Original scientific paper
Journal of Central European Agriculture, 2018, 19(3), p.530-543
DOI: /10.5513/JCEA01/19.3.2070

Biofertilizer (EM-1) effect on growth and yield of three bread wheat cultivars

Muwafaq Abdul-Razzaq Al-NAQEEB[1], Intsar Hadi Hameedi Al-HILFY[1], Jalal Hameed HAMZA[1*], Ammar Sadiq Mahdi AL-ZUBADE[1] and Hadi Mohamad Karim Al-ABODI[2]

[1]University of Baghdad, College of Agriculture, Department of Field Crops, Al-Jadiriya, Baghdad, Iraq

[2]Ministry of Agriculture, State Board for Agricultural Research, Department of Crops, Abu-Ghraib, Baghdad, Iraq, *correspondence: j.hamza@coagri.uobaghdad.edu.iq

Abstract

Farmers keep trying to avoid using chemical fertilizer without losing high yield. A field experiment was conducted in the fields of Agriculture College, University of Baghdad during winter seasons of 2015 and 2016 to investigate the response of three bread wheat (*Triticum aestivum* L.) cultivars (Ibaa99, Abu-Ghraib3 and Buhooth22) to the frequency of spraying with biofertilizer (EM-1) (one time at tillering stage, twice at tillering and stem elongation stages and three times at tillering, stem elongation and booting stages) in addition to the control (without spraying), to the increase of grain yield. Randomized complete block design (RCBD), in split plots arrangement and four replications, was used. Spraying treatments were placed as main plots and cultivars as subplots. The results showed that Ibaa99 cultivar, three times of EM-1 spraying and their interaction gave the highest averages of grain yield (3.89 and 4.31), (3.85 and 4.36) and (4.11 and 4.58 ton*ha^{-1}), respectively, for both seasons. It can be concluded that yield responded significantly to the frequency of EM-1 spraying during vegetative stages.

Keywords: booting, effective microorganisms, spraying, stem elongation, tillering, *Triticum aestivum* L.

Introduction

The results of modern scientific studies have mentioned the threat of agriculture industrialized that uses some of the chemical fertilizer because it has a harmful effect on the environment and human health. Therefore, there is a new trend toward minimizing using these chemical fertilizers and herbicides and focusing on the technology of bio-organic farming which is also known by sustainable agriculture. Bioorganic farming uses organic matter and beneficial microorganism to provide healthy food associated with high quantity and quality while maintaining the environment (Mallik and Williams, 2008). Biofertilizer defines, as all the additives with bio sources such as microbial inoculants that provide the plant with its need of nutrients. Sources of these fertilizers are spontaneous and inexpensive in comparison with the chemical fertilizers. Production concept of biofertilizer rely on that, the soil is full of beneficial microbes that help in decomposing of complex material and providing the plant with reliable and absorbable elements. Soil microbes have a significant role in keeping the biological balance of the soil, so they produce the essential CO_2 for compensating the resulting shortage of photosynthesis in the plant and keeping the gases equilibrium in the atmosphere. EM-1 is an effective microorganism, which is a natural compound, consists of a combination of useful microorganism, in addition to its effective role in enhancing the soil fertility. It doesn't contain any herbicide or harmful chemical substance (Youssef, 2011; Al-Janaby, 2014). EM-1 consists of more than 60 kinds of useful organism that have many groups of microorganisms such as bacteria and fungi (Higa, 1996).

Many studies have been performed on different crops using biofertilizer to increase grain yield. Al-Juboory et al. (2011) studying the effect of fertilizing with biofertilizer EM-1 and comparing it with chemical fertilizer in growth and yield of three wheat cultivars. The best yield was obtained using EM-1 in the concentration of 1:500. Esmailpour et al. (2013) noticed that using biofertilizer *Azotobacter* had led to an increase in grain yield of wheat (3,360 $kg*ha^{-1}$) in comparison to the control (2,839 $kg*ha^{-1}$). Ghaderi-Daneshmand et al. (2012) found that using biofertilizer (biophospor and nitroxin) had led to significant differences in grain numbers per spike, and grain weight compared to the control, which eventually increased the final yield, in addition to the decreasing of the environmental pollution. El-Habbasha et al. (2013) noticed a significant effect on vegetative growth, yield, and its components when using a combination of biocide, nutrient solution and *Azospillium* in wheat. Al-niemi and Ibrahim (2005) noticed a significant difference among wheat cultivars when a high vegetative growth related to the Intisar compared to Tummooz2 and Abu-Ghraib3. Hamza (2011) studied five cultivars of bread wheat (Alfeteh, Ibaa99, Sham6, Clare, and Caxton) and found that Ibaa99 and Alfeteh were significantly surpassed in comparison to the other cultivars, in the performance of seed germination. Jaddoa and Baqir (2012) found that the Ibaa99 cultivar was significantly supremacy in grain number per spike, grain weight, and grain yield compared to Flamour, Abu-Ghraib3, Alfeteh, Ibaa95, and Sham6. The results of Al-amery (2014) showed that Aliraq (wheat cultivar) gave the highest of plant height, number of tillers per square meter, flag leaf area, number of grain per spike, grain weight and grain yield compared to Alfeteh, Altahadi, Sally and Adnanah cultivars.

Wheat is considered the main source of food for human being and animals. It's productivity in Iraq still lower in comparison to the world production. One of the reasons is; the agriculture in Iraq does not rely on modern techniques for nutrients supplementation. Thus, using the application of biofertilization technique is needed to raise the efficiency of plant-feeding which is reflected in production increase per unit area. The aim of this study was to investigate the response of three bread wheat cultivars to the frequency of spraying with biofertilizer (EM-1) to enhance final grain yield.

Materials and methods

A field experiment was conducted in the fields of Agriculture College, University of Baghdad during 2015 and 2016 winter growth seasons to investigate the response of three bread wheat cultivars (Ibaa99, Abu-Ghraib3 and Buhooth22) to the frequency of spraying with biofertilizer (EM-1) (one time at tillering stage, twice at tillering and stem elongation stages and three times at tillering, stem elongation and booting stages) in addition to control (without spraying). The randomized complete block design (RCBD) in split plots arrangement was used with four replications. Spraying treatments were placed as main plots and cultivars as subplots. Area of subplot was 12 m^2 (3*4 m) including twenty rows. Soil and crop were subjected to the routine management such as 100 $kg*ha^{-1}$ of monophosphate fertilizer (P 20%) added at once after tillage, and mixed with soil, 150 $kg*ha^{-1}$ of urea fertilizer (N 46%) divided into four equal amounts and added at three fully emerged leaves, the second node on steam and booting stages, seed rate was 120 $kg*ha^{-1}$, distance between each row was 20 cm, planting depth was 5 cm, and planting date was in 25^{th} and 22^{nd} of November of 2015 and 2016, respectively, (Al-Hassan, 2011). The physical and chemical characteristics of field experiment soil were shown in Table 1.

Table 1. Some of the physical and chemical characteristics of experiment's soil during 2015 and 2016 growing seasons

Soil components	Season of 2015	Season of 2016
Silt ($g*kg\ soil^{-1}$)	530.6	551.6
Clay ($g*kg\ soil^{-1}$)	341.5	329.6
Sand ($g*kg\ soil^{-1}$)	127.9	118.8
Soil texture	Silty clay loam	Silty clay loam
Soil pH	8.1	8.3
Nitrogen ($mg*\ kg\ soil^{-1}$)	29	35
Available phosphorus ($mg*\ kg\ soil^{-1}$)	45	41
Available potassium ($mg*\ kg\ soil^{-1}$)	36	32
Organic matter (%)	8.6	8.3
Ec ($dS*m^{-1}$)	4.3	4.8

Soil analysis was done by central laboratories of College of Agriculture, University of Baghdad.

The biofertilizer was sprayed on shoot system with a rate of 4 cm^3*l^{-1} for each time. Water chlorine free was used. The extraction of EM-1 contained more than 60 types of beneficial microorganisms as photosynthesis bacteria, lactic acid bacteria, yeast, and funguses.

The plants were sprayed in the early morning using a portable sprayer with a capacity of 18 litters and pressure of 405 bars. A soluble substance was added to the spraying solution (0.5 ml*l^{-1}) to increase the efficiency of the spraying solution by reducing the adhesion on the leaf surface and reaching full wetting.

Plants were harvested in full maturity stage in May of 2015 and 2016. One square meter was chosen from each plot at harvesting to calculate the following traits:

Plant height (cm) was calculated from the ground to the top of the main spike, excluding the awn, as an average for the main stem. It was randomly taking from ten plants for one square meter.

Tillers number per square meter (tiller*m^{-2}) was randomly calculated from one square meter of each plot without counting the main steam and was differentiated by colored plastic rings.

Percentage of tillers carrying spikes (%) was calculated after the harvesting by the following equation:

Percentage of tillers carrying spikes (%) = (No. of spikes per square meter) / (Whole No. of tillers per square meter) × 100

Flag leaf area (cm^2) was measured from the average of ten flag leaves of the same main stems used in calculating the plant height according to the following equation (Thomas, 1975).

Flag leaf area = Length of flag leaf × Width of flag leaf in its middle × 0.95

Spikes number per square meter (spike*m^{-2}) was calculated through an average of spikes number for one square meter harvested from each plot.

Grain number per spike (grain*spike^{-1}) was calculated through an average of grain number of randomly taking ten spikes from each plot.

Weight of 1,000 grain (g) was manually calculated from each plot and weighted with four-digit balance.

Grain yield (ton*ha^{-1}) was calculated after manual threshing of harvested plants of one square meter of each plot, isolating the straw from the grain, and purifying them well, the grain were calculated and combined with the grain used in calculating 1,000 grain for the same treatment and weighted. Then, the weight was converted from g*m^{-2} to ton*ha^{-1} and grain weights were corrected according to the safe moisture content (12%).

Statistical analysis of collected data was done according to the analysis of variance at P≤0.05 by using the Genstat software. The test of least significant differences (LSD) was used to compare calculated averages of traits (Steel and Torrie, 1981).

Results and discussions

Plant height (cm)

The effect of cultivars, frequency of EM-1 spraying and their interaction was significant on plant height for both seasons. The highest plant height related to the cultivar of Abu-ghraib3 (109.4 and 114 cm), three times of EM-1 spraying (114 and 117.5 cm) and their interaction (115.4 and 120.5 cm) and Abu-ghraib3 × three times didn't significantly different with Ibaa99 × three times for both seasons (Table 2). This variation maybe due to the genetic difference of cultivars in the internodes length, especially the upper internodes and that in agreement with Amaya et al. (1972) who indicated that plant height is one of the characteristics dominated by an additional gene. It also agreed with the results of El-Kalla et al. (2002); Amanullah et al. (2012). All indicated that wheat cultivars varied in plant height trait. This increase in plant height is because of the role of biofertilizer in enhancing growth through increasing photosynthesis, producing materials that having a biological effect as hormones or enzymes. The results were similar to Amal et al. (2011); Kandil et al. (2011); Muhammed et al. (2013).

Table 2. Effect of frequency of biofertilizer (EM-1) spraying on plant height (cm) of three bread wheat cultivars during 2015 and 2016 growth seasons

Frequency of EM-1 spraying during growth stages	Cultivars of bread wheat							
	Winter season of 2015				Winter season of 2016			
	Abu-Ghraib3	Ibaa99	Buhooth22	Average	Abu-Ghraib3	Ibaa99	Buhooth22	Average
Control	102.1^{fg}	100.5^{gh}	98.3^{h}	100.3^{d}	104.2^{e}	103.3^{e}	100.6^{f}	102.7^{d}
One time	109.3^{de}	108.7^{e}	104^{f}	107.3^{c}	111.8^{c}	107.8^{d}	103.4^{e}	107.7^{c}
Twice	110.6^{cde}	111.1^{cd}	109.3^{de}	110.3^{b}	119.6^{a}	114.4^{b}	106.3^{d}	113.4^{b}
Three times	115.4^{a}	113.8^{ab}	112.7^{bc}	114^{a}	120.5^{a}	118.9^{a}	113^{bc}	117.5^{a}
Average	109.4^{a}	108.5^{b}	106.1^{c}		114^{a}	111.1^{b}	105.8^{c}	

Averages followed by the same letters didn't differ significantly at P≤0.05

Tillers number per square meter (tiller*m^{-2})

The effect of cultivars, frequency of EM-1 spraying and their interaction were significant on tillers number for both seasons. The Abu-Ghraib3 cultivar, three times of EM-1 spraying and their interaction gave the highest tillers number (516 and 518.9, (524.2 and 527.1, and 545.9 and 547.2 tiller*m^{-2}), respectively, for both seasons (Table 3). These results were in agreement with El-Sayed and Hammad (2007); Badr et al. (2009). They had a significant difference in a tillers number of wheat cultivars, which was due to the genetic differences, in addition to the possible role of biofertilizer in stimulating plant growth regulators and increase cells division, stimulating of buds growth and eventually an increment in tillers number. These results agreed with Al-Juboory et al. (2011); Abd El-Lattief (2012); Youssef et al.

(2013) when they found the increase in tillers number of wheat as a response to the biofertilizer application.

Table 3. Effect of frequency of biofertilizer (EM-1) spraying on tillers number per square meter (tiller*m^{-2}) of three bread wheat cultivars during 2015 and 2016 growth seasons

Frequency of EM-1 spraying during growth stages	Cultivars of bread wheat							
	Winter season of 2015				Winter season of 2016			
	Abu-Ghraib3	Ibaa99	Buhooth22	Average	Abu-Ghraib3	Ibaa99	Buhooth22	Average
Control	476.3^{fg}	451.8^{h}	440.6^{h}	456.3^{d}	483.6^{f}	468.4^{g}	462.7^{g}	471.6^{d}
One time	510^{cd}	498.2^{de}	472.3^{g}	493.5^{c}	512.5^{d}	498.5^{e}	483^{f}	498^{c}
Twice	531.9^{b}	517.4^{c}	487.7^{ef}	512.3^{b}	532.3^{b}	515.3^{cd}	498.5^{e}	515.4^{b}
Three times	545.9^{a}	532.2^{b}	494.5^{e}	524.2^{a}	547.2^{a}	527.1^{bc}	506.9^{de}	527.1^{a}
Average	516^{a}	499.9^{b}	473.8^{c}		518.9^{a}	502.3^{b}	487.8^{c}	

Averages followed by the same letters didn't differ significantly at P≤0.05

Percentage of tillers carrying spikes (%)

Results in Table 4 showed no significant difference among cultivars. However, there was a significant effect among frequencies of EM-1 spraying and among interactions on this trait for both seasons. The highest percentage of tillers carrying spikes was for three times of EM-1 spraying (83.3 and 85.8%) and Ibaa99 cultivar × three times (84.3 and 86.3%) for both seasons (Table 4). That is maybe due to an increase in the availability of assimilating supply of photosynthesis for the benefit of tillers emergence, which contributes to increase their productivity, and subsequently an increase in the number of tillers carrying spikes. These results were in agreement with El-Sayed and Hammad (2007); Youssef et al. (2013).

Flag leaf area (cm^2)

The effect of cultivars, frequency of EM-1 spraying and their interaction were significant in the area of flag leaf for both seasons. The highest average belonged to the Ibaa99 cultivar (40.4 and 41.8 cm^2), three times of EM-1 spraying (39.8 and 39.2 cm^2) and their interaction (42.4 and 44.4 cm^2) for both seasons (Table 5).

Table 4. Effect of frequency of biofertilizer (EM-1) spraying on tillers carrying spikes (%) of three bread wheat cultivars during 2015 and 2016 growth seasons

Frequency of EM-1 spraying during growth stages	Cultivars of bread wheat							
	Winter season of 2015				Winter season of 2016			
	Abu-Ghraib3	Ibaa99	Buhooth22	Average	Abu-Ghraib3	Ibaa99	Buhooth22	Average
Control	73.2^{d}	74.4^{d}	73.4^{d}	73.6^{d}	76.2^{e}	76.9^{e}	76.5^{e}	76.5^{d}
One time	80.2^{c}	81.5^{abc}	79.7^{c}	80.5^{c}	82.1^{d}	82.8^{cd}	83^{cd}	82.6^{c}
Twice	80.9^{bc}	83.1^{ab}	82^{abc}	82.3^{b}	84.2^{abcd}	84.7^{abc}	83.5^{bcd}	84.1^{b}
Three times	82.1^{ab}	84.3^{a}	83.6^{ab}	83.3^{a}	85.2^{abc}	86.3^{a}	85.9^{ab}	85.8^{a}
Average	79.4^{a}	80.8^{a}	79.7^{a}		81.9^{a}	82.7^{a}	82.2^{a}	

Averages followed by the same letters didn't differ significantly at P≤0.05

Table 5. Effect of frequency of biofertilizer (EM-1) spraying on flag leaf area (cm^2) of three bread wheat cultivars during 2015 and 2016 growth seasons

Frequency of EM-1 spraying during growth stages	Cultivars of bread wheat							
	Winter season of 2015				Winter season of 2016			
	Abu-Ghraib3	Ibaa99	Buhooth22	Average	Abu-Ghraib3	Ibaa99	Buhooth22	Average
Control	31.3^{g}	37.1^{de}	28^{h}	32.1^{d}	32.9^{de}	39.4^{b}	31.1^{e}	34.5^{c}
One time	35.6^{ef}	39.7^{bc}	31.8^{g}	35.7^{c}	33.6^{d}	40.1^{b}	33.4^{d}	35.7^{b}
Twice	38.3^{cd}	41.3^{ab}	34.2^{f}	37.9^{b}	36.9^{c}	43.3^{a}	35.7^{c}	38.6^{a}
Three times	39.9^{bc}	42.4^{a}	36.2^{ef}	39.8^{a}	37^{c}	44.4^{a}	36.2^{c}	39.2^{a}
Average	36.3^{b}	40.4^{a}	32.5^{c}		35.1^{b}	41.8^{a}	34.1^{c}	

Averages followed by the same letters didn't differ significantly at P≤0.05

The difference in this trait referred to the differences in cultivars genetic variation. Amal et al. (2011); Muhammad et al. (2014) agree with these results. They pointed to the differences in wheat cultivars regarding flag leaf area. The increase in flag leaf area was accompanied by the increase in the frequency of EM-1 spraying. That could explain by the effect of EM-1 in stimulating the growth regulators of plants, and an increase nitrogen fixation, which led to an increase in the vegetative growth through elongating and division of cells that reflected an increase in the flag leaf area. These results in agreement with Kabesh et al. (2009); Youssef et al. (2013); Muhammad et al. (2014). They indicated a significant role for biofertilizer in increasing vegetative growth of wheat.

Spikes number per square meter (spike*m^{-2})

The effect of cultivars, frequency of EM-1 spraying and their interaction were significant on the number of spikes per square meter for both seasons. The highest average belonged to the Ibaa99 cultivar (418.1 and 429.9 spike*m^{-2}), three times of EM-1 spraying (437 and 450.4 spike*m^{-2}) and their interaction (460 and 472.1 spike*m^{-2}), for both seasons (Table 6). This is referred to the transcendence of Ibaa99 cultivar in the percentage of tillers carrying spikes per square meter (Table 4). These results in agreement with Al-Juboory et al. (2011); El-Habbasha et al. (2013). The increase in a number of spikes per square meter was in correspondence with an increase in the frequency of EM-1 spraying. This is explained by the increase in a number of tillers and percentage of tillers carrying spikes (Tables 3 and 4) as a response to the frequencies of EM-1 spraying. These results in agreement with El-Sayed and Hammad (2007), Kandil et al. (2011); Amanullah et al. (2012); Muhammad et al. (2014).

Grain number per spike (grain*spike^{-1})

The effect of cultivars, frequency of EM-1 spraying and their interaction were significant on grain number per spike for both seasons. The highest average related to Ibaa99 cultivar (43.9 and 46.4 grain*spike^{-1}), three times of EM-1 spraying (45.1 and 48.3 grain*spike^{-1}) and their interaction (45.7 and 48.7 grain*spike^{-1}) for both seasons (Table 7). This could explain by genetic variation in the number of spikelet*spike^{-1} which is determined by the number of fertile flowers on a spike (Amanullah et al., 2012; Pandey et al., 2013). The reason behind the increase of grain number per spike accompanied with the increase of frequencies of spraying with EM-1 is, because of the capacity of sink in wheat depends on well vegetative growth, that response more to increase spraying frequency (Tables 3 and 5). These results go along with El-Kalla et al. (2002), El-Habbasha et al. (2013); Muhammed et al. (2013); Muhammad et al. (2014).

Weight of 1,000 grain (g)

Results in Table 8 indicated no significant effect for the cultivars, frequency of EM-1 spraying and their interaction on the weight of 1,000 grain for both seasons.

Table 6. Effect of frequency of biofertilizer (EM-1) spraying on spikes number per square meter (spike*m^{-2}) of three bread wheat cultivars during 2015 and 2016 growth seasons

Frequency of EM-1 spraying during growth stages	Cultivars of bread wheat							
	Winter season of 2015				Winter season of 2016			
	Abu-Ghraib3	Ibaa99	Buhooth22	Average	Abu-Ghraib3	Ibaa99	Buhooth22	Average
Control	331.6^{h}	354.4^{g}	322.3^{h}	336.1^{d}	353.5^{i}	372.1^{h}	352.3^{i}	359.3^{d}
One time	396.9^{e}	415.9^{cd}	378.9f	397.2^{c}	404.4^{fg}	424.4^{de}	400.8^{g}	409.9^{c}
Twice	424.2^{c}	442.1^{b}	399.4^{e}	421.9^{b}	428.6^{de}	451^{b}	416.6^{ef}	432.1^{b}
Three times	444.8^{a}	460^{a}	406.1^{de}	437^{a}	443.7^{bc}	472.1^{a}	435.5^{cd}	450.4^{a}
Average	399.4^{b}	418.1^{a}	376.7^{c}		407.6^{b}	429.9^{a}	401.3^{b}	

Averages followed by the same letters didn't differ significantly at P≤0.05

Table 7. Effect of frequency of biofertilizer (EM-1) spraying on grain number per spike (grain*spike^{-1}) of three bread wheat cultivars during 2015 and 2016 growth seasons

Frequency of EM-1 spraying during growth stages	Cultivars of bread wheat							
	Winter season of 2015				Winter season of 2016			
	Abu-Ghraib3	Ibaa99	Buhooth22	Average	Abu-Ghraib3	Ibaa99	Buhooth22	Average
Control	41.8^{gh}	42.5^{fgh}	41.6^{h}	42^{d}	43.3^{e}	43.1^{e}	42.7^{e}	43^{d}
One time	43.4^{cdef}	43.2^{cdefg}	42.8^{efgh}	43.1^{c}	46.7^{abcd}	47.1^{abcd}	45.7^{d}	46.2^{c}
Twice	44.1^{bcde}	44.3^{abcd}	43^{defgh}	43.8^{b}	46.5^{bcd}	47.8^{abc}	46.2^{cd}	46.8^{b}
Three times	45.1^{ab}	45.7^{a}	44.6^{abc}	45.1^{a}	48.3^{ab}	48.7^{a}	47.9^{abc}	48.3^{a}
Average	43.6^{b}	43.9^{a}	43^{c}		46.2^{a}	46.4^{a}	45.6^{b}	

Averages followed by the same letters didn't differ significantly at P≤0.05

The insignificant effect of studied factors may be due to the compensation among yield components of wheat when there was an increase in the number of spikes per square meter and the number of grain per spike (Tables 6 and 7) as a response to studied factors and their interaction.

Table 8. Effect of frequency of biofertilizer (EM-1) spraying on weight of 1,000 grain (g) of three bread wheat cultivars during 2015 and 2016 growth seasons

Frequency of EM-1 spraying during growth stages	Cultivars of bread wheat							
	Winter season of 2015				Winter season of 2016			
	Abu-Ghraib3	Ibaa99	Buhooth22	Average	Abu-Ghraib3	Ibaa99	Buhooth22	Average
Control	35.19	35.88	36	35.69	34.68	34.54	34.4	34.54
One time	36.2	35.46	35.58	35.74	34.17	34.37	34.33	34.29
Twice	35.87	35.38	35.65	35.63	34.9	34.46	34.88	34.74
Three times	35.11	35	35.19	35.1	34.49	34.31	34.9	34.56
Average	35.59	35.43	35.1		34.56	34.42	34.62	

Effect of cultivars, frequency of spraying and their interaction were not significant at $P \leq 0.05$ for both seasons

Grain yield (ton*ha^{-1})

The effect of cultivars, frequency of EM-1 spraying and their interaction were significant on grain yield for both seasons. Ibaa99 cultivar gave the highest grain yield (3.89 and 4.31 ton*ha^{-1}). It's not significantly different than the Abu-Ghraib3 cultivar for both seasons (Table 9). The reason behind transcendent Ibaa99 cultivar is because it gave the highest averages of the number of spikes per square meter and the number of grain per spike (Tables 6 and 7). These results go along with the results of El-Sayed and Hammad (2007); Amanullah et al. (2012); Pandey et al. (2013). Three times of EM-1 spraying gave the highest grain yield (3.85 and 4.36 ton*ha^{-1}) for both seasons (Table 9). This is maybe due to the increase in the number of spikes per square meter and the number of grain per spike (Tables 6 and 7). These results were similar to the Al-Juboory et al. (2011), Ghaderi-Daneshmand et al. (2012); El-Habbasha et al. (2013); Esmailpour et al. (2013) who confirmed the response of wheat to spraying with biofertilizer. Ibaa99 cultivar which had three times of EM-1 spraying gave the highest grain yield (4.11 and 4.58 ton*ha^{-1}). This is maybe due to the significant transcendent of an interaction treatment of Ibaa99 cultivar with three times of EM-1 spraying on the number of spikes per square meter and the number of grain per spike (Tables 6 and 7).

Table 9. Effect of frequency of biofertilizer (EM-1) spraying on grain yield (ton*ha^{-1}) of three bread wheat cultivars during 2015 and 2016 growth seasons

Frequency of EM-1 spraying during growth stages	Cultivars of bread wheat							
	Winter season of 2015				Winter season of 2016			
	Abu-Ghraib3	Ibaa99	Buhooth22	Average	Abu-Ghraib3	Ibaa99	Buhooth22	Average
Control	3.43^{cde}	3.65^{abcd}	2.87^{f}	3.32^{c}	3.8^{cd}	3.91^{bcd}	3.56^{d}	3.76^{b}
One time	3.6^{bcde}	3.89^{abc}	3.15^{ef}	3.55^{bc}	4.23^{abc}	4.36^{ab}	3.98^{bcd}	4.19^{a}
Twice	3.75^{abc}	3.94^{ab}	3.27^{def}	3.66^{ab}	4.29^{abc}	4.41^{ab}	4.02^{bcd}	4.24^{a}
Three times	4^{ab}	4.11^{a}	3.43^{cde}	3.85^{a}	4.36^{ab}	4.58^{a}	4.14^{abc}	4.36^{a}
Average	3.69^{a}	3.89^{a}	3.18^{b}		4.17^{a}	4.31^{a}	3.92^{b}	

Averages followed by the same letters didn't differ significantly at P≤0.05

Conclusions

It can be concluded that spraying of EM-1 enhanced plant efficiency, increased an incidence of elongation and division of cells in all vegetative organs. Thus, stimulating branches led to producing a higher number of spikes, as well as an increment in grain number per spike which led to higher yield. According to the response of wheat cultivars toward spraying of biofertilizer, it can be recommended frequent spraying of biofertilizer at tillering, stem elongation and booting stages. Also, conduct further studies under a wide range of environmental conditions using different concentrations and frequencies for biofertilizer.

References

Abd El-Lattief, E. A. (2012) Improving bread wheat productivity and reduce use of mineral nitrogen by inoculation with *Azotobacter* and *Azospirillum* under arid environment in Upper Egypt. In: Internationnal Society for Applied Life Sciences (ISALS), International Conference on Applied Life Sciences. Konya, Turkey, 10-12 September 2012, IntechOpen, 393-398.

Al-amery, A. A. (2014) Genotype differences of bread wheat (*Triticum aestivum* L.) in some growth and yield indicators under salinity stress. Journal University of Kerbala, 12 (2), 203-211. Available at: https://www.iasj.net

Al-Hassan, M. F. H. (2011) Understanding of tillering in different wheat cultivars as influenced by seeding rate and nitrogen levels and its relationship to grain yield and its components. Ph.D. Thesis. Baghdad: University of Baghdad, College of Agriculture, Department of Field Crops.

Al-Janaby, M. A. A. (2014) Effect of magnetized irrigation water and bio-fertilizer spraying on growth and yields of maize (*Zea mays* L.). Journal of Genetic and Environmental Resources Conservation, 2 (1), 10-15. Available at: http://jgerc.com/wp-content/uploads/2017/11/2-3.pdf

Al-Juboory, Kh. Kh., Dawod, K. M., Al-Abedrabba, W. M. Sh. (2011) Using enriched bio EM1 technique on some important field crops. Journal of Tikrit University for Agricultural Sciences, 11 (2), 97-104. Available at: https://www.iasj.net

Al-niemi, S. N., Ibrahim, T. I. (2005) Effect of phosphorus application on the vegetative growth of three wheat varieties. Rafidain Journal of Science, 16 (6), 1-11. Available at: https://www.iasj.net

Amal, A. M. A., Magda, G. A., Mohamed, H., Tawfik, M. M. (2011) Integrated effect of organic and biofertilizers on wheat productivity in new reclaimed sandy soil. Research Journal of Agriculture and Biological Sciences, 7 (1), 105-114. Available at: http://www.aensiweb.net

Amanullah, A., Kurd, A. A., Khan, S., Ahmed, M., Khan, J. (2012) Biofertilizer - a possible substitute of fertilizers in production of wheat variety zardana in Balochistan. Pakistan Journal of Agricultural Research, 25 (1), 44-49. Available at: http://www.pjar.org.pk

Amaya, A. A., Busch, R. H., Lebsock, K. L. (1972) Estimates of genetic effects of heading date, plant height, and grain yield in durum wheat. Crop Science, 12 (4), 478-481. DOI: http://dx.doi.org/10.2135/cropsci1972.0011183X001200040026x

Badr, E. A., Ibrahim, O. M., El-Kramany, M. F. (2009) Interaction effect of biological and organic fertilizers on yield and yield components of two wheat cultivars. Egyptian Journal of Agronomy, 31 (1), 17-27. Available at: Google Scholar

El-Habbasha, S. F., Tawfik, M. M., El-Kramany, M. F. (2013) Comparative efficacy of different bio-chemical foliar applications on growth, yield and yield attributes of some wheat cultivars. World Journal of Agricultural Sciences, 9 (4), 345-353. DOI: http://www.idosi.org/10.5829/idosi.wjas.2013.9.4.1760

El-Kalla, S. E., Sharief, A. E., Leilah, A. A., Abdalla, A. M., El-Awami, S. A. K. (2002) Utilization of some agriculture practices to improve some wheat cultivars productivity: I- yield and its components. Mansoura Journal of Agricultural Science, 27 (10), 6583-6597. Available at: http://jas.mans.edu.eg

El-Sayed, S. A., Hammad, S. M. (2007) Effect of nitrogen and potassium levels on agronomic and quality traits in three bread wheat cultivars. Mansoura Journal of Agricultural Science, 32 (7), 5139-5153. Available at: http://jas.mans.edu.eg

Esmailpour, A., Hassanzadehdelouei, M., Madani, A. (2013) Impact of livestock manure, nitrogen and biofertilizer (*Azotobacter*) on yield and yield components of wheat (*Triticum aestivum* L.). Cercetări Agronomice în Moldova, 46 (2), 5-15. Available at: http://www.uaiasi.ro

Ghaderi-Daneshmand, N., Bakhshandeh, A., Rostami, M. R. (2012) Biofertilizer affects yield and yield components of wheat. International Journal of Agriculture Research and Review, 2 (6), 699-704. Available at: Google Scholar

Hamza, J. H. (2011) Relation of temperature in germination attributes of some bread wheat cultivars. The Iraqi Journal of Agricultural Sciences, 42 (2), 45-52. Available at: https://www.iasj.net

Higa, T. (1996) An Earth saving revolution. 2nd edition. Tokyo: Sunmark Publishing Inc.

Jaddoa, K. A., Baqir, H. A. (2012) Effect of sowing depth on grain yield and its components of six bread wheat cultivars. The Iraqi Journal of Agricultural Science, 43 (1), 25-37. Available at: https://www.iasj.net

Kabesh, M. O., El-Kramany, M. F., Sary, G. A., El-Naggar, H. M., Gehan, Sh. H. B. (2009) Effect of sowing methods and some bio-organic fertilization treatments on yield and yield components of wheat. Research Journal of Agriculture and Biological Sciences, 5 (1), 97-102. Available at: https://www.cabdirect.org

Kandil, A. A., El-Hindi, M. H., Badawi, M. A., El-Morarsy, S. A., Kalboush, F. A. H. (2011) Response of wheat to rates of nitrogen, biofertilizers and land leveling. Crop & Environment, 2, 46-51. Available at: Google Scholar

Mallik, M. A., Williams, R. D. (2008) Plant growth promoting rhizobacteria and mycorrhizal fungi in sustainable agriculture and forestry. In: Zeng, R. S., Mallik, A. U., Luo S. M., eds. Allelopathy in sustainable agriculture and forestry. New York: Springer, 321-345. Available at: https://www.mssoy.org/uploads/files/mallik-and-williams-2008.pdf

Muhammad, H., Zaman, A., Khalil, S. K., Shah, Z. (2014) Effect of beneficial microbes (BM) on the efficiency of organic and inorganic N fertilizers on wheat crop. Sarhad Journal of Agriculture, 30 (1), 7-14. Available at: http://www.aup.edu.pk

Muhammed, A. S. S., Memon, H. UR. R., Baloch, S. K., Osman, K. A., Ali, S. A. I. (2013) Effect of bio-organic and inorganic fertilizers on the growth and yield of wheat (*Triticum aestivum* L.). Persian Gulf Crop Protection, 2 (4), 15-24. Available at: Google Scholar

Pandey, B., Basnet, K., Bhatta, M., Sah, S., Thapa, R., Kandel, T. (2013) Effect of row spacing and direction of sowing on yield and yield attributing characters of wheat cultivated in Western Chitwan, Nepal. Agricultural Sciences, 4 (7), 309-316. DOI: http://dx.doi.org/10.4236/as.2013.47044.

Steel, R. G. D., Torrie, J. H. (1981) Principles and procedures of statistic. New York: McGraw-Hill Book Co., Inc.

Thomas, H. (1975) The growth response of weather of simulated vegetative swards of single genotype of *Lolium perenne*. The Journal of Agricultural Science, 84, 333-343. DOI: https://doi.org/10.1017/S0021859600052485

Youssef, M. A. (2011) Synergistic impact of effective microorganisms and organic manures on growth and yield of wheat and marjoram plants. Assiut, Egypt: University of Assiut, Faculty of Agriculture. Available at: http://www.academia.edu

Youssef, M. A., EL-Sayed, M. M., Sadek, I. I. (2013) Impact of organic manure, bio-fertilizer and irrigation intervals on wheat growth and grain yield. American-Eurasian Journal of Agricultural and Environmental Sciences, 13 (11), 1488-1496. DOI: http://www.idosi.org/10.5829/idosi.aejaes.2013.13.11.76183