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The effect of biofertilizer and nitrogen rates on quantitative and qualitative properties of strawberry cultivar 'Paros'

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Abstract

In order to investigate the effect of EM (Effective Microorganisms) application and various nitrogen levels on qualitative and quantitative traits of strawberry cultivar 'Paros'. The layout of the experiment was based on randomized complete block with three replications. The research was conducted in an experimental planting of strawberry cv. 'Paros' at the University of Mohaghegh Ardabili, during 2014-2015. EM was used at four rates (0, 1, 2, and 3%) in two ways (soil and foliar application) and nitrogen was used in three rates (50, 100 and 150 kg*ha^{-1} soil application). Leaf area, number of flowers per plant, number of fruit per plant, length, volume, fresh and dry weight of fruit, yield per plant, vitamin C content, total soluble solids content and total acidity were measured. There was significant difference between the EM and nitrogen treatments for all the traits except fruit shelf life. Mean comparison revealed that 2% concentration of EM had positive effect on most of the traits. Regarding nitrogen treatments, the best yield was obtained at application of 100 kg*ha^{-1}.

Keywords: application type, fruit shelf life, soluble solids, urea, yield

Introduction

The useful microorganisms contained in effective microorganisms (EM) mix include photosynthetic bacteria (*Rhodopseudomonas palustris, Rhodobacter sphacrodes*), lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus casei, Streptococcus*), yeasts (*Saccharomyces spp*.), actinomycetes (*Streptomyces spp*.) and fermentation fungi (*Aspergillus, Penicillium*) (Higa and Parr, 1994). EM are increased in soil and plants by combination the EM, this material is used in organic farming to improve the yield and quality of crops (Xu et al., 2001). EM promotes health and yield by enhancing photosynthesis, producing bioactive compounds such as hormones and enzymes, controlling soil-borne diseases and facilitating organic matter decay in the soil. When the combination of EM spray on plants or with soil be used to can increase the population of photosynthetic bacteria and the nitrogen stabilizer. This phenomenon causes plant growth, yield and higher quality by increasing the efficiency of photosynthesis and nitrogen fixation levels (Higa, 2000). Many authors documented favorable effects of using EM biostimulant on growth yield of several crops. Application of EM together with mineral fertilizers increased crop growth indices, yield of fruit, physical quality and chemical composition of strawberry (Hassan, 2015). Application of EM in strawberry improved quality, soluble solids content, ascorbic acid, firmness, tissue color, titratable acidity, total sugars content and also decreased weight loss, decay percentage and anthocyanin content of the fruits. EM application is recommended for improving fertility, fruit quality and storage of strawberry (Hassan and Emam, 2015). As a result of EM application, fruit yield and quality including weight, size, total sugar percentage and total soluble solids percentage at harvest time was increased compared to control (Mohamed et al., 2007).

EM inoculation together with chicken manure enhanced photosynthesis and fruit yield and quality (vitamin C content) of tomato plants (Xu et al., 2001). Application of EM in soil reclaimed with manure, NPK and ½ NPK significantly increased stem biomass of mung bean (*Viagna radiate* (L.) Wilczek var. NIAB Mung 98) (Javaid and Bajwa, 2011).

The present study was conducted to investigate the effect of EM treatments practiced as foliar application or irrigation at various concentrations of nitrogen on qualitative traits and shelf life of fruits of strawberry cultivar 'Paros'.

Material and methods

The layout of the experiment was based on complete randomized block with three replications (total of 72 experimental units). EM were applied in two ways (soil irrigation and foliar application) each at four rates (0, 1, 2, and 3%) and nitrogen treatment as urea fertilizer at three rates (50, 100 and 150 kg*ha^{-1}) in University of Mohaghegh Ardabili during 2014-2015. Nitrogen rate was set at main plots, EM application type was set at sub plots and EM concentration was set at sub-sub plots. After analysis of soil and manure based on 50 t*ha^{-1}, the manure was added to the soil. Land was prepared in late August and potassium and phosphorous fertilizers were added as 150 and 50 kg*ha^{-1}, respectively. Strawberries were planted in early November 2014 in double rows on beds covered with black polyethylene foil. Plant spacing was 25 cm. Strawberry cultivar 'Paros' were taken from Zaribar strawberry nursery in Marivan and transported to Ardebil. EM biofertilizer was purchased from Emkanpazir Pars Company, the agency of EM Research Organization (EMRO) in Iran. According to the catalogue, the EM contains bokashi compounds, water, cane molasses, aloe vera, a natural mix of photosynthetic bacteria, lactic acid bacteria, yeasts and EM_1 (containing filamentous fungi). Half of nitrogen amount was added at the time of planting, 25% was added at late April and 25% was added at late May by irrigation water. EM treatments were carried out weekly after initiation of plant for two mounts through irrigation systems or foliar application (control plants were treated with distilled water). Plant irrigation was performed weekly and equally (25 l per plot).

Leaf area index was measured by leaf area meter device (ADC BioScientific Ltd AM300, UK) and data were expressed in mm^2. Flower and fruit number was measured in each plot and divided by the number of plants per plot. Fruit length was measured by digital caliper and data were expressed in mm. Fruit volume was estimated by graded cylinder and data were expressed in terms of cm^3. Fresh weight was measured by digital balance immediately after delivering to laboratory. For measuring dry weight, the fruits were dried in oven at 37 ^oC for 72 h and then the weight was measured by digital balance (AND, EK1200i, Japan). The fruits harvested from each plant were weighed by digital balance and average yield of each stand was calculated and data were expressed in g. To measure vitamin C (mg*100 g^{-1} fw) based on titration was conducted using 2,6-dichlorophenol indophenols (AOAC, 1989). Refractometer (OE-ATC France) was used to measure soluble solids. Total acidity was measured by titration with 0.1N sodium hydroxide.

Data were analyzed by SAS 9.3 software and mean values were compared by LSD test ($P \leq 0.05$).

Results and discussion

All the measured traits were significantly affected by EM application ($P \leq 0.01$) with the exception for fruit formation percentage. Different application method (soil and foliar application) of EM had no significant effect on qualitative and quantitative traits of strawberry cultivar 'Paros'. Nitrogen rates had significant effect on leaf area, leaf number, fruit number, length, fresh and dry weight, yield and vitamin C content ($P \leq 0.01$) and total soluble solids content and fruit acidity ($P \leq 0.05$) (Tables 1 and 2).

Table 1. Effect of application EM and various rates of N on qualitative and quantitative traits of strawberry cultivar 'Paros'

SOV	Leaf area (mm^2)	Number of flowers per plant	Number of fruits per plant	Fruit length (mm)	Fruit volume (cm^3)
N	2,083,897.13**	13.79**	8.33**	17.14**	12.12**
EM	1,644,919.1**	10.6**	16.86**	25.65**	12.27**
EM application method	201,519.37^{ns}	0.03^{ns}	0.0006^{ns}	0.25^{ns}	1.12^{ns}
N×EM	148,074.04^{ns}	0.08^{ns}	0.2^{ns}	1.59^{ns}	0.69^{ns}
N×EM application method	80,828.03^{ns}	0.49^{ns}	0.5^{ns}	0.66^{ns}	1.05^{ns}
EM×EM application method	3,231.8^{ns}	0.54^{ns}	3.6^{ns}	1.15^{ns}	3.33^{ns}
N×EM×EM application method	373,239.42^{ns}	0.19^{ns}	0.49^{ns}	0.78^{ns}	0.35^{ns}

ns- not significant, *P≤0.05, **P≤0.01

Table 2. Effect of application EM and various rates of N on qualitative and quantitative traits of strawberry cultivar 'Paros'

SOV	Fruit weight (g)	Fruit dry weight (g)	Yield (g)	Vitamin C (mg* 100 g^{-1} fw)	Total soluble solids (°brix)	Total acidity (%)
N	23.68**	0.11**	11,442.39**	130.06 **	4.81*	0.14*
EM	10.4**	0.61**	8,609.04**	70.7**	5.46**	0.22**
EM application method	1*	0.0004ns	308.6ns	4.5ns	2.98ns	0.01ns
N×EM	0.56*	0.01ns	142.03ns	5.99ns	1.1ns	0.01ns
N×EM application method	0.31ns	0.0004ns	270.24ns	2.18ns	0.84ns	0.01ns
EM×EM application method	1ns	0.001ns	164.78ns	0.2ns	0.43ns	0.01ns
N×EM×EM application method	0.12ns	0.0006ns	44.96ns	0.78 ns	0.18 ns	0.006ns

ns- not significant, *P≤0.05, **P≤0.01

Effect of EM on the quantitative and qualitative parameters of strawberry fruit

Mean comparison results indicated that the highest leaf area was achieved in 2% and 3% of EM (6,175.2 and 6,097 mm^2, respectively), indicating a significant difference with that of control (Table 3). This finding is in accordance with those reported by other authors (Mohamed et al., 2007; Shokouhian et al., 2013). Increased leaf area can be attributed to profound effect of the growth regulators synthesized by these microorganisms and improvement of soli nutrients uptake (Martin et al., 1989).

Table 3. Effect of different rates of EM on quantitative traits of strawberry cultivar 'Paros'

EM	Leaf area (mm^2)	Flower number per plant	Fruit number per plant	Fruit length (mm)	Fruit volume (cm^3)	Fruit weight (g)
Control	5,571[b]	16.85[c]	15.46[c]	32.74[c]	4.57[c]	8.81[c]
1	5,669.1[b]	17.66[b]	16.44[b]	34.32[b]	5.7[b]	9.45[b]
2	6,175.2[a]	18.48[a]	17.48[a]	35.39[a]	6.45[a]	10.52[a]
3	6,097[a]	18.42[a]	17.35[a]	35.12[a]	6.16[a]	10.17[a]

Common letters in each column show insignificance of the difference in LSD test (P≤0.05).

The highest flower and fruit number was obtained in 2% and 3% EM whereas the lowest number of 15.46 was observed in control. Treatments of 2% and 3% EM resulted in the longest fruits (35.39 and 35.12 mm, respectively) while the shortest fruit of (32.74 mm) length was observed in control. Considering fruit volume, the highest volumes of 6.45 and 6.16 cm^3 were obtained in 2% and 3% EM and the lowest value was obtained in control. There was significant difference between EM application and control regarding fruit fresh/dry weight. The highest fresh/dry weight of 10.52 and 1.73 g were obtained in 2% EM, whereas the lowest values of 8.81 and 1.33 g were observed in control. The highest yield per plant was observed in 2% EM and the lowest yield per plant was observed in control. Numerous reports indicate an increase in yield by application of EM in various crops (Mohamed et al., 2007; Kleiber et al., 2014).

Table 4. Effect of different rates of EM on qualitative traits of strawberry cultivar 'Paros'

EM	Fruit dry weight (g)	Yield per plant (g)	Vitamin C (mg*100 g^{-1} fw)	Total soluble solids (°brix)	Total acidity (%)
Control	1.33^{d}	136.45^{c}	46.05^{c}	8.75^{b}	1.15^{a}
1	1.41^{c}	155.98^{b}	48.01^{bc}	9.17^{ab}	1.05^{ab}
2	1.73^{a}	185.2^{a}	50.24^{a}	9.81^{a}	0.89^{c}
3	1.62^{b}	176.75^{a}	50.04^{ab}	9.92^{ab}	0.96^{bc}

Common letters in each column show insignificance of the difference in LSD test (P≤0.05).

Lactic acid bacteria contained in EM are particularly effective in degradation of cellulosic and lignin compounds (Gao et al., 2008). Indeed, EM facilitates chemical degradation and mineralization process (Hussain et al., 1999) and incorporates the nutrients into the soil (Daly and Stewart, 1999). Inoculation of soil by EM promotes biodiversity of soil microorganisms and not only provides more nutrients, but also acts as a factor for controlling pathogenic microorganisms and promoting plant growth by production of hormones (cytokinins, gibberellins, etc) (Avis et al., 2008). Improved growth and yield can be due to activities of photosynthetic bacteria such as *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides* in EM solution. These form an independent group and are able to synthesis amino acids, polysaccharides, nucleic acids, bioactive compounds and sugars from root exudates, organic matter and poisonous gases such as hydrogen sulfide using soil temperature and sunlight as energy resource (Kim et al., 2004). These compounds can be directly taken up by the plant and paly role in plant growth and development (Higa, 2000; Ranjith et al., 2007). Yeasts play critical role in synthesis of compounds such as vitamins and cytokinins and are regarded as important factor in metabolite transportation from leaves to reproductive organs (Jagnow et al., 1991; Attala et al., 2000). Positive influence of EM on plant growth and yield can be attributed to increased availability of nutrients in the presence of organic matter, increased microbial biomass of the soil and thereby increased rate of biological nitrogen fixation via increase in *Azotobacter*, synthesis of plant hormones, bioactive compounds and antioxidants that facilitate solubility of nutrients (Hussain et al., 1999; Yaduvanshi, 2003).

Mean comparison indicated that EM application improved vitamin C content of strawberry cultivar 'Paros'. The highest vitamin C content (50.29 mg*100 g^{-1} fw) was obtained in 2% EM which was significantly different from control and 1% EM but not different from 3% EM treatment (Table 4). This finding agrees with those reported by other authors (Xu et al., 2001; Hassan and Emam, 2015). According to the results obtained in this research, 3% EM resulted in the highest total soluble solids. This value is not significantly different from those obtained in other treatments. However, this value was significantly different from control as presented in Table 4. These results are in accordance with those reported by other authors (Mohamed et al., 2007; Hassan, 2015; Hassan and Emam, 2015).

The highest total acidity was observed in control group which was not significantly different from those observed in EM treatments. The lowest values were obtained in 2% and 3% EM (0.89% and 0.96%, respectively) which were not significantly different from each other. Significant differences were not determined between the treatments considering fruit shelf life. However, the highest shelf life was obtained in 2% EM. This finding accordance with those reported by Mohamed et al. (2007). Phytohormones and other bioactive compounds synthesized by EM can postpone plant senescence (Yamada and Xu, 2000).

Effect of nitrogen on the quantitative and qualitative parameters of strawberry fruit

Mean comparison results indicated that the highest leaf area was obtained in 150 $kg*ha^{-1}$ N and lowest leaf area was obtained in treatment with 50 $kg*ha^{-1}$ N (6,156.90 mm^2 and 5,569.26 mm^2, respectively) (Table 5).

Table 5. Effect of different rates of N on quantitative traits of strawberry cultivar 'Paros'

N ($kg*ha^{-1}$)	Leaf area (mm^2)	Flower number per plant	Fruit number per plant	Fruit set (%)	Fruit length (mm)	Fruit volume (cm^3)
50	5,569.29^c	17.07^c	16.4^b	95.93^a	33.44^b	4.92^b
100	5,908.79^b	18.59^a	17.39^a	93.51^b	35.06^a	6.28^a
150	6,156.9^a	17.9^b	16.33^b	91.28^b	34.68^a	5.96^a

Common letters in each column show insignificance of the difference in LSD test ($P≤0.05$).

By increase in nitrogen rate, vegetative growth of rice was promoted (Hao et al., 2007). Moreover, in accordance with the results obtained in the present study, it has been reported that vegetative growth of strawberry is improved by increase in nitrogen rate (Lolaei, 2012). Optimal application of nitrogen increases protein content and leaf growth which is follows by increased photosynthesis, accelerated production of photosynthetic compounds and enhanced yield. However, excess amount of nitrogen increases leaf area which results in shading on other leaves and thereby decreases photosynthetic pigments and photosynthesis efficiency (Marschner, 1995).

The highest number of flower was obtained in 100 $kg*ha^{-1}$ nitrogen treatment and the lowest number of flower was observed in control plants. According to mean comparison results, the highest number of fruits was observed in 100 $kg*ha^{-1}$ nitrogen treatment showing significant difference with that of 150 kg*ha^{-1} and 50 kg*ha^{-1}. Treatments with 100 and 150 kg*ha^{-1} N resulted in formation of fruits with length of 35.06 mm and 34.68 mm which are longer than the fruits formed in control. The highest fruit volume was obtained in 100 kg*ha^{-1} nitrogen which was significantly different from that of 50 kg*ha^{-1} but not different from that obtained in 150 kg*ha^{-1} nitrogen (Table 5).

The highest values of fruit fresh/dry weight of 10.82 g and 1.6 g were obtained in 100 kg*ha^{-1} nitrogen. Other treatments were equal considering fruit weight. Moreover, the highest fruit yield per plant (188.68 g) was obtained in 100 kg*ha^{-1} nitrogen. However, by increase in nitrogen rate to 150 kg*ha^{-1}, the yield per plant was decreased. The lowest value of fruit yield (148.81 g) was achieved by 50 kg*ha^{-1} nitrogen (Table 6).

Table 6. Effect of different rates of N on quantitative and qualitative traits of strawberry cultivar 'Paros'

N (kg*ha^{-1})	Fruit fresh weight (g)	Fruit dry weight (g)	Yield per plant (g)	Vitamin C (mg*100 g^{-1} FW)	Total soluble solids (°brix)	Total acidity (%)
50	9.05^{b}	1.46^{b}	148.81^{b}	46.05^{c}	8.99^{b}	1.15^{a}
100	10.82^{a}	1.6^{a}	188.68^{a}	48.01b^{c}	9.88^{a}	1.05^{ab}
150	9.34^{b}	1.5^{b}	153.31^{b}	50.24^{a}	9.36^{ab}	0.96^{c}

Common letters in each column show insignificance of the difference in LSD test (P≤0.05).

Increase in nitrogen rate significantly increased strawberry yield (Papadopoulos, 1987), which is in agreement with the results obtained in the present study. Nitrogen deficiency results in reduction of leaf area, leaf number, crown formation, fruit size and the yield (Mass, 1984). By permanent application of chemical fertilizers in crop production, especially in fruit production, not only natural balance of the elements is disrupted, but also economic productivity is diminished (Pešakovič et al., 2013). Increase in nitrogen rate significantly increased vegetative growth of eggplant. Moreover, suitable application of nitrogen had positive effect on flower number and the days to flowering (Aminifard et al., 2010).

By increase in nitrogen rate from 50 kg to 150 kg*ha^{-1}, vitamin C content was increased but further increasing in nitrogen to 150 kg*ha^{-1}, vitamin C content was decreased (Table 6). Reduction of ascorbic acid content of fruit due to increase in nitrogen rate has been described by Lisiewska and Kmiecik (1996) which confirms the result obtained in the present study. In general, since vegetative growth is improved by nitrogen, nutrients stored in plant tissues are depleted. Moreover, nitrogen fertilizers increase shoot growth and development.

According to the results obtained in this research, 100 kg*ha^{-1} nitrogen resulted in the highest total soluble solids content. This value was decreased to 9.36^{o} Brix by increase in nitrogen rate to 150 kg*ha^{-1}. Moreover, the lowest soluble solids content was obtained in 50 kg*ha^{-1}. The highest acidity value was obtained in 50 kg*ha^{-1} nitrogen indicating significant difference with those obtained in other nitrogen rates. Fruit acidity in 100 and 150 kg*ha^{-1} nitrogen treatments were 0.96 and 0.98% (Table 6).

Conclusions

The application of EM increased the morphological traits and yield in strawberry cultivar 'Paros'. EM concentratioin of 2% showed better effect on most parameters than other concentrations. According to the results of this study, EM of 2% and nitrogen treatments of 100 kg*ha^{-1} are recommended for improvement of growth, yield and quality of strawberry cultivar 'Paros'.

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