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EFFECT OF VERMICOMPOST ON SOME PHYSIOLOGICAL ATTRIBUTES INVOLVED IN CARBON AND NITROGEN METABOLISM AS WELL AS NUTRIENT STATUS IN LEAVES OF TOBACCO (*NICOTIANA TABACUM* L.)

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Abstract

A pot experiment was carried out to examine the influence of vermicompost application on some key enzymes and metabolites involved in carbon (C) and nitrogen (N) metabolism as well as nutrient status in the leaves of tobacco (*Nicotiana tabacum* L.). Two types of vermicompost with two application rates were used in this study. Regardless of application rate, both types of vermicompost significantly increased total N, phosphorus (P) and potassium (K) contents in the leaves. They also caused enhancements in contents of total soluble carbohydrates, reducing sugars, starch and total organic C as well as amylase and invertase activities involved in C metabolism, contents of soluble protein and nicotine in N metabolism in the leaves. With an increase in application rate, each vermicompost type had an increasing effect on almost all measured parameters except nitrate reductase activity. Regardless of vermicompost type, the high rate (50%) of application showed the best effects compared with controls. The effects of V1 type vermicompost were superior to those of V2 at the same application rate. Therefore, the above effects might appear to be dependent on both type and dose. Vermicompost could be considered as an effective organic matter for attaining improved plant nutrition as well as C and N metabolism.

Key word: Vermicompost; Carbon metabolism; Nitrogen metabolism; Nutrient status; tobacco (*Nicotiana tabacum* L.)

Introduction

Tobacco (*Nicotiana tabacum* L.) is an important cash crop in China (Zhang *et al*., 2015). Soil quality is one of the most vital factors which influences the growth and production of tobacco plant (Chen, 2013). Misuse of chemical fertilizers and pesticides has led to the deterioration of soil quality resulting in suppression of plant growth and production. In recent year, using organic fertilizers instead of some chemical fertilizers has become one of the effective measures to improve soil quality and plant growth (Nicolodellia *et al*., 2016).

Organic fertilizer is an essential and better resource as a soil fertilizer and a soil amendment because it not only increases organic matter content, but improves soil properties (Audette *et al*., 2016). It could release a great amount of mineral elements for regulation of physiological metabolisms and plant growth. However, the use of organic fertilizers such as animal manure can cause some environmental problems due to its excessive contents of heavy metals and antibiotics together with its irrational application to agricultural fields (Zucco *et al*., 2015).

Among the various organic fertilizers, vermicompost has been regarded as a safe, clear and efficient soil fertilizer. Vermicompost is derived from agricultural wastes such crop straw, cow dung, leftover food and so on (Arancon *et al*., 2005). Its application to plants is believed to be highly beneficial for plant growth (Rakesh *et al*., 2015). Vermicompost contains a lot of mineral nutrients, active soil enzymes and microbes (Dominguez, 2004). Being a fine organic material it can improve soil physical properties due to its high porosity, crumbly structure, great capacity of drainage and water-holding capacity (Pandya *et al*., 2014).

Some studies on impacts of vermicompost show that it could increase macropore space and improve soil texture which affect the soil enzyme activities, and release capacity of soil nutrients required for plant growth (Marinari *et al*., 2000). The main nutrients contained in vermicompost are present in readily available forms for plant uptake, such as nitrates, available phosphorus (P) and potassium (K) (Chaudhuri *et al.*, 2000). Many studies concerned on the beneficial effects of vermicompost have been conducted in relation to growth, yield and quality of plants (Rakesh *et al*., 2015), however, there are few studies on tobacco plant. Thus, the purpose of this investigation was to evaluate the effects of application of different rates of vermicompost with two types on some key enzyme activities, metabolite levels and nutrient status involved in carbon (C) and nitrogen (N) metabolisms in the tobacco leaves to find an effective organic fertilizer in the form of vermicompost for improving plant growth of the crop.

Materials and Methods

Plant materials and trial location: A pot experiment was conducted using tobacco cultivar Yunyan 99 as the test material under a greenhouse at the College of Life Sciences, Northwest A&F University (Yangling, P.R. China). The chemical properties of the soil used in this study were as follows: pH 7.56, EC 102.2µS /cm, organic matter content 8.74 g/kg, NO_3^--N 32.84 mg/kg, NH_4-N

5.43 mg/kg, available P ($NaHCO_3^-$ extractable) 10.41 mg/kg and available K 79.7 mg/kg.

The two types of vermicompost namely V1 and V2 were produced from cow manure mixed with maize straw at a ratio of 7:3 and 5:5, respectively. The mixture composition was adjusted to 70% moisture content, and placed in a wooden frame. Two hundred gram earthworms (*Eisenia fetida*) were added to each wood-frame for two months. The properties of the above two types of vermicomposts are shown in Table 1.

Experimental design and sampling : Five treatments were used in this experiment. The control treatment (CK) had no application of vermicompost. Two application rates i.e., 25% (low rate, L) and 50% (high rate, H) fraction of total mass were applied for each type of vermicompost. Ten seedlings were planted in the mixture medium in a plot (120 cm length and 50 cm diameter). Each treatment had three replications in a completely randomized block design. After 60 d of transplant, the typical leaves were sampled for measurement.

Leaf nutrients' measurement: Total leaf N, P, and K contents were measured by the micro-Kjeldahl method, the molybdenum blue-ascorbic acid method, and flame photometry, respectively (Nicolodellia, 2016; Olsen & Sommers, 1982).

Key enzyme activities and carbon and nitrogen metabolisms: Total C content was determined by the combustion method following Yan *et al.* (2015). Starch content was assayed by the iodine method as described by Rufty & Huber (1983). Soluble sugar content was analyzed following the anthrone colorimetric method following Dai *et al.* (2011). Reducing sugar was analyzed using colorimetric determination of 3,5-dinitro-salicylic acid, and soluble protein content was assayed by the Coomassie brilliant blue staining method (Gao, 2006). Nicotine content was assayed following the method of active carbon extraction and decolorization following Wang (2003). The amylase activity was determined using starch as a substrate according to Joel & Bhimba (2012). The nitrate reductase activity in the freshly harvested samples was measured by the *In vitro* method (Srivastava & Ormrod, 1984). The invertase activity was measured by measuring the reducing sugar content following Liu *et al*. (2005).

Statistical analysis: Multiple ANOVA and comparison were carried out to determine significant differences between treatments at the 95% level of probability. Statistical analysis of all data was carried out using the SPSS 20.0 software package.

Results

Some key enzymes' activities involved in C and N metabolisms: The vermicompost application significantly affected the activities of leaf amylase, invertase and nitrate reductase involved in C and N metabolism, respectively (Table 2). The activities of all three enzymes were gradually increased with increase in vermicompost application rate. However, the nitrate reductase activities showed firstly an increasing and then a decreasing trend. The above key enzymes activities were greater for V1 than those for V2 type under the same level of treatment. The best effect was recorded at the high rate for each type of vermicompost.

Nitrogen metabolites involved in N metabolism: The effects of vermicompost on contents of N metabolite are shown in Table 3. With the increase in application rate of the two types of vermicompost, all measured metabolites contents were gradually increased. The highest content of soluble protein was recorded at the high application rate, approximately an increase by 48.8% and 35.1% for V1 and for V2 types, as compared with the control. There were significant differences between the two types of vermicompost at each level.

Carbon metabolites involved in C metabolism: Total soluble sugars, reducing sugar content, starch, and total organic carbon were positively influenced by the addition of two types of vermicompost. All the four parameters increased with increase in application rate. Regardless of type, the best effects were obtained at the high application rate with respect to contents of total soluble sugars, reducing sugars, starch, and total organic C. Compared with V2 type, V1 showed better effects on all four metabolite, especially, the total soluble sugar content which increased by 67.0% compared with the control (Table 4).

Leaf nutrients: Vermicompost application increased the contents of total N, P, and K to different degrees irrespective of application rate. With an increase of application rate of each type of vermicompost, the above parameters exhibited an increasing trend. The effects of vermicompost treatment with V1 type were slightly better than those with V2. The best effects were recorded at the high rate of application, especially for the total N content which increased 1.2-fold compared with the controls (Table 5).

Discussion

Plants employ different strategies to increase C and N storage by improving their metabolism so as to encounter adverse situation (Dietze *et al*., 2014). Bio-fertilizers, including microorganisms, could improve physiological metabolism and plant growth by adding more efficient nutrients to soil (Huseyin *et al*., 2007). However, to develop an effective type of growth substrate is the key for achieving enhanced productivity of crop (Xiao *et al*., 2014). In our study, two types of vermicompost were better growth substrate by added to soil, which produced from the mixture of cow manure and maize straw with different proportions. The purpose of our study was to explore their effects on improvement of C and N metabolism as well as nutrition status in tobacco leaves (Table 1).

Table 1. The physical and chemical characteristics of two types of vermicompost.

Type	pH	Conductivity (μS/cm)	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	Organic C (%)
V1	7.12	985	8.28	9.67	3.70	35.56
V2	7.02	1064	3.89	6.42	2.91	31.93

Table 2. Some key enzymes' activities involved in C and N metabolisms in tobacco leaves.

Vermicompost type	Vermicompost rate	Amylase activity ($mg\cdot g^{-1}FW\cdot min^{-1}$)	Invertase activity (mg glocose$\cdot g^{-1}FW\cdot min^{-1}$)	Nitrate reductase activity ($\mu g\ NO_2^-\cdot g^{-1}\ FW\cdot h^{-1}$)
CK	None	3.42 ± 0.36 c	2.87 ± 0.21 c	4.86 ± 0.41 bc
V1	Low rate	4.34 ± 0.63 b	3.32 ± 0.39 bc	6.22 ± 0.56 a
	High rate	5.71 ± 0.43 a	5.15 ± 0.44 a	3.54 ± 0.22 c
V2	Low rate	3.98 ± 0.36 bc	3.09 ± 0.21 c	5.27 ± 0.18 b
	High rate	2.04 ± 0.24 b	4.52 ± 0.15 b	3.12 ± 0.31 c

Different low-case letters in the column indicate significant differences between all treatments at $p<0.05$. The same should be followed in all following tables

Table 3. Effect of vermicompost on nitrogen metabolites in tobacco leaves.

Vermicompost type	Vermicompost rate	Soluble protein content (%)	Nicotine content (%)
CK	None	4.14 ± 0.22 d	2.28 ± 0.04 d
V1	Low rate	5.09 ± 0.16 bc	2.46 ± 0.11 bc
	High rate	6.16 ± 0.14 a	3.08 ± 0.07 a
V2	Low rate	4.88 ± 0.31 c	2.33 ± 0 .05 cd
	High rate	5.51 ± 0.23 b	2.71 ± 0.22 b

Table 4. Effect of vermicompost on carbon metabolites in tobacco leaves.

Vermicompost type	Vermicompost rate	Total soluble carbohydrate content (%)	Reducing sugar content (%)	Starch content (%)	Total organic carbon (%)
CK	None	3.42 ± 0.36 c	2.87 ± 0.21 c	24.59 ± 0.13 d	44.39 ± 0.66 d
V1	Low rate	4.34 ± 0.63 b	3.32 ± 0.39 bc	26.04 ± 0.32 c	46.01 ± 0.22 bc
	High rate	5.71 ± 0.43 a	5.15 ± 0.44 a	31.19 ± 0.24 a	49.88 ± 0.36 a
V2	Low rate	3.98 ± 0.36 bc	3.09 ± 0.21 c	25.82 ± 0.31 cd	45.86 ± 0.33 cd
	High rate	4.39 ± 0.45 b	3.64 ± 0.24 b	29.12 ± 0.33 b	47.05 ± 0.59 b

Table 5. Effect of vermicompost on nutrient content in tobacco leaves.

Vermicompost type	Vermicompost rate	Total N (%)	Total P (%)	Total K (%)
CK	None	3.54 ± 0.08c	0.31 ± 0.04b	4.1 ± 0.65b
V1	Low rate	3.65 ± 0.14c	0.33 ± 0.06b	4.35 ± 0.36b
	High rate	4.27 ± 0.24a	0.47 ± 0.10a	5.32 ± 0.37a
V2	Low rate	3.53 ± 0.09c	0.34 ± 0.03b	4.17 ± 0.10b
	High rate	4.00 ± 0.10b	0.37 ± 0.03ab	4.38 ± 0.19b

Nitrogen status and its metabolism might be closely related to carbon assimilation in photosynthesis, resulting in an extremely important roles in regulating plant growth and development (Ibrahim & Jaafar, 2011). Of the two key enzymes involved in C and N metabolisms, invertase activity can limit reducing sugar buildup (Derek *et al*., 2016; Herman *et al*., 2016; Liu *et al*., 2005). Nitrate reductase (NR) could regulate the absorbing ability of NO_3^- and NH_4^+ in soil (Kan *et al*., 2016; Liu *et al*., 2005). The activities of the above two enzymes are crucial for accumulation of organic C and N in plant leaves (Arancon *et al*., 2006; Liu *et al*., 2005). Many studies showed that organic fertilizer can notedly influence N metabolism and nutrient status in plants (Pramanik *et al*., 2007; Yuan *et al*., 2016). The vermicompost could contribute to increasing total N content, even promoting physiological metabolism in plants (Xiao *et al*., 2014; Rakesh *et al*., 2015). In the present study, we observed obvious effects of vermicompost application on N metabolism and its metabolites status in tobacco (Table 3). Activities of invertase and nitrate reductase were increased significantly resulting in enhancement of total nutrients in leaves. The effects of high application rate were greatest among all treatments (Tables 2 and 5).

In soils, the addition of organic fertilizer may promote the mobilization of soil N, while in soils with high N availability could result in the synergistic mobilization of C (Nicolodellia *et al*., 2016; Molina-Herrera & Romanyà, 2015). The main function of amylase activity is the hydrolysis of starch and glycogen (Molina-Herrera & Romanyà, 2015). In our study, the amylase activity was significantly improved by high application rate of vermicompost (Table 2). The dynamic changes of C metabolites measured in the present study were similar for the two types of vermicompost (Table 3). The improvement in C metabolism was greater by V1 type than by V2 under the same application rate. The enhancement of C metabolism level eventually formed a lot of organic compounds that could be readily used in the synthesis of energy and release of nutrients in leaves (Li *et al*., 2016). For example, the enhancement of starch content was associated to an increase in chlorophyll content and photosynthetic ability during the leaf growth and development (Collier, 1997).

It is well documented that vermicompost can maintain and improve soil fertility, soil structure as well as nutrient status of leaves and hence high plant productivity (Riley *et al*., 2008). Nitrogen, P and K are important physiological indicators of plants (Pramanik *et al*., 2007). In the present study, total N, P and K contents of tobacco leaves were obviously increased by the additions of V1 type vermicompost, particularly at high rate. This may be due to the reason that it contains a well-balanced composition of nutrients. Otherwise, Arancon *et al.* (2006) reported that vermicompost application exhibited a limited impact on total N contents of strawberries (*Fragaria ananassa* Duch.). Different types of vermicompost contain different amounts of nutrients which may affect the levels of soluble nutrients, absorption rates and microbial immobilization of nutrients in soils (Arancon *et al*., 2006). Vermicompost could significantly increase the microbial biomass, and enhance the absorption of nutrients in soil in order to improve C and N metabolisms in plants (Padmavathiamma *et al*., 2008).

Conclusions

The current study indicated that the two types of vermicompost might be quite useful for increasing the activities of some key enzymes and levels of metabolites involved in C and N metabolisms as well as N, P and K in the tobacco leaves. The effects of V1 type was generally better than V2. The high application rate of vermicompost was beneficial for improving the C and N metabolisms and nutrition status of the leaves. Therefore, we recommend that vermicompost could be a better and efficient organic manure for tobacco production, especially for V1 type. The specific impact of vermicompost on tobacco needs further studies under field conditions.

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