0971-4693/94 Euro 10.00
Allelopathy Journal 39 (2): 153-164 (2016) *International Allelopathy Foundation* 2016
Table: 2, Figs : 5

Impacts of additives on quality of *Rehmannia* grown in soils previously used for its cultivation

Y.P. He[1], N.X. Zhao[2], H.P. Yang[1], Y.T. Yang, J.L.Wang[1*] and X.D. Wu[1*]
College of Agriculture & Biotechnology, Zhejiang University,
Hangzhou 310029, China
E. Mail: wangjinlong@tjau.edu.cn, wuxidong@tjau.edu.cn

(Received in revised form : October 21, 2016)

ABSTRACT

Pot experiments were conducted to study the impact of different soil additives on the quality of *Rehmannia glutinosa* (Gaetn.) Libosch. exFisch. etMey. and on soil properties, in the soils previously used for cultivating Rehmannia. Adding the vermicompost improved the total catapol content in *Rehmannia* tubers to 7.22 mg in soils previously used for 1-cultivation of *Rehmannia* and 6.70 mg in soils used for 2-cultivations. Superphosphate increased significantly the total catapol content in tubers in soils used for 2-cultivations. The improvement in catapol content may be due to the lower ratio of fungi to bacteria in rhizosphere soil, better improvement of soil micro-ecological environment and better fertility status. Activated carbon did not have any significant effect. Regulating the root soil microbial environment and nutrients status may be an effective way to improve the quality of *Rehmannia* cultivated in soils previously used for its cultivation.

Key words: Activated carbon, additives, alleviation, catapol, cultivation, *Rehmannia glutinosa*, successive cropping problem, superphosphate, vermicompost

INTRODUCTION

Rehmannia (Family Scrophulariaceae) is a medicinal herb and its tubers are used widely in food and health products (13,29). However, the land suitable for its cultivation is limited in Henan, Shanxi, and other parts of Hebei province, China. Generally, the crop is sown in spring and harvested in autumn and the land is left fallow for 8-10 years before next crop of Rehmania is cultivated. This is followed, because there are many oil sickness problems in cultivating *Rehmannia* in the same land repeatedly. Due to its high demand and limited growing area, overcoming the constraints of soil sickness in successive cropping has become important. Successive cropping on the same land leads to poor tuber germination, unhealthy plants, as well as more pests and diseases. It also decreases the tuber growth, increases fibrous root development and decreases yield and quality of tubers (6,24). These effects are attributed to soil microbial ecological imbalance (11,19,24,26,32), nutrients shifts in the rhizosphere (24,25,30) and toxicity by root exuded allelochemicals (3,11,24). Therefore, eliminating these problems may help in improving the conditions for *Rehmannia* growth in soils previously used for its cultivation. Studies have shown that continuous cropping can be successful by manipulating the soil conditions.

*Correspondence author: [1]College of Agronomy & Resources and Environment, Tianjin Agriculture University, Tianjin 300384, China, [2]College of Life Science, Nankai University, Tianjin 300071, China

Photograph 1. The *Rehmannia glutinosa* plants with roots

For example, addition of vermicompost effectively improve the soil fertility and helps in successive cropping of cucumber by increasing the population of beneficial microorganisms (18,23). Similarly, addition of activated carbon also alleviates the toxicity of phenolic acids (15,20) and addition of superphosphate improves the soil phosphorus status (1). However it is not known, whether these additives are helpful in successive cultivation of Rehmannia. This study aimed to determine the effects of 3-additives (vermicompost, superphosphate and activated carbon) on the microbial status, amounts of phenolics in the soils and catalpol yield of Rehmannia, planted in the soils that was previously used for its cultivation.

MATERIALS AND METHODS

The experiment was done in greenhouse in Nankai University, Tianjin Province, China, (Latitude 39°06′4.47″'N, Longitude 117°09′41.23″'E. Altitude : 2-5 m above sea level, Annual rainfall : 520-660 mm, continental climate). Rehmannia, cv "Beijing 3" was used in this study. Three types of soils were used: (i) S0- Soil not used for *Rehmannia* cultivation for the past 10 years], (ii) S1 - Soil used for Rehmannia cultivation only once in past 10 years and (iii) S2- Soil used for Rehmannia cultivation twice successively in past 10 years. All these soils were used for growing one crop of Rahmania in pot culture in greenhouse.) The soil samples were collected from the top 40 cm soil in Yuncheng city, Shanxi Province and were transported in bags in 2012, from Yuncheng to Tianjing. The physico-chemical properties of experimental soil were: PH : 6.7, OM: 10.7 ($g \cdot kg^{-1}$), available N, P, K were: 57.5, ,9.5 and 182,6 $mg \cdot kg^{-1}$ respectively. The fresh Vermicompost was purchased from Jia Liming Earthworm Inc. Tianjin, activated carbon from Hongyan Chemical Reagent Factory, Tianjin, and single superphosphate (16 % P) from Huayuan Bird and Flower Market nursery.

Pot Culture: The experimental treatments consisted of three factors: I. Soil types : 3 [(i) S0 -soil not used for Rehmannia cultivation in past 10 years, (ii) S1 -soil used for Rehmannia cultivation only once in past 10 years and (iii) S2 -soil used for Rehmannia cultivation 2-times, in past 10 years, II and III. Additives and their doses: 3 (Vermicompost 2 doses: !0 % and 30 %, Activated carbon 2 doses: 0.8 % and 1.6 %, and Superphosphates 2 doses : 0.16 % and 0.32 %). The treatments were replicated 9 times in Randomized block design. Pots (18 x 19 cm), with a gauze at the bottom to prevent soil loss, were filled with 2.5 Kg soil mixed with the additives. The Rehmannia tubers were cut into uniform pieces (each piece with 2 or 3 eyes). Cut tubers were soaked in 1% carbendazim solution for 20 min and then sown 2-3 cm deep in the soil. All pots were kept in greenhouse under natural light conditions and were watered once in 4 days. The tubers were harvested after 6 months and these were dried in shade till constant dry weight.

Rhizosphere Soil Analysis: The soil in the vicinity of plant roots (about 0.5 cm) was collected as the rhizosphere soil. The rhizosphere microorganisms in the rhizosphere soils samples were determined by dilution plate technique (8). Beef extract-peptone agar medium, Martin agar medium and Improved Gaoshi No.1 agar medium were used to determine the number of Bacteria, fungi and actinomycestes, respectively (9).

Chemical analysis: The amount of phenolic acids in the rhizosphere soils (31) and of catalpol in rehmannia tubers (14) was determined by HPLC using a Shimadzu LC-20AT HPLC, All standards used were of chromatographic grade (>98%). The ferulic acid, p-hydroxybenzoic acid and vanillic acid were from ShijiAoke Co in Beijing. Vanilline standard was from Xiya Company in Sichuan. All the mobile phase solutions and samples were filtered through millipore filters (0.45μm).

Phenolic acids extraction and determination: Twenty g air dried rhizosphere soil was added to 20 mL of 1 mol/L NaOH**,** sonicated for 30 min and left over night. Next day it was again sonicated for 30 min and the mixture was centrifuged at 3000 rpm for 20 min. The supernatant was adjusted to pH 2.5 with 12 M HCl and allowed to stand for 2 h and then centrifuged to remove humic acid. The supernatent solution was extracted thrice with an equal volume of n-butanol. The butanol fractions were combined and dried using a rotary evaporator (EYEL4OSB-2000). It was then mixed with 2 ml methanol and filtered using a 0.45 μm filter. Liquid chromatography of this methanol preparation was done using an HPLC fitted with a Venusll XBP-C18 column (5μm, 100A, 4.6 × 250 mm). The volume of sample injected was 5 μL The mobile phase was 0.01mol/LNaAc:HAc:n-butanol:NH4OH 100:0.15:2:0.05 and the flow rate was 0.7mL/min. Fractions were monitored by UV detector at 277 nm. Phenolic acids content was in $\mu g.g^{-1}$of air dry soil.

Catalpol extraction and determination: Dried and ground *Rehmannia* tuber slices (0.50g) were sonicated in 10 mL methanol for 30 min. The volume was increased to 10 mL and then the solution was filtered through 0.45 μm filter. The filtrate was analysed by HPLC using a Unitary-C18 column (5 μm, 100A, 4.6× 250 mm), with acetonitrile: water (1:99 v:v) as mobile phase, with a flow rate of 1.0 mL /min. Injection volume was 20 μL and detection at 210 nm (14). The total amount of catalpol in *Rehmannia* roots per plant was calculated as under:

Total catalpol (mg) = Concentration of catalpol (mg g^{-1}) × Total dry weight of roots (g).

Statistical analysis

One-way ANOVA with a Duncan multiple comparison was used to evaluate the differences ($P< 0.05$) between the treatments. Figures were made by EXCEL 2007. A two-factor analysis using SAS 9.3 indicated a significant interaction between the two factors.

RESULTS AND DISCUSSION

Catalpol content

In the treatment, where no additive was added [CK (control) group], the total amount of catalpol in tubers was 8.77 mg per plant, it decreased to 3.64 mg and 0.26 mg in the tubers of Rehmannia planted in soil S1 and soil S2, respectively. Thus the total amount of catalpol in tubers decreased significantly with successive cultivation of Rehmannia in the same soil (Figure 1). This was consistant with the results in other studies (33).

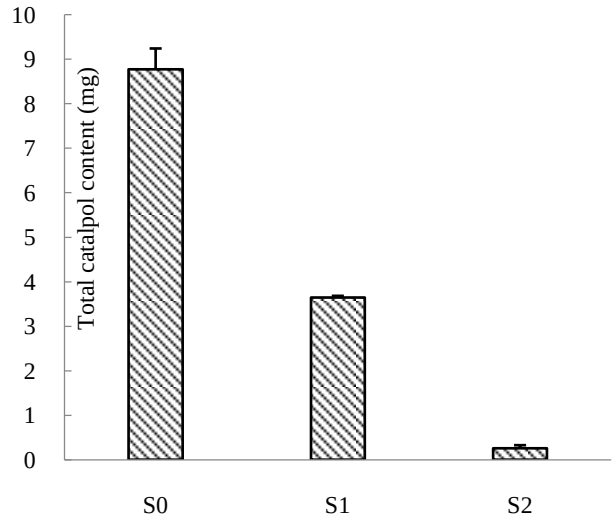

Figure 1. The total catalpol content (mg/pot) in the root tuber of Rehmannia.

In pots applied with additives (the treatment group), the 30% vermicompost dramatically increased the total amount of catalpol in tubers from 3.64 mg to 7.22 mg in Rehmannia planted in soil S1 and from 0.26 mg to 6.70 mg in Rehmannia planted in soil S2.

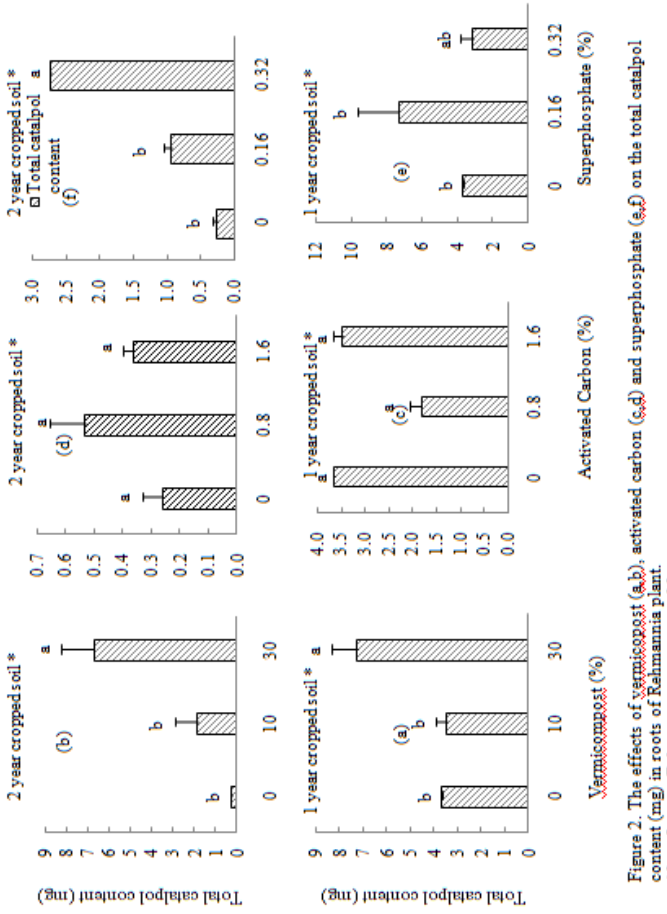

Figure 2. The effects of vermicompost (a,b), activated carbon (c,d) and superphosphate (e,f) on the total catalpol content (mg) in roots of Rehmannia plant.
*1 or 2 years Rehmannia cropping in 10 years.

Application of superphosphate at 0.32% also increased the total catalpol content in the tubers of Rehmannia planted in soil S2 from 0.26 mg to 2.71 mg. However, this was much lower than when vermicompsot was added. Active carbon had no effect on the catalpol yield of Rehmannia planted in soil S1 and S2. Thus among the three additives, vermicompost at 30%, was the best (Figure 2).

Soil Microorganisms

Compared to the control soil (CK), the ratio of fungi to bacteria in soils increased significantly with successive cultivation of Rehmannia, from 0.56 in S0 to 1.58 in S2, indicating that its successive cultivation of Rehmannia in the same soil led to an imbalance in the micro-environment of the soil (Table 1).

Table 1. Effects of different additives on soil microorganisms

Additives	Additive dose	Fungi	Bacteria	Fungi/Bacteria
S0 soil (Control)*	-	61.90^{c}	111.500^{a}	0.56
	S1 soil			
S1 soil Control	-	146.85^{a}	133.85^{b}	1.10
Vermicompost	10%	116.35^{ab}	211.15^{a}	0.55
	30%	85.65^{b}	166.85^{ab}	0.51
Activated carbon	0.8%	110.65^{b}	137.65^{b}	0.80
	1.6%	90.50^{c}	163.00^{a}	0.56
Superphosphate	0.16%	103.70^{b}	124.25^{b}	0.83
	0.32%	89.35^{b}	140.80^{b}	0.63
	S2 soil			
S2 soils Control		189.35^{a}	119.80^{c}	1.58
Vermicompost	10%	69.20^{b}	227.30^{b}	0.30
	30%	74.00^{b}	281.50^{a}	0.26
Activated carbon	0.8%	182.20^{a}	265.15^{b}	0.69
	1.6%	138.00^{b}	144.65^{c}	0.95
Superphosphate	0.16%	112.50^{b}	128.65^{c}	0.87
	0.32%	64.00^{c}	179.00^{b}	0.36

*Control (soils without additives)
Bacteria 10^{4}cuf g^{-1} soil, fungi 10^{3}cuf g^{-1} soil.
Additive doses: 10% : 200 Kg/ha, 30% : 600 Kg/ha, 0.8%: 16 Kg/ha, 1.6% : 32 Kg/ha, 0.16% : 3.2 Kg/ha 0.32% : 6.4 Kg/ha
All values are presented as the mean ± standard error.
In controls,(CK) different letters in the same column indicate significant differences ($P < 0.05$) between different successive cropping years;
In "1st (2nd) successive cropping" different letters in the same column indicate significant differences ($P < 0.05$) between 1st (2nd) year CK and additives (low add and high add).

All additives reduced the number of fungi and the ratio of fungi to bacteria. Vermicompost significantly increased the number of bacteria in the S1 and S2 soils. The vermicompost at 30 % dose increased the number of bacteria by 24.7% and 135% in soil S1 and S2 over the control, respectively. Contrarily, the number of fungi was significantly inhibited by vermicompost. The vermicompost at 10 % decreased the number of fungi by

20.8% and 63.5% in soil S1 and S2 over the control, respectively. While vermicompost at 30 % reduced the number of fungi by 41.7% and 60.9% in soil S1 and S2 over the control, respectively (Figure 3). The vermicompost at 30 % dose was most effective in reducing the ratio of fungi to bacteria, with 0.51 and 0.26 in S1 and S2, respectively (Table 1).

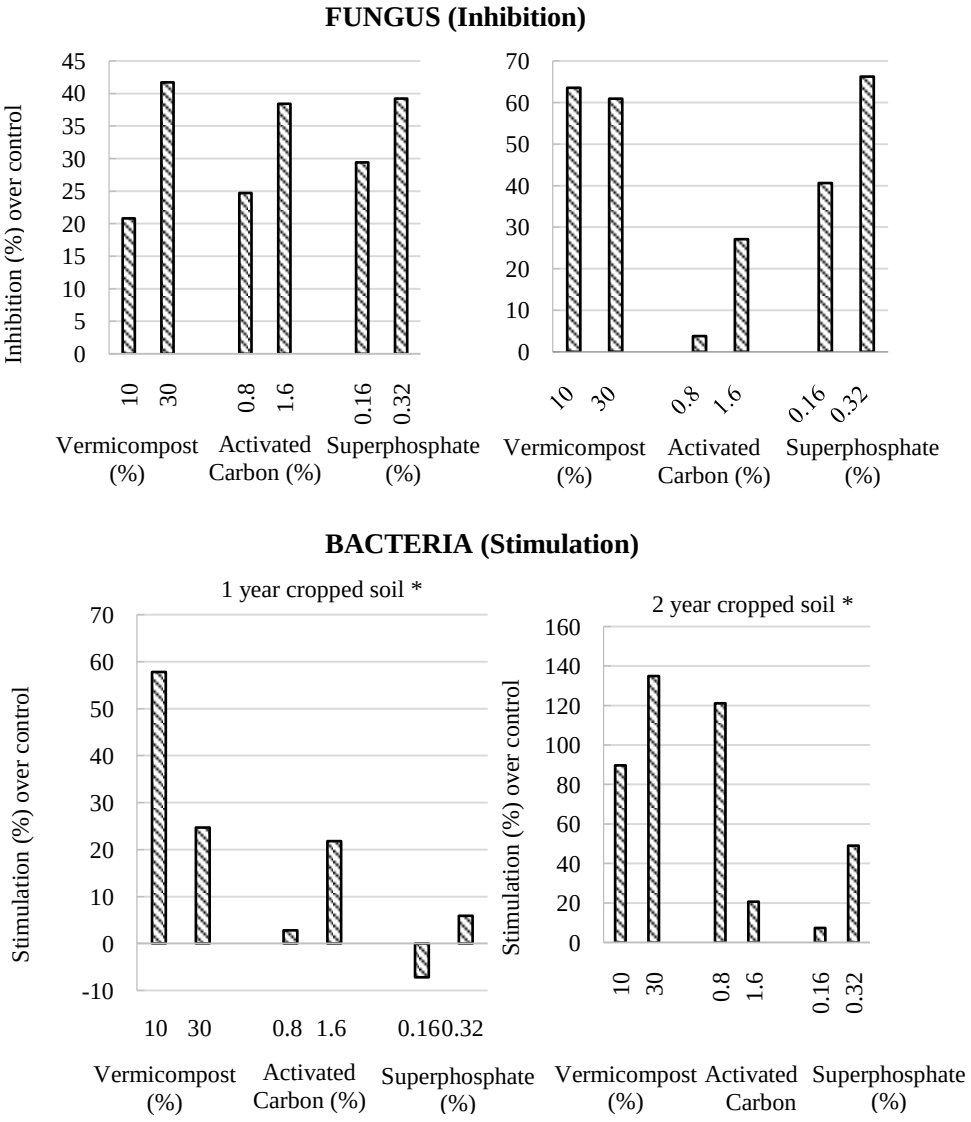

Figure 3. Inhibition/stimulation effect of vermicopost, activated carbon and superphosphate on the fungus and bacteria in Rehmannia grown soils. Additive doses: 10% : 200 Kg/ha, 30% : 600 Kg/ha, 0.8%: 16 Kg/ha, 1.6% : 32 Kg/ha, 0.16% : 3.2 Kg/ha 0.32% : 6.4 Kg/ha
*1 or 2 years Rehmannia cropping in 10 years.

The increase in the number of bacteria was perhaps due to the presence of abundant beneficial bacteria originally present in the vermicompost (10,21,27). This led to a reduction in the ratio of fungi to bacteria, which contributed to the improvement in the soil micro-ecological environment. Earlier studies (12,22) have shown that an increase in fungi and a decline in soil fertility are closely related. Since the vermicompost contains abundant plant nutrients (16,17), hence can effectively improve the soil fertility. This may be the reason, why the vermicompost effectively improved the catalpol content in Rehmannia tubers.

Activated carbon can also largely stimulate the number of bacteria in soil S1 and S2. Activated carbon at 1.6 % increased the number of bacteria by 21.8% in soil S1, and activated carbon at 0.8 % increased the number of bacteria by 121.2% in soil S2. High dose (1,6 %) of activated carbon inhibited the number of fungi in soils, reducing by 38.4% in soil S1 and 27.1% in soil S2 (Figure 3). The decreased number of fungi and a decreased ratio of fungi to bacteria might be due to the decrease in phenolic acids content of soils, which led to reduction in energy resources creating conditions unsuitable for optimal fungal growth.

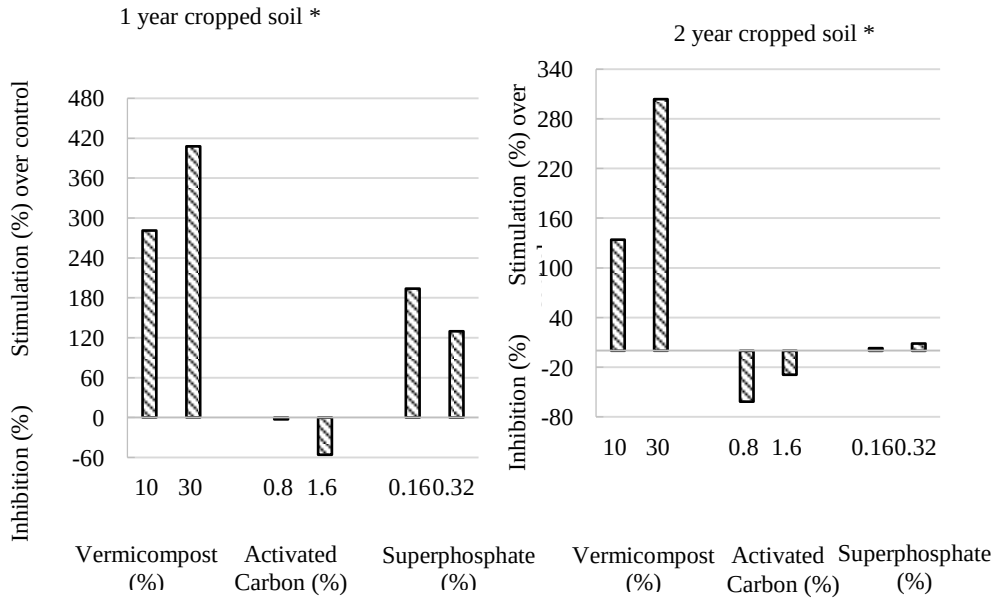

Figure 4. Inhibition/stimulation effect of vermicopost, activated carbon and superphosphate on the total polyphenol content in Rehmannia grown soils. Additive doses: 10% : 200 Kg/ha, 30% : 600 Kg/ha, 0.8%: 16 Kg/ha, 1.6% : 32 Kg/ha, 0.16% : 3.2 Kg/ha 0.32% : 6.4 Kg/ha
*1 or 2 years Rehmannia cropping in 10 years.

Superphosphate also significantly inhibited the number of fungi in soils. Superphosphate at 0.32 % decreased the number of fungi by 39.2% and 66.2% in soil S1 and S2, respectively. And notably increased the number of bacteria by 49.1% in soil S2 (Figure 3). This might due to the improvement of soil fertility by superphosphate.

Soil Phenolic Acids Content

As the number of Rehmannia croppings increased, the content of phenolic acids [ferulic acid, p-hydroxybenzoic acid (PHBA), vanillic acid and vanilline] and their total amount also increased in the soils (19,31). The content of ferulic acid increased from 0.331 μg g^{-1} to 1.176 μg g^{-1} in S1 soil and was 0.937 μg g^{-1} in S2 soil. The content of PHBA increased from 0.136 μg g^{-1} to 0.172μg g^{-1} in S2 soil. Vanillic acid increased from 0.189 μg g^{-1} to 0.272 μg g^{-1} in S1 soil and vanilline increased from 0.064 μg g^{-1} in S0 soil, to 0.139 μg g^{-1} in S2 soil. The content of total phenolic acids in S0 soil was 0.719 μg g^{-1} and increased to 1.761 μg g^{-1} in S1. These results are consistent with Du (7), who confirmed the content of PHBA in soils S2 was 8.75 times higher than soil S0, and the content of vanillic acid in soils S2 was 1.84 times more than soil S0.

Zhu *et al*. (34) reported that plant root exudates provide energy for rhizosphere microorganisms and Zhang (31) found that phenolic acids stimulated the growth of some soil fungi. An increase in the phenolic acids content stimulated the fungal growth significantly, increase the ratio of fungi to bacteria and alter the soil micro-ecological balance. Earlier studies (12,22) have shown that an increase in fungi and a decline in soil fertility are closely related, hence, successive cultivation of one crop in the same soil can also reduce soil fertility.

Table 2. Effects of successive cropping and additives on phenolic acids content in Rehmannia rhizosphere soils

Additives	Additive dose	PHBA	Vanillic acid	Vanilline	Ferulic acid	Total polyphenols
S0 soil (Control)*	-	0.136c	0.189b	0.064a	0.331b	0.719b
	S1 soil					
S1 soils Control	-	0.190^{a}	0.272^{b}	0.121^{b}	1.176^{a}	1.761^{a}
Vermicompost	10%	1.445^{a}	0.480^{b}	0.228^{b}	2.799^{a}	4.949^{a}
	30%	1.424^{a}	1.179^{a}	0.433^{a}	4.144^{a}	7.179^{a}
Activated carbon	0.8%	0.211^{a}	0.301^{b}	0.038^{c}	1.165^{a}	1.713^{a}
	1.6%	0.163^{b}	0.189^{c}	ND	0.422^{b}	0.776^{b}
Superphosphate	0.16%	0.450^{a}	0.407^{b}	0.207^{b}	2.348^{a}	3.413^{a}
	0.32%	0.217^{a}	0.328^{b}	0.124^{b}	1.617^{a}	2.284^{a}
	S2 soil					
S1 soils Control	-	0.172^{a}	0.260^{b}	0.139^{b}	0.937^{b}	1.508^{b}
Vermicompost	10%	0.642^{a}	0.525^{b}	0.203^{b}	2.158^{ab}	3.528^{ab}
	30%	0.783^{a}	1.248^{a}	0.632^{a}	3.428^{a}	6.089^{a}
Activated carbon	0.8%	0.171^{a}	0.217^{b}	ND	0.538^{b}	0.926^{b}
	1.6%	0.190^{a}	0.210^{b}	0.042^{b}	0.628^{b}	1.072^{b}
Superphosphate	0.16%	0.185^{a}	0.286^{b}	0.145^{b}	0.940^{b}	1.556^{b}
	0.32%	0.180^{a}	0.271^{b}	0.114^{b}	1.074^{b}	1.639^{b}

All values are presented as the mean ± standard error. Additive doses: 10% : 200 Kg/ha, 30% : 600 Kg/ha, 0.8%: 16 Kg/ha, 1.6% : 32 Kg/ha, 0.16% : 3.2 Kg/ha 0.32% : 6.4 Kg/ha

In "CK" different letters in the same column indicate significant differences ($P < 0.05$) between different successive cropping years; In "1st (2nd) successive cropping" different letters in the same column indicate significant differences ($P < 0.05$) between1st (2nd) year CK and additives (low add and high .PHBA :p-hydroxybenzoic acid. ND: Not detected

Activated carbon reduced the total amount of these four phenolic acids (Table 2). Activated carbon at 0.8 % significantly reduced the content of total polyphenol by 61.4% over the control in the soil S2, and 1.6 % activated carbon largely decreased the amount of the four phenolic acids by 55.9% and 28.9% over the control in soil S1 and S2, respectively (Figure 4). This is consistent with other studies (4,15) and may be also due to the high adsorption capacity of activated carbon (4,15) and the increased number of bacilli which degrades the phenolic acids (5). The decrease in phenolic acid content of soil however, led to a reduction in energy resources creating conditions unsuitable for optimal fungal growth, resulting in decreased number of fungi and a decreased ratio of fungi to bacteria.

The addition of vermicompost increased the phenolic acids by 281.1% and 134.1% over the control in soil S1 and S2, when the additive dose was 10 %, and increased by 407.7% and 303.9% when vermicompost at 30 %. This is because of the high content of phenolic acids in vermicompost (2).

Soil microorganisms play an important part in the soil previously used for cultivating Rehmannia. The successive cultivation in the same soil could significantly reduce the species and number of soil bacteria (28), especially the bacilli, which have a positive effects on the degradation of phenolic acids and rhizosphere ecological environment. Vermicompost contains abundant plant nutrients (16,17) and beneficial bacteria (10,21,27), hence, effectively improves the soil fertility and increase the number of bacteria, and reduce the ratio of fungi to bacteria, which contributed to the improvement in the soil micro-ecological environment. Therefore, the improvement of soil fertility and soil micro-ecological conditions play major role in the positive effect of vermicompost in improving the quality of Rehmannia tubers from successive harvests from the same soil.

CONCLUSIONS

Our results showed that vermicompost, was superior to superphosphate and activated carbon in improving the quality of *Rehmannia* planted in soils previously used for cultivating this crop. This may be due to its impact on soil fertility and soil micro-ecological environment. Hence, regulating the root soil microbial ecology and soil nutrition may be an effective way to improve the quality of *Rehmannia* tubers from the soils used for its successive cultivation.

ACKNOWLEDGEMENTS

This work was financially supported by Tianjin “131” Talents Engineer Scheme for the First-Level Candidates “Effects of Drought Stress and Mowing disturbance on Competition- Facilitation Interaction of Key Species in Typical Steppe”, Scheme for the Excellent Young Teachers of Tianjin Universities “Research to Alleviate the Effects of Exogenous Additives on Continuous Cropping Obstacle of *Rehmannia glutinosa*” and Innovation Teams Scheme of Tianjin Universities “Genetic Improvement of Crops Traits in Qualities and Stress Resistance” (TD12-5017)

REFERENCES

1. Achmad Arivin, R. and Russ Williams, T. (2010). Phosphorus fertilizer induced changes in the soil available P, the P nutrition and the growth of Pinus radiata seedlings grown in association with understory. *Journal of Forestry Research* **21** : 129-136.
2. Aremu, A.O., Stirk, W.A., Kulkarni, M.G., Tarkowska, D., Tureckova, V., Gruz, J., Subrtova, M., Pencik, A., Novak, O., Dolezal, K., Strnad, M. and Van Staden, J. (2015). Evidence of phytohormones and phenolic acids variability in garden-waste-derived vermicompost leachate, a well-known plant growth stimulant.*Plant Growth Regulation* **75** : 483-492.
3. Bennett, A.J., Bending, G.D., Chandler, D., Hilton, S. and Mills, P. (2012). Meeting the demand for crop production: The challenge of yield decline in crops grown in short rotations.*Biological Reviews* **87** : 52-71.
4. Cai, Y., Liao, Z., Zhang, J.E., Kong, W. and He, C. (2003). Effects of ecological organic fertilizer on tomato bacterial wilt and soil microbial diversities.*Yingyong Shengtai Xuebao* **14** : 349-353. (Chinese)
5. Chen, H. (2008). *Effects of Suceessively Cropping for Rehmannia glutinosa on Microflora in the Rhizospheric Soil*. Master Thesis. Fujian Agriculture and Forestry University Pp. 22-45. (Chinese)
6. Chen, H., Hao, H.R., Xiong, J., Qi, X.H., Zhang, C.Y. and Lin, W.X. (2007). Effects of successive cropping *Rehmannia glutinosa* on rhizosphere soil microbial flora and enzyme activities.*Yingyong Shengtai Xuebao* **18**: 2755-2759. (Chinese)
7. Du, J. (2009). *Research of Phenolic Acids Allelopathy on Soils Around the Rhizosphere of Rehmannia glutinosa* Libosch. Master Thesis. Henan Agricultural University Pp. 28-32. (Chinese)
8. Gu, L., Xu, B., Ling, Q. and Yin, T. (2013). Impact and colonisation ability of Trichoderma biocontrol on lawn soil microflora. *Acta Prataculturae Sinica* **22**: 321-326. (Chinese)
9. He, J., Fu, X., Ma, D., Jia, X. and Wang, M. (2008). Changes of phenolic acid content and rhizosphere microbial population in soil during tuberous root expansion of *Rehmannia glutinosa* Libosch. *Henan Science* **26**: 1369-1372. (Chinese)
10. Hu, Y.Y., Sun, Z.J. and Cheng, W.L. (2016). The study on the inhibition of plant soil-borne diseases by earthworm. *Northwestern Horticulture* (1): 11-13. (Chinese).
11. Huang, L.F., Song, L.X., Xia, X.J., Mao, W.H., Shi, K., Zhou, Y.H. and Yu, J.Q. (2013). Plant-soil feedbacks and soil sickness: from mechanisms to application in agriculture. *Journal of Chemical Ecology* **39**: 232-242.
12. Li, J., He, X., Li, J. and Li, B. (2012). Impacts of soil factors on AM fungi of invasive plants - *Flaveria bidentis*. *Journal of Anhui Agriculture Science* **40** : 8922-8925. (Chinese)
13. Li, J., Wang, Y. and Fan, H. (2013). An overview of medicinal research on *Rehmannia*. *Biology Teaching* **3**: 4-6. (Chinese)
14. Liu, H., Li, J. and Lei, Y. (2010). The study of catalpol assaying and its extraction and separation processes in rehmannia. *Information on Traditional Chinese Medicine* **27**(1): 86-88. (Chinese)
15. Morvillo, C.M., de, la Fuente, E.B., Gil, A., Martinez-Ghersa, M.A. and Gonzalez-Andujar, J.L. (2011). Competitive and allelopathic interference between soybean crop and annual wormwood (*Artemisia annua* L.) under field conditions. *European Journal of Agronomy* **34**: 211-221.
16. Najafi-Ghiri, M. (2014). Effects of zeolite and vermicompost applications on potassium release from calcareous soils. *Soil and Water Research* **9**(1): 31-37.
17. Papathanasiou, F., Papadopoulos, I., Tsakiris, I. and Tamoutsidis, E. (2012). Vermicompost as a soil supplement to improve growth, yield and quality of lettuce (*Lactuca saliva* L.). *Journal of Food Agriculture & Environment* **10** : 677-682.
18. Pathma, J. and Sakthivel, N. (2012). Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential.*Springerplus* **1**: DOI 10.1186/2193-1801-1-26.
19. Qi, J., Yao, H., Ma, X., Zhou, L. and Li, X. (2009). Soil microbial community composition and diversity in the rhizosphere of a Chinese medicinal plant. *Communications in Soil Science and Plant Analysis* **40**: 1462-1482.
20. Satoru, M., Eiji, N., Naoshi, T., Hermann, L. and Yutaka, S. (2007). Effects of activated carbon to reduce allelopathy during raising the seedling stage. *Horticultural Research* (Japan) **6** : 603-609.
21. Tian, G. and Zhang, L. (2016). Alleviation of vermicompost to obstacle in sterilized continuous cropping soil in strawberry production. *Journal of Plant Nutrition and Fertilizer* **22** : 759-767. (Chinese)
22. Wang, C., Wu, F., Liu, X. and Liu, B. (2005). Tobacco rhizosphere microorganism in different fertility of soil. *Chinese Tobacco Science* **2**:12-14.

23. Wang, M., Jing, D., Zhang, H., Li, S. and Zheng, F. (2016). Effects of vericompost on active organic carbon and microbial activity in cowpea soil. *Acta Agriculturae Nucleatae Sinica* **30**: 1404-1410. (Chinese)
24. Wu, L., Wang, H., Zhang, Z., Lin, R., Zhang, Z. and Lin, W. (2011). Comparative metaproteomic analysis on consecutively *Rehmannia glutinosa*- monocultured rhizosphere soil. *Plos One* **6**(5): 1-12.
25. Wu, L., Li, Z., Li, J., Khan, M.A., Huang, W., Zhang, Z. and Lin, W. (2013). Assessment of shifts in microbial community structure and catabolic diversity in response to *Rehmannia glutinosa* monoculture. *Applied Soil Ecology* **67**: 1-9.
26. Wu, L.K., Wu, H.M., Chen, J., Wang, J.Y. and Lin, W.X. (2016). Microbial community structure and its temporal changes in *Rehmannia glutinosa* rhizospheric soils monocultured for different years. *European Journal of Soil Biology* **72**: 1-5.
27. Yang, M. (2008). Using wormcast prevent vegetable soil-borne disease. *Journal of Changjiang Vegetables* **17**: 21-22. (Chinese).
28. Zhang, J.X. (2016). Discussion on the reasons and prevention methods of continuous cropping of horticultural crops. *Rural Economy and Science-Technology* 27 (14): 46-47. (Chinese)
29. Zhang, R.X., Li, M.X. and Jia, Z.P. (2008). *Rehmannia glutinosa*: Review of botany, chemistry and pharmacology. *Journal of Ethnopharmacology* **117**: 199-214.
30. Zhang, S. and Gao, Z. (2000). Continuous cropping obstacle and rhizospheric microecology II. Root exudates and phenolic acids. *Chinese Journal of Applied Ecology* **11**: 152-156. (Chinese).
31. Zhang, S., Gao, Z. and Liu, H. (2000). Continuous cropping obstacle and rhizospheric microecology III. Soil phenolic acids and their biological effect. *Chinese Journal of Applied Ecology* **11**: 741-744. (Chinese).
32. Zhang, Z.Y., Lin, W.X., Yang, Y.H., Chen, H. and Chen, X.J. (2011). Effects of consecutively monocultured *Rehmannia glutinosa* L. on diversity of fungal community in rhizospheric soil. *Agricultural Sciences in China* **10** : 1374-1384. (Chinese)
33. Zhongyi, Z., Gailing, L.I., Miaomiao, N., Huamin, F., Juan, L.I. and Wenxiong, L. (2011). Responses of physiological ecology and quality evaluation of Rehmannia gltinosa in continuous cropping. *Chinese Journal of Chinese Materia Medica* **36** : 1133-1136. (Chinese)
34. Zhu, L., Zhang, J. and Liu, W. (2003). Review of studies on interactions between root exudates and rhizopheric microorganisms. *Ecology and Environment* **12**: 102-105. (Chinese)