



Full Length Article

Bio-organic Fertilizer Promotes Plant Growth and Yield and Improves Soil Microbial Community in Continuous Monoculture System of *Chrysanthemum morifolium* cv. Chuju

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Abstract

Chuju, a cultivar of *Chrysanthemum morifolium*, has been growing for both drink and medicinal uses for hundreds of years in China. In recent years, however, continuous monoculture of Chuju has caused declines in growth and yields. In this study, we investigated the plant growth and yields, soil microbial community composition dynamics, and the improvements of bio-organic fertilizer on plant growth and yield in Chuju continuous cropping systems. Results showed that the yield of Chuju flowers was decreased by 11-37%, but *Fusarium oxysporum* and total fungus populations were respectively increased by 34-79% and 53-107% in soils continuously monocultured with Chuju for two to four years, as compared to those for one year. Bio-organic fertilizer significantly reduced *F. oxysporum* population and the ratio of *F. oxysporum* to total fungus population, but enhanced shoot biomass, flower diameter, and flower yields, in comparison with synthetic fertilizer. These results indicate that bio-organic fertilizer could be used to alleviate soil problems and to sustain crop production in continuous cropping systems of Chuju. © 2017 Friends Science Publishers

Keywords: Monocropping system; Soil sickness; Soil pathogen; Sustainable production; Yield reduction

Introduction

Chrysanthemums are perennial flowering plants. They were first cultivated in China as early as the 15th century BC (Cumo, 2013). Nowadays, chrysanthemums are grown not only for ornamental uses, but also for drink as well as pharmaceutical purposes (Cai *et al.*, 2004). Chuju, a cultivar of *Chrysanthemum morifolium* Ramat, is grown for both drink and medicinal uses, and is ranked the first among the four famous medicinal chrysanthemums in China (Xie *et al.*, 2012). The area for growing Chuju is about 6000 hectares, with annual yield of 3.6×10^7 kg (fresh weight) and revenue of 1.2×10^8 US dollars in China (Dai, 2014). However, growing Chuju continuously in the same field has resulted in yield decline and quality degradation (Chen *et al.*, 2011).

Continuous growing of the same crop in the same field has led to problems of poor establishment, stunted growth and reduced yields. This phenomenon of declines in plant growth and yield caused by continuous monocropping is commonly called as “soil sickness” (Nishizawa *et al.*, 1971). Much research on soil sickness has been documented on many upland crops such as aerobic rice (Ventura *et al.*, 1981), maize (Horst and Hardter, 1994), barley (Delogu *et al.*, 2003), wheat (Kirkegaard *et al.*, 2004), and vegetables

(John *et al.*, 2010). Soil sickness may involve soil-borne abiotic and/or biotic stresses including the buildup of soil-borne nematodes, pathogens and depleted nutrients (Ventura *et al.*, 1981; Huang *et al.*, 2006), poor soil structure (Huang *et al.*, 2006; Prasad, 2011) or allelopathic substances (Huang *et al.* 2006; John *et al.*, 2010). Soil sickness problems have impacted agricultural sustainability. Therefore, research on alleviation of soil sickness has drawn wide attention. Nie *et al.* (2007) have effectively remedied soil sickness from monocropping using soil heating. Fumigation with methyl bromide has alleviated soil sickness (Kirkegaard *et al.*, 1995). Crop rotation and fertilization are employed to overcome the problems caused by continuous cropping to certain extent (Horst and Hardter, 1994). However, it has not been well understood how to improve growth and yields of Chuju under continuous monocropping so far.

Bio-organic fertilizer is organic composts from organic wastes (farm waste, city waste, poultry litter and industrial wastes) blended with living cells of beneficial microorganisms (Kramany *et al.*, 2007; Ahmad *et al.*, 2008; Luo *et al.*, 2010; Naveed *et al.*, 2015). The microorganisms could increase the uptake of soil nutrients by plants (Ahmad *et al.*, 2008), improve soil microbial community and

activities (Ding *et al.*, 2013); and/or inhibit soil borne pathogens (Luo *et al.*, 2010; Ding *et al.*, 2013), thus enhancing plant growth and yields (Naveed *et al.*, 2015). Srivastava and Govil (2007) found that biofertilizers significantly enhanced vegetative growth and improved floral characters of gladiolus cv. American Beauty, and increased the total rhizospheric bacterial population, as compared to control. Luo *et al.* (2010) observed that applying bio-organic fertilizer effectively suppressed cotton verticillium wilt via improving fungal community structure in rhizospheric soils.

The objectives of this study were to examine (1) changes of plant growth and yield of Chuju, and the dynamics of soil microbial community in continuous monocropping system, and (2) effects of applying bio-organic fertilizer on Chuju growth and yield and soil microbial community in field conditions.

Materials and Methods

Study 1: Field Investigation

In March, 2009, surface soil (0-20 cm) samples were collected from fields (118°7'8"E, 32°16'55"N) under continuous monocropping of *Chrysanthemum morifolium* Ramat var. Chuju for four years and the neighboring ones without growing Chuju. Eight replicate fields were investigated for each cropping system. During sampling, 5-10 sampling points were randomly selected in each field and at each point, 5 soil cores of 2.5 cm diameter and 15 cm depth were taken within a 50-cm radius of the point. The soil cores (25-50) from each field were bulked, crumbled and thoroughly mixed with in a plastic bag. One composite subsample (~500 grams) per field was taken and then transported to laboratory on ice in a cooler and immediately stored in sealed plastic bags at 4°C. All microbial determinations were done within 1 week of sampling.

Study 2: Effect of Chuju Continuous Monocropping on Soil Microbial Community and Plant Growth and Yield

A plot experiment was initiated in 2009 to further examine whether Chuju continuous monocropping alters microbial community composition and affect plant growth and yield over time at the Chuju Planting Base of Anhui Jutai Chuju Science and Technique Inc., Chuzhou, Anhui. Prior to this experiment, wheat-soybean had been grown in the field for three years. The soil was classified as yellow-brown soil according to the Chinese Soil Genetic Classification (Zhang *et al.*, 2014). Each plot measured 10 m × 5 m. There was 50 cm space between plots. Experimental design is given in Table 1. Three replicates were used for each treatment. All treatment plots were arranged randomly.

In late May of each year, young Chuju plants with 12 true leaves and about 20 cm tall, propagated from cuttings, were transplanted into plots with planting space 90 cm by

10 cm. Beginning from October, flowers were harvested and their weights were recorded. In November 2012, soil samples in all plots were collected from the rhizosphere of Chuju plants for microbial measurements. Five sampling points within each plot were randomly selected. About 100 grams of soil at each point were taken. All soils from each plot were bulked into one composite sample and stored at 4°C for microbial determinations, which were done within 1 week of sampling.

Study 3: Effects of Bio-organic Fertilizer on Chuju Growth and Yield and Soil Microbial Community

This study involved two field trials on whether applying bio-organic fertilizer enhanced Chuju growth and yield and improved soil microbial community.

Trial 1: This trial was performed on the plots from Study 2. In 2012, when Chuju was grown the first to the fourth season, the plots with the same number of growing years were equally split into two groups. One group was supplied with synthetic fertilizer (SF) as before. Another was applied with bio-organic fertilizer (BOF). To ensure that both groups received the same amount of major nutrients (N, P and K), BOF was balanced by adding certain amount of chemical fertilizers. Bio-organic fertilizer (Commercial Name: BIO) was purchased from Jiangsu New World Amino Fertilizers Inc.(Yixing, Jiangsu, China). It contained 2.0% nitrogen (N), 3.0% phosphorus (P₂O₅), 1.9% potassium (K₂O), ≥23.8% organic C, and pH 6.0. The microbial community composition of this bio-organic fertilizer was 4.36 × 10⁸ CFU/g of bacteria, 3.28 × 10⁶ CFU/g of fungi, and 1.58 × 10⁶ CFU/g of actinomycetes.

Trial 2: This field trial was carried out in fields that had previously been in Chuju production for 4 years at the Chuju Planting Base of Anhui Jutai Chuju Science and Technique Inc., Chuzhou, Anhui. The experiment consisted of two fertilization treatments with eight replicates: synthetic fertilizers (SF) and bio-organic fertilizers (BOF). For SF plots, a local farmer-used rate of 2250 kg/ha of mixed compound fertilizer (N-P₂O₅-K₂O: 12-12-6) was applied before planting, then urea of 75 kg/ha in early or middle June, and again urea of 150 kg/ha in middle July annually. For BOF plots, only 1500 kg/ha BOF was applied. This rate was chosen according to our previous study (Wang *et al.*, 2014). The area of the trial plot was 60-100 m². All flowers were collected to obtain the yields. At peak flowering stage, one composite soil (0-20 cm) sample was collected randomly from each plot and stored at 4°C for soil microbial analysis, which were done within 1 week of sampling.

Microbial Analyses

Soil microbial community was enumerated using serial dilution plating technique. Bacteria, Actinomycete, fungi, and *F. oxysporum* were isolated and incubated with nutrient agar (Beef extract peptone agar by Sinopharm Chemical

Table 1: Growing and fertilizing history of Chuju plants in different plots during 2009-2012

Plot ID	Year of continuous cropping	Application of synthetic fertilizer (SF)*	Application of bio-organic fertilizer (BOF)†
Y1W	1	2012	None
Y1B	1	2012	2012
Y2W	2	2011 through 2012	None
Y2B	2	2011 through 2012	2012
Y3W	3	2010 through 2012	None
Y3B	3	2010 through 2012	2012
Y4W	4	2009 through 2012	None
Y4B	4	2009 through 2012	2012

Notes: * N-P₂O₅-K₂O (12-12-6) applied at 225 0 kg/ha before planting, then urea at 75 kg/ha in early or middle June, and again urea at 150 kg/ha in middle July annually; †Bio-organic fertilizer applied at a rate of 1500 kg/ha

Reagent Co., Ltd, Shanghai, China), Gause's synthetic agar (Borgmeyer and Crawford, 1985), Martin's rose bengal agar, and *Fusarium* selective medium, respectively (Komada, 1975). After incubation, the colony-forming units (CFU) of microbes were counted and expressed as CFU/g of dry soil.

Statistical Analysis

Differences in plant biomass and yield among treatments were assessed with an analysis of variance (ANOVA). If the ANOVA revealed significant differences ($p < 0.05$), Duncan's multiple range test (DMRT) was used to separate the means. For microbial populations (CFU/g soil), data from microbial enumeration were logarithmically transformed and subsequently analyzed by ANOVA. However, microbial data presented in tables are the actual average values. All statistical analyses were performed using SAS 9.3 software (SAS Institute Inc. 2011).

Results

Soil Microbial Population under Continuous Monocropping of Chuju

Monocropping Chuju did not change the populations of bacteria, actinomycete and total microorganisms, but significantly increased total fungus and *F. oxysporum* populations by 123 and 457%, respectively, compared with the control without growing Chuju (Table 2). The ratio of *F. oxysporum* to total fungi was 156% greater under continuous cropping than in the control (Fig. 1).

Effects of Number of Chuju Monocropping on Soil Microbial Population and Chuju Flower Yield

As the number of continuous cropping year increased, bacterium and actinomycete populations did not change but fungus and *F. oxysporum* populations increased, especially under no bio-organic fertilizer (Table 3). In the plots of four-year cropping, the populations of fungi and *F. oxysporum* increased by 79% and 110% relative to the first year in SF-applied plots, respectively. The ratio of *F.*

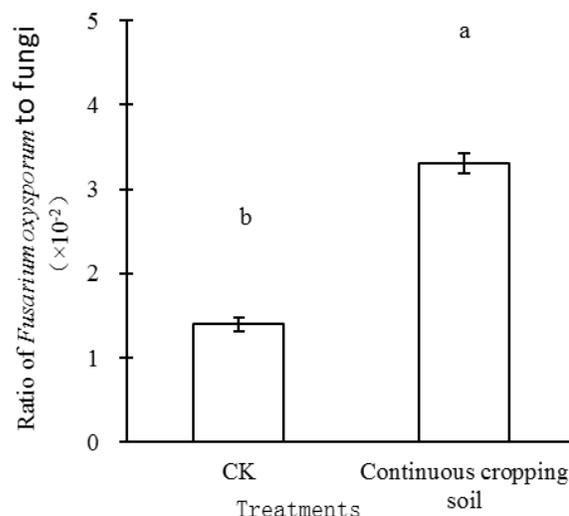


Fig. 1: Effects of Chuju continuous cropping on ratios of *F. oxysporum* to fungus population in soils. Means with different letters indicate significant difference at $p < 0.05$ level

oxysporum to total fungi tended to increase with increasing number of continuous growing year. Applying bio-organic fertilizer enhanced total fungus population but inhibited *F. oxysporum*. However, bacterial and actinomycetic populations were not affected by bio-organic fertilizer.

Yield of Chuju flowers decreased as increasing number of continuous cropping years. Compared with one year cropping under SF, the Chuju yield reduction was 11, 19 and 37% for two-, three- and four-year cropping, respectively (Table 4).

Effects of Bio-organic Fertilizer Application on Chuju Growth and Yield and Soil Microbial Community

In Field Trial 1, applications of BOF significantly improved Chuju yields in the plots grown with Chuju for three and four years (Table 4). In the plots applied with BOF, the Chuju yield was respectively 92, 89 and 85% in the second-, third-, and fourth-year plots as compared to that in the first-year plot. In the plots with SF, however, the Chuju yields in the second-, third-, and fourth-year plots were 89, 81 and 63% of that in the first-year plot.

In Field Trial 2, applying BOF at 1500 kg/ha increased plant yield by 10 % over that in the synthetic fertilizer (Table 5). The BOF application mainly increased both flower and petal numbers per plant (Table 6).

In the experiments, applying BOF tended to increase bacterium, actinomycete and fungus populations, but SF application increased actinomycetes only, compared with those prior to the experiment (Table 7). However, both *F. oxysporum* population and the ratio of *F. oxysporum* to total fungi were significantly reduced by applying BOF.

Table 2: Microbial community composition in soils under Chuju continuous cropping

Soil sample	Bacteria ($\times 10^7$ CFU/g)	Actinomycetes ($\times 10^6$ CFU/g)	Fungi ($\times 10^4$ CFU/g)	<i>F. oxysporum</i> ($\times 10^3$ CFU/g)	Total microorganism ($\times 10^7$ CFU/g)
Control	1.4 \pm 0.16 a*	1.6 \pm 0.24 a	3.9 \pm 1.35 b	0.6 \pm 0.23 b	1.7 \pm 0.15 a
Continuous cropping	1.4 \pm 0.24 a	1.6 \pm 0.33 a	8.7 \pm 2.24 a	3.1 \pm 0.79 a	1.5 \pm 0.24 a

Note: * Data (mean \pm SE). Means with different letters within a column indicate significant; difference at $p < 0.05$ level. n=8

Table 3: Soil microbial populations under continuous cropping and bio-organic fertilizer

Year of continuous cropping	SF	SF+BOF
	Bacteria ($\times 10^7$ CFU/g soil)	
1	1.5 \pm 0.07 aA	1.6 \pm 0.08 aA
2	1.3 \pm 0.04 aB	1.6 \pm 0.12 aA
3	1.2 \pm 0.12 aA	1.3 \pm 0.06 aA
4	1.2 \pm 0.10 aA	1.3 \pm 0.15 aA
	Actinomycetes ($\times 10^6$ CFU/g soil)	
1	1.7 \pm 0.12 aA	1.7 \pm 0.19 aA
2	1.6 \pm 0.19 aA	1.7 \pm 0.23 aA
3	1.6 \pm 0.14 aA	1.7 \pm 0.07 aA
4	1.5 \pm 0.09 aA	1.6 \pm 0.10 aA
	Fungi ($\times 10^4$ CFU/g soil)	
1	4.6 \pm 0.27 cB	9.3 \pm 0.54 aA
2	6.1 \pm 0.40 bB	10.0 \pm 1.02 aA
3	7.4 \pm 0.22 aB	10.0 \pm 0.48 aA
4	8.1 \pm 0.28 aB	11.1 \pm 0.77 aA
	<i>F. oxysporum</i> ($\times 10^3$ CFU/g soil)	
1	2.9 \pm 0.29 cA	2.2 \pm 0.46 bA
2	4.4 \pm 0.11 bA	2.9 \pm 0.19 aB
3	5.6 \pm 0.35 aA	3.3 \pm 0.05 aB
4	6.0 \pm 0.23 aA	3.5 \pm 0.33 aB

Note: *SF-- Synthetic fertilizer; BOF--Bio-organic fertilizer; †Data (mean \pm SE). Different lowercase letters mean significant difference between; growth years within a column, while different uppercase letters indicate significant; difference between fertilizers within a row ($P < 0.05$). n=3

Table 6: Effects of bio-organic fertilizer on characteristics of Chuju flowers in continuous cropping system

Treatments	Flower number per plant)	Flower diameter (cm)	Petal number per plant)	Petal length (cm)	Petal width (cm)
SF	93.5 \pm 2.66b	5.4 \pm 0.22 a	231.0 \pm 17.29 a	2.3 \pm 0.09 a	0.6 \pm 0.03 a
SF+BOF ₁₅₀₀	157.0 \pm 12.36a	5.5 \pm 0.14 a	217.0 \pm 18.44 a	2.3 \pm 0.09 a	0.6 \pm 0.04 a

Note: See the footnotes in Table 2

Table 7: Effects of bio-organic fertilizer on soil microbial populations under continuous cropping

Treatments	Bacteria ($\times 10^7$ CFU/g)	Actinomycetes ($\times 10^6$ CFU/g)	Fungi ($\times 10^4$ CFU/g)	<i>F. oxysporum</i> ($\times 10^3$ CFU/g)	Total microorganisms ($\times 10^7$ CFU/g)
Reference soil ‡	1.4 \pm 0.16 a	1.6 \pm 0.24 ab	3.9 \pm 1.35 c	0.6 \pm 0.23 c	1.6 \pm 0.15 a
SF	1.3 \pm 0.18 a	2.3 \pm 0.54 a	8.3 \pm 2.05 ab	4.0 \pm 0.59 a	1.6 \pm 0.23 a
SF+BOF ₁₅₀₀	1.5 \pm 0.30 a	1.7 \pm 0.45 ab	10.2 \pm 1.60 a	0.9 \pm 0.26 b	1.6 \pm 0.34 a

Note: See the footnotes in Table 2; ‡Reference soil collected from a neighboring field with the same soil type but without growing Chuju

Discussion

Results obtained in this study showed that continuous monocropping of Chuju reduced plant growth and yield. Such effects were exacerbating with increasing number of monocropping year (Table 4). This is in agreement with the

Table 4: Chuju yields (kg fresh weight /50 m²) as influenced by continuous cropping and bio-organic fertilizer

Year of continuous cropping	Fertilization	
	SF *	SF+BOF
1	52.3 \pm 0.76 Aa†	53.8 \pm 0.79 Aa
2	46.5 \pm 0.56 Ab	49.3 \pm 0.44 Ab
3	42.5 \pm 0.46 Bc	47.7 \pm 0.84 Ab
4	32.7 \pm 1.39 Bd	45.5 \pm 0.52 Ac

Note: See the footnotes in Table 3

Table 5: Effects of bio-organic fertilizer on biomass and flower yield of Chuju in continuous cropping system

Treatments	Yield (kg FW/ha)	Fresh weights		
		Flowers (g/plant)	Leaves (g/plant)	Stems (g/plant)
SF	3550.5 \pm 169.5a	67.6 \pm 7.45a	45.5 \pm 5.98a	65.2 \pm 2.25a
SF+BOF ₁₅₀₀	3873.0 \pm 234.0a	73.8 \pm 6.31a	56.1 \pm 4.45a	66.6 \pm 6.31a

Note: See the footnotes in Table 2

results reported on other plants (Delogu *et al.*, 2003; Nayyar *et al.*, 2009; John *et al.*, 2010). John *et al.* (2010) summarized that continuous cropping of vegetables in same field causes reduction in their growth, yield and quality. Over the three years of trials, Delogu *et al.* (2003) observed that repeated growing of barley reduced growth and yield. Plants can influence soil properties and soil organisms via

releasing low-molecular mass compounds(sugars, amino acids and organic acids) and dead roots, in turn modifying plant performance through mutualistic interactions, nutrition availability, or pathogenic activity, etc. (Berg and Smalla, 2009; Jones *et al.*, 2009; Huang *et al.*, 2013). Although the reasons for poor growth and reduced yields caused by

continuous monocropping are complicated and have not been clearly defined, they may be summarized as follows: (1) deterioration of soil physical and chemical properties, (2) proliferation of soil-borne diseases, and (3) accumulation of toxic substances (autopathy).

Our data have showed that continuous monocropping of Chuju significantly increased the populations of *F. oxysporum* and total fungi and their ratios, but not those of bacteria and actinomycetes in soil (Fig.1, Tables 2 and 3). Similar results have been documented (Zhou and Wu, 2012; Xiong *et al.*, 2014). Zhou and Wu (2012) found that monocropping of cucumber increased the population sizes rather than the diversity of fungus and *Fusarium* communities in soil. Long-term continuous cropping led to the reduction of the beneficial microbes and the accumulation of the fungal pathogen (Xiong *et al.*, 2014). As we know, soil microbes obtain carbon and energy sources mainly from the plants. Plants may in turn impact soil microbial communities either directly through root exudates or indirectly via altering soil properties (Garbeva *et al.*, 2004). As microbial food sources, root exudates released from different plants might vary in their nutrient contents and therefore prefer different types of soil microbes (Bais *et al.*, 2006). Recent studies have showed that genetically modified plants affect the composition of soil fungus communities (Hannula *et al.*, 2014).

F. oxysporum, one of the most ubiquitous genera, is pathogenic to a wide range of plants such as cucumber (Zhou and Wu, 2012), corn and bean (Okoth and Siameto, 2010), and white lupin (Mohamed *et al.*, 2012). *F. oxysporum* has also been demonstrated to cause fusarium wilt of chrysanthemum (Woltz and Engelhar, 1973).

It is most interesting that our data also showed that application of bio-organic fertilizer significantly suppressed *Fusarium* population and improved the yield of Chuju (Tables 3, 5 to 7). In the plot grown with Chuju for four seasons, applying bio-organic fertilizer increased Chuju yield by 39% but reduced *Fusarium* population by 42% as compared to synthetic fertilizer. Such effects of bio-organic fertilizers on pathogenic suppression and plant growth improvements have been reported in other plants such as banana (Shen *et al.*, 2013), muskmelon (Zhao *et al.*, 2011), and watermelon (Ling *et al.*, 2010). Bio-organic fertilizer is an organic amendment inoculated with living beneficial micro-organisms. It has showed better roles in improving plant growth and suppressing disease than common organic amendments have (Ahmad *et al.*, 2008; Luo *et al.*, 2010; Ding *et al.*, 2013). In a comparative study on different organic amendments, Shen *et al.* (2013) found that bio-organic fertilizer was the most effective in improving soil microbial communities, suppressing *Fusarium* wilt disease in banana, and enhancing yield and quality of banana. Luo *et al.* (2010) also showed that application of bio-organic fertilizer formulated with *Bacillus subtilis* biological control agent significantly improved fungal population composition in rhizosphere soils and effectively

controlled cotton verticillium wilt. In our present experiments, bio-organic fertilizer showed significant roles in enhancing Chuju yield and quality and suppressing *Fusarium* population, but it is necessary to further examine bio-organic fertilizer impact on microbial community dynamics and its duration.

It is worth noticing that adding bio-organic fertilizer did not significantly promote Chuju yields in the first two planting years (Table 4), suggesting that bio-organic fertilizer at the application rate may not contribute other nutrients much to the plant. Therefore, the improvements of Chuju yields by bio-organic fertilizer in both trial 1 and trial 2 would be due to the suppressiveness of fungal pathogens.

Conclusion

Applying bio-organic fertilizer could alleviate the continuous monocropping problems in Chuju production. However, further research is necessary on the mechanisms of Chuju production improvement and pathogenic suppression by and the optimal application rates of bio-organic fertilizers.

Acknowledgments

This study was supported by the Transformation Program in Agricultural Scientific and Technological Achievements of Anhui Province (No: 10140306017) and the Public Benefit Research Foundation of the Ministry of Agriculture of China (No: 201103004).

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(Received 25 January 2016; Accepted 22 February 2017)