Effect of Yield and Quality of Erythropalum Scandens Bl. Under Different Dosage and Proportion of Chicken Manure and Cow Manure

Dao-Cheng Ma
Forestry College, Guangxi University, Guangxi Zhuang Autonomous Region
Xiang Tian
Nanning Arboretum, Guangxi Zhuang Atonomous Region
Pinxiang Guo
Forestry College, Guangxi University, Guangxi Zhuang Autonomous Region
Ling-hui Wang (wanglinghui97@163.com)
Forestry College, Guangxi University, Guangxi Zhuang Autonomous Region
Wei-Chao Teng
Forestry College, Guangxi University, Guangxi Zhuang Autonomous Region

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Research Article

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Abstract

Erythropalum scandens Bl. is a kind of leafy vegetable which has high edible and medicinal value in Southern China. However, it has been treated as a kind of wild vegetable for a long time and there is still little researches on its yield and quality after fertilization. This study aimed to assess the effect of yield and quality of *E. scandens* after using two kinds of organic fertilizer: chicken manure and cow manure, which find out the responses of the edible part in *E. scandens* after using different dosage and proportion of chicken manure and cow manure and find out a best fertilization treatment. We investigated the yield and quality of the edible part in biennial *E. scandens* including average yield, titratable acid content, sugar acid ratio, crude protein content, ascorbic acid content, tea polyphenols content, nitrate content, general flavone content and macronutrient content (including total nitrogen, phosphorus and potassium) after using different dosage and proportion of chicken manure and cow manure, which to find out a best fertilization treatment via the yield, nutrient and taste. Compared with CK group, proper fertilization can increase the average yield of *E. scandens* and promote its growth and macronutrient accumulation at the same time. The content of all kinds of nutrients are higher than CK group after using organic fertilizers and they can be accumulated during the process of fertilization. The *E. scandens* seedlings had the highest yield and best quality under T_6 treatment (0.8kg/plant chicken manure+1.2 kg/plant cow manure). However, nitrate can be accumulated in the edible part of *E. scandens* during the process of fertilization. Compared with CK group, organic fertilization can increase the yield and quality of *E. scandens*, but excessive fertilization can lead to a significant increase in nitrate levels in plants, even exceeding food safety standards. The nitrate level of *E. scandens* is a little bit high in this research and better solution is still needed to ensure the quality and food security of *E. scandens* in the near future.

1 Introduction

Proper fertilization is important during the process of plant growth and vegetable production, which can improve vegetable’s yield, quality and increase soil fertility. But the soil residual of excessive fertilizers can cause lots of problem, such as greenhouse gas emission, crop yield decrease, soil texture recession (Reay et al., 2012; Wang et al., 2019a), ground water pollution, surface water eutrophication (Gu et al., 2013; Le et al., 2010) and so on. Therefore, using fertilizers properly is important of crop cultivation. As one of the common organic fertilizers, animal manures (such as cattle manure, chicken manure, sheep manure, swine manure, etc.) are widely used on vegetable cultivation and many other aspects in agriculture. As a kind of fertilizer, manure can improve soil fertility, nutrient content, soil carbon and pH (Cai et al., 2019), and it can also reduce the reliance of inorganic fertilizers (Du et al., 2020). Unlike inorganic chemical fertilizers, most kinds of manures (like chicken manure) decompose slowly in soil and it can provide nutrients to plants for a long time (Yang et al., 2015). In conclusion, manure has been widely used to improve the yield and quality of vegetables (Adekiya, 2019; Zhang et al., 2019), crops (Hoover et al., 2019; Li et al., 2019; Wang et al., 2020) and many other fruits and economic crops.

Wild vegetables were common in the rural areas or underdeveloped areas in some countries (such as South Africa, South Kivu, Mediterranean and so on) (*Wivine* et al., 2017; Uusiku et al., 2010; Bvenura and

Afolayan, 2015). People treated them as a kind of food when famine and food shortage came because it can provide vitamins, microelements, fiber and more nutrient to human (*Łukasz* and *Katija*, 2015; *Sajid* and *Joachim*, 2015). Fertilization is rarely studied on these plants, which has been found in some kinds of wild leafy vegetables. For example, organic fertilizers can improve the yield of pumpkin and nightshade (Azeez et al., 2010). Some other kinds of leafy vegetables (such as *Moringa oleifera* (Sarwar et al., 2020), *Calamus gracilis* (Wang et al., 2019b) and so on) also have some researches on fertilizers. There are a number of resources survey (*Łukasz* and *Katija*., 2015), phytochemistry and pharmacological studies review (Bello et al., 2019), nutrient composition testing (*Garcı ´a-Herrera*, 2014) researches about these plants. *Erythropalum scandens* Bl. is a kind of perennial woody lianas distributed naturally in the south and southeast of China (*Xi* et al., 2009). Its young leaves and stems is always used as a kind of eatable wild leafy vegetable in tropical and subtropical China because of its unique flavor. The content of its nutrients are higher than those common vegetables in Guangxi Zhuang Autonomous Region in China (Long et al., 2017). Besides, its leaves, roots and stems can be used as a kind of medicine, which can treat gout, relieve edema for human beings (Xu et al., 2019). Besides, it also has the function of being interlaminar plant, conserving soil and water and so on (Qi and Tang, 2008; Jiang et al., 2014.). Zhu et al (2018) extracted its DNA and analyzed its genetic and genomic information in order to have a further promotion of its medicinal and edible value; Besides, there are some researches about its essential oil extraction (Feng et al., 2014). But there has been no research on fertilization, growth regulation and other aspects up till now.

According to few fertilization researches in leafy vegetables nowadays, chicken manure and cow manure were selected as two kinds of organic fertilizers to increase the yield and quality of *E. scandens* seedlings in this experiment. A series of field experiments were conducted to measure the average yield, titratable acid content, sugar acid ratio, crude protein content, vitamin C content, tea polyphenols content, nitrate content, general flavones, amino acid content in its eatable part and total N, P, K content in its tender buds to reflect the change of *E. scandens* seedlings after using different dosage and ratio of these two kinds of organic fertilizers. These all indices can also be the basis of *E. scandens* cultivating under different fertilizers, so as to promote its salesment and economic development in the countryside. To some extent, the enhancement of its sale can even improve farmers' annual income in these areas.

2 Materials And Methods

2.1 Ethics statement

The *Erythropalum scandens* Bl. is a common kind of leafy vegetable in China. It's not concluded in the "IUCN Red List of Threatened Species". This experiment was conducted in Nanning Arboretum and has been approved. The process of plant transplanting, maintenance and leaves collecting are conducted in accordance with ethical guidelines, in order to ensure its normal growth.

2.2 The introduction of test site, plants, soil and fertilizers

The field experiment was conducted at Liangfengjiang Forest Research Center, Nanning Arboretum (22°43'N, 108°17'E), Guangxi Zhuang Autonomous Region, China. The total area of the test site is about 4863.7 hactare. The average annual temperature is 21.6℃, the average annual temperature of the hottest and coldest months is 28.3℃ and 12.8℃ respectively, and the average annual precipitation is about 1300.6mm. The climate is the south Asian tropical monsoon climate, which is suitable for the growth of *E. scandens*. The soil sample which is used for this study was partial acid red soil, with a pH of 5.5. The other basic physical and chemical properties of the tested soil were: total nitrogen (N) 0.68g/kg, total phosphorus (P) 0.26 g/kg, total potassium (K) 4.09 g/kg, available nitrogen (N) 87.25mg/kg, available phosphorus (P) 10.14mg/kg, available potassium (K) 85.12mg/kg, organic matter 14.67g/kg.

The biennial *Erythropalum scandens* Bl. seedlings (43.9±7.4cm in the length of branches and 9.43±1.35mm in diameter at the base) were provided by Liangfengjiang Forest Research Center and Nanning Arboretum. In January 2018, a flat open field was chosen to plant the annual *E. scandens* seedlings and they were planted as a row spacing of 60cm (Figure 1). Before planting the seedlings, the soil in the field should be loosen, and the planting pattern is shown in Figure 1 below. Before the official experiment, the tested seedlings should be carefully managed, the frequency of irrigation should be formulated properly according to the soil condition and weeding should be done once a week. Automatic spraying 2-3 times per day in drought weather to ensure the healthy growth of the tested seedlings.

The chicken manure was obtained from Nanning Liangfeng Agriculture and Animal Husbandry Co. LTD, which contained total nitrogen 2.25%, total phosphorus 2.92%, total potasium 2.13%, organic matter 29.82% and its ratio of carbon and nitrogen was 8.44; the cow manure was obtained from cattle farm in Guangxi University, which contained total nitrogen 1.41%, total phosphorus 0.97%, total potasium 1.04%, organic matter 43.69% and its ratio of carbon and nitrogen was 17.97. Both of these manures were fully rotten before the formal experiment. Besides, some of the harmful elements, such as arsenic (As), chromium (Cr), cadmium(Cd) and plumbum (Pb) are also tested before the experiment. The chicken manure contained arsenic (As) 2.17mg/kg, chromium (Cr) 6.81mg/kg, cadmium (Cd) 0.83mg/kg and plumbum (Pb) 3.84mg/kg; The cow manure contained arsenic (As) 1.04mg/kg, chromium (Cr) 5.63mg/kg, cadmium (Cd) 0.44mg/kg and plumbum (Pb) 1.22mg/kg.

2.3 Experimental design

The biennial *E. scandens* seedlings were recovered for about two months after transplanting and were carefully managed during the period of their recovery. The whole process of the experiment was conducted from September 2018 to September 2019. A pre-experiment was done before the formal experiment began. 5-level treatments of chicken manure and cow manure (0.4g/plant, 0.8 g/plant, 1.2 g/plant, 1.6 g/plant, 2.0 g/plant) were set respectively to test the effect of fertilization. After the pre-experiment, we found that the growth of *E. scandens* seedlings will be inhibited under the treatment of 1.6 g/plant, 2.0 g/plant. Combined with the previous pre-experiment, some examples in some wooden plants and the calculation of the reasonable amount of fertilization applied, we set the final 3 levels of the two kinds fertilizer (0.4kg/plant, 0.8kg/plant and 1.2kg/plant).

While doing formal experiment, treatments were arranged in a randomized block test design, being an exploratory research, three replications were used in each treatment, 20 plots were repeated for each plot, and a total of 600 plants were used in the whole experiment. Two kinds of organic fertilizers-chicken manure and cow manure-were used as fertilizers and set at 3 levels (0.4kg/plant, 0.8kg/plant and 1.2kg/plant, respectively). A total of 10 groups of treatments were set up, with a control treatment to which no fertilizers were added (CK). Fertilizer formula of each group is shown in the table below (Table 1).

Table 1
Fertilization test design table during this experiment

Number	Treatment	Chicken manure (g/plant)	Cow manure (g/plant)	Amount of fertilizers (g/plant)
T_1	J_1N_1	0.4	0.4	0.8
T_2	J_1N_2	0.4	0.8	1.2
T_3	J_1N_3	0.4	1.2	1.6
T_4	J_2N_1	0.8	0.4	1.2
T_5	J_2N_2	0.8	0.8	1.6
T_6	J_2N_3	0.8	1.2	2.0
T_7	J_3N_1	1.2	0.4	1.6
T_8	J_3N_2	1.2	0.8	2.0
T_9	J_3N_3	1.2	1.2	2.4
T_{10}	CK	0.0	0.0	0.0

Note: Among all he treatments: J=level of chicken manure usage; N=level of cow manure usage; The subscript “1” represents the first level-0.4g/plant; The subscript “2” represents the second level-0.8g/plant; The subscript “3” represents the third level-1.2g/plant;

2.4 Experimental procedures

Two rows of *E. scandens* seedlings without any treatment were set between different groups. At the same time, trenches were dug in the soil and plastic film was embedded in order to prevent the interference and influence among different fertilization treatments. Two fertilization treatments were conducted in March and June 2019. During fertilization, dig pits with a size of 35cm×25cm at the corresponding plants of each plot, weigh the organic fertilizer used by each group, mix it with the excavated soil, and fill the pits again. After the completion of landfill, level the corresponding positions. During the experiment, the watering frequency in summer and cloudy and rainy days was 2-3 times a day and 1 to 2 times a day in turn (the soil and seedlings in the field of watering frequency were appropriately fine-tuned).

2.5 Indices determine

During the experiment period, the edible parts of tender stems and leaves of *E. scandens* after each fertilization treatment were cut over 15cm each month and placed on the electronic balance, and their fresh weight (accuracy was 0.01g) was measured and the value was recorded. After the recording was completed, the total output increment was calculated and recorded; Titratable acid was measured according to the method described by (Abid et al. 2013.). Sugar acid ratio can be calculated after assessing the content of soluble sugar and titratable acid. Crude protein content can be assessed by the Chinese Standard SN/T 2115-2008; Ascorbic acid content was assessed by the method described by Kampfenkel et al. 1995. Tea polyphenols content can be assessed by the Chinese National Standard GB/T 8313-2018. Nitrate content can be assessed by the Chinese National Standard GB5009. 33-2016. Total N P K content can be assessed by indigo-phenol blue colorimetry, vanadium-molybdenum yellow colorimetry and flame photometry, respectively (Rukun L, 1999).

2.6 Data statistics and analysis

All data were statisticed by Excel 2016 and analyzed by SPSS version 17.0 (IBM Corp., Armonk, NY, USA). Analysis of variance was used to analyze the difference between different groups under different fertilizer treatments. Duncan's new complex range method was used for multiple comparisons. Generalized linear models are used to determine the main factors influencing these same parameters and to quantify the effect of the interactions between two different kinds of organic fertilizers (chicken manure and cow manure).

3 Results

3.1 Average yield

It can be seen from Figure 2 that different types of fertilization treatments can improve the average yield of *E. scandens*, and the average yield of *E. scandens* has an extremely significant improvement than CK group after using chicken manure and cow manure ($p < 0.01$). The average yield of CK group has a decrease due to the lack of fertilization. Besides, under the condition of the same application amount of chicken manure (0.4kg/plant and 0.8kg/plant), the total increase yield of *E. scandens* with the increase of the application amount of cow manure ($T_3>T_2>T_1$, $T_6>T_5>T_4$). However, when the application amount of chicken manure was 1.2kg/plant, the average yield of *E. scandens* first increased and then decreased with the increase of the application amount of cow manure. Under the condition of the same application amount of cow dung, the increased yield of *E. scandens* first increased and then decreased with the increase of application amount of chicken manure ($T_4>T_7>T_1$, $T_5>T_8>T_2$, $T_6>T_9>T_3$). Among all the treatments, T_6 group (chicken manure 0.8kg/plant+ cow manure 1.2kg/plant) has the maximum average yield: 20.58g/area (Figure 2).

3.2 Titratable acid content

It can be seen from Table 2 that the titratable acid content has no significant difference at first, but the titratable acid content of *E. scandens* can be significantly improved under different types of fertilization treatments ($p < 0.05$) during the period of the experiment (the metaphase and the anaphase). The content of titratable acid increased in the same set of treatment, but the degree of increments varies with the amount of fertilizer applied. At the metaphase and anaphase, titratable acid content increased with the increase amount of cow manure when the amount of chicken manure is the same ($T_3>T_2>T_1$, $T_6>T_5>T_4$, $T_9>T_8>T_7$), and vice versa($T_7>T_4>T_1$, $T_8>T_5>T_2$, $T_9>T_6>T_3$). In other words, when the amount of one fertilizer is the same, increasing the use of another fertilizer enhances the accumulation of titratable acid. Among all the treatments, at the metaphase and anaphase, T_9 group (chicken manure 1.2kg/plant+ cow manure 1.2kg/plant) has the maximum titratable acid content among all the treatments, and the results are: 0.751% and 0.995%, and these two results were 27.94% and 66.67% higher than the CK group, respectively (Table 2).

Table 2
Effects of different fertilization treatments on titratable acid content of *E. scandens* seedlings.

Number	Treatment	Prophase(%)	Metaphase(%)	Anaphase(%)
T_{10}	CK	0.581±0.02a	0.587±0.02e	0.597±0.04f
T_1	J_1N_1	0.582±0.03a	0.601±0.02e	0.627±0.04f
T_2	J_1N_2	0.614±0.01a	0.648±0.02d	0.691±0.03def
T_3	J_1N_3	0.596±0.02a	0.696±0.02bc	0.836±0.05bc
T_4	J_2N_1	0.587±0.02a	0.612±0.02e	0.645±0.05ef
T_5	J_2N_2	0.612±0.02a	0.675±0.02cd	0.777±0.05cd
T_6	J_2N_3	0.609±0.02a	0.731±0.01ab	0.904±0.04b
T_7	J_3N_1	0.608±0.02a	0.655±0.02d	0.733±0.03de
T_8	J_3N_2	0.602±0.02a	0.708±0.02bc	0.877±0.05b
T_9	J_3N_3	0.587±0.03a	0.751±0.02a	0.995±0.04a

Note: Different lowercase letters indicate significant difference ($p < 0.05$) among different fertilization treatments on one-way ANOVA, followed by a Duncan test. The same below.

3.3 Sugar acid ratio

It can be seen from Table 3 that the sugar acid ratio has no significant difference at first, but it can be significantly improved under different types of fertilization treatments ($p < 0.05$) during the period of the experiment (the metaphase and the anaphase), but the sugar acid ratio of *E. scandens* seedlings showed a trend of first increasing and then decreasing in CK group. After fertilization, the sugar acid ratio increased in the same set of treatment differently. At the metaphase and anaphase, there was no obvious relationship between the sugar acid ratio and the consumption of chicken manure and cow manure. Among all the treatments, at the metaphase and anaphase, T_5 group (chicken manure 0.8kg/plant+ cow manure 0.8kg/plant) has the maximum sugar acid ratio among all the treatments, and the results are: 6.03 and 7.29, and these two results were 53.05% and 92.35% times higher than the CK group, respectively (Table 3).

Table 3
Effects of different fertilization treatments on sugar acid ratio of *E. scandens* seedlings.

Number	Treatment	Prophase	Metaphase	Anaphase
T_{10}	CK	3.79±0.08a	3.94±0.33d	3.79±0.31d
T_1	J_1N_1	3.83±0.08a	4.49±0.35c	4.99±0.12c
T_2	J_1N_2	3.68±0.15a	4.66±0.15bc	5.25±0.23c
T_3	J_1N_3	3.71±0.09a	4.67±0.08bc	4.81±0.11c
T_4	J_2N_1	3.74±0.30a	4.90±0.13bc	6.04±0.08b
T_5	J_2N_2	3.63±0.09a	6.03±0.27a	7.29±0.55a
T_6	J_2N_3	3.68±0.08a	5.72±0.22a	6.38±0.32b
T_7	J_3N_1	3.68±0.08a	5.09±0.17b	6.21±0.22b
T_8	J_3N_2	3.75±0.12a	4.96±0.53bc	5.58±0.20bc
T_9	J_3N_3	3.73±0.09a	4.94±0.20bc	5.07±0.14c

3.4 Crude protein content

It can be seen from Table 4 that the crude protein content has no significant difference at first, but it can be significantly improved under different types of fertilization treatments ($p < 0.05$) during the period of the experiment (the metaphase and the anaphase), but the crude protein content of *E. scandens* seedlings showed a trend of first increasing and then decreasing in CK group. At the metaphase and anaphase, the crude protein of *E. scandens* seedlings increased with the increasing of cow manure under the condition of the same treatment of chicken manure ($T_3>T_2>T_1$, $T_6>T_5>T_4$, $T_9>T_8>T_7$), but there was no significant relationship between the amount and increasing of fertilizer and the crude protein content in other treatments. At the metaphase, T_6 treatment had the maximum content of crude protein (26.34%), and at the anaphase, T_6 and T_9 treatments had the maximum value (27.79%). These two results were 8.93% and 19.12% higher than CK group, respectively (Table 4).

Table 4
Effects of different fertilization treatments on crude protein content of *E. scandens* seedlings.

Number	Treatment	Prophase(%)	Metaphase(%)	Anaphase(%)
T_{10}	CK	23.96±1.69a	24.18±0.72g	23.33±2.41g
T_1	J_1N_1	23.78±2.11a	24.95±2.00f	25.82±2.08f
T_2	J_1N_2	23.69±1.94a	25.08±1.78ef	25.91±2.01f
T_3	J_1N_3	23.67±1.96a	25.52±1.69cd	26.41±1.94e
T_4	J_2N_1	23.61±1.83a	25.21±1.29def	26.79±1.69d
T_5	J_2N_2	23.84±1.48a	25.93±2.10b	27.31±1.52bc
T_6	J_2N_3	23.91±2.19a	26.34±1.94a	27.79±1.88a
T_7	J_3N_1	23.61±2.07a	25.37±2.30de	27.14±1.59cd
T_8	J_3N_2	23.74±2.38a	25.73±1.45bc	27.62±2.88ab
T_9	J_3N_3	23.76±1.08a	26.28±2.20a	**27.79±2.72a**

3.5 Ascorbic Acid (Vitamin C) content

It can be seen from Table 5 that the content of vitamin C can be significantly improved under different treatments of chicken manure and cow manure ($p < 0.05$), but in CK group the Vc content increased at first and then decreased. With the process of fertilization, Vc in *E. scandens* seedlings can be accumulated in the same treatment except CK group. When using 0.4g/plant chicken manure and cow manure, the Vc content of *E. scandens* seedlings can be improved with the increasing of cow manure and chicken manure respectively, but with the increasing of chicken manure and cow manure, there was a trend of first rising then falling, even continually decline at last with the increasing dosage of another manure. For example, at metaphase, when using 0.8g/plant chicken manure, the Vc content showed a trend of first rising and then falling (T5>T6>T4), but when the dosage of chicken manure was up to 1.2g/plant, the Vc content of *E. scandens* decreased continuously with the increasing of cow manure (T_7>T_8>T_9). At the metaphase and anaphase, T_5 treatment (0.8g/plant chicken manure+0.8g/plant cow manure) had the maximum of Vc content (25.02mg/100g and 35.05mg/100g), and they were 134.88% (34.88%) and 189.25% (89.25%) higher than CK group (Table 5).

Table 5
Effects of different fertilization treatments on vitamin C content of *E. scandens* seedlings.

Number	Treatment	Prophase(mg/100g)	Metaphase(mg/100g)	Anaphase(mg/100g)
T_{10}	CK	18.56±1.99a	18.55±2.17c	18.52±1.61e
T_1	J_1N_1	18.13±1.67a	20.17±1.63bc	22.73±2.66d
T_2	J_1N_2	19.02±1.90a	24.16±1.60a	32.05±2.50ab
T_3	J_1N_3	19.12±1.23a	24.27±2.38a	33.75±2.51ab
T_4	J_2N_1	18.62±1.92a	24.35±2.76a	30.22±2.56bc
T_5	J_2N_2	17.59±1.02a	25.02±2.68a	35.05±2.04a
T_6	J_2N_3	18.83±0.68a	24.41±2.06a	32.63±2.74ab
T_7	J_3N_1	19.73±1.90a	24.70±2.05a	29.43±2.75bc
T_8	J_3N_2	19.64±1.27a	23.11±1.36ab	26.59±2.84cd
T_9	J_3N_3	18.98±2.41a	20.88±2.36abc	23.53±2.62d

3.6 Tea polyphenols content

It can be seen from Table 6 that the tea polyphenols content increased continually in the same group with the process of fertilization. Although the final content at anaphase can be significantly different under different fertilization combination ($p < 0.05$), in CK group the content of tea polyphenols showed a trend of increasing first and then staying the same. At the metaphase and anaphase, the content of tea polyphenols increased continually with the increasing dosage of chicken manure when using the same dosage of cow manure. The trend of change in *E. scandens* seedlings with the increasing of cow manure is similar at metaphase when using the same dosage of chicken manure, but when using 1.2g/plant chicken manure, the tea polyphenols content would stay the same. But there was no obvious relationship between tea polyphenols content and the dosage change of fertilizers in other conditions. At the metaphase and anaphase, the tea polyphenols content had the maximum under T_5 treatment (0.8g/plant chicken manure+0.8g/plant cow manure), which the values were 0.34% and 0.45% respectively, and they were 78.95% and 136.84% higher than CK group (Table 6).

Table 6
Effects of different fertilization treatments on tea polyphenols content of *E. scandens* seedlings.

Number	Treatment	Prophase(%)	Metaphase(%)	Anaphase(%)
T_{10}	CK	0.18±0.04a	0.19±0.05b	0.19±0.05c
T_1	J_1N_1	0.22±0.04a	0.29±0.04a	0.35±0.06b
T_2	J_1N_2	0.21±0.01a	0.31±0.06a	0.37±0.06ab
T_3	J_1N_3	0.21±0.02a	0.30±0.03a	0.41±0.05ab
T_4	J_2N_1	0.21±0.04a	0.31±0.05a	0.45±0.05a
T_5	J_2N_2	0.23±0.03a	0.34±0.05a	0.45±0.04a
T_6	J_2N_3	0.21±0.03a	0.32±0.05a	0.44±0.05ab
T_7	J_3N_1	0.19±0.04a	0.30±0.05a	0.39±0.06ab
T_8	J_3N_2	0.17±0.03a	0.27±0.06ab	0.41±0.03ab
T_9	J_3N_3	0.23±0.03a	0.27±0.04ab	0.41±0.04ab

3.7 Nitrate content

It can be seen from Table 7 that the nitrate content increased continually in the same group under different fertilization, but the content of nitrate decreased without any fertilizers (CK group). There was also a significant difference between groups in nitrate content at metaphase and anaphase ($p < 0.05$). At the metaphase and anaphase, the nitrate content increased continually with the increasing dosage of chicken manure and cow manure when using the same dosage of another manure. The content of nitrate had the maximum under T_9 treatment (1.2g/plant chicken manure+1.2g/plant cow manure) at both metaphase and anaphase, which the values were 1638.92mg/kg and 2087.50 mg/kg respectively, and they were 73.16% and 152.75% higher than CK group (Table 7). Therefore, nitrate content increases with the increase of fertilizer application.

Table 7
Effects of different fertilization treatments on nitrate content of *E. scandens* seedlings.

Number	Treatment	Prophase(mg/kg)	Metaphase(mg/kg)	Anaphase(mg/kg)
T_{10}	CK	1034.85±54.11a	946.45±42.37e	825.93±122.00g
T_1	J_1N_1	1023.32±77.17a	1047.75±75.21de	1065.29±77.17f
T_2	J_1N_2	1059.91±62.36a	1153.53±116.81cd	1212.81±90.56ef
T_3	J_1N_3	991.25±37.19a	1189.64±84.72cd	1295.46±129.47e
T_4	J_2N_1	1008.34±60.26a	1180.06±61.05cd	1349.59±24.78de
T_5	J_2N_2	981.47±44.60a	1268.13±118.52bc	1457.41±95.41d
T_6	J_2N_3	1039.84±35.27a	1424.73±70.47b	1677.90±127.14bc
T_7	J_3N_1	1105.24±98.42a	1365.94±83.44b	1624.43±30.76c
T_8	J_3N_2	1024.05±72.03a	1407.99±59.36b	1787.02±32.06b
T_9	J_3N_3	987.66±70.95a	1638.92±144.20a	2087.50±79.84a

3.8 General flavone content

It can be seen from Table 8 that the general flavone content can be continually improved with the process of using organic fertilizers, even there was a little bit increase of the general flavone content with the growth of *E. scandens* seedlings in CK group. At the metaphase and anaphase, when using the same amount of cow manure, the content of general flavone first increasing, then decreasing and then increasing, and finally gradually decreasing with the increasing of the chicken manure (T_3>T_2>T_1, T_6>T_4>T_5, T_7>T_8≥T_9). Accordingly, when using the same dosage of cow manure at metaphase, the content of general flavone increased with the increasing of chicken manure (T_7=T_4>T_1, T_8>T_5>T_2, T_9>T_6>T_3), and when it came to the anaphase, the general flavone content showed a trend of first increasing, then decreasing with the increasing of cow manure (T_7>T_4>T_1, T_8>T_5>T_2, T_6>T_9>T_3). The general flavone content of *E. scandens* had the maximum under the T_7 treatment (1.2g/plant chicken manure+ 0.4g/plant cow manure) in both metaphase and anaphase, which the values were 0.075% and 0.091%, and they were 31.58% and 54.24% higher than CK group, respectively.

Table 8
Effects of different fertilization treatments on general flavone content of *E. scandens* seedlings.

Number	Treatment	Prophase(%)	Metaphase(%)	Anaphase(%)
T_{10}	CK	0.056±0.0036a	0.057±0.0035d	0.059±0.0044d
T_1	J_1N_1	0.057±0.0040a	0.062±0.0040cd	0.069±0.0056c
T_2	J_1N_2	0.058±0.0052a	0.065±0.0046bc	0.075±0.0060c
T_3	J_1N_3	0.061±0.0026a	0.068±0.0050abc	0.077±0.0036bc
T_4	J_2N_1	0.062±0.0036a	0.075±0.0036a	0.088±0.0060a
T_5	J_2N_2	0.058±0.0030a	0.069±0.0026abc	0.086±0.0056ab
T_6	J_2N_3	0.057±0.0026a	0.072±0.0046ab	0.089±0.0053a
T_7	J_3N_1	0.06±0.0026a	0.075±0.0017a	0.091±0.0062a
T_8	J_3N_2	0.056±0.0030a	0.073±0.0053a	0.089±0.0044a
T_9	J_3N_3	0.06±0.0030a	0.073±0.0020a	0.088±0.0070a

3.9 Total nitrogen(N), phosphorus(P) and potassium(K) content

It can be seen in Table 9 that the total content of nitrogen(N), phosphorus(P) and potassium(K) in tender buds of *E. scandens* seedlings can be improved gradually with the process of fertilization and they can accumulate in the buds. There were no significant difference on these three macronutrient content at first ($p > 0.05$), but different organic fertilization can affect the content of them significantly ($p < 0.05$). At metaphase and anaphase, the content of total phosphorus in the tender buds of *E. scandens* seedlings can be improved gradually with the increasing dosage of chicken manure and cow manure when using the same dosage of the other kind of fertilizer (T_3>T_2>T_1, T_6>T_5>T_4, T_9>T_8>T_7, T_7>T_4>T1, T_8>T_5>T_2, T_9>T_6>T_3), and the trends in total kalium was similar. But when using 1.2g/plant chicken manure or cow manure at anaphase, there was a trend of first increasing then decreasing with the increase of the other fertilizer of the total kalium content in the tender buds of *E. scandens* seedlings (T_8>T_9>T_7, T_6>T_9>T_3). But as the content of total nitrogen, the situation was quite different. When using the same dosage of cow manure at metaphase and anaphase, the total nitrogen content in tender buds of *E. scandens* seedlings increased with the increased of chicken manure (T_9>T_6>T_3 at metaphase, $T_9 \geq T_6$>T_3 at anaphase), but when using the same dosage of chicken manure, there was no significant relationship between the increment of cow manure and total nitrogen content in the tender buds of *E. scandens* seedlings. It can be seen in the Table 9 that the total nitrogen content in the tender buds of *E. scandens* seedlings had the maximum under T_6 treatment (0.8g/plant chicken manure+ 1.2g/plant cow manure) at both metaphase and anaphase, and as for total phosphorus and total kalium can be best improved under T_9 treatment (1.2g/plant chicken manure+ 1.2g/plant cow manure), and the maximum value of total N, P, K at anaphase were 44.47g/kg, 1.21g/kg and 26.29g/kg, respectively.

Table 9
Effects of different fertilization treatments on total nitrogen, phosphorus and kalium content of tender buds on *E. scandens* seedlings.

Number	Treatment	Period	Total nitrogen(g/kg)	Total phosphorus(g/kg)	Total potassium(g/kg)
T_{10}	CK	Prophase	38.34±0.27a	0.76±0.04a	17.26±1.01a
		Metaphase	38.69±0.12g	0.75±0.03d	17.33±0.89f
		Anaphase	37.32±0.39f	0.74±0.03d	17.52±1.13e
T_1	J_1N_1	Prophase	38.05±0.34a	0.74±0.03a	18.43±1.13a
		Metaphase	39.92±0.32f	0.83±0.05c	19.61±0.31e
		Anaphase	41.32±0.33e	0.91±0.05c	21.21±0.57d
T_2	J_1N_2	Prophase	37.91±0.31a	0.71±0.05a	18.80±0.98a
		Metaphase	40.12±0.28ef	0.85±0.05c	20.06±0.29de
		Anaphase	41.45±0.32e	0.93±0.04c	22.54±0.49cd
T_3	J_1N_3	Prophase	37.87±0.31a	0.74±0.05a	17.09±0.96a
		Metaphase	40.83±0.27cd	0.88±0.04bc	20.47±0.93cde
		Anaphase	42.26±0.31de	1.01±0.04b	23.87±0.79bc
T_4	J_2N_1	Prophase	37.78±0.29a	0.76±0.04a	18.73±1.20a
		Metaphase	40.34±0.21def	0.88±0.05bc	21.02±0.50bcd
		Anaphase	42.86±0.27cd	1.02±0.03b	23.93±0.65bc
T_5	J_2N_2	Prophase	38.14±0.24a	0.71±0.03a	17.48±1.23a
		Metaphase	41.48±0.34b	0.90±0.06abc	21.25±0.80abcd
		Anaphase	43.70±0.24abc	1.06±0.04b	25.19±0.93ab
T_6	J_2N_3	Prophase	38.26±0.35a	0.78±0.05a	18.06±0.64a
		Metaphase	42.14±0.31a	0.95±0.05ab	21.73±0.86abc
		Anaphase	44.47±0.30a	1.18±0.02a	26.29±1.04a
T_7	J_3N_1	Prophase	37.77±0.33a	0.77±0.03a	17.55±1.08a
		Metaphase	40.59±0.37de	0.95±0.03ab	21.86±0.58ab
		Anaphase	43.42±0.26bc	1.18±0.02a	26.25±1.11a
T_8	J_3N_2	Prophase	37.98±0.38a	0.71±0.03a	17.48±0.91a

Number	Treatment	Period	Total nitrogen(g/kg)	Total phosphorus(g/kg)	Total potassium(g/kg)
		Metaphase	41.17±0.23bc	0.96±0.03a	22.13±0.91ab
		Anaphase	44.20±0.46ab	1.19±0.02a	26.31±1.37a
T_9	J_3N_3	Prophase	38.02±0.17a	0.73±0.03a	18.78±1.12a
		Metaphase	42.05±0.35a	0.97±0.05a	22.42±0.54a
		Anaphase	44.47±0.44a	1.21±0.05a	26.28±0.94a

4 Discussions

Organic fertilization is a common way to improve the yield and quality of vegetables during the whole process of plant growing (Gai et al., 2016), but some research showed that using organic fertilizer can be beneficial to the environment but it can cause the decrease of vegetable yield (Zhuang et al., 2019). In this research, the yield of *E. scandens* are improved greatly after using chicken manure and cow manure, which is consistent with most studies. Reasonable using of fertilizer can promote organic growing in many kinds of plants.

Titrate acid is an important index to reflect the taste of vegetables, and it can be influence by many aspects, such as soil conditions, planting seasons and so on (Ren et al., 2016). Sometimes using fertilizer (or increasing the supplement of N, P and K) can decrease the content of acids, which can improve the taste of fruit and juice (Zhang et al., 2012; Parvizi and Sepaskhah, 2015). The content of titrate acid can be improved after using organic fertilizer, which is consistent with the findings of Cen et al., 2020 and many other researches. Organic fertilization can increase the content of titrate acid compared with common management. Some components of manure can transfer into titrate acid during the process of vegetable growing, which can influence the taste of *E. scandens*.

The result indicate that the content of crude protein is higher than CK after using chicken manure and cow manure. Using pig manure can improve the crude protein content of peanut kernels (Wang et al., 2020). Organic fertilizer can improve the content of crude protein in seeds, vegetables and many other foods.

Ascorbic acid, also known as Vitamin C, is a main vitamin in food, fruits and many kinds of vegetables. It is a strong antioxidant and can promote the absorption of Fe in human, which is good for human's health and prevent human from chronic diseases (Bruno et al., 2006; Traber and Stevens, 2011; Andarwulan et al., 2012). The content of ascorbic acid increased after using manure.

Nitrate is very common in many kinds of vegetables and it be can transferred into nitrite by micro-organisms in the saliva, which is a harmful substance for human bodies (Cassens, 1995). The nitrite can cause cancers, methaemoglobinaemia and many other diseases (Cassens, 1997; Chan, 2011). From our experiment we can found that the content of nitrate is higher than CK after using manure. The main reason is that the N in chicken manure and cow manure can be transferred into nitrate via Nitrate reductase (NR). According to the standard of (GB 2762-2017 national food safety standards of food contaminants in limited) and some researches (Shen et al., 1982; Tang et al., 2007), the nitrate content in vegetables should be no higher than 3000 mg/kg.

According to the scientific risk assessment on nitrate in vegetables, requested by the European Commission and adopted by the European Food Safety Authority (EFSA, 2008), nitrate per se is relatively harmless, since the toxic threshold (>7–35 g) for nitrate is much higher (100-fold) than the acceptable daily intake of 3.7 mg nitrate kg^{-1} body weight adopted by the joint FAO/WHO Expert Committee on Food Additives (FAO/WHO, 2003a, 2003b) and the European Union (Kyriacou et al., 2019).

Vegetables can be generally classified into four classes according to the nitrate content according to Shen et al., 1982 and Tang et al., 2007. When the content is below 432mg/kg, the vegetables can be eaten raw; Between 432mg/kg and 785mg/kg, it cannot be eaten raw but can be salted or cooked food; Between 786mg/kg and 1440mg/kg, not raw food or salt, but cooked food; Between 1441mg/kg and 3100mg/kg, or more than 3100mg/kg, the vegetables should not be eaten. Although an accurate evaluation standard has not been obtained, only T1, T2, T3 and T4 fertilization treatments can meet the edible requirements in this experiment, and all of them are suitable for cooked food. Although some researches indicate that the way of storage and handling can influence the content of nitrate in vegetables (Leszczynska et al., 2008. Chang et al., 2013), it still need to be controlled in all kinds of foods and vegetables.

N, P and K are three main elements which can provide the main nutrients and improve the plants growth. The results indicate that the content of N, P and K are higher after using the organic fertilizer. Manure application increased the content of orthophosphate and myo-inositol hexaphosphate (myoIHP), especially the orthophosphate content exceeded 95% (Qin et al., 2020.). The increment of orthophosphate can provide more phosphorus to the plant. Single and combining application of N, P, and K fertilizers could enhance fruit yield and juice content in aril and increase TSS, total sugar and vitamin C content but decrease total acid content in aril juice of pomegranate (Zhang et al., 2012). In short, although using organic fertilizer can improve the yield and quality of *E. scandens*, long-term organic fertilizer using can significantly improve the content of heavy metals, trace elements (TEs) and antibiotics (ABs) and many other kinds of harmful substances in soils, which can influence the vegetable safety and do harm to human health (Ning et al., 2017; Margenat et al., 2020).

5 Conclusion

According to the result of a series of experiments, we found that in contrast to CK group, using chicken manure and cow manure appropriately can promote the growth of *E. scandens* and improve its yield, and the T_6 treatment (0.8g/plant chicken manure +1.2 g/plant cow manure) has the best yield and quality among all the treatments. But it was found that using too much fertilizer does not necessarily promote the growth of *E. scandens*. Besides, it was concluded that the content of some indices (such as nitrate) can be significantly increased after using too much fertilizers. In summary, how to ensure the quality and food security of *E. scandens* as a kind of leafy vegetable still need further research.

Declarations

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Figures

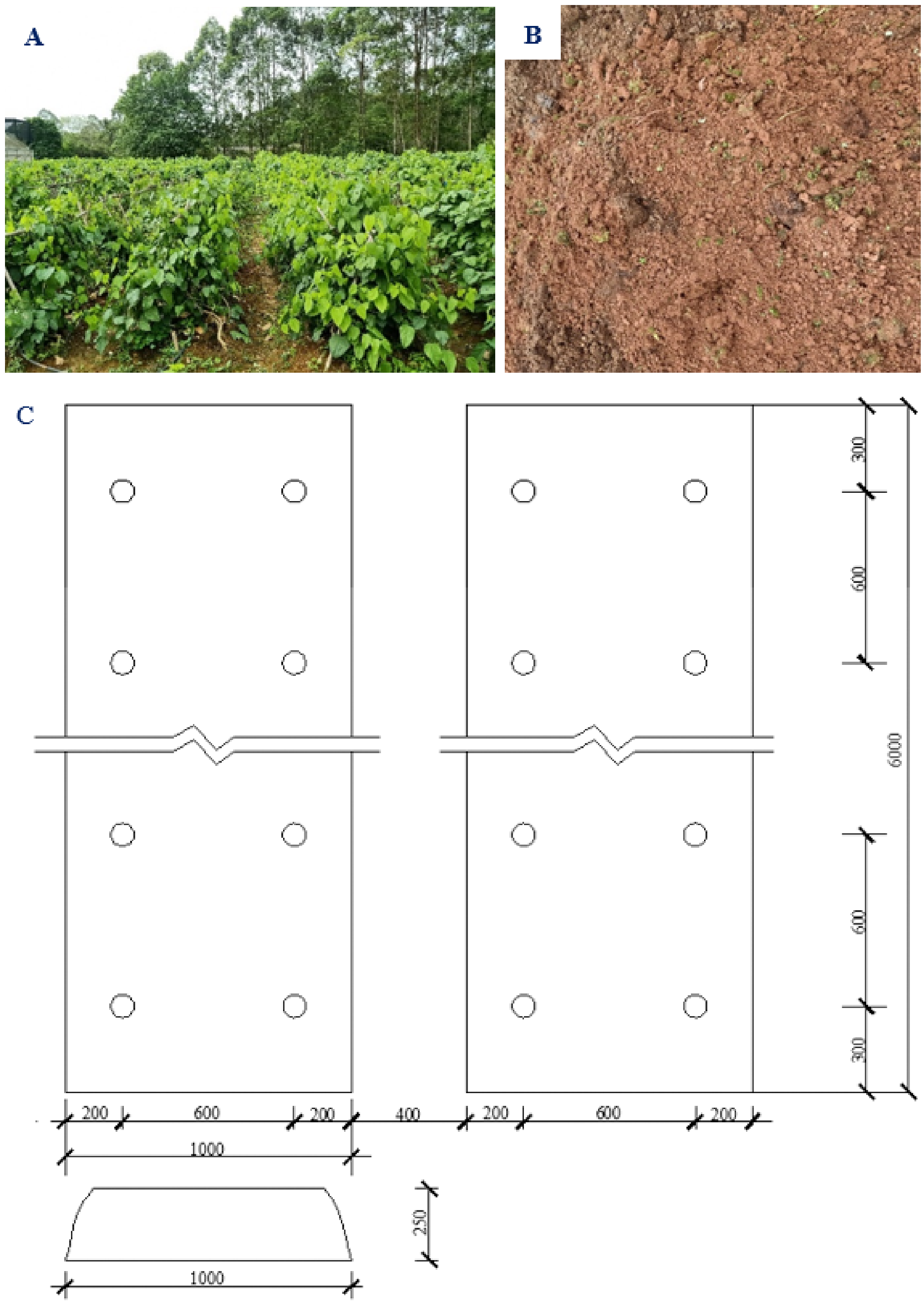

Figure 1

Plants and soil materials and planting pattern in this experiment (A, B: the biennial E. scandens seedlings and soil material, C: planting pattern)

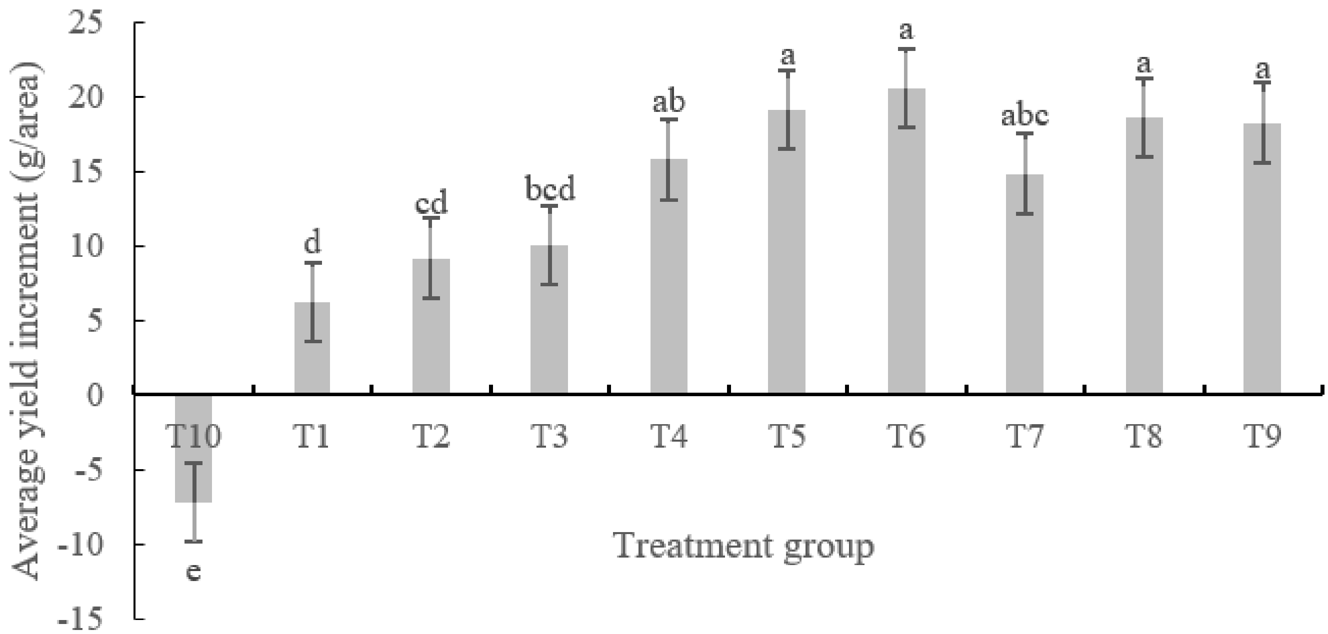

Figure 2

Effects of different fertilization treatments on yield increment of E. scandens seedlings.