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EFFECTS OF VERMICOMPOST APPLICATION ON QUALITY OF FLUE-CURED TOBACCO

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Abstract

In this study, the field experiments were conducted to study the effects of different proportions of vermicompost (without vermicompost, 1800 kg ha^{-1} vermicompost, 2400 kg ha^{-1} vermicompost, 3000 kg ha^{-1} vermicompost, 3600 kg ha^{-1} vermicompost) on the flue-cured tobacco economic traits and tobacco internal quality. Results showed that the application of 3000 kg ha^{-1} vermicompost in the field significantly increased the contents of nicotine, total nitrogen and potassium, enhanced the ratio of potassium to chloride, coordinated the chemical ingredients of tobacco leaves, improved the sensory quality, and made flue-cured tobacco as high-quality tobacco. Therefore, application of 3000 kg ha^{-1} vermicompost proved to be more effective in improving economic benefits and internal quality of tobacco. Such effects were associated with increased contents of carotene degradation products, Maillard reaction products, cembratriendid alkyl degrading products and other aromatic compositions in flue-cured tobacco leaves by vermicompost and with improved intrinsic quality of tobacco.

Key words: Economic characters, Quality, Flue-cured tobacco, Chemical components, Aaromatic compositions.

Introduction

Tobacco is an important economic crop with high yield and large planting area. It is a broad-leaved crop with low planting density (Song *et al*., 2016). Long-term field trials and treatments with different fertilizers changed the nutrient composition and content of the soil, which affected the yield and quality of crops. There were increasing evidences that when organic fertilizers were used as basic fertilizers, the yield and quality of crops were effectively improved (Ortas *et al*., 2014).

As a high-quality organic fertilizer, vermicompost contained high levels of different plant growth regulators and soil enzymes (Datta *et al.*, 2018), which promoted the growth and development of plants (Atiyeh *et al.*, 2000). Nurhidayati *et al*., (2018) found that vermicompost effectively improved soil quality, increased microbial activity and nutrient cycling speed and produced high-quality crops. Replacing about 20% to 40% of the substrate with earthworm droppings promoted plant rooting and growth (Rosa *et al.*, 2013). Thus, this study comprised field experiments to explore the effects of different concentrations of vermicompost on the economic characteristics, internal and sensory aroma quality of flue-cured tobaccos, and it signified that vermicompost played a role in promoting the quality and yield of flue-cured tobaccos, so as to provide a theoretical reference for the application of vermicompost in the actual production of tobacco.

Materials and Methods

Field experiment: An experiment was carried out in Yongfeng Town (34°25′N,110°40′E), Shaanxi, China. It was located in temperate humid monsoon climate zone, the average annual temperature was 11.1℃ and the annual precipitation was 760 mm. Tobacco soil was yellowish brown, pH value 5.96, organic matter content 25.32 g kg^{-1}, available nitrogen content 39.73 mg kg^{-1}, available phosphorus content 25.86 mg kg^{-1}, and available potassium content was 110.57 mg kg^{-1}.

Experimental treatments: A randomized block design was used in this experiment and contained five treatments (no vermicompost; 1800 kg ha^{-1} vermicompost; 2400 kg ha^{-1} vermicompost; 3000 kg ha^{-1} vermicompost; 3600 kg ha^{-1} vermicompost). In addition, 450 kg ha^{-1} special tobacco fertilizers (N: P_2O_5: K_2O=10: 9: 23) were applied as a standard management practice in each treatment. Each plot area of each repetition was 91.2 m^2, 160 tobacco plants were assigned to a group, with three replicates. The vermicompost was supplied by the Xi'an Dillon Company.

The appearance and sensory quality of tobacco: The harvested tobacco leaves were roasted in the same curing barn, and the cured tobacco leaves were classified according to the standard method GB2635-92. Each plot separately counted the output and the percentage of superior and middle-class tobacco leaves, and calculated the average price and output value. For each treatment, 2 kg of C3F grade tobacco leaves were selected; the appearance and sensory quality of tobacco were evaluated in Qingzhou Research Institute of China Tobacco Corporation with the standard methods YC/T159-2002, YC/T160-2002, YC/T161-2002, YC/T162-2002.

Estimation of total sugars and reducing sugars: The total sugars were determined by the anthrone method of Fales (1951). The content of reducing sugars was determined by the 3,5- dinitrosalicylic acid method under alkaline conditions, and the absorbance was determined at 483 nm (Breuil *et al*., 1985).

Nicotine measurement: 0.5 g of flue-cured tobacco leaves were ground into powder, boiled with 30 ml of 0.05 mol/L hydrochloric acid for 5 minutes, and filtered while hot. The filtrate was extracted with dichloromethane under the indicator of methyl orange, and the absorbance was determined at 435 nm.

The determination of total nitrogen, potassium and chlorine: The contents of total nitrogen and potassium were determined by concentrated sulfuric acid-hydrogen peroxide method; dry powdered samples (each 0.03 g) were digested with a sulfuric acid and hydrogen peroxide mixture (V:V =5:2), and determined with continuous flow analyzer (Hach *et al*., 1985). The determination of chlorine content referred to the Mohr method, K_2CrO_4 was used as the indicator in the Weakly alkaline solution, and the $AgNO_3$ standard solution was used for direct titration (Yoder *et al*., 1919).

Volatile aroma components measurement: Tobacco leaves were extracted by vacuum distillation, and the main components in tobacco leaves were detected by gas chromatography-mass spectrometry (GC-MS) (Bartle *et al*., 1970).

Statistical analyses: One-way analysis of variance test (ANOVA) was used to analyze the collected data. Duncan's multi-range test (DMRT) was performed using SPSS 20.0 software to calculate the average significant difference.

Results

Economic characteristics of flue-cured tobacco leaves: The treatment of vermicompost with different application levels had increased the main indicators of economic characters of cured tobacco to varying degrees (Table 1). Among them, the average price of 3000 kg ha^{-1} vermicompost treatment and the proportion of upper and middle-grade tobacco reached the maximum, which increased by 24.91% and 87.82% compared with no vermicompost, respectively. The results showed that vermicompost had a significant effect on the tobacco leaf grade. Application of vermicompost (3600 kg ha^{-1}) significantly increased the output value compared with no vermicompost treatment, but the average price was no significant difference.

The appearance quality of flue-cured tobacco leaves: There were no significant differences in tobacco leaf color, maturity, leaf structure and identity between different treatments after the application of vermicompost in the field grown tobacco plants (Table 2). Although the oil content and aroma of tobacco leaves treated with vermicompost were not significantly different from each other, the oil content and aroma of tobacco plants treated with 1800 or 2400 kg ha^{-1} vermicompost were significantly higher than those without the vermicompost group. Overall, the appearance and quality of tobacco leaves treated with 1800 kg ha^{-1} vermicompost were better than the other treatments.

Table 1. Effect of vermicompost on economic characters of flue-cured tobacco.

Treatment	Yield ($kg \cdot hm^{-2}$)	Output value ($yuan \cdot hm^{-2}$)	Average price ($yuan \cdot kg^{-1}$)	Percentage of superior tobacco (%)	Percentage of medium and superior tobacco (%)
No vermicompost	152.4c	3782.57c	24.82a	35.45a	87.63a
1800 kg ha^{-1} vermicompost	151.2c	3733.13c	24.69b	35.39a	87.52a
2400 kg ha^{-1} vermicompost	154.60b	3806.25b	24.62b	35.61a	87.68a
3000 kg ha^{-1} vermicompost	155.80b	3880.98b	24.91a	35.42a	87.82a
3600 kg ha^{-1} vermicompost	158.40a	3929.90a	24.81a	35.77a	87.63a

Data are means of three biological replicates. Statistical difference (p<0.05) between the treatments are indicated by different lowercase letters

Table 2. Effect of vermicompost on appearance quality of flue-cured tobacco.

Treatment	Color-maturity-structure-identity + grade	Oil	Chroma	Comprehensive evaluation
No vermicompost	Orange-mature-loose-medium grade-C3F	contain+	medium+	Bright and lustrous, uniform leaf color, oily, loose structure
1800 kg ha^{-1} vermicompost		contain+	medium+	Oily, bright and shiny, loose structure
2400 kg ha^{-1} vermicompost		contain+	medium+	A few leaves are slightly on the second shed, the gloss is bright, there is oil
3000 kg ha^{-1} vermicompost		contain	medium	The oil content is relatively small, the gloss is brighter, but the uniformity of the leaf surface is slightly worse, and the structure is slightly tight
3600 kg ha^{-1} vermicompost		contain	medium	Some foliar oils are slightly less, the leaves are slightly ashed, and some leaves have less uniformity

Data are means of three biological replicates. Statistical difference (p<0.05) between the treatments are indicated by different lowercase letters

Chemical compositions of flue-cured tobacco leaves: As was evident from Table 3, the contents of total soluble sugars, reducing sugars, nicotine, total nitrogen and potassium in flue-cured tobacco leaves were significantly improved after vermicompost application. In addition to the potassium content, the contents of other chemical compositions showed a decreasing trend with increase in the amount of vermicompost applied. Compared with no vermicompost, the treatment with 1800 kg ha^{-1} vermicompost significantly increased the total soluble sugars and reducing sugars by 20.67% and 11.10%, respectively. The treatment with 1800 kg ha^{-1} earthworm manure also significantly increased the chlorine content, which was 75% higher than that of the control. Compared to the control treatment, the nicotine content in the tobacco leaves was significantly increased by 35.16% and 15.38% respectively, with 2400 and 3000 kg ha^{-1} vermicompost, and the total nitrogen content in tobacco leaves was significantly increased by 6.21% and 2.26%, respectively. The accumulation of potassium in the tobacco leaves was directly proportional to the amount of vermicompost applied. When 3600 kg ha^{-1} of vermicompost was applied, the potassium content of flue-cured tobacco leaves was the highest, which was 1.84%.

The coordination of chemical ingredients in tobacco leaves: The ratio of total sugars to nicotine in all treatments exceeded the appropriate value. The ratio of total sugar to nicotine was significantly reduced with the application of 2400 and 3000 kg ha^{-1} vermicompost compared with the control, and the ratio was close to the standard value (Table 3). In addition to the treatment of 3600 kg ha^{-1} vermicompost in the field, the other vermicompost concentrations reduced the total nitrogen to nicotine ratio of the flue-cured tobacco. The ratio of reducing sugars to total sugars in each treatment was greater than 0.75, which was in the appropriate range. Moreover, the application of 3000 and 3600 kg ha^{-1} vermicompost in the field significantly increased the K/Cl ratio of the tobacco leaves by 67.00% and 236.37% respectively compared to the control.

The sensory quality of flue-cured tobacco leaves: The results in Table 4 showed that vermicompost treatment improved the sensory quality of flue-cured tobacco leaves. Aroma type, strength, flammability and gray of each treatment were with no difference. The leaves of tobacco plants applied with 3000 kg ha^{-1} vermicompost had the highest aroma quality, aroma volume, aftertaste, and quality grade score. Moreover, the irritant difference in the tobacco leaves due to vermicompost application was not large. Overall, the sensory quality of the tobacco leaves at 3000 kg ha^{-1} treatment was the best. The tobacco leaves with reference to sensory quality were ranked in order of superiority to inferiority as 3000 kg ha^{-1} vermicompost > 3600 kg ha^{-1} vermicompost > 2400 kg ha^{-1} vermicompost > no vermicompost > 1800 kg ha^{-1} vermicompost.

Table 3. Effect of vermicompost on chemical composition of flue-cured tobacco.

Treatment	Total soluble sugar content (%)	Reducing sugar content (%)	Nicotine content (%)	Total nitrogen content (%)	Potassium content (%)	Chlorine content (%)	Ratio of total sugar to nicotine	Ratio of total nitrogen to nicotine	Ratio of reducing sugar to total sugar	Ratio of chlorine to potassium
no vermicompost	30.63±0.15d	25.5±0.1b	1.82±0.03c	1.77±0.01c	1.62±0.005d	0.32±0.05c	16.80±0.36c	0.97±0.01a	0.83±0.003a	5.00±0.11c
1800 kg ha^{-1} vermicompost	36.96±0.15a	28.33±0.05a	1.71±0.01d	1.53±0.005e	1.76±0.01b	0.37±0.05b	21.58±0.16a	0.89±0.007b	0.77±0.002c	4.71±0.05c
2400 kg ha^{-1} vermicompost	32.3±0.1b	25.23±0.15b	2.46±0.01a	1.88±0.005a	1.67±0.005c	0.56±0.05a	13.11±0.10e	0.76±0.002d	0.78±0.005b	3.00±0.03d
3000 kg ha^{-1} vermicompost	31.3±0.2c	23.63±0.05d	2.10±0.01b	1.81±0.005b	1.78±0.01b	0.21±0.05d	14.88±0.02d	0.86±0.004c	0.76±0.005d	8.35±0.26b
3600 kg ha^{-1} vermicompost	32.06±0.21b	24.66±0.15c	1.69±0.02d	1.64±0.01d	1.84±0.01a	0.11±0.01e	18.90±0.34b	0.97±0.01a	0.77±0.002c	16.82±1.57a

Data are means of three biological replicates. Statistical difference (P<0.05) between the treatments are indicated by different lowercase letters

Table 4. Effect of vermicompost on sensory quality of flue-cured tobacco.

Treatment	Odor type strength combustibility gray	Quality of aroma	Volume of aroma	Aftertaste	Hetero zygosity	Imitation	Score	Concentration	Quality grade
No vermicompost	Intermediate type moderate 3.00 3.00	11.25	16.25	18.63	12.50	8.88	73.5	medium +	medium+
1800 kg ha^{-1} vermicompost		11.10	16.00	18.40	12.30	8.60	72.4	medium +	medium
2400 kg ha^{-1} vermicompost		11.20	16.20	18.80	12.90	8.80	73.9	medium	medium+
3000 kg ha^{-1} vermicompost		11.40	16.50	19.10	13.20	8.90	75.1	medium	better-
3600 kg ha^{-1} vermicompost		11.30	16.30	19.00	13.10	8.90	74.6	medium	medium+

Data are means of three biological replicates. Statistical difference (p<0.05) between the treatments are indicated by different lowercase letters

Table 5. Effect of vermicompost on every aroma substance content of flue-cured tobacco leaves.

Aroma substance (μg·g^{-1})	No vermicompost	3000 kg ha^{-1} vermicompost	Change rate
Carotenoid degradation products	56.81 ± 0.56a	58.26 ± 0.36a	2.56%
Maillard reaction products	17.74 ± 0.29b	19.15 ± 0.15a	7.99%
Aromatic amino acids degradation products	13.90 ± 0.55a	13.66 ± 0.11a	-1.77%
Cembratriendiod alkyl degradation products	14.85 ± 0.36b	29.70 ± 0.24a	100.02%
Chlorophyll degradation products	658.69 ± 48.58a	622.17 ± 59.41a	-5.54%
Others	2.86 ± 0.10b	3.180 ± 0.15a	10.94%
Total (except neophytadiene)	106.16 ± 2.67b	123.94 ± 0.96a	16.76%

Data are means of three biological replicates. Statistical difference (P<0.05) between the treatments are indicated by different lowercase letter

The content of volatile aromatic matter of flue cured tobacco: In order to further verify that the intrinsic quality of flue-cured tobacco leaves treated with 3000 kg ha^{-1} vermicompost was significantly higher than the control, the aroma substances of the treatment with no vermicompost and 3000 kg ha^{-1} vermicompost were determined (Table 5). Among six categories, Maillard reaction products and cembratriendid alkyl degradation products were significantly increased with vermicompost application. Moreover, a major increase in aroma substances was found to be due to a maximal increase (100% increase) in cembratriendid alkyl degradation products. In different treatments, there was almost no change in the degradation products of aromatic amino acids. However, chlorophyll degradation products were decreased due to the application of vermicompost, but not significantly. Moreover, aroma substances from others category also increased substantially.

Discussion

Tobacco is an important cash crop. Its economic importance is related to leaf growth, accumulations of nicotine and nornicotine, and the synthesis of sensory flavoring compounds (Moghbel *et al.*, 2017). Nicotine is biosynthesized in the ornithine and arginine pathway operating in root cells, then it is translocated to the leaves via xylem, and stored in the vacuole (Leete, 1980; Taiz *et al*., 2015). Moreover, the biosynthesis and accumulation of nicotine are affected by various environmental factors such as soil fertility, plant hormones, etc. (Chen *et al.*, 2013; Zhang *et al.*, 2016). This study indicated that the application of vermicompost significantly increased the accumulation of nicotine in flue-cured tobacco leaves. This effect was the result of the application of vermicompost to promote the absorption, accumulation, assimilation and metabolism of nitrogen in tobacco plants (Joshi *et al*., 2015; Chen *et al*., 2016; Qin *et al*., 2016). Vermicompost was rich in humic acid, which significantly increased plant nutrients, such as nitrogen, phosphorus, and potassium in the soil, increased plant root vitality and nitrogen fixation capacity, and promoted the ability of carbon and nitrogen metabolism (Nagavallemma *et al.*, 2004; Tanaka *et al.*, 1998; Theunissen *et al.*, 2010).

Chemical component, as an essential material basis of the inherent quality of flue-cured tobacco leaves, was closely related to the sensory quality of tobacco. Nicotine was the main composition of tobacco alkaloids, accounting for more than 95% of the total alkaloids. The total nitrogen content in the tobacco leaves was much higher, and the smoke was pungent and bitter; the total nitrogen content was too low, and the smoke was insipid and tasteless (Tokuzo *et al.*, 2008). The potassium content mainly affected the combustion of flue-cured tobacco, and also affected the appearance quality such as elasticity and color, which was directly related to sensory quality (Schwamberger *et al.*, 1991). The low content of chloride ions made the tobacco leaves softer and less broken, and improved the quality of flue-cured tobacco (Reisenauer, 1950). In this study, the application of high-concentration vermicompost significantly increased the nicotine, total nitrogen and potassium contents of flue-cured tobacco, reduced the chlorine content, and made the flue-cured tobacco leaves soft. The coordination of chemical composition was also one of the main reasons for the high

sensory quality of tobacco leaves. The application of high-concentration vermicompost (3000 and 3600 kg ha^{-1}) effectively raised the ratio of chlorine to potassium, balanced the ratio of total sugars to nicotine and total nitrogen to nicotine, and improved the aroma quality and irritation of flue-cured tobacco.

In addition to volatile aroma developing compounds, high quality aroma cigarettes contain sugar, organic acids, amino acids and alkaloids produced by Maillard reaction (Song *et al.*, 2007). In this study, the application of vermicompost in tobacco fields improved the sensory quality of the chemical ingredients of flue-cured tobacco leaves (Table 3). This effect was more prominent at high concentrations vermicompost application. Several studies described that aroma type could be categorized as light, medium and heavy type based on several compounds such as potassium, chlorine, sugars, nitrogen, nicotine etc. And the tobacco leaves treated with 3000 and 3600 kg ha^{-1} vermicompost had the best quality and volume of aroma. In addition, based on aroma sensory characteristics (Odor type-Strength-Combustibility-Gray, Aftertaste, Heterozygosity and Imitation), the sensory characteristics of the tobacco leaves treated with 3000 kg ha^{-1} vermicompost had the highest score of sensory characteristics and better grade quality (Table 4). Pathma *et al*., (2012) found that the role of vermicompost in improving crop yield and quality could be due to the reason that vermicompost contains a large amount of amino acids and plant growth hormones, which could promote plant root growth.

The volatile compounds are main constituents of flavor of cigarettes as they directly influence senses (Chen *et al.*, 2016; Zhang *et al.*, 2016). However, other compounds such as sugars, amino acids and organic acids react during the leaf curing process (maillard reaction) and produce different products to increase aroma of the product such as (DDMP), an important flavor formation component (Song *et al.*, 2007; Mitsui *et al.*, 2015; Yin *et al.*, 2019). Carotenoids, as precursors of terpenoids, are important aroma components in flue-cured tobacco, which directly determin the appearance and sensory quality, and affect the economic benefits of tobacco (Popova *et al*., 2019). Weeks (1985) found that the sensory quality of tobacco leaves increased with the content of carotenoid degradation products. The results showed that the threshold of aroma substances produced by carotenoid degradation was relatively low, the irritation small, the aroma quality good, and the contribution rate to the aroma was large. This study showed that 3000 kg ha^{-1} vermicompost application increased the content of carotenoid degradation products and aroma components in flue-cured tobacco. Chlorophyll content was also the main indicator to assess the inner quality of flue-cured tobacco (Zelena *et al*., 2009). The higher chlorophyll content led to the poor appearance grade of tobacco, and the raw green odor was obvious (Wahlberg *et al.*, 1977). Although chlorophyll degradation products were reduced, but not so significant. All of these reports suggest that application of vermicompost significantly increased the content of carotenoid degradation products, Maillard reactants, cembratriendiod alkyl degradation products and other aroma substances in flue-cured tobacco, improved the sensory quality, and increased economic benefits (Table 5).

Conclusions

This study showed the effects of different concentrations of vermicompost on economic traits, appearance quality, intrinsic chemical composition and coordination of flue-cured tobacco leaves. It was evident that application of 3000 kg ha^{-1} vermicompost significantly improved the appearance and intrinsic quality of cured tobacco leaves, enhanced the economic value, coordinated the chemical constituents, and improved the sensory quality of tobacco leaves.

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