ENHANCEMENT OF HEAT STRESS TOLERANCE IN WHEAT USING A NEW MODIFIED FORMULA OF EFFECTIVE MICROORGANISMS-5

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Two field experiments were conducted in Six October Farm, East Oweinat, New Valley Governorate during 2009/2010 and 2010/2011 successive winter seasons, to study the effect of different new modified formulas of EM_5 named as $EM_{5\text{-micronutrient plus}}$ on the plant tolerance to two heat stress treatments (simulated as two irrigation methods; i.e. pivot and sprinkler) in wheat (*Triticum aestivum* L. var. Gemiza 9). Heat stress as a global warming element substantially affects grain setting, duration and rate, and ultimately grain yield. Nonetheless the timing, duration and intensity of heat stress determine its impact on grain yield. Results obtained indicated that under heat stress conditions, pivot irrigation system was the most effective irrigation method compared to sprinkler irrigation system. Similarly, spraying the $EM_{5\text{-micronutrient plus}}$ in the concentration of 8000 ppm gave the best results either at heading stage (110 days after sowing date); i.e. plant height (cm), leaf area (cm^2) and chlorophyll (a+b) as ppm/m^2 or at harvest (150 days from sowing date); i.e. spike length (cm), number of tillers/m^2, number of spikes/m^2, number of grain/spike, weight of 1000 grains (g), biological yield (ton/fed), grain yield (ton/fed), and straw yield (ton/fed), while the best interaction treatments was obtained from the implementation of pivot irrigation system × $EM_{5\text{-micronutrient plus}}$ (8000 ppm). In contrary, only free proline (µmole proline/g fresh weight), which gave the high values under either sprinkler irrigation system or spraying with water as a control treatment or the interaction between sprinkler irrigation system × water.

Keywords: *Triticum aestivum* L., global warming, pivot irrigation system, sprinkler irrigation system, EM_5 new formula, growth characters, yield and yield attributes

Increased incidences of abiotic and biotic stresses impacting productivity in principal crops are being witnessed all over the world. Extreme events like prolonged droughts, intense rains and flooding, heat waves and frost damages are likely to further increase in future due to climate change (Alexander et al., 2006). Besides influencing the physico-chemical properties of soil rhizosphere through production of exo-polysaccharides and formation of biofilm, microorganisms such as EM_1 and EM_5 can also influence higher plants response to abiotic stresses like drought, chilling injury, salinity, metal toxicity and high temperature, through different mechanisms like induction of osmo-protectants and heat shock proteins.. etc, in plant cells (Higa, 1999).

The effective microorganisms (EM_1), is a formulas that contain a mixture of microorganisms groups that includes lactic acid bacteria, photosynthetic bacteria, yeasts, actinomycetes and fermenting fungi. The basis for using these EM species of microorganisms is that they contain various organic acids due to the presence of lactic acid bacteria, which secrete organic acids, enzymes, antioxidants, and metallic chelates (Higa, 1999). Consequently, EM_5 is a modification product from EM_1 by adding vinegar and ethyl alcohol during the fermentation process in order to have multifunction product such as foliar fertilizer and insect repellent (Higa, 2000). Wisselinka et al. (2002) reported that several heterofermentative lactic acid bacteria produce mannitol, which is a sugar alcohol in large amounts, using fructose as an electron acceptor, whereas homo-fermentative lactic acid bacteria only produce small amounts of mannitol, therefore mannitol is produced in EM_1 and EM_5 naturally as a byproduct of the fermentation process during EMx manufacture. The use of these microorganisms can alleviate stresses in crop plants thus provide excellent models for understanding the stress tolerance, adaptation and response mechanisms that makes crop plants more capable to cope with climate change induced stresses.

Wheat (*Triticum aestivum* L.) is very sensitive to high temperature (Slafer and Satorre, 1999) and trends in increasing growing season temperatures have already been reported for the major wheat-producing regions (Gaffen and Ross, 1998; Alexander et al., 2006 and Hennessy et al., 2008). Wheat experiences heat stress to varying degrees at different phenological stages, but heat stress during the reproductive phase is more harmful than during the vegetative phase due to the direct effect on grain number and dry weight (Wollenweber et al., 2003). End of season or ‘terminal’ heat stress is also likely to increase for wheat in the near future (Mitra and Bhatia, 2008 and Semenov, 2009). Thus the main focus is on responses to elevated temperatures during reproductive and grain-filling stages and processes that affect grain yield.

As indicated by Colaizzi et al. (2006), wheat seemed to be more reproductive under central pivot irrigation system compared with sprinkler system, this may due to minimize the probability of heat stress that temperature was reduced at least two Celsius in fields irrigated with pivot system compared with sprinkler ones (Hai-Jun and Kang, 2006).

Sainz et al. (2010) reported that drought and heat stress stimulated the degradation of Photosystem II (PSII) in *Lotus japonicas*. They added that heat is also induced degradation of chloroplast Cu/Zn superoxide dismutase, therefore degradation of PSII could be caused by the loss of components of chloroplast antioxidant defense systems and subsequent decreased function of PSII. Similarly, Maksymiec (1997) reported that Cu is an indispensable component of oxidative enzymes or of particular structural component of cells. At elevated concentrations, Cu can act strongly on chromatin, the photosynthetic apparatus, growth and senescence processes.

Manganese is an essential element for plants, intervening in several metabolic processes, mainly in photosynthesis and as an enzyme antioxidant-cofactor as reported by Millaleo et al. (2010), while regarding Boron, Metwally et al. (2014) reported that B is an essential element for soil micro-biomes and enzyme activities but in a very low concentrations.

This work aimed to study the effects of global warming on wheat production under field conditions and to suggest a physiological method to enhance the wheat adaptation to heat stress using a new modified formula of effective microorganisms five (EM_5) that later on will be known as EM-5 micronutrient plus.

MATERIALS AND METHODS

Two field experiments were conducted in Six October Farm and East Oweinat - New Valley Governorate during 2009/2010 and 2010/2011 successive winter seasons, to study the effect of different new formulas of EM_5 on plant tolerance to two heat stress treatments (simulated as two irrigation methods according to Colaizzi et al. (2006) as it will be described later on) in wheat (*T. aestivum* L. var. Gemiza 9).

The experimental soil was tilled three overlapping times. During soil preparation, the animal dung was added into the soil as a source of organic matter at the rate 20 m^3/fed, while calcium super – phosphate (15.5% P_2O_2) was added into the soil at the rate of 100 kg/fed. Both the nitrogen fertilizer as ammonium nitrate (33.5% N) at the rate of 60 kg/fed and the Potash fertilizer as potassium sulphate (48% K_2O) were added during the growth stages through the fertigation process. The chemical analysis of the used compost was presented in table (1), while the chemical and physical properties of the experimental soil was presented in tables (2 and 3). However, the chemical properties of the irrigation water were presented in table (4).

Table (1). The chemical properties of the compost used in the two seasons.

Moisture content (%)	Organic matter (%)	C/N ratio	pH	Available						S %
				(%)			(ppm)			
				N	P	K	Z	Mn	Fe	
8.3	30.2	18.1	7.3–7.1	2.15	1.14	1.25	2.1	3.9	4.2	0.25

Table (2). The physical and chemical properties of the experimental soil**.**

Field	Sand (%)	Silt (%)	Clay (%)	Texture	pH	EC (ppm)	O.M.
Pivot	45.91	23.76	22.15	Sandy Clay	8.74	614	0.58
Sprinkler	46.12	23.17	22.95	Sandy Clay Loam	8.69	587	0.67

Table (3). Other chemical properties of the experimental soil**.**

Field	Cations (meg/L)				Anions (meg/L)				Micronutrients (ppm)				
	Na^+	K^+	Ca^+	Mg^{+2}	Cl^-	SO_4	HCO_3	CO_3	B	Fe	Cu	Mn	Zn
Pivot	23.7	1.67	7.5	2.79	21.3	6.35	5.1	-	0.55	66.08	1.86	3.91	5.66
Sprinkler	28.4	1.88	6.8	3.12	23.4	5.74	6.5	-	0.47	71.44	1.78	3.86	5.82

Table (4). The chemical properties of the irrigation water.

EC (ds/m)	pH	B (ppm)	Cations (mg/L)				Anions (mg/L)			
			Ca^+	Mg^{+2}	K^+	Na^+	Cl^-	SO_4^{-2}	HCO^-_3	CO_3^{-2}
1.14	7.7	0.068	19.68	1.45	31.37	11.82	29.94	283.64	73.22	-

Following the common agricultural practices in the region, seeds of wheat (*T. aestivum* L. var. Gemiza 9) were drilled mechanically at 10^{th} of November in the two seasons at the rate of 30 kg/fed. The heat stress treatments were simulated as two irrigation systems; the first is under the conditions of central pivot sprinkler system (VXP, Irrifrance, Paulhan, France) where temperature is always two Celsius less than under the second irrigation system conditions, which was the fixed sprinkler system (NAAN Sprinkler 233 A-S, Israel) referring to Colaizzi et al. (2006). The metrological data of the two fields of wheat growth period during the two seasons were recorded within two in-site metrological booths; i.e. mean temperature (ºC), maximum temperature (ºC), minimum temperature (ºC) and mean humidity (%).

The NAAN Sprinkler 233 A-S, Israel system irrigated using 360º angle sprinklers, the sprinkler is a metal impact sprinkler 3/4" male (NAAN Sprinkler 233 A-S, Israel) with a discharge of 1.170 m^3/h, wetted radius of 13.5 m, working pressure of 300 kPa and irrigation intensity of 8.10 mmh^{-1}. The irrigation system's control unit had a two sand filters (Amiad, Israel) 3″ inlet/outlet diameter, 36″ vessel diameter, 35-50 m^3/h and 200 kg vessel weight (empty), and screen filter 200 mesh. A flow-meter and a pressure regulated valve were installed at the head of the irrigation system to measure the applied water and to control the system pressure with a splay rate of 2500 L/min. After the filtration system, the solid set sprinkler irrigation system had 27 laterals, 60 m long, installed on a 1.944 ha field (approximately 324 m long and 60 m wide) with an average slope of 0.0%. The VXP, Irrifrance, Paulhan, France irrigation system has a hose-fed valmout model 6000 central-move irrigation system equipped with a CAMA computerized controller. The system had three, 39 m long spans with twenty four, 1.52 m spaced drops under each span. Pressurized water, on demand from a deep well, was supplied to the irrigation system through an underground water pump. Senninger (Quest and Sons) 360º spray nozzles placed above the LEPA socks metered the flow to drag socks at same rate as the other irrigation system. All application devices were spaced 1.25 m apart and discharged 19.0 L/min equivalent to the discharge rate at the end of 400 m center pivot with a supply rate of 2500 L/min. Pressure to the sprinkler irrigation system was 207 kPa. The LEPA devices were equipped with pressure regulators for the irrigation. Irrigation amount was controlled by changing the speed of the central move irrigation system.

The term effective microorganisms (EM_1) as proposed by Higa (1999) contains selected species of microorganisms including predominant populations of lactic acid bacteria and yeasts, and smaller numbers of photosynthetic bacteria, actinomycetes and other types of organisms such as mycorrhizae. Inoculation of EM cultures to the soil/plant ecosystem can improve soil quality, plant health, growth, yield, and quality of crops. Consequently, (EM_5) is a modification product from EM_1 that created by adding vinegar and ethyl alcohol during the fermentation process in order to have multifunction product such as foliar fertilizer and insect repellent (Higa, 2000). Later on, the new modification of $EM_{5\text{-micronutrients}}$ was made by myself through different experiments for series of concentrations from each micronutrient which fermented in the classical EM_5; i.e. Cu, Zn, Mn, B and their interactions to select the most proper concentration as active ingredient and total concentration of the modified formulas of EM_5 to apply on wheat plants under heat stress conditions. The selected concentration for the micronutrients as active materials in each formula were as follows: EM_5; Cu 2.5 ppm, Zn 1.8 ppm, Mn 1.8 ppm, B 0.1 ppm and for the micronutrient plus (all together). While the applied formulas were sprayed into wheat plants in two concentrations (6000 and 8000 ppm) at the pre-heading stage (90 days after sowing date). The thirteen tested formulas were five new formulas of EM_5 i.e. $EM_{5\text{-cu}}$, $EM_{5\text{-Zn}}$, $EM_{5\text{-Mn}}$, $EM_{5\text{-B}}$, $EM_{5\text{-micronutrients}}$, besides EM_1, (in two concentrations), while water was the control treatment.

The experimental design used in this experiment was spilt plot design in three replicates, where heat stress treatments (irrigation treatments) occupied the main plots and the different EM_5 formulas were been arranged in the sub ones. The area of the experimental unit was 12 m^2 (3 m × 4 m) with 15 rows, 20 cm apart and 4 m length.

Ten guarded wheat plants have been taken from each treatment at heading stage (110 days after sowing date) as sample to determine growth characters; i.e. plant height (cm), and leaf area (cm^2), in addition to total chlorophyll using Minolta SPDA-502 leaf chlorophyll meter, then converted into total chlorophyll (a+b) as ppm/m^2 referring to John et al. (1988) and free proline (µmole proline/g fresh weight) as indicted by Bates (1975).

At harvest (150 days from sowing date), one square meter plants were harvested then converted into ton per feddan to determine yield and its attributes; i.e. spike length (cm), number of tillers/m^2, number of spikes/m^2, number of grain/spike, weight of 1000 grains (g), biological yield (ton/fed), grain yield (ton/fed) and straw yield (ton/fed).

Pooled data were subjected to the combined statistical analysis after passing the homogeneity test using M-STAT C (Russell, 1991), while Duncan's multiple range test was used to verify the significant differences between treatments means as described by Duncan (1955).

RESULTS AND DISCUSSION

1. Metrological Data

The observed meteorological data that shown in table (5) indicates that the growing season of 2009/2010 and 2010/2011 of wheat were relatively high in temperature and humidity. This was in agreement with what reported by IPCC (2011) that during 2010 and 2011 the hotter, moister atmosphere is an atmosphere primed to trigger disasters, besides, there is high confidence that both maximum and minimum daily temperatures have increased on a global scale due to the increase of greenhouse gases. It also proved that the air temperature was lower two Celsius under the conditions of central pivot sprinklers than the conditions of the fixed sprinkler system as reported by Colaizzi et al. (2006).

2. Effect of Heat Stress Treatments Simulated as Two Irrigation Methods

Data that presented in tables (6 and 7) indicated that except of the free proline (µmole proline/g fresh weight), which highly accumulated under sprinkler irrigation conditions, all the other studied characters were negatively affected under the heat stress conditions of the dominant air temperature of the region (sprinkler irrigation system), either at heading stage (110 days after sowing date); i.e. plant height (cm), leaf area (cm^2) and chlorophyll (a+b) as ppm/m or at harvest (150 days from sowing date); i.e. spike length (cm), number of tillers/m^2, number of spikes/m^2, number of grain/spike, weight of 1000 grains (g), biological yield (ton/fed), grain yield (ton/fed) and straw yield (ton/fed) compared to under the conditions of two

Celsius lower than the dominant air temperature in the region (pivot irrigation system). This agrees with what described by Colaizzi et al. (2006) and was proved by the data presented in table (5).

Table (5). The metrological data of East Owinat during wheat growing seasons in 2009/2010 and 2010/2011.

I. Under sprinkler irrigation system								
	Maximum temperature (ºC)		**Minimum temperature (ºC)**		**Mean temperature (ºC)**		**Mean humidity (%)**	
Month	**2009/10**	**2010/11**	**2009/10**	**2010/11**	**2009/10**	**2010/11**	**2009/10**	**2010/11**
Nov.	28.6	32.2	10.6	11.2	19.1	21.2	43.7	44.2
Dec	25.2	30.1	13.9	14.3	19.1	21.7	50.4	51.3
Jan	25.5	28.8	7.8	8.9	16.2	18.4	43.3	43.2
Feb	25.6	26.7	10.2	11.5	17.4	18.6	41.9	42.9
Mar	30.1	33.5	13.8	15.1	21.4	23.8	32.7	33.4
Apr	35.5	37.4	18.6	19.6	26.5	28.2	25.6	26.1
May	38.3	40.3	22.9	23.4	30.1	31.3	23.6	24.8

II. Under pivot irrigation system								
	Maximum temperature (ºC)		**Minimum temperature (ºC)**		**Mean temperature (ºC)**		**Mean humidity (%)**	
Month	2009/10	2010/11	2009/10	2010/11	2009/10	2010/11	2009/10	2010/11
Nov.	26.6	29.8	8.4	10.1	17.3	18.6	44.2	46.5
Dec	22.9	26.2	10.2	12.4	18.7	19.3	52.4	52.6
Jan	23.2	27.1	7.2	7.9	15.2	16.3	44.4	45.3
Feb	23.6	23.9	8.3	9.6	15.3	17.5	42.3	44.8
Mar	28.7	30.8	10.2	11.7	18.9	19.6	34.1	36.7
Apr	34.1	34.8	16.5	17.4	23.5	25.3	27.2	28.2
May	37.6	37.9	21.3	22.2	28.8	30.2	25.4	26

Data was recorded automatically through the meteorological booths in site.

As reported by Stone and Nicolas (1995), heat stress is an important constraint to wheat productivity affecting different growth stages, specially anthesis and grain filling. Thus, to stay green is a trait that has been used to indicate heat tolerance in hot environment. It is confirmed that photosynthetic rate is maximum at 20-22ºC and decreases abruptly at 30-32ºC, so heat stress injuries of the photosynthetic apparatus during reproductive growth of wheat diminish source activity and sink capacity, which results in reduced productivity as reported by Harding et al. (1990) and Aziz et al. (2009), who added that accumulation a small quantity of free proline in the proper time may plays an important role in accumulation of assimilates and enhance source-think relationships, thus, it is an important criteria when breeding for stresses. Yet, the very high concentration of free proline indicates that the plant is challenging a severe stress and the net gain soon will be the dominancy of the senescence hormones; such as ethylene and abscisic acid by the meaning that the plant will have a shorter growth period and maybe fail to produce a valued yield (Bates, 1975). Therefore, wheat production is more appreciated under pivot irrigation system than sprinkler irrigation system when heat stress out of global warming is present.

Table (6). Effect of heat stress tolerance (simulated as two irrigation methods on wheat (*Triticum. aestivum* L. var. Gemiza 9) characteristics at heading stage (combine analysis of 2009/2010 and 2010/2011 seasons).

[1] Irrigation method	Studied characteristics: Plant height (cm)	Leaf area (cm^2)	Proline (μ mole proline /g f. wt.)	Chlorophyll (ppm)
[2] Pivot	63.3 A	50.6 A	7.06 B	395.35 A
[3] Sprinkler	44.3 B	35.4 B	10.09 A	275.74 B

[1] Heat stress treatments were simulated as two irrigation methods referring to Colaizzi et al. (2006)
[2] Lower 2 º C than the dominant average temperature in the area.
[3] The dominant average temperature in the area.
• Means having similar latters at same column has no significant differences at $P \geq 0.05$.

Table (7). Effect of heat stress tolerance (simulated as two irrigation methods) on wheat (*Triticum. aestivum* L. var. Gemiza 9) yield and its attributes at harvest (combine analysis of 2009/2010 and 2010/2011 seasons).

[1] Irrigation method	Studied criteria: Spike length (cm)	No. of tillers /m^2	No. of spikes /m^2	No. of grains /spike	1000 grains weight (g)	Biological yield (ton/ fed)	Grain yield (ton/ fed.)	Straw yield (ton/ fed)
[2] Pivot	8.9 A	291.4 A	278.4 A	60.4 A	29.13 A	4.35 A	2.12 A	2.23 A
[3] Sprinkler	6.2 B	203.9 B	194.9 B	42.3 B	20.39 B	3.03 B	1.83 B	1.20 B

[1] Heat stress treatments were simulated as two irrigation methods referring to Colaizzi et al. (2006)
[2] Lower 2 º C than the dominant average temperature in the area.
[3] The dominant average temperature in the area.
• Means having similar latters at same column has no significant differences at $P \geq 0.05$.

3. Effect of Different New Formulas of EM_5

Generally and as shown in tables (8 and 9), spraying EM_1 and EM_5 (either as traditional formula or as the new formulas) into the heat stressed wheat plants increased all the studied characters either at heading stage (110 days after sowing date); i.e. plant height (cm), leaf area (cm^2), chlorophyll (a+b) as ppm/m^2 and free proline (µmole proline/g fresh weight), or at harvest (150 days from sowing date); i.e. spike length (cm), number of tillers/m^2, number of spikes/m^2, number of grain/spike, weight of 1000 grains (g), biological yield (ton/fed), grain yield (ton/fed) and straw yield (ton/fed) compared to spraying with water as the control treatment. The highest observations were obtained from spraying $EM_{5\text{-micronutrients}}$ with the concentration of 8000 ppm, followed by spraying with the concentration of 4000 ppm, followed by $EM_{5\text{-Cu}}$ in 8000 ppm then 6000 ppm, EM_1 in 8000 ppm then 6000 ppm, $EM_{5\text{-Zn}}$ in 6000 ppm, then after $EM_{5\text{-Mn}}$ in 8000 ppm then 6000 ppm, then after $EM_{5\text{-B}}$ in 8000 then after 6000 ppm. While the lowest observations were obtained from spraying with water as the control treatment. Many investigators obtained similar trends for the effect of spraying of EM_1, EM_5 and Cu, Zn, Mn and B as micronutrients on the plants growth, yield and metabolism.

Table (8). Effect of different new formulas of EM_5 on wheat (*Triticum. aestivum* L. var. Gemiza 9) characteristics at heading stage (combine analysis of 2009/2010 and 2010/2011 seasons).

EM_5 formulas	**Studied characteristics**			
	Plant height (cm)	**Leaf area (cm^2)**	**Proline (µ mole proline /g f. wt.)**	**Chlorophyll (ppm)**
Water	22.9 L	18.3 L	15.5 A	143.50 L
EM_1 (6000 ppm)	55.2 F	44.1 F	8.7 F	344.41 F
EM_1 (8000 ppm)	56.5 E	45.1 E	8.4 G	352.61 E
EM_5-Cu (6000 ppm)	59.1 D	47.2 D	6.2 I	369.00 D
EM_5-Cu (8000 ppm)	56.7 C	52.4 C	5.6 I	410.01 C
EM_5-Zn (6000 ppm)	52.6 G	42.0 G	8.4 G	328.01 G
EM_5-Zn (8000 ppm)	56.5 E	45.1 E	7.7 H	352.61 E
EM_5-Mn (6000 ppm)	46.0 I	36.7 I	9.6 D	287.01 I
EM_5-Mn (8000 ppm)	49.9 H	39.9 H	9.3 E	311.61 H
EM_5-B (6000 ppm)	39.4 K	31.5 K	11.4 B	246.00 K
EM_5-B (8000 ppm)	42.7 J	34.1 J	10.8 C	266.51 J
EM_5-micronutrients (6000 ppm)	68.0 B	54.3 B	5.41 J	424.36 B
EM_5-micronutrients (8000 ppm)	85.4 A	68.2 A	4.64 K	355.01 A

- Means having similar latters at same column has no significant differences at *$P \geq 0.05$*.

Table (9). Effect of different new formulas of EM_5 on wheat (*Triticum aestivum* L. var. Gemiza 9) yield and its attributes at harvest (combine analysis of 2009/2010 and 2010/2011 seasons).

EM_5 formulas	Studied criteria							
	Spike length (cm)	No. of tillers /m^2	No. of spikes /m^2	No. of grains /spike	1000 grains weight (g)	Bio-logical yield (ton/ fed)	Grain yield (ton/ fed.)	Straw yield (ton/ fed)
Water	3.2 L	105.8 L	101.1 L	21.9 L	10.57 L	1.58 L	0.95 K	0.61K
EM1 (6000 ppm)	7.7 F	253.8 F	242.6 F	52.6 F	25.37 F	2.78 F	2.28 E	1.50 E
EM1 (8000 ppm)	7.9 E	259.9 E	248.3 E	53.8 E	25.99 E	3.89 E	2.32 E	1.57 E
EM_5-Cu (6000 ppm)	8.3 D	271.9 D	259.9 D	56.3 D	27.19 D	4.03 D	2.43 D	1.62 D
EM_5-Cu (8000 ppm)	9.2 C	302.2 C	288.8 C	62.6 C	30.21 C	4.50 C	2.72 C	1.80 C
EM_5-Zn (6000 ppm)	7.4 G	241.8 G	231G	50.1 G	24.17 G	3.60 G	2.18 F	1.43 F
EM_5-Zn (8000 ppm)	7.8 E	259.9 E	248.3 E	53.8 E	25.98 E	3.88 E	2.31E	1.57 E
EM_5-Mn (6000 ppm)	6.4 I	211.5 I	202.2 I	43.8 I	21.14 I	3.13 I	1.90 H	1.27 H
EM_5-Mn (8000 ppm)	7 H	229.7 H	219.5 H	47.6 H	22.96 H	3.42 H	2.06 G	1.37 G
EM_5-B (6000 ppm)	5.5 K	181.3 K	173.3 K	37.5 K	18.12 K	2.70K	1.62J	1.07 J
EM_5-B (8000 ppm)	6 J	196.4 J	187.7 J	40.7 J	19.63J	2.92 J	1.78 I	1.17 I
EM_5-micronutrients (6000 ppm)	9.5 B	312.7 B	298.9 B	64.8 B	31.26 B	4.65B	2.80 B	1.85B
EM_5-micronutrients (8000 ppm)	11.9 A	392 A	375A	81A	39.27 A	5.85A	3.52 A	2.33A

- Means having similar latters at same column has no significant differences at $P\geq 0.05$.

Higa (1999 and 2000) reported that spraying both EM_1 and EM_5 on the stressed plants resulted in stress tolerance for its positive effects on the metabolic consequences of the plants, particularly for the presence of sugar alcohol (polyol), which can be a source of metabolic carbon under stressful conditions (Wisselinka et al., 2002). He added that polyols act as osmoprotectants under stress conditions, because the water-like hydroxyl groups in polyols allow them to form an artificial sphere of hydration around macromolecules, thus preventing metabolic inactivation under these conditions. Moreover, given a comparative advantage from its linear shape, mannitol can enter the semi-closed stomata carrying the chelated micro elements, thus the efficiency of the fertilization with the micronutrients would be increased under the stressful conditions, where the other configuration will fail (Stoop et al., 1994). Cupper as a micronutrient plays a very important role in photosynthesis that increases atmospheric CO_2 absorbance by the plant. It also involves in the plastocyanin protein formation in the plastids and acts like electron carrier within the photosynthesis process, in addition to it involves in phenolase enzyme formation and activity as reported by Alam (1999). He added that zinc as well is a very important microelement in photosynthesis and protein formation, it inhibits the activity of the enzyme tryptophan synthetase and promotes the activity of the enzyme anhydrase and triosphate dehydrogenase, thus stimulates the protein formation. Manganese encourages the photosynthesis process within promotion of the oxygen molecule from the water to the chlorophyll molecule during photosynthesis (Millaleo et al., 2010). Boron as reported by Metwally et al. (2014), is an essential element but in a very low concentrations for enzyme activities, accelerate the source-think relationship and act like osmoregulator under stress conditions. When take into account the integration between the above mentioned features of mannitol and the micronutrients involved in this study; i.e. Cu, Zn, Mn, and B, the potentiality of the new formula $EM_{5\text{-micronutient plus}}$ can be understood.

4. Effect of the Interaction Between Heat Stress Treatments and New Formulas of EM_5

Results in tables (10 and 11) indicate that the application of the $EM_{5\text{- micronutrient plus}}$ to mitigate the bad consequences of heat stress on wheat growth and productivity is highly significant. Except for the free proline (µmole proline/g fresh weight) that highly accumulated under the heat stress conditions of the dominant air temperature of the region (sprinkler irrigation system), the highest observation for the other studied characters either at heading stage (110 days after sowing date); i.e. plant height (cm), leaf area (cm^2) and chlorophyll (a+b) as ppm/m^2 or at harvest (150 days from sowing date); i.e. spike length (cm), number of tillers/m^2, number of spikes/m^2, number of grain/spike, weight of 1000 grains (g), biological yield (ton/fed), grain yield (ton/fed) and straw yield (ton/fed) were obtained from the interaction between the conditions of two Celsius lower than the dominant air temperature in the region (pivot irrigation system) × $EM_{5\text{- micronutrientplus}}$ 8000 ppm followed by the same treatment with 4000 ppm concentration. The other interactions were scattered between pivot irrigation system × $EM_{5\text{- micronutrient plus}}$ 8000 ppm and spraying with water as the control treatment.

Table (10). Effect of the interaction between heat stress tolerance (simulated as two irrigation methods) and different new formulas of EM_5 on wheat (*Triticum. aestivum* L. var. Gemiza 9) characteristics at heading stage (combine analysis of 2009/2010 and 2010/2011 seasons).

[1] Irrigation method	EM_5 formulas	Studied characteristics			
		Plant height (cm)	**Leaf area (cm^2)**	**Proline (μ mole proline /g f. wt.)**	**Chlorophyll (ppm)**
[2] Pivot	Water	27 Q	21.6 Q	12.73 C	168.83 Q
	EM_1 (6000 ppm)	64.9 E	51.8 E	4.13 KL	405.18 E
	EM_1 (8000 ppm)	66.4 E	53.1 E	6.87 L	414.83 E
	EM_5-Cu (6000 ppm)	69.5 D	55.5 D	5.09 O	434.13 D
	EM_5-Cu (8000 ppm)	77.3 C	61.7 C	4.58 P	482.36 C
	EM_5-Zn (6000 ppm)	61.8 F	49.4 F	6.87 L	385.89 F
	EM_5-Zn (8000 ppm)	66.4 E	53.1 E	6.36 M	414.83 E
	EM_5-Mn (6000 ppm)	54.1 I	43.2 I	7.89 J	337.66 I
	EM_5-Mn (8000 ppm)	58.8 G	46.9 G	7.64 J	366.60 G
	EM_5-B (6000 ppm)	46.4 K	37 K	9.43 H	289.41 K
	EM_5-B (8000 ppm)	50.2 J	40.1 J	8.91 I	313.54 J
	EM_5-micronutrients (6000 ppm)	80 B	63.9 B	4.45 P	499.25 B
	EM_5-micronutrients (8000ppm)	100.4 A	80.2 A	3.82 Q	627.07 A
[3] Sprinkler	Water	18.9 R	15.1 R	18.19 A	118.18 R
	EM_1 (6000 ppm)	45.5 K	36.3 K	10.19 F	283.63 K
	EM_1 (8000 ppm)	46.5 K	37.1 K	9.82 G	290.38 K
	EM_5-Cu (6000 ppm)	48.7 J	38.9 J	7.28 K	303.89 J
	EM_5-Cu (8000 ppm)	54.1 I	43.2 I	6.55 M	337.66 I
	EM_5-Zn (6000 ppm)	43.3 L	34.6 L	9.83 G	270.12 L
	EM_5-Zn (8000ppm)	46.5 K	37.1 K	9.09 I	290.38 K
	EM_5-Mn (6000 ppm)	37.9 N	30.2 N	11.28 D	236.36 N
	EM_5-Mn (8000 ppm)	41.1 M	32.8 M	10.92 E	256.62 M
	EM_5-B (6000 ppm)	32.5 P	25.9 P	13.47 B	202.59 P
	EM_5-B (8000 ppm)	35.2 O	28.1 O	12.74 C	219.47 O
	EM_5-micronutrients (6000 ppm)	56 h	44.7 H	6.36 M	349.47 H
	EM_5-micronutrients (8000 ppm)	70.3 D	56.1 D	5.45 N	438.95 D

[1] Heat stress treatments were simulated as two irrigation methods referring to Colaizzi et al. (2006).
[2] Lower 2 º C than the dominant average temperature in the area.
[3] The dominant average temperature in the area.
- Means having similar latters at same column has no significant differences at *P*≥ *0.05*.

Table (11). Effect of the interaction between heat stress tolerance (simulated as two irrigation methods) and different new formulas of EM_5 on wheat (*Triticum. aestivum* L. var. Gemiza 9) yield and its attributes at harvest (combine analysis of 2009/2010 and 2010/2011 seasons).

[1] Irrigation method	EM_5 formulas	Studied criteria							
		Spike length (cm)	No. of tillers/ m^2	No. of spikes/ m^2	No. of grains /spike	1000 grains weight (g)	Bio-logical yield (ton/ fed)	Grain yield (ton/ fed)	Straw yield (ton/ fed)
[2] Pivot	Water	3.8 R	124 Q	119 Q	25.8 Q	12.4 Q	1.8 Q	1.1 P	0.7 P
	EM1 (6000 ppm)	9.1 E	298 E	285 E	61.9 E	29.9 E	4.5 E	2.7 E	1.8 E
	EM1 (8000 ppm)	9.3 E	306 E	292 E	63.3 E	30.6 E	4.6 E	2.7 E	1.9 O
	EM_5-Cu (6000ppm)	9.8 D	320 D	305 D	66.2 D	32 D	4.8 D	2.9 D	1.9 O
	EM_5-Cu (8000 ppm)	10.8 C	355 C	340 C	73.6 C	35.5 C	5.3 C	3.2 C	2.1 C
	EM_5-Zn (6000 ppm)	8.7 F	284 F	271 F	58.9 F	28.4 F	4.2 F	2.6 F	1.6 F
	EM_5-Zn (8000 ppm)	9.3 E	305 E	292 E	63.3 E	30.6 E	4.6 E	2.7 E	1.9 E
	EM_5-Mn (6000 ppm)	7.6 I	248 I	238 I	51.6 I	24.9 I	3.7 H	2.2 H	1.5 H
	EM_5-Mn (8000 ppm)	8.2 G	270 G	258 G	56 G	27 G	4 G	2.7 G	1.4 G
	EM_5-B (6000 ppm)	6.5 L	213 K	204 K	44.2 K	21.3 K	3.2 JK	1.9 JK	1.3 JK
	EM_5-B (8000 ppm)	7.0 J	231 J	221 J	47.9 J	23.1 J	3.4 I	2.1 I	1.3 JK
	EM_5-micronutrients (6000 ppm)	11.2 B	368 B	352 B	76.2 B	36.8 B	5.5 B	3.3 B	2.2 B
	EM_5-micronutrients (8000 ppm)	14.1A	462 A	442 A	95.7 A	46.2 A	6.9 A	4.1 A	2.8 A
[3] Sprinkler	Water	2.7 S	87 R	83 R	18 R	8.7 R	1.3 R	0.7 Q	0.6 Q
	EM1 (6000 ppm)	6.4 L	209 K	200 K	43.3 K	20.9 K	3.1 K	1.9 JK	1.2 K
	EM1 (8000 ppm)	6.5 L	214 J	205 K	44.3 K	21.4 K	3.2 JK	1.9 JK	1.3 JK
	EM_5-Cu (6000 ppm)	6.8 K	224 J	214 J	46.4 J	22.4 J	3.3 J	2 J	1.3 JK
	EM_5-Cu (8000 ppm)	7.6 I	249 I	238 I	51.6 I	24.9 I	3.7 H	2.2 H	1 H
	EM_5-Zn (6000 ppm)	6.1M	199 L	190 L	41.2 L	19.9 L	2.9 L	1.8 K	1.1 K
	EM_5-Zn (8000 ppm)	6.5 L	213 K	205 K	44.3 K	21.4 K	3.2 JK	1.9 JK	1.3 JK
	EM_5-Mn (6000 ppm)	5.3 O	174 N	167 N	36.1 N	17.4 N	2.6 N	1.6 M	1 h
	EM_5-Mn (8000 ppm)	5.8 N	189 M	181 M	39.2 M	18.9 M	2.8 M	1.7 L	1.1 K
	EM_5-B (6000 ppm)	4.6 Q	149 P	143 P	30.9 P	14.9 P	2.2 P	1.3 O	0.9 O
	EM_5-B (8000 ppm)	4.9 P	162 O	155 O	33.5 N	16.1 O	2.4 O	1.5 N	0.9 O
	EM_5-micronutrients (6000 ppm)	7.9 H	257 H	246 H	53.4 H	25.7 H	3.8 H	2.3 H	0.6 O
	EM_5-micronutrients (8000 ppm)	9.9 D	323 D	309 D	67 D	32.3 D	4.8 D	2.9 D	1.9 D

[1] Heat stress treatments were simulated as two irrigation methods referring to Colaizzi et al. (2006).

[2] Lower 2 º C than the dominant average temperature in the area.

[3] The dominant average temperature in the area.

- Means having similar latters at same column has no significant differences at $P \geq 0.05$.

Similar trend was obtained under the heat stress conditions of the dominant air temperature of the region (sprinkler irrigation system). This may be due to integration between the potentialities of the pivot irrigation system to reduce the daily temperature in the field by two Celsius, the mannitol as polyol to fully opened stomata or the semi closed ones under the both stressful conditions for its liner configuration, besides mode of action as an osmoprotectants, and the micronutrients, which paly very important roles in promoting the photosynthesis, maintaining the vitality of chlorophyll, especially under stress conditions, in addition to promoting the source-think relationships as reported by Aziz et al. (2009), Kumar et al. (2009) and Millaleo et al. (2010).

CONCLUSION

High temperatures causing heat stress in wheat are expected to increase in frequency across the globe. Heat stress substantially affects grain setting, duration, rate and ultimately grain yield. Nonetheless, the timing, duration and intensity of heat stress determine its impact on grain yield. The adversities of heat stress can be minimized by applying the proper agronomic strategies. One of these strategies is the proper and efficient irrigation method and schedule, which significantly help in minimizing the bad consequences of heat stress as pivot irrigation system. Another agronomic strategy is applying of specific biotic compounds that has the potentiality to enhance the tolerance mechanisms in plants under stress conditions such as $EM_{5\text{-micronutrient plus}}$. Yet, the integration between one or more of agronomic strategies is more capable to mitigate the dramatic consequences of the stress factors similar to what achieved by the interaction between {(pivot irrigation system) × $EM_{5\text{-micronutrient plus}}$ 8000 ppm} in this study.

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تشجيع تحمل الإجهاد الحراري في القمح بإستخدام مستحضر جديد معدل من الكائنات الدقيقة الفعالة - (٥)

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أقيمت تجربتان حقليتان في مزرعة ستة أكتوبر النموذجية بشرق العوينات - محافظة الوادي الجديد خلال المواسم الشتوية المتتالية ٢٠٠٩/٢٠١٠ و٢٠١٠/٢٠١١، لدراسة تأثير عدد من التركيبات الجديدة من مادة EM_5 تحت إسم $EM_{5\text{-micronutrient plus}}$ على تاقلم النبات للإجهاد الحراري في القمح صنف جميزة ٩، حيث تم محاكاة الإجهاد الحراري من خلال طريقتين للري (الري المحوري والري بالرش). يعد الإجهاد الحراري واحداً من أهم عوامل تغير المناخ العالمي، حيث يؤثر إلى حد كبير في فترة ومعدل إمتلاء الحبوب، وبالتالي محصول الحبوب. ومع هذا فإن توقيت ومدة وشدة الإجهاد الحراري لها دور كبير في تحديد تأثير الاجهاد على محصول الحبوب. وقد أشارت النتائج المتحصل عليها من التجارب أن نظام الري المحوري كان أكثر فاعلية تحت ظروف الاجهاد الحراري مقارنة بالري بالرش. وبالمثل فإن رش مركب $EM_{5\text{-micronutrient plus}}$ بتركيز (٨٠٠٠ جزء في المليون) تحت ظروف الإجهاد الحراري قد أعطي أفضل النتائج سواء في مرحلة طرد السنابل (١١٠ يومًا بعد تاريخ الزراعة) وذلك للقياسات التالية؛ إرتفاع النبات (سم)، مساحة الورقة (سم^{٢}) والكلوروفيل (أ + ب) مقدر بالجزء في المليون/م^{٢}، أو عند الحصاد (١٥٠ يومًا بعد تاريخ الزراعة) أي طول السنبلة (سم)، عدد الأشطاء/م^{٢}، وعدد السنابل/م^{٢}، عدد الحبوب/سنبلة، وزن ١٠٠٠ حبة (جم)، المحصول البيولوجي (طن/فدان)، محصول الحبوب (طن/فدان) ومحصول القش (طن/فدان). أما بالنسبة للتفاعل، فقد تم الحصول على أفضل النتائج من خلال تطبيق الري المحوري × $EM_{5\text{-micronutrient plus}}$ بتركيز (٨٠٠٠ جزء في المليون). بينما محتوي البرولين الحر (ميكروجرام البرولين/جم من الوزن الغض) فقط هو الذي أعطي أعلي القيم تحت ظروف نظام الري بالرش أو عند رش النباتات بالماء كمعاملة مقارنة أو التفاعل بين نظام الري بالرش × الرش بالمياه.