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EFFECTS OF VERMICOMPOST APPLICATION ON SOIL PROPERTIES AND ROOT PHYSIOLOGICAL CHARACTERISTICS OF FLUE-CURED TOBACCO (*NICOTIANA TABACUM* L.) – A POTENTIAL ANIMAL FEED ADDITIVE

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Abstract

Flue-cured tobacco is not only a kind of economic crop, but it can also be used as a special unconventional functional feed additive, which plays an important role in animal health care. In the current study, we examined how vermicompost could promote root growth of flue-cured tobacco plants by regulating soil physical-chemical characteristics. A pot experiment was conducted to investigate the effects of different proportions of vermicompos (CK: 0%, T1: 5%, T2:10%, T3: 20%, T4: 50%, T5: 100%) on soil chemical and physical properties, root growth and physiological characteristics of flue-cured tobacco. Results showed that with increase in vermicompost dose, cation exchange capacity (CEC), soil organic matter (SOM) content, microbial biomass carbon (MBC) content, microbial biomass nitrogen (MBN) content, and contents of glomalin-related soil protein (GRSP) including total glomalin-related soil protein (T-GRSP) and easily extractable glomalin-related soil protein (EE-GRSP) increased in the tobacco planting soil, which resulted in increases in root length, fresh weight and volume of flue-cured tobacco plants. With increase in vermicompost does in soil, the root activity and nicotine content first showed an increasing trend and then a decreasing trend. All vermicompost treatments increased the plant hormonal balance i.e. ratio of indoleacetic acid (IAA) to abscisic acid (ABA) in flue-cured tobacco roots. High IAA/ABA ratio was found in the tobacco plants supplied with 5% vermicompost (T1), followed by T5 (100%) treatment. The findings showed that CEC and contents of SOM, MBC, MBN and GRSP could better reflect the effect of vermicompost on soil quality as appraised with the principal component analysis. The vermicompost treatment, i.e.T5 (100%), was the best treatment, followed by T4 (50%), T3 (20%), and T2(10%) treatments. Such improvements by applying appropriate amount of vermicompost were associated with improvement in soil fertility and structure as well as IAA/ABA hormonal regulation, cellular activity, nitrogen and carbon metabolisms in the roots of flue-cured tobacco.

Key words: Vermicompost, Soil property, Root vitality, Flue-cured tobacco, Feed additive.

Introduction

Tobacco (*Nicotiana tabacum* L.) is an important economic crop with a large planting area in the world. It not only has high biological yield and economic value, but also has many special uses (Song *et al.*, 2016). Although tobacco leaves are the main product used for cigarette (Li *et al.*, 2016), the roots and residues of flue-cured tobacco discarded during the field production can be a potential plant feed additive for animal as it can provide appropriate protein sources to meet the needs of the domestic feed market. These residues of flue-cured tobacco are rich in a variety of nutrients, of which the protein content is up to 15%, being much higher than that of soybean (*Glycine max* (L.) Merr.), and its water-soluble protein is similar to egg protein, nutrition exceeds milk and soybean protein, and insoluble protein is a good livestock and poultry feed (Fan *et al.*, 2016).

Long-term field trials and treatments with different fertilizers may change nutrients composition and contents of the soil, which affects root growth, yield and quality of crops. There is an increasing evidence that the yield and quality of crops can be effectively improved when organic fertilizers are used as basic fertilizers (Ortas & Lal, 2014). Earthworm composting technology is a new environmental composting technology developed based on traditional composting. Vermicompost as a product of earthworm decomposition waste, has uniform particles, and soil flavor. It is an excellent bio-organic fertilizer (Qin *et al.*, 2016; Bressanin & Andrade, 2020; Lin *et al.*, 2021). It is an exciting research in terms of establishing organic agriculture and developing the potential application value of tobacco roots in China (Zhang, 2018).

As a high-quality organic fertilizer, vermicompost contains high levels of plant growth hormones and soil enzymes (Datta *et al.*, 2018). The hormonally active secondary metabolites contained in the vermicrompost may also have a role in promoting plant growth (Atiyeh *et al.*, 2000). Nurhidayati *et al.*, (2018) found that vermicrompost can effectively improve soil quality, increase microbial activity and nutrient cycling speed, and produce high-quality crops. Replacing about 20% to 40% of the substrate with earthworm droppings can promote plant rooting and growth without any negative effects (Belda *et al.*, 2013). In view of all these reports, we hypothesized that vermicompost application could promote root growth of flue-cured tobacco plants by regulating soil characteristics. Thus, the present study focused on revealing the role of vermicompost in improving soil quality and promoting the growth and development of tobacco roots in southern Shaanxi province. It is of great significance to develop the potential application value of tobacco roots as a feed additive for animal in China.

Material and Methods

Pot experiment: The experiment was conducted in the greenhouse of Northwest A&F University, Yangling, China. Plastic buckets having 30 cm diameter and height were filled with 10.0 kg mixed sandy loam soil. The sandy loam soil used in this experiment was collected from local agricultural fields in Yangling, air-dried, ground and passed through a 2 mm sieve. The soil physical and chemical characteristics were as followed: pH 7.72, water holding capacity 32.21%, organic matter content 19.98 g kg^{-1}. The contents of available nitrogen, phosphorus, and potassium were 65.29 mg kg^{-1}, 27.83 mg kg^{-1} and 142.65 mg kg^{-1}, respectively. Before filling the plastic buckets with soil, 2 kg of pebbles were placed at the bottom of each bucket and inserted a PVC pipe of 40 cm length at the center for irrigation purpose. Moreover, a layer of gauze and a layer of newspaper were placed on the pebble layer to separate the mixture of stones and vermicompost soil, to maintain a slight space at the bottom of the plastic bucket. Finally, plastic buckets were filled with a mixture of 10.0 kg vermicompost and soil. Vermicompost was collected from the bio-health agriculture demostration garden of Northwest A&F University, after airdrying, it was crushed and sieved properly and used in this experiment.

Experimental treatment: A completely randomized design was used in this experiment and used six vermicompost treatments (CK, 100% soil; T1, 5% vermicompost + 95% soil; T2, 10% vermicompost + 90% soil; T3, 20% vermicompost + 80% soil; T4, 50% vermicompost + 50% soil; T5, 100% vermicompost, W/W). Each treatment was replicated six times. Tobacco seedlings of cultivar Yunyan 99 were raised in nursery beds directly from the seeds. Six tobacco seedlings at four leaf stage and of uniform size were transplanted in each of the plastic buckets. Each plastic bucket was irrigated through the PVC pipe at 18:00 o'clock every day to keep 70% water holding capacity. Moreover, standard management practices as being used in the Shaanxi province to produce quality tobacco. To assess the physical and chemical properties of soil, soil samples were collected at 0 (transplanting period), 60 (vigorous growth period), and 90 (maturity period) days after seedling transplantation. After 60 days of seedling transplantation, the root characteristics of flue-cured tobacco were measured.

Determination of cation exchange capacity (CEC): Air-dried soil sample (5.0 g) was passed through a 2 mm sieve into a 100 mL centrifuge tube, added 50 mL of 1 mol L^{-1} ammonium acetate solution, and stirred until the soil sample was in a uniform slurry state without particles, then centrifugated in a 4,000 r min^{-1} centrifuge for 3 min. The clear liquid after centrifugating was discarded. The alkaline soil was exchanged with ammonium acetate solution 2 times, and the remaining soil was exchanged with ammonium acetate solution 5 times. After washing off excess ammonium acetate with 95% ethanol, the soil was washed into an open bottle with water, added solid magnesium oxide for distillation, absorbed the fraction with boric acid solution, and then titrated with a standard hydrochloric acid solution to calculate the soil CEC (Bao, 2000).

Determination of content of soil organic matter (SOM): Air-dried soil sample (0.2 g) was passed through a sieve into a test tube, then accurately added 5 mL of 0.8 mol/L $K_2Cr_2O_7$ with a burette, and gently shaked the test tube to disperse the soil sample in the tube. Then slowly added 45 ml of concentrated H_2SO_4 along the tube wall, and added a small funnel to the mouth of the test tube to condense the steamed water vapor. The test tube was inserted into a wire cage and put it in the oil bath preheated to 180-190°C, and then allowed to boil for 5 minutes. The contents of the test tube were poured into a triangular flask. The total volume of the flask was not allowed to exceed 60-70 ml. The 2-3 drops of *O*-phenanthroline indicator and titrated with 0.2 mol L^{-1} $FeSO_4$. The color of the solution changed from orange to green and then to brown. The red color was the end point (Bao, 2000).

Determination of glomalin-related soil protein (GRSP) content: Air-dried soil sample (0.2 g) was passed through a sieve into a test tube, then accurately added 5 mL of sodium citrate solution of pH 8.0 and pH 7.0 for extraction of total glomalin-related soil protein (T-GRSP) and easily extractable glomalin-related soil protein (EE-GRSP). After extraction, the contents of the two proteins were determined by a modified Coomassie Brilliant Blue staining method following David *et al*., (2008) and Wright *et al.*, (1996).

Determination of content of microbial biomass carbon (MBC): Air-dried soil sample (0.2 g) was passed through a 10mm sieve in a small beaker, 30ml of 0.1 mol L^{-1} NaOH solution and 30ml of chloroform, sealled and incubated at 25°C in the dark for 24h, and then place the extract in a 100°C water bathing to remove off the residual chloroform, the TOC analyzer was used to determine MBC content in the extract (Bao, 2000).

Determination of content of microbial biomass nitrogen (MBN): Air-dried soil sample (20.0 g) was passed through a 10 mm sieve and placed it in a 200 mL Erlenmeyer flask, added 2 mol L^{-1} KCl solution and the mixture was shaken for 1 h. The supernatant was subjected to an AA3 continuous flow analyzer to determine MBN content in the extract (Bao, 2000).

Determination of the physical growth index: The main root length was measured by a measuring tape. The fresh weight was measured with an electrical balance. The root volume was measured by the drainage method, and the root activity measured using the TTC measurement method (Qiman *et al*., 2011).

Nicotine content measurement: A proportion of 0.5 g of flue-cured tobacco leaves was ground into powder, boiled with 30 ml of 0.05 mol L^{-1} hydrochloric acid for 5 minutes, and filtered while it was hot. The filtrate was extracted with dichloromethane under the indicator of methyl orange, and the absorbance was determined at 435 nm to determine nicotine content in the extract (Chen *et al*., 2016).

Determination of indoleacetic acid (IAA) and abscisic acid (ABA) concentrations: The ELLSA kit method was used to measure root IAA and BAA levels. A sample (100 mg) was accurately weighed, put into a mortar and ground in liquid nitrogen, then the mixture was transferred to a 2 ml centrifuge tube, added 10% PBS buffer, centrifuged for 20 minutes at 5000 r min^{-1}, and the supernatant was obtained by enzyme-linked immunosorbent assay kit. After diluting the standards, added them to the wells of the loading reaction plate one by one, added the diluent to the sample wells, incubated, repeated the washing 5 times, added the corresponding enzymes, and finally added the color reagent and stop solution. Taking the concentration of a hormone as the abscissa and the OD value as the ordinate, a standard curve was drawn, and the concentrations of each hormone were calculated following Gao (2000).

Statistical analysis

One-way analysis of variance test (ANOVA) was performed on the collected data that was repeated six times in a complete randomized design. The Duncan's multiple-range test (DMRT) was performed by SPSS 20.0 version at 5% level of significance to calculate the significant differences between the treatment means.

Results

Principal component analysis of all indicators during all periods: As shown in Table 1, from the principal component analysis (PCA) of tobacco planting soil indicators, components were extracted having values greater than 1, and first two components with their cumulative contribution larger than 85% were selected. The principal component equation is:

$F_1 = 0.413X_1 + 0.412X_2 + 0.411X_3 + 0.409X_4 + 0.408X_5 + 0.395X_6 - 0.002X_7$

$F_2 = -0.055X_1 + 0.083X_2 + 0.117X_3 + 0.137X_4 - 0.179X_5 - 0.242X_6 + 0.933X_7$

The principal component comprehensive model can be expressed as:

$F = 0.336X_1 + 0.358X_2 + 0.362X_3 + 0.364X_4 + 0.311X_5 + 0.290X_6 + 0.152X_7$

In the formula: X_1 represents EE-GRSP, X_2 represents CEC, X_3 represents T-GRSP, X_4 represents SOM, X_5 represents MBN, X_6 represents MBC, and X_7 represents available nitrogen.

The soil CEC: The application of vermicompost significantly increased soil CEC in the tobacco field . At 100% vermicompost application (T5), CEC of the soil was most obvious in the transplanting period, being considered as the vigorous growth period and the maturity period. At this vermicompost treatment, the CEC was significantly high being 11.00% higher than the blank control group (Fig. 1).

The content of SOM: Addition of different proportions of vermicompost excrement increased SOM content of the tobacco-growing soil to varying degrees, and with increase in the proportion of vermicompost excrement, the SOM content showed a gradual upward trend. However, compared with each period, contents of SOM did not change significantly (Fig. 2).

The content of glomalin-related soil protein (GRSP): The contents of T-GRSP and EE-GRSP in tobacco-growing soil increased with increase in the proportion of vermicompost. When treated with 100% vermicompost, the effect was significantly high (Figs. 3 and 4).

The contents of MBC and MBN in tobacco-growing soil: The soil MBC and MBN contents increased with the addition of vermicompost, and each treatment also showed a significant difference in each period, and the vigorous growth period being > maturity period > transplanting period. This may have been due to the reason that the tobacco plant itself has a strong growth and metabolism in the long-term, showing its maximum ability to fix carbon and nitrogen. The contents of soil MBC and MBN reached the highest at T5 (100% vermicrompost), MBC content increased by more than 100% than CK in each period, and MBN content increased by more than 200% compared with CK in each period (Figs. 5 and 6).

Physical growth indexes of flue-cured tobacco roots*:* Vermicompost application significantly enhanced the root fresh weight, root length and root volume of tobacco plants. This growth promoting effect on the roots of tobacco plants was maximum (19.17 cm) at the highest vermicompost (T5) treatment (Fig. 7).

Table 1. Principal component analysis of soil indices during three periods.

Item	F_1	Loading capacity	F_2	Loading capacity
Microbial biomass nitrogen (MBN)	0.423	0.983	0.080	0.086
Microbial biomass carbon (MBC)	0.416	0.967	0.089	0.097
Organic matter (OM)	0.410	0.952	0.125	0.135
Total glomalin-related soil protein (T-GRSP)	0.406	0.944	-0.200	-0.216
Easily extractable glomalin-related soil protein (EE-GRSP)	0.396	0.922	-0.285	-0.308
Cation exchange capacity (CEC)	0.389	0.904	-0.209	-0.226
Available-N	-0.013	-0.031	0.917	0.992
Characteristic value	5.409	/	1.17	/
Contribution (%)	77.267	/	16.716	/
Cumulative contribution (%)	77.267	/	93.983	/

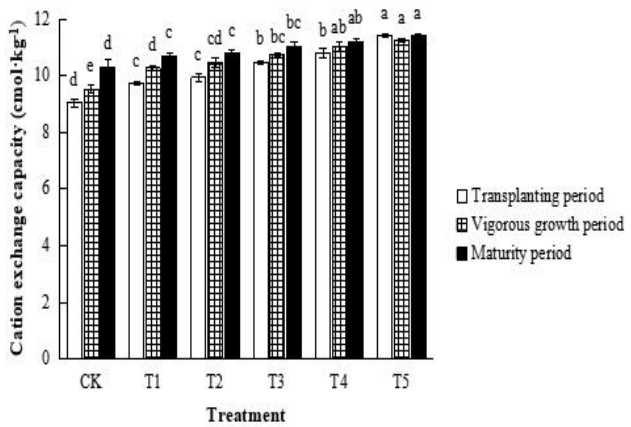

Fig. 1. Effect of vermicompost on soil cation exchange capacity(CEC). The values represent the mean ± standard error (n = 6) of six repeated tests, while the values with different letters indicate the significance level between the treatments at $p \leq 0.05$.
Note: CK, 100% soil; T1, 5% vermicompost + 95% soil; T2, 10% vermicompost + 90% soil; T3, 20% vermicompost + 80% soil; T4, 50% vermicompost + 50% soil; T5, 100% vermicompost. (All Figures below will follow the same treatments' detail)

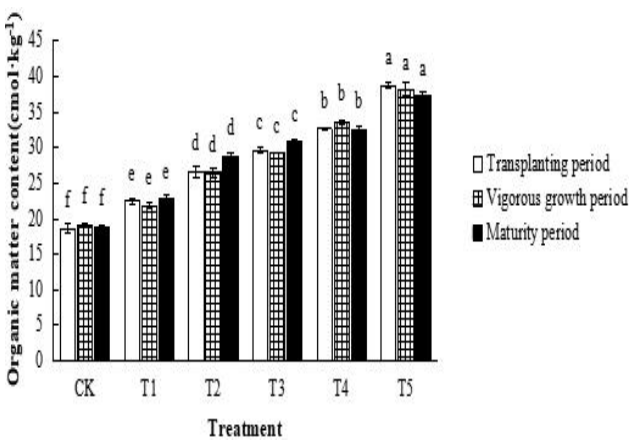

Fig. 2. Effects of vermicompost on organic matter content (SOM) in tobacco planting soil. The values represent mean ± standard error (n = 6) of six repeated tests, while the values with different letters indicate the significance level between the treatments at $p \leq 0.05$.

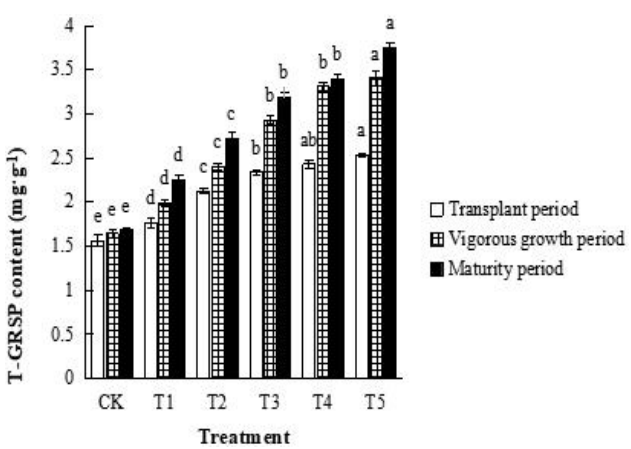

Fig. 3. Effects of vermicompost on content of T-GRSP in the tobacco planting soil. The values represent mean ± standard error (n = 6) of six repeated tests, while the values with different letters indicate the significance level between the treatments at $p \leq 0.05$.

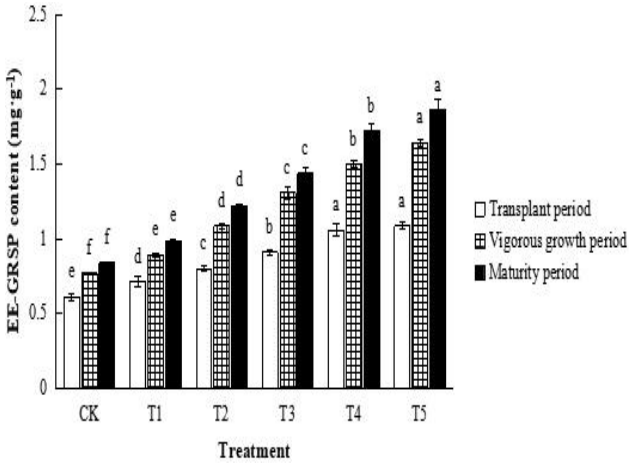

Fig. 4. Effects of vermicompost on content of EE-GRSP in the tobacco planting soil. The values represent mean ± standard error (n = 6) of six repeated tests, while the values with different letters indicate the significance level between the treatments at $p \leq 0.05$.

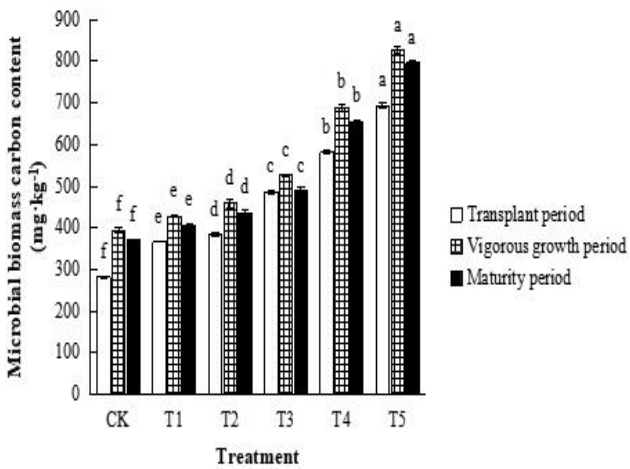

Fig. 5. Effects of vermicompost on content of microbial biomass carbon (MBC) in tobacco-growing soil. The values represent mean ± standard error (n = 6) of six repeated tests, while the values with different letters indicate the significance level between the treatments at $p \leq 0.05$.

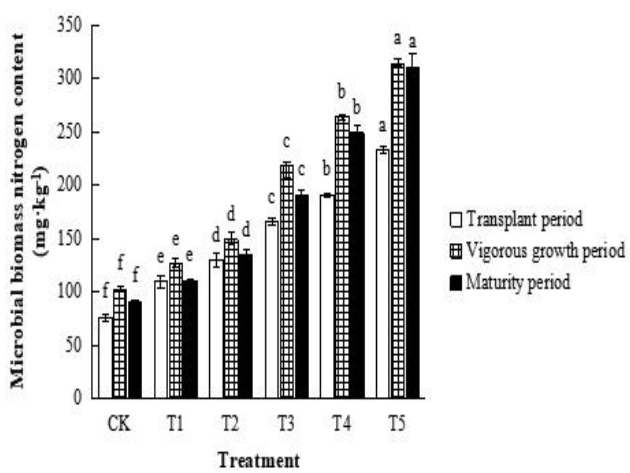

Fig. 6. Effects of vermicompost on content of microbial biomass nitrogen (MBN) in tobacco-growing soil. The values represent mean ± standard error (n = 6) of six repeated tests, while the values with different letters indicate the significance level between the treatments at $p \leq 0.05$.

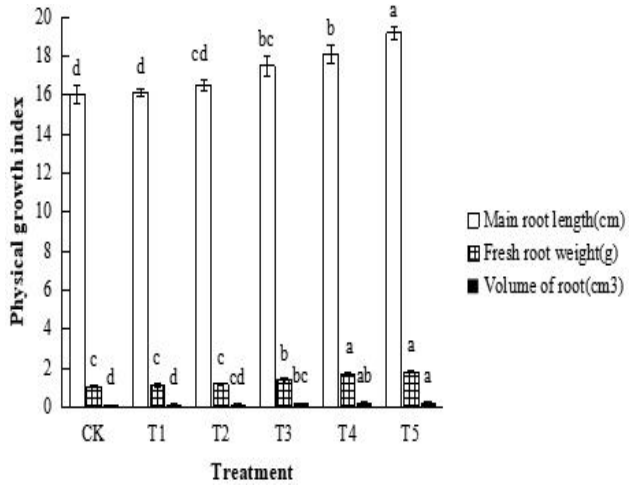

Fig. 7. Effects of vermicompost on physical growth indexes of flue-cured tobacco roots. The values represent mean ± standard error (n = 6) of six repeated tests, while the values with different letters indicate the significance level between the treatments at $p \leq 0.05$.

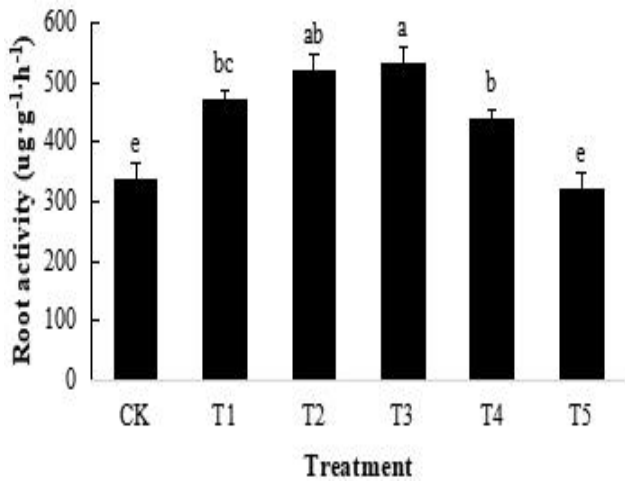

Fig. 8. Effects of vermicompost on root activity of flue-cured tobacco. The values represent mean ± standard error (n = 6) of six repeated tests, while the values with different letters indicate the significance level between the treatments at $p \leq 0.05$.

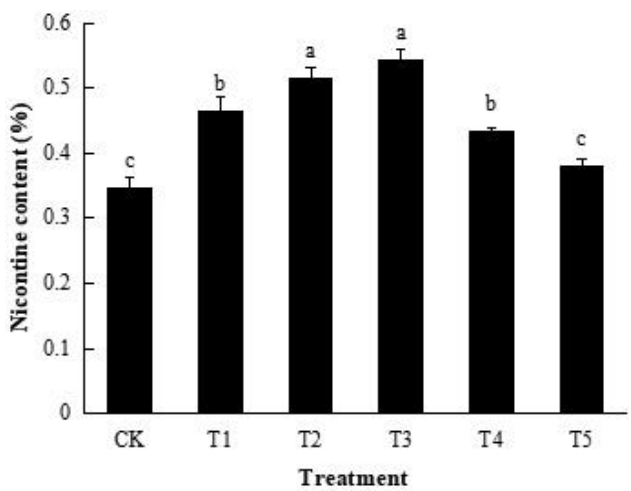

Fig. 9. Effects of vermicompost on nicotine content in flue-cured tobacco roots. The values represent mean ± standard error (n = 6) of six repeated tests, while the values with different letters indicate the significance level between the treatments at $p \leq 0.05$.

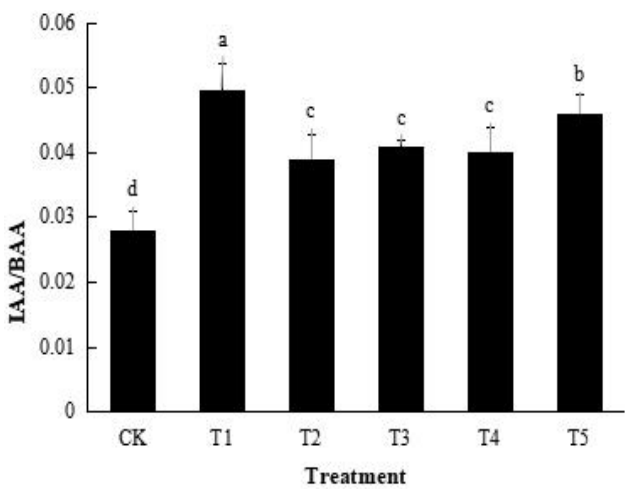

Fig. 10. Effects of vermicompost on ratio of indoleacetic acid (IAA) to abscisic acid (ABA) in flue-cured tobacco roots. The values represent mean ± standard error (n = 6) of six repeated tests, while the values with different letters indicate the significance level between the treatments at $p \leq 0.05$.

Root activity of flue-cured tobacco: Root activity significantly increased with increase in addition of vermicompost to the soil. However, tobacco root activity decreased when vermicompost fraction increased greater than 50%. Maximum root growth was found at 20% vermicrompost (T3) treatment (Fig. 8).

Nicotine content of flue-cured tobacco roots: With increase in the proportion of vermicompost in soil, nicotine content of tobacco roots firstly showed an increasing trend and then a decreasing trend. The root nicotine content of flue-cured tobacco treated with T1 (5% vermicompost), T2 (10% vermicompost), and T3 (20% vermicompost) was significantly higher than that of CK. However, afterwards, the root nicotine content of T4 (50% vermicompost) and T5 (100% vermicompost) began to decline, even there was no significant difference between T5 and CK (Fig. 9).

Ratio of indoleacetic acid (IAA) to abscisic acid (ABA) of flue-cured tobacco roots: All vermicompost treatments increased the plant hormonal balance (IAA/ABA) in the flue-cured tobacco roots. However, IAA/ABA ratio in the roots of control plants was too low. Maximum IAA/ABA ratio was found in the tobacco plants supplied with 5% vermicompost (T1), followed by T5 (100%). Although IAA/ABA ratio decreased with increase in fraction of vermicompost to the soil, it was greater than that in the control plants (Fig. 10).

Discussion

Flue-cured tobacco is the largest and higher-yielding cash crop in China. In addition to good quality leaves of tobacco used for production of cigarettes, in fact, abandoned tobacco leaves, stems and roots can be efficiently utilized for production of organic fertilizer,

unconventional animal feed additives is another more promising development direction (Song *et al.*, 2016). Application of organic fertilizer such as vermicompost, could increase the total biomass of flue-cured tobacco, which also increase the supply of discarded leaves, stems and roots of flue-cured tobacco for development and utilization (Ortas & Lal, 2014; Lin *et al.*, 2021).

Earthworm compost is not only harmless to the environment, but also can add some vital nutrients to the soil and reduce the use of chemical fertilizers. To a certain extent, it can alleviate a series of problems caused by the excessive use of chemical fertilizers (Bressanin & Andrade, 2020). This study explored the effects of vermicompost on soil characteristics, growth and development of tobacco roots, and quality and economic characteristics of flue-cured tobacco through pot and field experiments, similar to some previous studies (Wang *et al.*, 2018). The results of this study showed that vermicompost supplementation enhanced the CEC, SOM content, MBC content, MBN content, and GRSP content (Figs. 2-6). Vermicompost contains a variety of microorganism and plant growth regulators, which might favor the development of mycorrhizae thereby providing considerable nutrient availability in soil for plants (He *et al.*, 2020; Lin *et al.*, 2021). The principal component analysis extracted first two principal components. The contribution of each index to the two principal components was ranked from high to low in order of MBN content and MBC content, SOM content, T-GRSP content, EE-GRSP content, CEC, and available nitrogen content. These results suggested that soil structure improvement due to vermicompost addition is associated with SOM, and GRSP. This can be supported by the fact that GRSP are generally released by mycorrhizae, which along with soil organic nitrogen (SON) and soil organic carbon (SOC) may facilitate stabilization and aggregation of soils.Thus, it can be concluded that addition of vermicompost enhanced the soil structure and soil fertility (Tian *et al.*, 2020).

Tobacco plant growth measured as root fresh weight, root length, and root volume were found to be increased under vermicompost treatment (Fig. 7). Growth promotive effect of vermicompost has already been reported in *Panax ginseng* (Eo & Park, 2019), *Allium cepa* (Datta *et al.*, 2018), and tomato (*Solanum lycopersicum* L.) (Wang *et al.*, 2017). Root activity, an important parameter for assessing cellular metabolism, was also increased substantially due to vermicompost application. The application of vermicompost could promote the metabolism of nitrogen and carbon as well as nutrient status in tobacco plants, which might be helpful to root and plant growth (Qin *et al.*, 2016). Vermicompost contains enough amount of humic acid and amino acids, which could significantly increase absorption of plant nutrients in the soil, thereby enhancing root vitality and plant growth (Theunissen *et al.*, 2010). This suggests that vermicompost can increase the cellular activity, cell division and root growth (Bressanin & Andrade, 2020). It is well evidenced that plant growth is regulated by a variety of hormones including IAA, cytokinins (CTK), ABA, etc. ABA is an anti-stress hormone and at high concentrations it can inhibit root growth (Xie *et al.*, 2019). In contrast, auxins are known to promote root growth (Taiz *et al.*, 2015). In the present study, vermicompost treatment significantly increased the content of IAA in the root of flue-cured tobacco, while it reduced the levels of ABA. These results suggest that vermicompost addition modulated IAA/ABA ratio in the roots to regulate root growth. This argument can be supported by the fact that there is a cross talk between ABA and IAA for signal transduction as has been observed in many plant species (Xie *et al.*, 2019, Figs. 7-10).

Tobacco is an important economic crop, and its economic importance is related to leaf growth, nicotine and nornicotine accumulation, and accumulation of compounds related to aroma sensory characteristics (Moghbel *et al.*, 2017). Nicotine is biosynthesized in the ornithine and arginine pathway operating in root cells and then it is translocated to the leaves via xylem, where nicotine is stored in vacuole (Taiz *et al.*, 2015). Moreover, biosynthesis and accumulation of nicotine are affected by various environmental factors such as soil fertility and plant hormones (Chen *et al.*, 2016). In this study, vermicompost application increased nicotine accumulation to a great extent in the leaves of flue-cured tobacco (Fig. 9). These results can be related to some of earlier studies in which it has been demonstrated that vermicompost application enhanced the nitrogen uptake, accumulation, assimilation and metabolism (Chen *et al.*, 2016). Similarly, addition of plant growth regulators such as jasmonic acid or gamma-aminobutyric acid also enhanced nitrogen metabolism and nicotine accumulation in tobacco plants (Chen *et al.*, 2016).

Conclusions

In the present study, application of vermicrompost improved soil quality by increasing CEC and enhancing soil structure and soil fertility, which in turn resulted in adjusting the ratio of IAA/BAA, improving root cell activity as well as metabolism of nitrogen and carbon in the roots, thereby improving the growth of roots. These findings are of important value for the effective development and supply of tobacco as a special economic crop as well as a potential animal feed additive.

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