Europ. J. Agronomy 46 (2013) 63–67

Contents lists available at SciVerse ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Long-term effective microorganisms application promote growth and increase yields and nutrition of wheat in China

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ARTICLE INFO

Article history:
Received 23 September 2012
Received in revised form
19 December 2012
Accepted 19 December 2012

Keywords:
Effective microorganisms (EM)
Compost
Long-term fertilizer experiment
Crop growth
Grains yields

ABSTRACT

An eleven years long-term field experiment for soil fertility and crop yield improvement had been conducted at China Agricultural University's Qu-Zhou experiment station since 1993. The field experiment included three treatments: effective microorganisms (EM) compost treatment; traditional compost treatment; and unfertilized control. The results revealed that long-term application of EM compost gave the highest values for the measured parameters and the lowest values in the control plot. The application of EM in combination with compost significantly increased wheat straw biomass, grain yields, straw and grain nutrition compared with traditional compost and control treatment. Wheat straw biomass, grain yields, straw and grain nutrition were significantly higher in compost soils than in untreated soil. This study indicated that application of EM significantly increased the efficiency of organic nutrient sources.

1. Introduction

Wheat is one of the most important food crops in China and mainly is cultivated in North China Plain. The North China Plain covers an area of 178,700 km^2, of which 88,500 km^2 is cultivated cropland (Lin et al., 2000). This area is major food-producing region of China and typical agriculture characterized by a high input and high output. The mainly cropping system is winter wheat and summer maize rotation, covering up to 60% of arable land in this area. The sustainable utilization of cropland and maintaining soil fertility in this area could appease food demand in China. Traditionally, farmers use organic materials such as manure and crop residues to maintain soil fertility (Gong et al., 2009a). However, due to the rapid development of economy since the 1990s, mineral fertilizers were largely and widely applied. As a result, more mineral fertilizers and less organic fertilizers are now being used in this region (Gong et al., 2009b). In order to achieve the maximum crop yields, mineral fertilizer were excessively and unreasonably applied, which is not only wasting resource, but also contaminating environment. In order to meet the demand of food grain production and ensure China's food security, reasonable and optimal application of fertilizer, maintaining or improving soil fertility, and protecting the environment are essential in this region (Miao et al., 2011).

In the recent years, some successful efforts have been made to at least partially substitute chemicals with natural substances to minimize the adverse effects of the synthetic agrochemicals (Javaid, 2006; Singh et al., 2011). One successful example was found by Higa (1991), who isolated some beneficial microorganisms from the soil and named them effective microorganisms (EM). EM contains about 80 species of microorganisms, which included photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes, and fermenting fungi like *Aspergillus* and *Penicillium* (Higa and Parr, 1994). Daly and Stewart (1999) reported that effective microorganisms could improve crop growth and yield by increasing photosynthesis, producing bioactive substances such as hormones and enzymes, controlling soil diseases and accelerating decomposition of lignin materials in the soil.

Some studies had reported that crop growth and yield were increased due to the application of EM (Daly and Stewart, 1999; Yan and Xu, 2002; Khaliq et al., 2006; Javaid, 2011). However, there had some controversial reports about the effect of applying EM on crop growth and yield (Formowitz et al., 2007; Daiss et al., 2008). Some studies showed that the effect of EM on crop growth, yield or quality was not usually evident (Formowitz et al., 2007; Daiss et al., 2008). However, these studies were short-term (only one crop growth season) effect of EM on crop growth and yield. Some studies have shown that these drawbacks can be overcome through periodic repeated applications of effective microorganisms (Javaid, 2006). However, there were a few investigations on the long-term effect of application of EM on crop growth and yield. Therefore, we

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http://dx.doi.org/10.1016/j.eja.2012.12.003

conducted an eleven years field experiment to investigate the long-term effect of application of compost on crop growth and yield. The results could reveal the effect of long-term EM application on crop growth and soil property, and help the selection of the optimal fertilization mode for maintaining soil fertility and high crop yields.

2. Materials and methods

2.1. Experiment site and design

An eleven years long-term field experiment was started in 1993 at China Agricultural University's Qu-Zhou experimental station in Hebei Province (located in the centre of the North China Plain) (115°01′E and 36°52′N, 40 m a.s.l.). The experimental station situated at continental temperate monsoon tone and the climate is warm, semi-humid and consists of summer rainfall and dry-cold winters. The mean annual temperature is 13.2 °C and ranges from a minimum of −2.9 °C in January to a maximum of 26.8 °C in July. The mean annual precipitation is 542.7 mm, of which 60% occurs from July to September, and the annual non-frost period is 201 d. The soil, with silty loam texture, is classified as a Cambisol according to the FAO/WRB (FAO, 1998).

An eleven years field experiment was designed with three treatments and three replications, laid out in a randomized complete block design with nine plots (3 m × 10.5 m each plot), with a long-term double-cropping rotation of winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.), which is the typical cropping system in this region. The wheat was planted in October and harvested in June, followed by maize which was planted in June and harvested in October.

Three treatments consisted of applied effective microorganisms (1 ml of the effective microorganisms concentrate contains a minimum of 10^5 viable organisms of the species *Streptomyces albus*, *Propionibacterium freudenreichil*, *Streptococcus lactis*, *Aspergillus oryzae*, *Mucor hiemalis*, *Saccharomyces cerevisiae*, and *Candida utilis*, in addition to an unspecified number of *Lactobacillus* sp., *Rhodopseudomonas* sp., and *Streptomyces griseus*) compost treatment (EM) (15 t ha^{-1}), applied traditional compost treatment (TC) (15 t ha^{-1}) and a control (no any soil amendments) since 1993. The traditional compost was 60% straw, 30% livestock dung, 5% cottonseed-pressed trash, and 5% bran (with a mean nutrient content of 100.5 kg N ha^{-1}, 36 kg P_2O_5 ha^{-1} and 196.2 kg K_2O ha^{-1}), whereas EM compost were composed of 50 kg traditional composts appended with 200 ml concentrated effective microorganisms and 1 kg red sugar. Each fertilization treatment was applied twice annually: in June and October before sowing winter wheat or summer maize, respectively. The amount fertilizers applied and rates and times of application were typical for this region. The unfertilized plots were cultivated and harvested for wheat and maize similarly to the fertilization plots. The fertilized and unfertilized plots were the same type of tillage. The above ground crop was mowed and removed and no straw was returned to the soil. Besides the three fertilizer treatments, all other agronomic management was identical.

2.2. Soil and plant sampling

Soil samples were collected from 0 to 20 cm soil layer at experimental site, using a 2.5-cm diameter soil auger on June 8th 2004 after wheat have been harvested but maize have not been sowed. Each soil sample consists of fifteen cores (2.5 cm diameter × 20 cm deep), which were mixed to form a composite sample, and samples were collected from each plot. The soil samples were stored in insulated and tied plastic bags and was transported to the laboratory. All soil analysis was completed within a week of soil sampling.

Fifteen plants were excavated, washed, and separated into roots, stems and leaves in wheat seedling (on November 22nd, 2004), jointing (on March 12th 2005) stages, and ten plants were excavated, washed, and separated into roots, stem and leaf, and spike in the wheat maturity stages (on June 10th 2005). The plant samples were analyzed the same day.

2.3. Soil and plant analysis

The bulk density was expressed by dividing the weight of the dried soil by the volume of the core using the core volume and dry soil weight (stainless steel cylinders with diameter and height of 5 cm). Soil subsamples were air-dried for 14 d at room temperature, sieved through a 1-mm screen, mixed, and subsamples were assayed for alkaline hydrolysable nitrogen (N), available phosphorus (P), available potassium (K) and soil pH. The air-dried sub-samples were ground to pass through a 0.25-mm sieve to determine soil organic matter and total N content. The potassium dichromate external heating method was applied to determine soil organic matter content (Blakemore et al., 1972). The semi-micro Kjeldahl method and the alkaline-hydrolysable diffusion method were applied to determine total N and alkaline-hydrolysable N content (Bremner, 1996). Soil available P was extracted with 0.5 mol L^{-1} $NaHCO_3$ (soil:solution = 1:20) and measured with the Olsen method (Blakemore et al., 1972). Soil available K was extracted with 1 mol L^{-1} NH_4Ac (soil:solution = 1:10) and measured with the flame photometry method (Blakemore et al., 1972). Soil pH was measured in 0.01 mol L^{-1} $CaCl_2$ slurry (soil:solution = 1:2.5) using a glass electrode.

The wheat grains were separated from straw using a plot thresher. Grains were weighted after air drying and were recorded from a whole plot. Wheat was harvested from ground level manually by sickle on, June 10th 2005. At crop maturity, sub samples of root, stem, leaf and spike were collected from each plot and dried in an oven-dried at 65 °C for 72 h. Plant samples were ground to pass through a 0.5-mm sieve and analyzed for total N by a micro-Kjeldahl method (Bremner and Mulvaney, 1982). Phosphorus concentration of plant tissues digested in HNO_3 and $HClO_4$ was determined by the ammonium molybdate method (Olsen and Sommers, 1982) and that of K by flame photometry. All the data were expressed on dry mass basis.

2.4. Statistical analysis

All obtained data were subjected to statistical analysis of variance (ANOVA) using the SPSS 11.5 software package and were used to evaluate differences between separate means. Difference obtained at $p < 0.05$ level was considered as statistically significant using the LSD (least significant difference) test.

3. Results

3.1. Soil physical-chemical properties

The long-term soil amendments caused significant changes in soil physical-chemical properties (Table 1). Soil bulk density and pH were significantly ($p < 0.05$) lower in the two compost plots than in the control plot. Moreover, soil pH was significantly ($p < 0.05$) lower in the EM compost plot than in the traditional compost plot. Soil organic matter, total N, alkaline-hydrolysable nitrogen, and available K content was significantly ($p < 0.05$) higher in the two compost plots than in the control plot. Soil available P and K content was

Table 1
Soil physical-chemical properties.

Treatment	Soil organic matter (g/kg)	Total N (g/kg)	Alkaline N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	pH	Bulk density (g/cm^3)
EM	21.32a	1.29a	111.98a	50.69a	207.21a	7.15c	1.33b
TC	20.86a	1.20a	103.71a	36.29b	161.75b	7.26b	1.32b
Control	12.86b	0.81b	68.43b	4.07c	80.86c	7.53a	1.47a

EM: EM compost treatment; TC: traditional compost treatment; Control: no any soil amendment. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison.

Table 2
Plant height and biomass in wheat seedling stage.

Treatment	Plant height (cm)	Root dry biomass (g)	Stem and leaf dry biomass (g)	Root, stem and leaf dry biomass (g)
EM	15.40a	0.047b	0.357a	0.403a
TC	15.00a	0.057a	0.340a	0.397a
Control	11.67b	0.020c	0.100b	0.120b

EM: EM compost treatment; TC: traditional compost treatment; Control: no any soil amendment. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison.

significantly ($p < 0.05$) higher in the EM compost plot than in the traditional compost plot.

3.2. Wheat plant height and biomass in the growing stages

The long-term soil amendments significantly increased wheat plant height and biomass during wheat growing stages (Tables 2–4). During wheat seedling and jointing stages, wheat stem and leaf biomass, and whole plant biomass was significantly ($p < 0.05$) higher in the two compost plots than in the control plot. Wheat stem and leaf biomass, and whole plant biomass were higher in the EM compost plot than in the traditional compost plot, but the difference is not significant in wheat seedling and jointing stages. During wheat maturity stage, wheat plant height, root biomass, and stem and leaf biomass was significantly ($p < 0.05$) higher in the two compost plots than in the control plot. Wheat spike biomass and whole plant biomass were significantly ($p < 0.05$) higher in the EM compost plot than in the traditional compost plot, and was significantly ($p < 0.05$) higher in the traditional compost plot than in the control plot in wheat maturity stage.

3.3. Yield and yield components of wheat

Wheat productive spike per hectare, thousand grains weight and grain yields were significantly ($p < 0.05$) higher in the two compost plots than in the control plot (Table 5). Moreover, wheat productive spike per hectare, and grain yields were significantly ($p < 0.05$) higher in the EM compost plot than in the traditional compost plot.

3.4. NPK contents in wheat plant

The long-term fertilization altered nutrients content in wheat plant tissues (Table 6). N content of stem, leaf, spike, and grain was significantly ($p < 0.05$) increased in order from control, traditional compost to EM compost plots. P content of stem, leaf, and spike was significantly ($p < 0.05$) higher in the two compost plots than in the control plot. Moreover, P content of leaf, and grain was significantly ($p < 0.05$) higher in the EM compost plot than in the traditional compost plot. K content of stem, leaf, and grain was significantly ($p < 0.05$) higher in the two compost plots than in the control plot. Moreover, K content of grain was significantly ($p < 0.05$) higher in the EM compost plot than in the traditional compost plot.

Table 3
Biomass of wheat in wheat jointing stage.

Treatment	Root dry biomass (g)	Stem and leaf dry biomass (g)	Root, stem and leaf dry biomass (g)
EM	0.204a	1.315a	1.519a
TC	0.191ab	1.280a	1.471a
Control	0.150b	0.772b	0.922b

EM: EM compost treatment; TC: traditional compost treatment; Control: no any soil amendment. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison.

4. Discussions

Soil organic matter, total N, and available K were significantly affected by soil amendments, as was in accordance with the previous result of Chu et al. (2007), who reported that soil organic matter, total N, and available K contents were significantly increased with the application of organic manure compared to control plot in long-term fertilizer experiment. The main reason was that organic manure could release some nutrients into soil (Zhao et al., 2009). Gami et al. (2001) reported available P contents were increased with the long-term application of manure, as was similar to our result. Application of organic manures with and without mineral N resulted in a greater increase in Olsen P in the soil after eight years fertilizer experiment (Singh et al., 2007). We found that the saline-alkaline soil pH was decreased with application of organic manure (Gong et al., 2009a; Lee et al., 2009), as indicated that organic fertilizer could amend with the saline-alkaline soil (Marinari et al., 2000; Hati et al., 2006). Soil organic matter, total nitrogen, alkaline-hydrolysable nitrogen, available P, and available K content were higher in the EM compost plot than in the traditional compost plot, moreover the difference of available P and available K content were also significant. This manifested that improvement of soil fertility in EM compost was better than that in traditional compost. The possible reason was that there are many advantageous microbes in EM compost, which stimulated release of nutrient elements from soil (Higa and Parr, 1994), and accelerated decomposition of organic materials (Javaid, 2011).

Wheat growth was significantly influenced by long-term soil amendments, which was consistent with Hati et al. (2006). Crop stem and leaf biomass was significantly increased due to compost in wheat growing stages, and this was similar to the previous study (Wang et al., 2004). Wheat stem and leaf biomass, spike biomass, and whole plant biomass was significantly higher in fertilized soils than in untreated soil in wheat maturity stage. For example, Rasool et al. (2007) reported that wheat and maize straw yields were significantly increased in farmyard manure plot than in untreated plot. Similarly, Marinari et al. (2000) reported a significant increase of maize productivity in organic manure soil compared to unfertilized soil. The wheat spike biomass and whole plant biomass was significantly higher in the EM compost plot than in the traditional compost plot in wheat maturity stage. Similarly, Javaid and Mahmood (2010) observed that EM application significantly enhanced soybean shoot biomass in farmyard manure amendment and Javaid (2011) found that the effect of EM on rice shoot biomass was not appreciable at the 90 and 120 d growth stages in farmyard manure amendment. However, at the 150 d growth stage, a significant increase in rice shoot biomass was observed due to

Table 4
Plant height and biomass of wheat in wheat maturity stage.

Treatment	Plant height (cm)	Root dry biomass (g)	Stem and leaf dry biomass (g)	Spike dry biomass (g)	Root, stem and leaf dry biomass (g)
EM	67.20a	4.051a	20.936a	29.020a	54.008a
TC	66.10a	3.977a	19.010a	24.042b	47.029b
Control	49.47b	2.197b	5.697b	7.534c	15.429c

EM: EM compost treatment; TC: traditional compost treatment; Control: no any soil amendment. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison.

Table 5
Yield and yield components of wheat.

Treatment	Productive spike ($\times 10^4$/ha)	Grains/spike	Thousand grains weight (g)	Grain yield (t ha^{-1})
EM	474.81a	29.87a	42.67a	6.12a
TC	459.12b	28.50a	41.93a	5.84b
Control	388.33c	18.03b	36.87b	1.81c

EM: EM compost treatment; TC: traditional compost treatment; Control: no any soil amendment. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison.

EM application. Moreover, Javaid and Bajwa (2011) reported that shoot biomass of mungbean were significantly increased due to EM application in farmyard manure amendment. The promoted wheat growth could probably be attributed to activity of photosynthetic bacteria such as *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides* in EM solution, which are a group of independent, self-supporting microbes (Javaid and Bajwa, 2011). These bacteria could synthesize useful substances from secretions of plant roots, or soil organic materials (Kim et al., 2004). These useful substances produced by these microbes include amino acids, nucleic acids, sugars, polysaccharides, and bioactive substances, all of which accelerate crop growth (Javaid and Bajwa, 2011).

Wheat grains per spike and thousand grains were significantly increased due to compost application in comparison to control plot in present experiment. Nevertheless, wheat grains per spike and thousand grains were not significantly affected by EM application in this study, and Javaid (2011) observed also that the effects of EM were not significant for rice 100-grain weight.

Wheat grain yields were significantly increased due to compost application. Similarly, wheat and rice yields were increased due to long-term application of organic fertilizers (Gu et al., 2009). Blaise et al. (2005) reported that seed cotton yields were significantly increased due to application of farmyard manure in the first year of the study. Wheat grain yields were significantly higher in the compost plot than in the unfertilized plot, as was consistent with the result reported by Rasool et al. (2007, 2008), who found that wheat and maize yields were higher in the compost plot than in the unfertilized plot under long-term application fertilizers experiment. Wheat grain yields were significantly higher in the EM compost plot than in the traditional compost plot. These results support the findings of Javaid and Mahmood (2010), and Javaid and Bajwa (2011), who reported that grain yields of soybean and mungbean were significantly increased due to EM application in farmyard manure amendment. Similarly, EM application enhanced rice grain yields by 46% in the green manure amendment (Javaid, 2011). Khaliq et al. (2006) reported that EM application in combination with organic matter significantly enhanced cotton yields. Similarly, Hussain et al. (1999) reported that grain yields of wheat and rice were increased when EM in combination with farmyard manure was applied. When EM in combination with organic materials was applied, the higher crop grain yields in the present and previous studies (Hussain et al., 1999; Javaid and Mahmood, 2010) could be attributed largely to the activity of the introduced exotic beneficial microorganisms, which stimulated the decomposition of organic materials and the release of nutrients for plant uptake (Javaid and Bajwa, 2011).

Wheat N, P, K content of stem, leaf, spike, and grain were significantly enhanced due to organic manure application. Similarly, the wheat and maize uptake of N, P and K were significantly improved with the long-term application of farmyard manure (Rasool et al., 2008), and the same trend was observed in rice–wheat system (Rasool et al., 2007). Wheat N, K content of stem, leaf, and grain were significantly increased in the compost plot than in unfertilized plot, similarly Blaise et al. (2005) reported that cotton N, P, K uptake were higher in farmyard manure plot than in unfertilized plot. The N, P, K content of wheat stem, leaf, spike and grain were higher in the EM compost plot compared with in traditional compost plot. Similarly, EM application significantly enhanced mungbean N, P, K concentration at the flowering stage in farmyard manure amendments (Javaid and Bajwa, 2011) and application of EM in combination with organic materials significantly increased the N, P and K contents of cotton (Khaliq et al., 2006). Effective microorganisms stimulate the degradation of organic materials and accelerate the process of mineralization of organic matter (Hussain et al., 1999), releasing more nutrients for plant uptake into the soil (Daly and Stewart, 1999). Similar effects of EM application on wheat, rice, cotton, mungbean, cowpea, and capsicum N, P, K nutrition in soil organic amendment system have been reported (Hussain et al., 1999; Khaliq et al., 2006; Javaid and Bajwa, 2011).

Table 6
N P, K concentrations in wheat plant tissues.

Treatment	Stem (g/kg)			Leaf (g/kg)			Spike (g/kg)			Grain (g/kg)		
	N	P	K	N	P	K	N	P	K	N	P	K
EM	2.69a	1.06a	22.30a	4.95a	2.26a	13.55a	16.98a	4.15a	4.17a	20.30a	4.66a	4.15a
TC	2.47b	1.04a	21.95a	4.54b	2.04b	13.41a	15.99b	4.07a	3.88a	19.25b	4.33b	3.83b
Control	2.08c	0.72b	10.42b	3.80c	1.32c	6.59b	14.16c	3.68b	3.11b	16.93c	4.23c	3.42c

EM: EM compost treatment; TC: traditional compost treatment; Control: no any soil amendment. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison.

5. Conclusions

Based on the results, we concluded that long-term EM application in combination with compost enhanced wheat straw biomass, grain yields, and straw and grain nutrition. The highest NPK content in plant tissues with EM compost treatment demonstrated higher efficiency of the release of nutrients through organic and microbial application. We deduced that the effect of improving soil fertility was better in compost appending EM than alone application of compost.

Acknowledgements

We thank Mr. Aiming Shi of Qu-Zhou experimental station, China Agricultural University for her valuable field study assistance.

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