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**Research Article** 

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## Improvement of alkaline tobacco field soil by humic acid

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## ABSTRACT

In order to control the alkalization trend of tobacco field soil of Chenzhou city in Hunan province and provide good soil environment for tobacco growth, humic acid was applied to a slightly alkaline soil in Wutong country to research its effects on tobacco soil and plants. The experiment results showed that: (1) Applying humic acid to slightly alkaline soil could reduce the pH value of tobacco field soil, but only when the addition amount was higher than 5 g/kg, the humic acid reduced the soil pH value significantly. However, it was not enough to apply humic acid to slightly alkaline soil could improve soil fertility. Compared with untreated soil, application of humic acid at 10 g/kg increased the contents of available P, Mn, Cu, Zn and Fe by 87.7%, 52.0%, 88.3%, 45.2% and 74.0%, respectively, and the contents of Cu, Fe in tobacco leaves increased by 17.6%, 32.5%, respectively. (3) Compared with control, tobacco plant height, maximum leaf length, maximum leaf width and number of productive leaves had no significant difference after humic acid treatments. It indicated that applying humic acid to slightly alkaline soil did not have a negative impact on tobacco growth.

Keywords: humic acid, tobacco, pH value, soil fertility

## INTRODUCTION

As we know, tobacco has high economic value. It can grow in several kinds of soils, and is cultivated widely in Hunan and Yunnan province of China. In recent years, the demands for high-quality tobacco and high-grade cigarette are increasing with the improvement of people's living standard and consumptive level. But, the high-quality tobacco growth needs strict requirements for soil types and properties. The tobacco yield and quality depend on soil nutrition, space nutrition and the nutrition of tobacco plants. The soil nutrition is the most important factor. The nutritional status of tobacco soil can affect tobacco growth directly [1-3]. Chenzhou is always the main production area of high-quality tobacco, and its yield rank keeps high in China [4]. However, some recent investigation results showed that the soil pH value in Chenzhou tobacco-growing area has a rising trend and is much higher than that of obtained from the second national soil survey in this area. Moreover, it has deviated from the optimum range (5.5-6.5) seriously [5-7]. The increase of soil pH has negative effect on tobacco growth [8, 9], resulting in some tobacco diseases and quality drop. Therefore, it is necessary to regulate the alkali soil in this area. Humic acid is a kind of complex natural organic matter, which is composed of plant and animal residues after microorganism decomposition, translation and a series of geochemical process [10, 11]. It contains a large of acidic groups, such as carboxyl and phenolic OH functional groups [10]; and it also has abundant of nutrient elements, such as organic matter, nitrogen (N), phosphorus (P) and potassium (K) [12]. Some studies showed that humic acid had obvious effects on improving soil granular structure, soil available nutrient and fertilizer utilization efficiency [11, 13]. Jonas Pertusatti [14] indicated that humic acid had strong buffer capacity in a wide pH range from 5.5 to 8.0 because of its chemical adsorption on H<sup>+</sup> and OH<sup>-</sup>. It implies that humic acid can be used to adjust soil pH, but related studies have rarely been reported. This research, according to the actual condition of Chenzhou tobacco field, aimed to study the effects of humic acid on soil pH, fertility, tobacco growth and quality by field experiment and provide some technical supports to regulate the alkali soil, and then improve tobacco yield and quality.

#### EXPERIMENTAL SECTION

## 2.1 Setting and materials

The field experiment site was set in Guiyang county of Chenzhou city, southern of Hunan province in China. This area has a subtropical monsoonal climate with annual average temperature of 15.6-18.3  $^{\circ}$ C, and the annual precipitation is 1400-1700 mm. The soil of this area was alkaline, so humic acid was selected to ameliorate soil. The soil physical and chemical properties of this experimental field were showed in table 1. The 40 mesh humic acid, brought by Pingxiang Huli Humic Acid Plant in Jiangxi province, contained organic matter (dry basis) > 40%, humic acid > 30% and water <18%.

#### Table 1 Physical and chemical properties of the tested soil

Soil	pН	Organic matter	Total nitrogen	Total phosphorus	Total potassium	Total calcium	Total magnesium
type		(%)	(g/kg)	(g/kg)	(g/kg)	(g/kg)	(g/kg)
Red soil	8.10	2.57	3.17	0.81	27.5	17.6	1.51

## 2.2 Experiment methods

The humic acid was applied into soil when turning over and ploughing the tobacco field. And the addition amounts of humic acid were calculated based on the soil weight as  $15 \times 10^4$  kg per mu. The experiment included the control treatment (no addition of humic acid), and treatments with 2.5, 5.0, 10.0 g kg<sup>-1</sup> humic acid. Each treatment set a quadrat of  $4 \times 4$  m<sup>2</sup> and had 3 repeats. The field experiment, with randomized block arrangement, has 12 quadrats in total. After applying humic acid and mixed with soil, the tobacco seedlings were planted into quadrats. When tobacco grew mature, their agronomic traits (plant height, leaf length, leaf width) were investigated and recorded. And the tobacco leaves were sampled in each quadrat, and each sample was collected from 4 strains of plants. At the same time, the soil samples were collected from tilled soil layer (0-20cm) around tobacco roots. Plant samples were rinsed in deionized water, oven dried at 70 °C for 48h to a constant weight. After that, they were homogenized in particle size by grounding in a ball mill to prepare for determining the nutrient element contents of nitrogen (N), phosphorus (P), potassium (K) , copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), calcium (Ca) and magnesium (Mg). Soil samples were air dried, removed debris, ground and sifted through 10 and 100 meshes nylon sieve, preparing for determining soil pH value and the contents of organic matter, exchangeable Ca, Mg and available P, Cu, Zn, Fe, Mn.

#### 2.3 Chemical analysis

In soil, the pH value was determined by acidometer (PHS-3C, Shanghai Precision and Science Instrument Corp., China) with a solid-water ratio of 1:2.5 [15]. Soil organic matter was determined by the potassium dichromate method (NY/T 1121.6-2006). The soil available P was extracted by sodium bicarbonate (NaHCO<sub>3</sub>) (LY/T 1233-1999) and analyzed by uv spectrophotometry (UV-1700, Shimadzu). The exchangeable K was extracted by 1 mol/L neutral ammonium acetate (NY/T 889-2004). And the available Zn, Cu, Fe, Mn was extracted by diethylenetriaminepentaacetic acid (DTPA) (NY/T 890-2004). Moreover, the exchangeable Ca and Mg was determined by ammonium acetate method (NY/T 1121.13-2006). The K, Ca, Mg, Zn, Cu, Fe, Mn in plant samples were extracted by mix acid (nitric acid: perchloric acid = 4:1). At last, the K, Ca, Mg, Zn, Cu, Fe and Mn analyses in the filtrate were performed by AAS (Z-2000, Hitachi) and inductive coupling plasma emission spectrometer (ICP 6300, Thermo).

#### 2.4 Statistical analysis

The data analysis and chart plotting were performed using Microsoft excel version 2010 and origin 9. The Duncan test of Statistic Package for Social Science (SPSS) version 17.0 was exploited for ANOVA analysis (p<0.05).

## **RESULTS AND DISCUSSION**

#### 3.1 Effects of humic acid on soil pH

The effects of humic acid on soil pH are shown in Fig 1. As is known to all, the optimal soil pH for tobacco growth is 5.5-6.5. From Fig 1, the control soil pH without humic acid treatment was 8.10, higher than the optimal soil pH. With the increase of humic acid, soil pH was decreased. Compared with control, the treatments with 2.5, 5.0, 10.0 g/kg humic acid decreased the soil pH by 0.04, 0.16, 0.21, respectively. The statistical results showed that there was a significant decrease only at the additional amount higher than 5.0 g/kg. Obviously, humic acid regulated soil pH efficiently, but only once application of humic acid was insufficient to decrease soil pH to the optimal range.

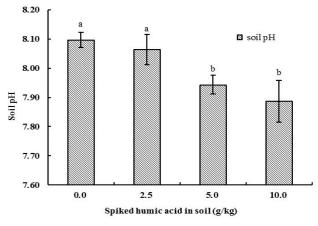


Fig.1 Effect on soil pH value after applying humic acid

3.2 Effects of humic acid on the availability of soil nutrients

The contents of soil organic matters, available P, available K, exchangeable Mg and exchangeable Ca after harvesting are shown in Table 2. With the increase of humic acid, the soil organic matter and available P were increased; the available K, exchangeable Mg and exchangeable Ca had no regulative changes. Compared with control, the treatments with 2.5, 5.0, 10.0 g/kg humic acid increased available P by 31.4%, 36.5% and 87.7%, respectively. The incremental P may be brought by additional humic acid.

Table 2 Effects of humic acid on the contents of organic matter, available P, available K, exchangeable Ca and Mg\*

Addition amount of humic acid (g/kg)	Organic matter (%)	Available P (mg/kg)	Available K (mg/kg)	Exchangeable Ca (cmol/kg)	Exchangeable Mg (cmol/kg)
0	2.57±0.35a	88.6±28.1b	97.01±0.31a	153.5±50.8a	2.30±0.44a
2.5	2.78±0.49a	116.4±53.8b	93.72±3.78a	141.7±68.5a	2.31±0.31a
5	2.74±0.32a	120.9±6.3b	102.48±1.84a	181.4±10.4a	2.76±0.49a
10	3.07±0.08a	166.3±25.4a	87.57±12.88a	144.0±18.8a	2.26±0.25a
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\* The contents were showed as average  $\pm$  standard deviation (n=3), the different alphabets (a, b) showed the significant difference (P<0.05) in a data column.

Mn, Cu, Zn and Fe are the essential trace elements for tobacco growth. As presented in Fig 2, the soil available Mn, Cu, Zn and Fe increased with the increasing amounts of humic acid. There was a significant increase in available Cu with the 5.0 g humic acid kg<sup>-1</sup> treatment and in available Mn, Zn and Fe with the 10.0 g humic acid kg<sup>-1</sup> treatment. Application of humic acid at 10 g/kg increased available Mn, Cu, Zn, Fe by 52.0% 88.3% 45.2%, 74.0%, compared with control. There were 2 reasons why higher dosage of humic acid increased the contents of soil available Mn, Cu, Zn, Fe: first, humic acid contains a certain amount of Mn, Cu, Zn, Fe; second, humic acid decreased soil pH, resulting in the dissolution of Mn, Cu, Zn, Fe from soil colloids. Generally, soil organic matters and nutrient elements are regarded as important indexes to evaluate soil fertility. These results indicated that humic acid could improve soil fertility obviously.

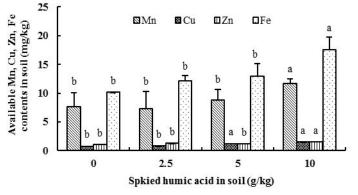


Fig.2 Effects of humic acid on the contents of bioavailability state of Mn, Cu, Zn and Fe in soil

3.3 Effects of humic acid on the contents of nutrient elements in tobacco leaves

The contents of N, P, K, Ca and Mg in tobacco leaves are shown in Table 3. After applying humic acid, the N and P contents in leaves were increased and K, Ca, Mg contents had no obvious regularity. Statistics analysis showed humic acid had no significant effect on the contents of N, P, K, Ca and Mg in tobacco leaves.

			-		
Addition amount of humic acid (g/kg)	N (%)	P (%)	K (%)	Ca (g/kg)	Mg (g/kg)
0	0.94±0.08a	0.13±0.01a	5.52±0.03a	17.60±1.77a	1.51±0.05a
2.5	1.13±0.23a	0.14±0.04a	5.49±0.02a	18.25±0.60a	1.57±0.10a
5	0.90±0.07a	0.14±0.03a	5.48±0.06a	18.46±0.61a	1.70±0.07a
10	0.98±0.21a	0.16±0.03a	5.50±0.03a	18.40±0.65a	1.50±0.30a
		1.00 1.1	1	1 1	1.00

\* The contents were showed as average  $\pm$  standard deviation (n=3), the different alphabets (a, b) showed the significant difference (P < 0.05) in a data column.

The effects of humic acid on the contents of Mn, Cu, Zn and Fe in tobacco leaves are shown in Fig 3. There were some increases in Mn, Cu and Fe contents in tobacco leaves, but based on the statistical analysis results, only Cu and Fe contents had significant changes. Compared with control, the treatment with 10 g/kg humic acid increased Cu, Fe by 17.6% and 32.5%, respectively. Humic acid had some effects on improving soil fertility, but the effects were just reflected in Cu, Fe contents in leaves.

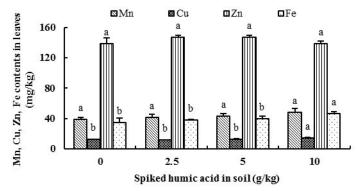


Fig.3 Effects of humic acid on the contents of Mn, Cu, Zn and Fe in tobacco leaves

#### 3.4 Effects of humic acid on tobacco agronomic traits

As presented in Table 4, humic acid did not affected the plant height, maximum leaf length, maximum leaf width, effective leaves and dry weigh of leaves significantly. No significant difference on the agronomic character of tobacco was visible at all humic acid treatments, indicating that humic acid had no positive or negative effect on tobacco growth.

Table 4 Effects	of humic acid on	tobacco	agronomic traits*
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Application of humic acid (g/kg)	Plant height (cm)	maximum leaf length (cm)	maximum leaf width (cm)	effective leaves (piece)	dry weigh of leaves (g/one piece)
0	96.5±3.4a	75.2±2.1a	26.5±1.8a	16.0±0a	6.95±1.08a
2.5	99.2±1.4a	73.6±4.6a	24.8±0.6a	15.9±0.5a	7.86±0.57a
5	96.5±6.5a	70.8±1.8a	26.4±0.6a	16.5±0.5a	7.52±0.88a
10	97.0±5.6a	73.9±3.5a	26.5±0.9a	16.7±1.1a	7.28±0.42a

\* The contents were showed as average  $\pm$  standard deviation (n=3), the different alphabets (a, b) showed the significant difference (P<0.05) in a data column.

#### CONCLUSION

(1) Humic acid decreased soil pH on certain degree. When the addition amount of humic acid was higher than 5 g/kg, the soil pH was increased significantly, but only once application of humic acid was insufficient to decrease soil pH to the optimal range.

(2) Humic acid increased the soil fertility. With the 10 g humic acid kg<sup>-1</sup> treatment, the available P, Mn, Cu, Zn and Fe contents were increased by 87.7%, 52.0%, 88.3%, 45.2% and 74.0%, respectively. Humic acid also increased the Cu, Fe contents in leaves slightly. For the 10 g humic acid kg<sup>-1</sup> treatment, Cu and Fe contents in tobacco leaves were increased by 17.6% and 32.5%, respectively.

(3) Humic acid had no obvious effect on plant height, maximum leaf length, maximum leaf width, effective leaves and dry weight of leaves. And at higher addition amount of humic acid, there were even no negative effect on tobacco growth under this experimental condition.

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