growth traits in 2017/2018 and 2018/2019 seasons.

Traits		Days to heading		Days to maturity		Plant height		Leaf area index (75 DAS)		Leaf area index (105 DAS)		Leaf Chlorophyll content (SPAD)		
		17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	
Seasons		17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	
Treatments	I ₁ (100%)	Control	92.50	94.00	139.00	140.25	111.25	118.75	4.19	4.74	6.73	7.13	43.58	43.73
		ME	93.50	94.75	140.00	141.25	114.50	122.75	4.34	4.92	6.92	7.39	44.75	45.60
		SA	94.75	95.00	142.00	141.50	116.00	120.25	4.41	5.01	7.06	7.58	45.90	46.90
		MF	95.00	95.25	142.00	142.50	114.50	122.00	4.33	4.90	6.88	7.37	46.60	46.86
		SA+MF	96.75	97.25	145.00	145.00	120.00	126.75	5.00	5.85	8.00	8.60	48.30	48.33
		ME+M	96.00	97.00	144.75	145.00	117.50	124.25	4.59	5.43	7.19	7.89	47.28	48.00
	I ₂ (80%)	Control	91.75	92.75	137.50	139.50	106.25	111.75	3.73	4.16	5.85	6.20	40.15	42.96
		ME	93.25	94.00	138.00	140.00	112.50	115.75	3.77	4.22	5.99	6.34	42.30	44.88
		SA	94.50	94.00	140.50	140.50	108.50	118.25	4.27	4.74	6.59	7.07	43.08	44.90
		MF	94.00	94.75	141.50	141.00	111.25	119.50	4.22	4.72	6.34	6.80	42.95	44.42
		SA+MF	96.00	95.50	143.50	143.25	116.75	123.00	4.57	5.15	7.44	7.99	45.40	46.65
		ME+M	95.75	95.50	142.75	142.75	116.75	123.00	4.46	5.14	7.22	7.76	44.09	46.40
	I ₃ (60%)	Control	89.00	91.50	136.25	137.00	98.25	102.75	3.51	3.91	5.56	5.87	39.50	41.45
		ME	92.00	92.50	136.75	138.50	103.00	108.25	3.72	4.15	5.82	6.17	40.03	43.88
		SA	93.75	93.25	137.75	138.50	107.75	110.25	4.17	4.60	6.32	6.80	42.65	44.35
		MF	93.25	94.00	138.25	139.75	110.00	116.25	3.94	4.40	6.02	6.47	41.60	43.35
		SA+MF	94.75	95.25	140.75	141.50	113.00	120.00	4.31	4.86	6.76	7.25	43.93	45.78
		ME+M	94.50	95.00	141.25	141.75	115.00	117.50	4.20	4.73	6.65	7.14	43.33	45.08
F test		ns	ns	ns	ns	*	*	*	**	*	**	ns	ns	
L.S.D 0.05		--	--	--	--	4.5	4.2	0.23	0.27	0.23	0.27	--	--	

ns, * and ** refer to non-significant and significant at 0.05 and 0.01 levels of probability, respectively

3.2. Yield and yield attributes traits

3.2.1. Irrigation levels effect

Results in **Table (3)** indicated that yield and its attributes significantly affected by different irrigation levels in both seasons. Reducing irrigation water amount (**Fig. 2**) from 100% (5476 m³ ha⁻¹) to 80% (4380 m³ ha⁻¹) significantly reduced all yield and its attributes traits of both seasons, except for number of kernels spike⁻¹ in the first season. While, decreasing irrigation water amount from 100% (5476 m³ ha⁻¹) to 60% (3285 m³ ha⁻¹) significantly decreased spike length by 11.67 and 10.93%, number of spikes m⁻² by 13.38 and 12.85%, number of spikelets spike⁻¹ by 11.98 and 9.56%, number of kernels spike⁻¹ by 6.81 and 6.67%, thousand kernel weight by 9.05 and 7.8%, grain yield by 12.87 and 10.99% and biological yield by 12.74 and 11.39% in the first and second seasons, respectively.

3.2.2. Melatonin, salicylic acid and mycorrhizal fungi treatments effect

Data presented in **Table (3)** illustrated that application of melatonin, salicylic acid and mycorrhizal fungi treatments under the three water irrigation levels had significant effects on yield and its attributes traits in the two growing seasons. Also, all treatments of melatonin (ME), salicylic acid (SA) and mycorrhizal fungi (MF) and their combinations significantly increased all studied traits compared to control treatment in both seasons, except for spike length and number of spikes m⁻²

in the first season and biological yield in both seasons under ME treatment as well as number of spikes m⁻² under MF treatment in the first season. The highest mean values of all yield and its attributes studied traits were recorded from plants which treated by SA+MF treatment in both seasons, except number of spikelets spike⁻¹ and number of kernels spike⁻¹ in both seasons and 1000- kernel weight in the first season which gave the highest mean values with plants treated by ME+MF treatment. However, SA + MF treatment and ME + MF were statistically similar for all studied traits. While the lowest mean values of all yield and its attributes studied traits were recorded with the control treatment in both seasons.

3.2.3. Interaction effect

Data in **Table (4)** showed that the interaction between irrigation water amount and melatonin, salicylic acid as well as mycorrhizal fungi applied treatments were significant for all yield and yield attributes traits in both seasons, except number of spikes m⁻² in the first season and biological yield in the second season. In the first season, the highest mean values of spike length (13 cm), thousand kernel weight (55.95 g), grain yield (8.02 ton ha⁻¹) and biological yield (16.16 ton ha⁻¹) were recorded under 100% of irrigation water amount and SA+MF treatment while, the highest mean values of number of spikelets spike⁻¹ (23.68 spikelet) and number of kernels spike⁻¹ (66.21 kernel) were obtained under I₁₀₀ and ME+MF treatment. In the second season, spike length, number of spikes m⁻², number of spikelets spike⁻¹ and grain yield had the highest mean values under I₁₀₀ and SA+MF treatment, whereas number

Table 3. Means of yield and its attributes traits as affected by melatonin (ME),salicylic acid (SA), and mycorrhizal fungi (MF) treatments under three irrigation levels in 2017/2018 and 2018/2019 seasons.

Trait	Spike length (cm)		Number of spikes m ⁻²		Number of spikelets spike ⁻¹		Number of kernels spike ⁻¹		Thousand kernels weight (g)		Grain yield (ton ha ⁻¹)		Biological yield (ton ha ⁻¹)	
	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19
Irrigation levels														
I ₁ (100%)	12.68	13.17	361.42	393.75	22.87	22.69	62.55	64.17	54.15	63.55	7.69	8.37	15.39	20.01
I ₂ (80%)	11.98	12.67	338.29	376.08	21.80	21.83	60.35	62.62	51.78	61.32	7.25	7.97	14.54	19.07
I ₃ (60%)	11.20	11.73	313.08	343.17	20.13	20.52	58.29	59.89	49.25	58.59	6.70	7.45	13.43	17.73
F test	**	**	**	**	**	**	*	**	**	**	**	**	**	**
LSD 0.05	0.41	0.42	7.15	7.4	0.97	0.53	2.50	1.43	1.23	1.18	0.20	0.27	0.70	0.30
Melatonin (ME),salicylic acid (SA) and mycorrhizal fungi (MF) treatments														
Control	11.18	11.63	319.67	340.67	19.71	20.18	54.76	56.10	48.45	54.82	6.49	7.00	13.43	17.51
ME	11.58	12.15	335.17	359.00	20.83	20.78	57.10	60.58	50.42	59.39	6.95	7.48	14.02	18.30
SA	12.08	12.53	338.42	376.00	21.39	21.59	60.39	63.17	51.49	61.49	7.10	8.05	14.28	19.24
MF	12.05	12.58	334.50	364.83	22.04	22.04	61.58	61.59	51.78	61.88	7.35	8.01	14.54	19.12
SA+MF	12.56	13.15	351.42	396.08	22.75	22.71	63.92	65.34	54.06	64.94	7.79	8.56	15.32	19.88
ME+MF	12.27	13.10	346.42	389.42	22.88	22.78	64.63	66.56	54.15	64.40	7.61	8.50	15.13	19.59
F test	*	**	*	**	**	**	**	**	**	**	**	**	**	**
LSD 0.05	0.71	0.37	17.04	12.10	0.69	1.10	2.11	1.83	1.27	2.34	0.25	0.46	0.63	0.89

** refer to significant at 0.01 level of probability

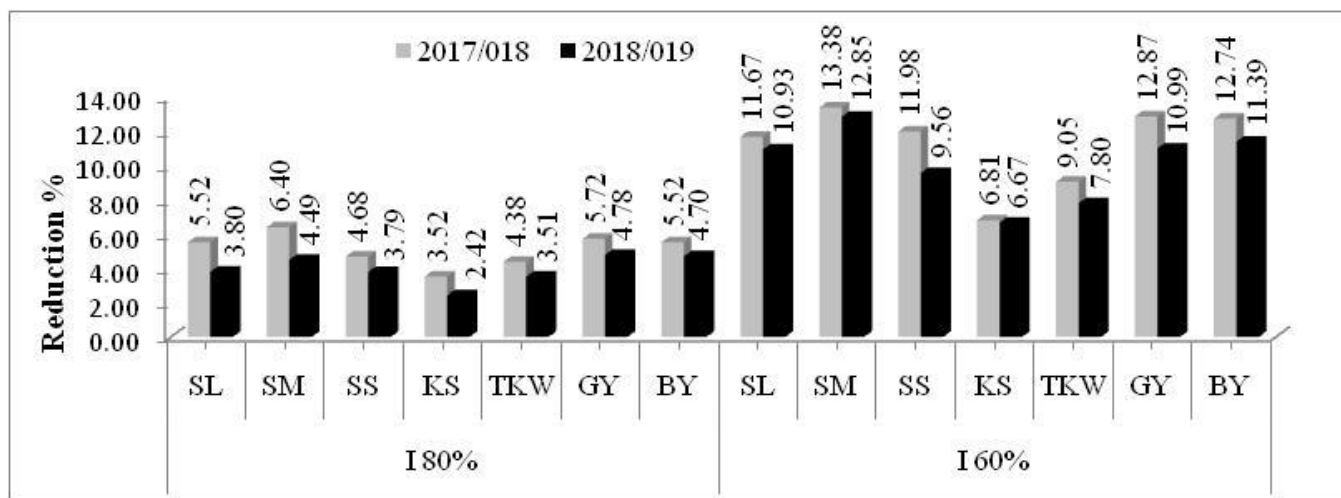


Fig. 2. Reduction of SL= spike length in cm. , SM = number of spikes m⁻², SS= number of spikelets spike⁻¹, KS⁻¹ = number of kernels spike⁻¹, TKW = thousand kernels weight in g., GY = grain yield (ton ha⁻¹) and BY= biological yield (ton ha⁻¹)under I_{80%} and I_{60%} as compared to I_{100%} irrigation level in 2017/018 and 2018/019 seasons.

of kernels spike⁻¹ and thousand kernel weight had the highest mean values under I₁₀₀ and ME+MF treatment but, it was statistically at par with I₁₀₀ and SA+MF treatment. In contrast, the lowest mean values for yield and yield attributes studied traits were recorded fewer than 60% of irrigation water amount and control in both seasons.

3.3. Grain quality

3.3.1. Irrigation levels effect

Data presented in Fig. 3 showed significant effects in

response to irrigation levels for grain protein and carbohydrate contents in both seasons. As compared to I_{100%} treatment, the I_{80%} and I_{60%} treatments significantly increased grain protein content by 6.20 and 11.45 in the first season and by 4.13 and 8.43 in the second season, respectively. On the other hand, grain carbohydrate content decreased by 4.13 and 3.88% under I_{80%} treatment and by 9.97 and 9.18% under effect of I_{60%} treatment compared to the I_{100%} treatment in the first and second seasons, respectively.

3.3.2. Melatonin, salicylic acid and mycorrhizal fungi treatments effect

Results presented in Fig. 4 revealed that melatonin, salicylic acid and mycorrhizal fungi treatments differed significantly for grain protein and carbohydrate content in the two growing seasons. Also, all treatments of melatonin (ME), salicylic acid (SA) and mycorrhizal fungi (MF) and their combinations significantly increased grain protein and carbohydrate contents compared to control in both seasons. The highest mean values of grain protein and carbohydrate contents were obtained from plants which treated by SA+MF treatment in both seasons, except grain protein content in the first season which gave the highest mean value with plants treated by ME+MF treatment. However, SA + MF treatment and ME+MF were statistically similar for grain protein and carbohydrate content traits. While the lowest mean values were obtained with the control treatment in both seasons. On the other hand,

insignificant differences between the SA and MF treatments for grain protein and carbohydrate content in both seasons. (in complete sentence).

3.3.3. Interaction effect

The interaction between irrigation levels and melatonin, salicylic acid, and mycorrhizal fungi applied treatments (Table 5) was significant for grain protein content in the first season and grain carbohydrate content in both seasons. The highest mean value, 13.33% of grain protein content, was obtained under the combination of I₆₀ and ME+MF treatment in the first season. While the highest mean values of grain carbohydrate content were obtained under the combination of I₁₀₀ and SA+MF treatment in both seasons. In contrast, the lowest mean values were obtained under I₆₀ and control treatment for the grain carbohydrate content and I₁₀₀ and control treatment for grain protein content in both seasons.

Table 4. Effect of the interaction between irrigation levels and melatonin (ME), salicylic acid (SA), and mycorrhizal fungi (MF) treatments for yield and its attributes traits in the 2017/2018 and 2018/2019 seasons

Traits		Spike length (cm)		Number of spikes m ⁻²		Number of spikelets spike ⁻¹		Number of kernels spike ⁻¹		Thousand kernels weight (g)		Grain yield (ton ha ⁻¹)		Biological yield (ton ha ⁻¹)		
		17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	
Seasons		17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	17/18	18/19	
Treatments	I ₁ (100%)	Control	12.30	12.73	345.00	367.50	21.85	22.05	59.09	61.10	52.06	58.64	7.34	7.70	14.78	19.12
		ME	12.35	12.98	362.00	382.25	22.73	22.55	60.60	63.10	53.78	63.10	7.53	8.02	15.31	19.66
		SA	12.68	12.93	365.50	395.25	22.88	22.28	61.48	63.93	54.25	64.43	7.63	8.34	15.45	20.41
		MF	12.80	13.13	348.00	400.00	22.55	22.88	62.87	62.38	53.40	63.38	7.73	8.46	15.14	19.62
		SA+MF	13.00	13.98	383.00	413.75	23.55	23.28	65.08	67.13	55.95	65.63	8.02	8.96	16.16	21.03
		ME+MF	12.95	13.31	365.00	403.75	23.68	23.10	66.21	67.40	55.46	66.11	7.90	8.74	15.53	20.24
	I ₂ (80%)	Control	11.13	11.78	324.00	341.00	20.00	20.48	54.74	55.61	47.81	55.11	6.31	7.25	13.69	17.91
		ME	11.75	12.35	331.50	372.25	21.35	21.08	56.79	61.69	51.29	60.64	6.90	7.59	14.46	18.49
		SA	12.38	12.55	342.50	383.00	21.65	22.05	60.45	63.75	52.35	60.75	7.20	7.96	14.30	19.16
		MF	11.85	12.80	337.50	366.25	22.40	21.78	61.48	62.19	51.34	62.57	7.42	7.96	14.69	19.13
		SA+MF	12.68	13.30	349.25	392.50	22.55	22.88	64.05	65.17	53.78	65.17	7.99	8.54	15.11	19.62
		ME+MF	12.13	13.23	345.00	401.50	22.85	22.73	64.59	67.29	54.09	63.69	7.71	8.55	14.98	20.12
	I ₃ (60%)	Control	10.13	10.38	290.00	313.50	17.28	18.00	50.46	51.59	45.49	50.72	5.83	6.05	11.81	15.51
		ME	10.63	11.13	312.00	322.50	18.40	18.70	53.93	56.97	46.18	54.44	6.44	6.82	12.30	16.74
		SA	10.88	12.10	307.25	349.75	19.65	20.45	59.26	61.84	47.86	59.28	6.46	7.86	13.10	18.16
		MF	11.50	11.83	318.00	328.25	21.18	21.48	60.40	60.20	50.59	59.70	6.90	7.60	13.79	18.60
		SA+MF	12.05	12.18	322.00	382.00	22.15	21.98	62.62	63.72	52.46	64.01	7.36	8.17	14.69	18.99
		ME+MF	12.00	12.75	329.25	363.00	22.13	22.50	63.09	65.01	52.90	63.40	7.20	8.23	14.87	18.41
F test		*	*	ns	*	*	**	*	*	*	*	*	*	*	ns	
L.S.D 0.05		0.90	0.66	--	18.0	1.46	1.43	3.46	2.88	2.29	3.21	0.37	0.47	1.08	--	

ns, * and ** refer to non-significant and significant at 0.05 and 0.01 levels of probability, respectively

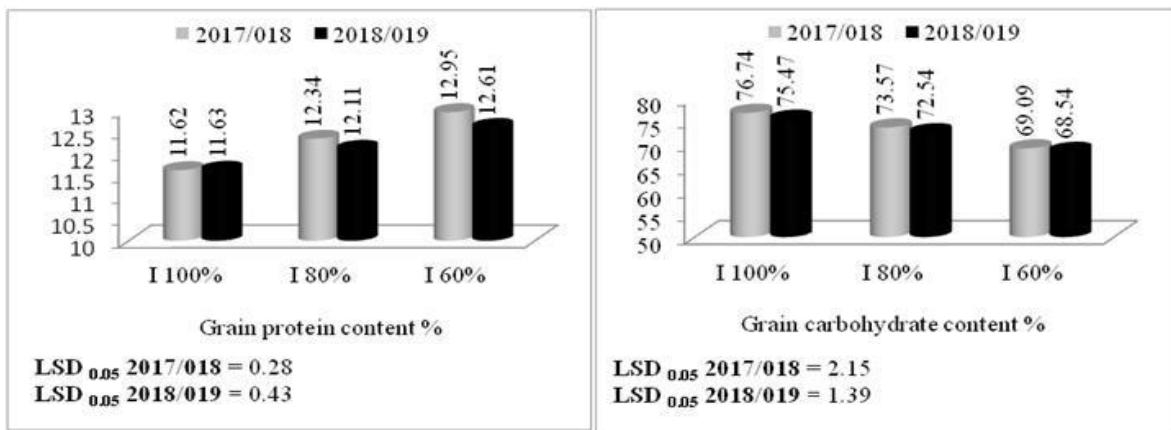


Fig. 3. Means of grain protein and carbohydrate content as affected by three irrigation levels in 2017/2018 and 2018/2019 seasons.

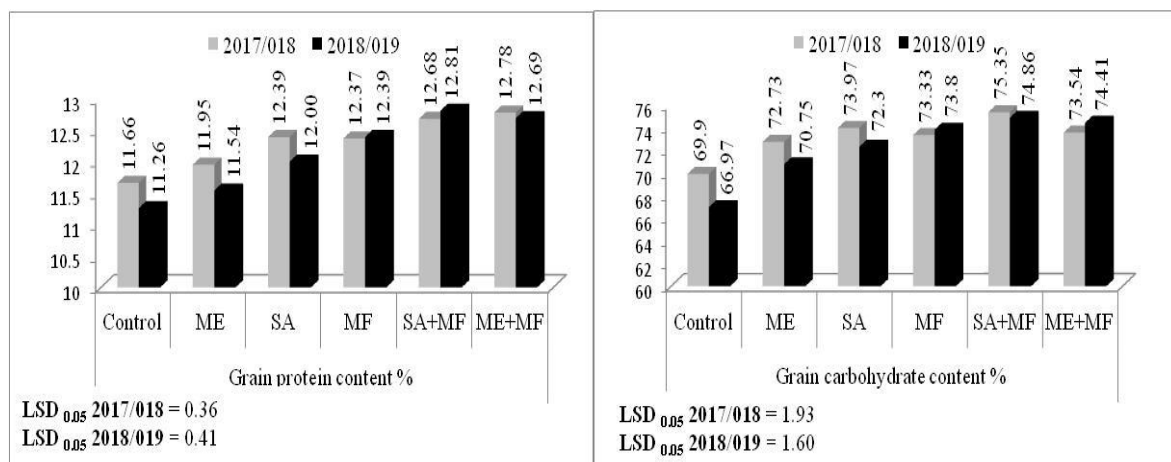


Fig. 4. Means of grain protein and carbohydrate content as affected by melatonin (ME), salicylic acid (SA) and mycorrhizal fungi (MF) treatments under three irrigation levels in 2017/2018 and 2018/2019 seasons.

Table 5. Effect of the interaction between irrigation levels and melatonin (ME), salicylic acid (SA) and mycorrhizal fungi (MF) treatments for grain protein and carbohydrate content in 2017/2018 and 2018/2019 seasons.

Traits	Grain protein content (%)			Grain Carbohydrate content (%)		
	I ₁ (100%)	I ₂ (80%)	I ₃ (60%)	I ₁ (100%)	I ₂ (80%)	I ₃ (60%)
2017/2018						
Treatments	I ₁ (100%)	I ₂ (80%)	I ₃ (60%)	I ₁ (100%)	I ₂ (80%)	I ₃ (60%)
Control	10.83	11.43	12.72	74.89	69.82	64.99
ME	11.13	11.89	12.84	76.38	73.06	68.74
SA	11.74	12.45	12.98	78.98	75.95	66.99
MF	12.03	12.26	12.83	74.44	74.66	70.88
SA+MF	12.17	12.87	13.00	79.44	74.53	72.08
ME+MF	11.82	13.18	13.33	76.34	73.42	70.86
F test	*			*		
LSD 0.05	0.57			2.45		
2018/2019						
Treatments	I ₁ (100%)	I ₂ (80%)	I ₃ (60%)	I ₁ (100%)	I ₂ (80%)	I ₃ (60%)
Control	10.78	11.04	11.96	72.14	66.60	62.18
ME	11.20	11.38	12.03	73.48	71.91	66.86
SA	11.55	12.01	12.45	77.54	73.15	66.22
MF	11.78	12.47	12.91	75.86	74.66	70.88
SA+MF	12.20	13.00	13.24	77.09	74.39	73.08
ME+MF	12.26	12.73	13.07	76.69	74.51	72.03
F test	ns			*		
LSD 0.05	--			2.92		

ns,* and ** refer to non-significant and significant at 0.05 and 0.01 levels of probability, respectively

4. Discussion

Drought stress, among abiotic stresses, is the most challenging threats to agricultural system and economic yield of crop plants [41]. Water stress conditions cause severe reductions in growth, development and overall productivity of plants even in tolerant genotypes. Recently, wheat production has been greatly affected by climate change around the globe with the increasing unavailability of water resources. Thus, the present study was conducted to elucidate the protective role of application of melatonin, salicylic acid, mycorrhizal fungi treatments and their combinations in ameliorating the water stress through enhancing the productivity of wheat plants under the three amounts 5476 ($I_{100\%}$), 4380 ($I_{80\%}$) and 3285 ($I_{60\%}$) $m^3 ha^{-1}$ of irrigation water. In the present study, decreasing irrigation water quantity from $I_{100\%}$, $I_{80\%}$ and up to $I_{60\%}$ caused a significant decrease of all studied traits except grain protein content % but, $I_{60\%}$ treatment had more adverse effect. This result indicated that an adequate irrigation water supply was an important prerequisite for high yield of wheat. Irrigation practices and amount of irrigation water are factors which have always limited wheat productivity [42]. The decrease in phenological, growth traits under water stress may be due to decreasing the internodes elongation and cell division as well decreasing the pigments concentration in wheat leaves which results in inhibition of photosynthetic activity and this in turn leads to less accumulation of carbohydrates in mature leaves and consequently may decrease the growth period and the rate of transport of carbohydrates from leaves to the developing grains. Decreasing irrigation requirements from 100% to 50% significantly reduced plant height, flag leaf area and total chlorophyll in wheat [13]. Machado et al. [43] indicated that drought stress at near anthesis and grain filling stage decreased translocation of photosynthetic products, and resulted in wrinkled grains which decreased grain yield. Decrease in grain yield under drought stress condition may be due to the reduction in the duration and rate of grain filling processes which resulted in the production of small grains and consequently reducing grain yield. Drought stress affects the pollination which results in the poor grain yield [44]. Singh et al. [11] found that grain yield and yield components of wheat were decreased with decreasing irrigation water amounts as well as its quality. The decrease in carbohydrate content under stress conditions is related to the reduction of pigment and photosynthesis resulted from low expression of enzymes involved in photosynthesis under drought stress [45]. On the other hand, the obtained results revealed that, grain protein content (%) increased with decreasing irrigation water level. Increase irrigation water may decrease flour protein content by dilution of N with carbohydrates [46]. Decreasing irrigation water decreased carbohydrate content and increased protein content in wheat [14]. Application of Melatonin, salicylic acid and mycorrhizal fungi or their combinations was significant in alleviating the adverse effects of irrigation water deficit on all studied traits. The combined application of SA + MF, and of ME + MF improved all the studied traits under different irrigation water amounts greater than when applied separately. Melatonin is considered as a plant master regulator which is widely involved in regulating plant growth, development and stress responses [47]. The previous study showed that

exogenous application of ME increased wheat drought tolerance by alleviating photosynthetic inhibition and oxidative damage induced by drought stress [48]. Treated wheat seedling with 500 μM melatonin at 60% and 40% of field capacity were significantly enhanced the drought tolerance of wheat seedling by decreased membrane damage, more intact grana lamella of chloroplast, higher photosynthetic rate, and maximum efficiency of photosystem II, as well as higher cell turgor and water holding capacity in melatonin-treated seedlings [49]. In our study, ME (30 ppm) application showed increase in all studied traits except for biological yield under different irrigations levels as compared by control treatment, but this increase was more under irrigation deficient conditions than that under irrigation sufficient conditions. Foliar spray of melatonin under salinity stress significantly improved the growth and yield and it was effective in improving the activities of catalase, peroxidase and superoxide dismutase under stress conditions in wheat genotypes compared to non-treated ones [26]. Moreover, grain yield, grain number and thousand grain weight were increased by ME application under N deficient conditions [50]. Salicylic acid (SA) is a plant growth regulator which enhances plant defense against various biotic and abiotic stresses through morphological, biochemical, and physiological mechanisms [27]. The application of SA separately or combined with MF enhanced phenological, growth, yield and its components and grain quality traits under the three irrigation water amounts as compared to control treatment. Aldesuquy et al. [51] and El-Mantawy et al. [14] reported marked increase in plant height, flag leaf area, chlorophyll content yield and its components and grain quality traits with application of SA on wheat under irrigation water deficit. Furthermore, the beneficial effect of SA on grain yield may be due to translocation of more photo-assimilates to grains during grain filling, thereby increasing grain weight. Application of SA enhanced grain yield, grain starch content and crude protein content under well-irrigated as well as drought stress conditions compared to SA-untreated plants [52]. Mycorrhizal fungi (MF) are obligate symbiotic soil microorganisms that colonize the roots of the majority of plants and this symbiosis enhances the performance of crop plants in stress condition such as water stress. In present study, inoculation with the MF fungi separately or combined with ME significantly affected days to heading and maturity, plant height, leaf area index (75 and 105 DAS), leaf chlorophyll content and yield and its components as well as grain protein and carbohydrate content under stress and non-stress irrigation water amount as compared to control treatment. The mean values for number of grains spike⁻¹, 100-grain weight, spike length, number of spikelets spike⁻¹ and grain yield plant⁻¹ in the mycorrhizal inoculated wheat cultivars were greater than the results from their non-inoculated counterparts [53]. Rashwan et al., [54] found that mycorrhizal inoculation gave the highest yield related traits i.e., number of grains spike⁻¹, number of spikes m⁻² and 1000-grain weight in wheat. The higher increase in wheat grain and biomass yield under water stress due to MF inoculation might be due to the small size of stressed plants [55] or to increased dependence of wheat on MF fungi for mineral and water uptake [56]. Increase growth in wheat plants inoculated with mycorrhizal fungi than non-inoculated plants

were probably indirectly due to mycorrhizal enhancement of P uptake, which enhance photosynthesis [57]. The mycorrhizal inoculation in wheat significantly increased the content of crude protein and carbohydrate content under water stress [58]. This increase was considered by the authors to be a beneficial mycorrhizal effect. In the present study, application of melatonin, salicylic acid and mycorrhizal fungi reduces the negative effects of water stress in wheat by improving various agronomic and grain quality traits. In general, the combined application of SA + MF or ME + MF were the greatest treatments in increase drought tolerance and improve all studied traits under different irrigation levels and these combinations could be a promising practice in water deficient regions for enhancing wheat productivity under drought stress conditions.

5. Conclusion

Water scarcity adversely affects all phases of growth, most strikingly noted at the reproductive phase and grain filling, leading to fewer grains and smaller grain size in cereal crops including wheat. Generally, managing the adverse effects of water stress, plants induce several physiological mechanisms. Our study has shown that the application of SA, melatonin and mycorrhizal inoculation are a strong tools in reducing the adverse effects of water stress in wheat plants. Decreasing irrigation water amount gradually from 5476 ($I_{100\%}$), 4380 ($I_{80\%}$) and up to 3285 ($I_{60\%}$) $m^3 ha^{-1}$ caused a significant decrease of days to heading, days to maturity, plant height, leaf area index at 75 and 105 DAS, spike length, number of spikes m^{-2} , number of spikelets spike $^{-1}$, number of kernels spike $^{-1}$, thousand kernel weight, grain yield, biological yield and grain carbohydrate content. On the other hand, it caused a significant increase in grain protein content. Application of melatonin, salicylic acid and mycorrhizal fungi reduces the negative effects of water stress in wheat by improving various growth, yield and its attributes as well as grain quality traits. In general, the combined application of SA + MF or ME + MF were the greatest treatments to reduce the negative effect of water stress, and these combinations can play a significant role in the reclamation of drought-affect land in arid and semi-arid regions. Even if irrigation water is available, it can use 80% of irrigation water with the combined application of SA + MF or ME + MF which can save about 20% of irrigation water to irrigate more field as well as field trials can also validate the practical usability of these combinations in drought tolerance.

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