In situ earthworm breeding in orchards significantly improves the growth, quality and yield of papaya (*Carica papaya* L.)

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The aim of this study was to compare the effects of four fertilizer applications—control (CK), chemical fertilizer (FL), compost (CM), and in situ earthworm breeding (EB)—on the growth, quality and yield of papaya (*Carica papaya L.*) . The EB treatment had the highest growth parameters over the whole growth period. At 127 days after transplantation, the plant heights were ordered EB > FL > CM > CK, and the stem diameters were EB > FL > CM > CK. The soluble-solid, sugar, vitamin C, and protein content significantly increased in the EB treatment. In addition, the total acid and the electrical conductivity of the fruit significantly decreased in the EB treatment. Fruit firmness clearly increased in the CM treatment, and it decreased in the FL treatment. The fresh individual fruit weights, fruit numbers, and total yields were greatly improved in the FL and EB treatments, and the total yield of the EB treatment was higher than that in the FL treatment. In conclusion, the in situ earthworm breeding treatment performed better than conventional compost and chemical fertilizer treatments. Furthermore, in situ earthworm breeding may be a potential organic fertilizer application in orchards because it not only improves the fruit quality and yield but also reduces the amount of organic wastes from agriculture as a result of the activities of earthworms.

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Abstract

The aim of this study was to compare the effects of four fertilizer applications—control (CK), chemical fertilizer (FL), compost (CM), and in situ earthworm breeding (EB)—on the growth, quality and yield of papaya (*Carica papaya L.*). The EB treatment had the highest growth parameters over the whole growth period. At 127 days after transplantation, the plant heights were ordered EB > FL > CM > CK, and the stem diameters were EB > FL > CM > CK. The soluble-solid, sugar, vitamin C, and protein content significantly increased in the EB treatment. In addition, the total acid and the electrical conductivity of the fruit significantly decreased in the EB treatment. Fruit firmness clearly increased in the CM treatment, and it decreased in the FL treatment. The fresh individual fruit weights, fruit numbers, and total yields were greatly improved in the FL and EB treatments, and the total yield of the EB treatment was higher than that in the FL treatment. In conclusion, the in situ earthworm breeding treatment performed better than conventional compost and chemical fertilizer treatments. Furthermore, in situ earthworm breeding may be a potential organic fertilizer application in orchards because it not only improves the fruit quality and yield but also reduces the amount of organic wastes from agriculture as a result of the activities of earthworms.

Keywords: Earthworm in situ breeding; Papaya; Plant growth; Fruit quality; Total yield

1. Introduction

Papaya (*Carica papaya L.*) is one of the most important fruit crops in tropical and subtropical areas. It is rich in nutrition, sugar, vitamin C, protein, and amino acids, and it is the primary raw material that contains papain. Papaya is also widely planted in southern China, especially in Guangdong, Yunnan, and Hainan Provinces. However, papaya production is frequently low and unstable. Although chemical fertilizer application is a common method for improving papaya yields, it is not environmentally friendly and can impair soil fertility by reducing the carbon and nitrogen content (Ngo et al., 2012). Moreover, chemical fertilizer application can also seriously affect animal and human health (Vu et al., 2007). For these reasons, the importance of organic fertilization has been increasing in recent years, and suitable organic amendments including composting, vermicomposting and in situ earthworm breeding have become promising biological ways to improve the growth, fruit quality and yield of papayas.

The addition of compost to soil has been described as an ideal alternative method for improving soil fertility and plant nutrition (Cantanazaro et al., 1998; Caravaca et al., 2002), and this method is especially appropriate for sustainable agriculture. Recent studies showed that vermicomposting takes advantage of the presence of epigeic earthworms during composting to generate an organic material that may be physically, nutritionally and biochemically improved when compared with compost (Pramanik et al., 2007; Ngo et al., 2012). It was shown that vermicompost could improve soil fertility and plant growth and increase crop yield (Atiyeh et al., 2000a; Singh et al., 2008; Jouquet et al., 2010; Jouquet et al., 2011; Ngo et al., 2012). Among the soil organisms favored by organic fertilization, earthworms have been identified as a key functional group (Jouquet et al., 2006). Earthworms have a great ability to consume all organic wastes, reducing the volume by approximately 50% and expelling the digested materials as castings, which are useful for soil amendments and may be easily stored for agricultural use (Tomati et al., 1985). In comparison with vermicomposting, in situ earthworm breeding in orchards usually has three important advantages. First, this method can be used to manage a large amount of organic wastes from agriculture. Earthworms have attracted a great deal of attention as an efficient and low-cost means of composting organic wastes such as animal wastes and crop residues (Ndegwa and Thompson, 2001; Singh et al., 2008). They not only reduce organic waste pollution but also improve the environment of rural areas. Second, this method produces a large amount of high-quality compost, known as “vermicompost,” which comes from the biological degradation of organic wastes by earthworms (Chaoui et al., 2003). Third, earthworm activities improve the soil structure, microbial activity and biodiversity, and soil OM dynamics (Jongmans et al., 2001; Pulleman et al., 2005; Jouquet et al., 2007; Bottinelli et al., 2010; Bernard et al., 2011). Furthermore, earthworm activity is also an important factor that controls vegetation dynamics and has a positive influence on plant growth (Doan et al., 2013). However, there is still a lack of knowledge about the effects of in situ earthworm breeding in orchards on the growth, quality, and yield of fruits.

Thus, the aim of our study was to evaluate the effects of chemical fertilizer, compost, and in situ earthworm breeding in orchards on the growth, fruit quality, and yield of papayas and to explore a valid application of organic fertilizer that can not only be used as a substitute for chemical fertilization but also improve papaya yield and quality.

2 Materials and methods

2.1. Site description

This study was conducted at Yinghuwan reclamation land, Xinhui district, Jiangmen city (23° N, 113°E), which is located in the southwestern Pearl River Delta in Guangdong Province, China. The area is characterized by a typical subtropical monsoon climate. The mean annual air temperature is 21.8 °C, with the lowest and highest monthly mean temperatures in January and July, respectively. The average annual precipitation is 1,763 mm, of which approximately 80%

falls during the wet season between May and September. The annual effective accumulated temperature is 7,693 °C. The annual solar radiation is 110 kcal cm^{-2}. The background values for the soil pH, soil organic matter, total nitrogen (N), total phosphorus (P), total potassium, available N, available P, and available potassium are 6.72 g kg^{-1}, 24.26 g kg^{-1}, 1.21 g kg^{-1}, 0.72 g kg^{-1}, 22.29 g kg^{-1}, 80.92 mg kg^{-1}, 62.80 mg kg^{-1}, and 286.42 mg kg^{-1}, respectively.

2.2. Experimental design and treatments

This experiment was conducted in a Hawaiian papaya orchard from March to December of 2008. Hawaiian papaya plants were transplanted on March 31, with a planting space of 3.1 m × 2.7 m. Four treatments were used in our study. These treatments consisted of a control (CK), chemical fertilizer (FL), compost (CM), and in situ earthworm breeding (EB). All treatments were repeated three times during the experiment. The CK was added at 5 g $plant^{-1}$ urea (CH_4N_2O, %N = 46.3%) to each plot in April 28, and then there was no chemical or organic fertilizer applied afterwards. The CM was prepared by using cow manure. The EB field pattern can be found in Fig. 1. An earthworm bed (length: 16 m, above width: 40 cm, below width: 60 cm, and height: 30 cm) was prepared approximately 50 cm from the papaya plant in each plot. We added sufficient organic waste to the bottom of the bed, and then we put earthworms (*Eisenia fetida*) into it at a density of 8 g earthworms per cm^2. Next, we put rice straw and sun shading net on the bed, and water and organic wastes were added regularly so that we could provide a better environment for the earthworms' growth and reproduction. The chemical properties of CM and EB are listed in Table 1.

On April 28, 5 g $plant^{-1}$ urea (CH_4N_2O, %N = 46.3%) was added to each plot. On May 24, 100 g plant^{-1} compound fertilizer (%N-%P-%K = 15%-15%-15%) was applied to the FL treatment, and 1 kg plant^{-1} cow manure was applied to the CM treatment. On June 12, the application of FL and CM was the same as that on May 24. On July 4, 40 g plant^{-1} urea and 100 g plant^{-1} phosphate (%P_2O_5, P = 12%) were applied to the FL treatment, and 2 kg plant^{-1} cow manure was applied to the CM treatment. On August 25, 100 g plant^{-1} compound fertilizer was applied to the FL, and 3 kg plant^{-1} cow manure was applied to the CM. Simultaneously, 100 g plant^{-1} microelement fertilizer was applied to each treatment. On September 12, 200 g plant^{-1} compound fertilizer was distributed over the FL, and 3 kg plant^{-1} cow manure was distributed over the CM. The total amounts of N, P, K, organic matter and microelements that were included in the fertilizer application for each treatment were analyzed (Table 2).

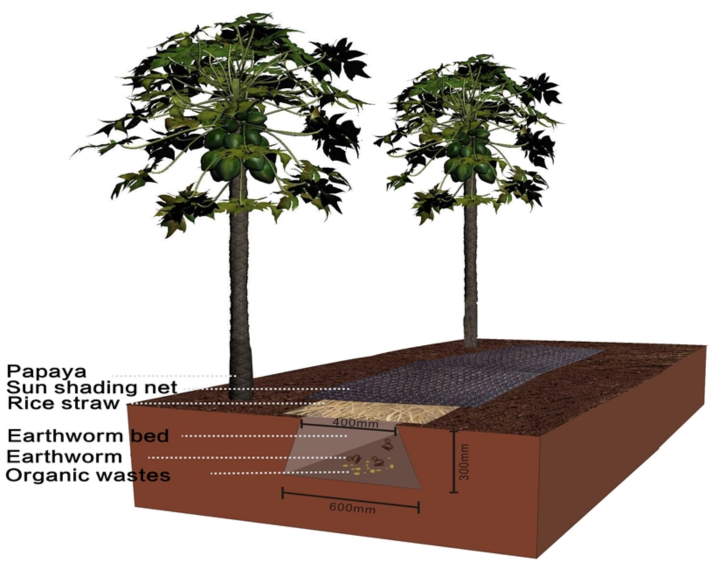

Figure 1: The EB field pattern in a papaya orchard. EB: in situ earthworm breeding.

Table 1 Chemical properties of compost and in situ earthworm breeding. CM-compost, EB-in situ earthworm breeding.

Treatment	pH	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	Organic matter (g/kg)

CM	6.12	9.58	4.23	4.03	193.22
EB	5.98	11.64	6.64	7.60	179.70

Table 2 The total amounts of N, P, K, organic matter and microelements in the fertilizer application for each treatment. CK-control, FL-chemical fertilizer, CM-compost, and EB-in situ earthworm breeding.

Treatment	Total N (g)	Total P (g)	Total K (g)	Organic matter (kg)	Microelements (g)
CK	2.32	0.00	0.00	0.00	50.00
FL	110.88	102.00	90.00	0.00	50.00
CM	98.12	42.30	40.30	1.93	50.00
EB	2.32	0.00	0.00	0.00	50.00

2.3. Measurements

Measurements of the plant height (cm) and stem diameter (cm) were recorded for seven plants from each replication at 38, 55, 76, 94, and 127 days after transplanting the papayas. Quality parameters such as the total acid and soluble-solid content were determined in accordance with the AOAC (1989). The vitamin C content was assessed as described by Bessey and King (1933). Fifty grams of papaya flesh was well homogenized with 50 mL of 2% (w/v) oxalic acid by using a kitchen blender, 20 mL of homogenate was diluted to 50 mL with 2% oxalic acid, and 10 mL of the solution was titrated with 2,6-dichlorophenolindophenol solution until it appeared pink in color.

The total sugar content was measured as follows: 1 g of fleshy tissue was ground in 5 mL of ethanol, and the mixture was then centrifuged at 12,000 × *g* for 10 min at 4 °C. After that, 0.1 ml of ethanol extract was mixed with 1 ml of 2 g/L anthrone in 706 g/L H_2SO_4. The mixture was incubated at 100 °C for 15 minutes and cooled in a water bath, and the total sugar content was determined at 625 nm. The protein content was measured according to a method described by Bradford (1976) with bovine serum protein as the standard, and the results were expressed in mg

g^{-1}. Electrical conductivity was measured by using an Orion Star Plus pH meter (Thermo Fisher Scientific Inc., Singapore). Firmness of 25 fruit samples from each replicate was determined with a texture analyzer (KM-1, Stable Micro Systems, Japanese) with a 2 mm diameter stainless steel probe. The fruits were tested equatorially at their maximum diameter with a cross-head speed of 50 cm min^{-1}. The force was expressed in Newtons (N). The fruit numbers were counted at harvest. The individual fruit weights and total yields were measured from the fresh weights of the fruits.

2.4. Statistical Analysis

Experimental data were evaluated by analysis of variance (ANOVA), and significant differences between the means of three replicates ($p \leq 0.05$) were determined by Duncan's multiple range tests with SPSS 13.0 for Windows. All figures were created in Origin version 8.

3. Results

3.1. Plant growth

Different fertilizer applications significantly increased the plant height and stem diameter growth parameters, and these positive effects were strengthened over time (Fig. 2a). For the plant height, a significant difference was found only between the EB and the CK treatments on June 10 (55 days after planting), and on July 3 (76 days after planting), the plant heights of the FL and EB treatments were significant higher than that of the CK treatment. The differences between the fertilizer applications and the CK treatment reached their peak on August 24 (127 days after planting). The plant heights of the EB, FL, and CM treatments were 195.70, 188.70, and 172.60 cm, respectively, which were 24.70%, 20.23%, and 9.98% higher than the CK treatments, respectively. The stem diameters showed similar increasing trends under different fertilizer applications (Fig. 2b). On Aug 24 (127 days after planting), the five treatments were also ordered EB > FL > CM > CK, and there was a significant difference between all treatments.

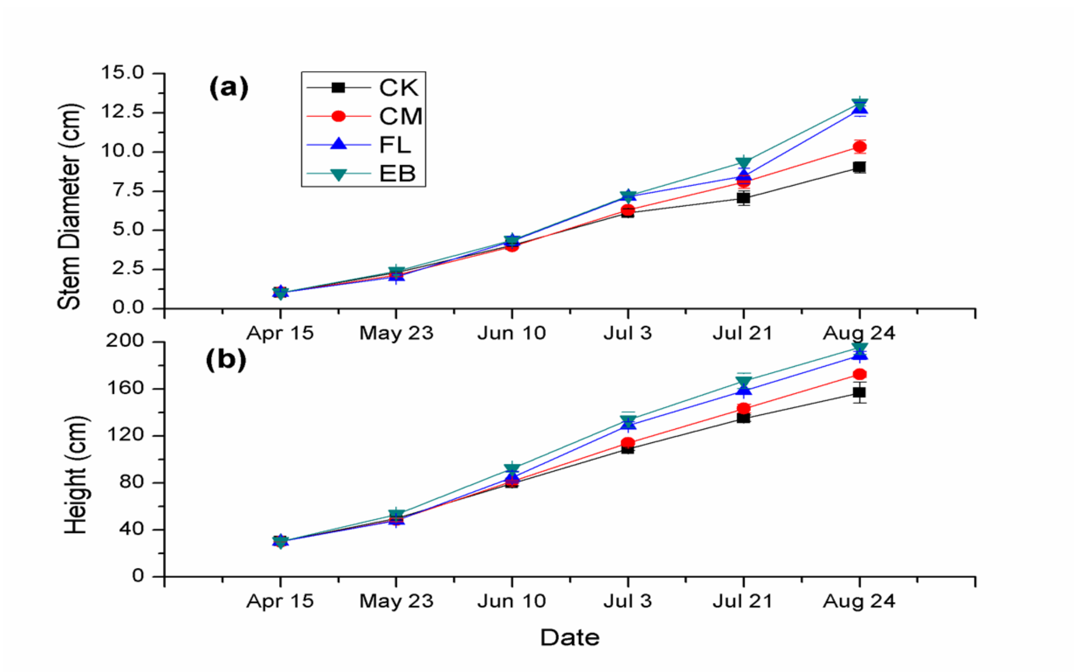

Figure 2: Plant heights and stem diameters under different fertilizer applications (mean ± standard error). CK: control; CM: compost, FL: chemical fertilizer, and EB: in situ earthworm breeding.

3.2. Fruit quality

Different fertilizer applications had distinct effects on fruit quality parameters such as the soluble-solid, sugar, vitamin C, and protein content. The soluble-solid content in each of the four treatments was ordered EB > FL > CM > CK, and this parameter was markedly improved in the EB treatment (Fig. 3a). The soluble-solid content in the EB treatment were 12.96%, 18.22%, and 28.22% higher than the content of the FL, CM, and CK treatments, respectively. The sugar content in the EB (8.18%) treatment was also clearly increased, at 5.68%, 31.09% and 19.21% higher than the sugar in the FL, CM, and CK treatments, respectively (Fig. 3b). The vitamin C content in the EB treatment was 132.95 mg kg^{-1}, which was slightly higher than that of the CK treatment (Fig. 3c). The protein content in the EB (2.45 g kg^{-1}) was also increased; it was 11.36% and 21.89% higher than the content of the FL and CK treatments (significantly different with $p < 0.05$) (Fig. 3d).

The total acid, electrical conductivity and firmness were also affected by the different treatments. The total acid was dramatically decreased in the EB treatment (Fig. 4a) and was reduced by 44.28%, 46.86% and 65.31% compared with that of the CK, CM and FL treatments, respectively. The electrical conductivity in the CM and EB treatments was obviously lower than that of the CK; they dropped by 31.88% and 28.26% relative to the CK (Fig. 4b). The fruit firmness was significantly enhanced in the CM, but it decreased in the FL treatment (Fig. 4c).

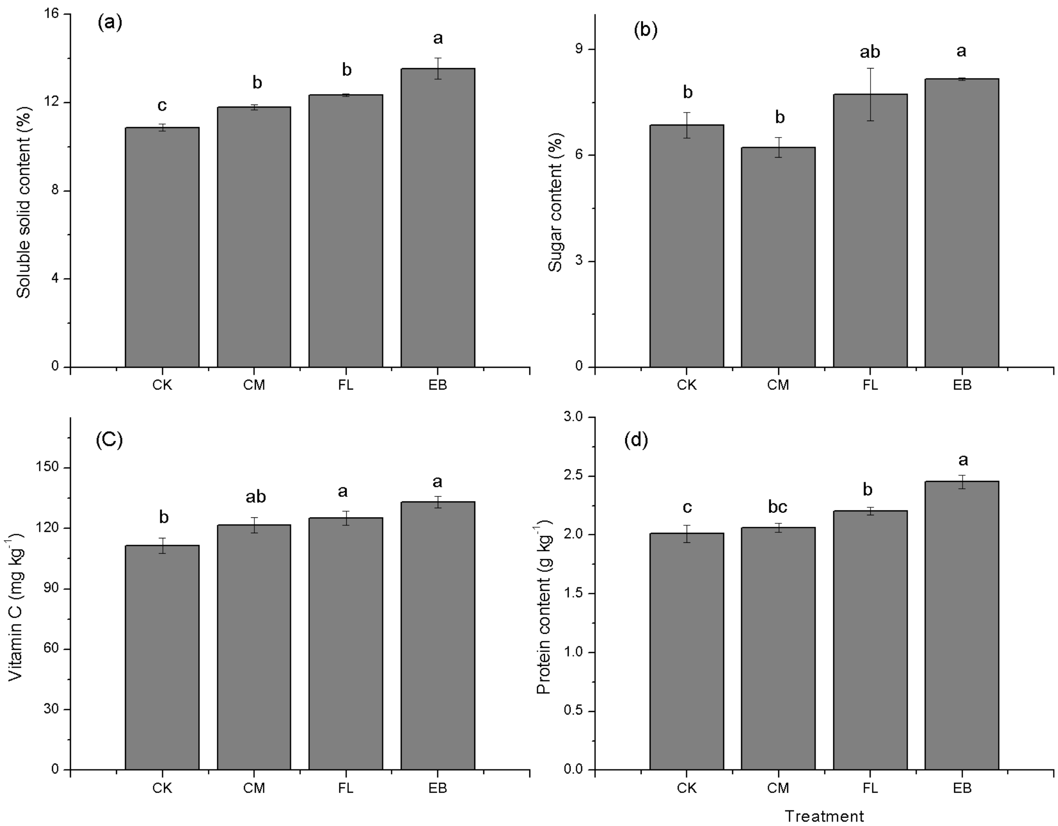

Figure 3: Soluble-solid, sugar, vitamin C and protein content of papaya fruit under different fertilizer applications (mean ± standard error). Different letters indicate significant differences between treatments at $p \leq 0.05$. CK: control; CM: compost, FL: chemical fertilizer, and EB: in situ earthworm breeding.

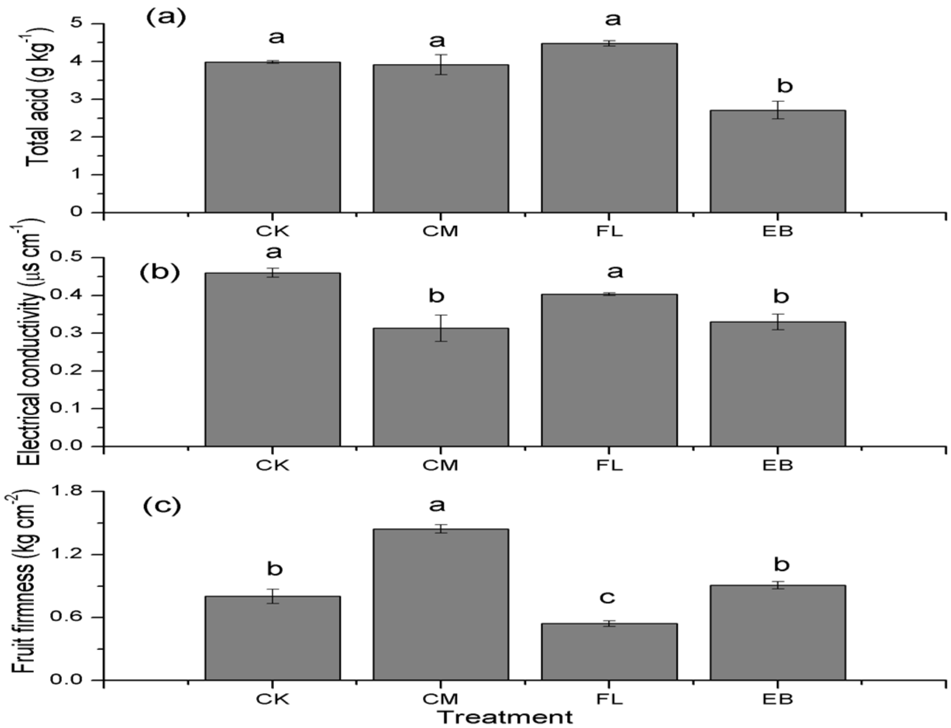

Figure 4: Electrical conductivity and firmness of papaya fruit under different fertilizer applications (mean ± standard error). Different letters indicate significant differences between treatments at $p \leq 0.05$. CK: control; CM: compost, FL: chemical fertilizer, and EB: in situ earthworm breeding.

3.3. Fruit yield

The EB treatment significantly enhanced the fresh weight per fruit, the fruit number, and the total yield (Table 3). The individual fruit weights for the four treatments were 373.48 (FL), 359.17 (EB), 299.47 (CM), and 241.92 g (CK). Compared with the CK treatment, the FL and EB treatments were increased by 54.38% and 48.47%, respectively. In addition, the fruit numbers and total yields were also significantly increased in the FL and EB treatments, and the EB was higher than the FL. The quantities of fruits in the FL and EB treatments were 39.86% and 47.59% higher than that of the CK treatment. The total yields of the FL and EB treatments were 116.60% and 120.62% higher than that of the CK treatment, and the EB yield was improved by 1.85%

relative to the FL treatment.

Table 3 Effects of different fertilizer applications on the papaya fruit number, weight, and total fruit yield of Hawaiian papaya.

Treatments	Individual papaya weights (g)	No. of fruits/plant	Total fruit yield (kg hm^{-2})
CK	241.92c	18.67b	4835.51c
CM	299.47b	21.44b	6927.87b
FL	373.48a	26.11a	10477.19a
EB	359.17a	27.56a	10671.04a

The means within the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$. CK: control; CM: compost, FL: chemical fertilizer, and EB: in situ earthworm breeding.

4. Discussion

4.1 Effects of different fertilizer applications on plant growth

In this study, the EB treatment significantly promoted papaya plant growth, and this treatment exhibited the highest plant heights and stem diameters of the four treatments over the whole growth period. Our result is consistent with other studies on earthworms in aboveground plant communities (Piearce et al., 1994; Wurst et al., 2005). The following mechanisms may be related to the results. First, the earthworm activities in the EB treatment can improve the soil structure (such as the porosity) and increase the soil nutrients, and thus they provide a better root growth medium. Derouard et al. (1997) found that earthworms significantly affect soil aggregation and water infiltration. Lee (1985) also noted that earthworms alter the physical, chemical, and biological properties of soil, which can in turn modify the plant growth. Second, the plant-growth hormones included in the fresh earthworm casts stimulated papaya growth. Numerous studies showed that earthworm casts contain plant-growth-regulating materials such as humic acids (Senesi et al., 1992; Masciandaro et al., 1997; Atiyeh et al., 2002) and plant-growth regulators such as auxins, gibberellins, and cytokinins (Krishnamoorthy and Vajrabhiah, 1986; Grappelli et al., 1987; Tomati et al., 1990), which contribute to increases in plant growth for many crops (Atiyeh et al., 2002). Thus, our study suggested that in situ earthworm breeding in orchards can result in better plant growth.

4.2 Effects of different fertilizer applications on the fruit quality

Soluble-solid, sugar, vitamin C, and protein content are very important parameters of fruit nutrition. Increases in the content of these indices can indicate the enhancement of fruit quality. During this one-year field experiment, the EB treatment significantly improved the fruit quality because it increased the soluble-solid, sugar, vitamin C, and protein content. The primary reason may be the presence of earthworm casts, which are also known as vermicompost, in the EB treatment. Vermicompost could improve the fruit quality, and our results are consistent with previous studies. For example, Premuzic et al. (1998) reported that the fruits of tomatoes grown on organic vermicompost substrates contained significantly higher vitamin C than those grown in hydroponic media. Gutiérrez-Miceli et al. (2007) suggested that the addition of sheep manure vermicompost decreased the titratable acidity and increased the soluble and insoluble solids in tomato fruits, compared with those harvested from plants cultivated in unamended soil. The beneficial effects of vermicompost utilization for improving the fruit quality in other horticulture settings have also been reported (Tomati et al., 1987; Hidalgo, 1999; Saciragic and Dzelilovic, 1986).

Moreover, the total acid, electrical conductivity, and fruit firmness were another three important indicators of fruit quality. The decreasing total acid content denoted an improvement in fruit flavor, the lower electrical conductivity indicates a longer period of fruit storage, and the higher fruit firmness represents easier storage. In this study, the total acid and electrical conductivity decreased, but the fruit firmness was increased in the EB treatment. Therefore, the EB treatment that was incorporated into soil could effectively improve the fruit quality.

4.3 Effects of different fertilizer applications on the fruit yield

Our results suggested that the FL and EB treatments significantly improved the papaya yield. Several field studies have also found significant increases in fruit yields under earthworm inoculation and vermicompost application (Goswami et al., 2001; Gutiérrez-Miceli et al., 2007; Fragoso et al. 1997). Goswami et al. (2001) observed that vermicompost addition rates of 0, 20, 30, and 40 t ha^{-1} produced tomato yields of 114, 138, 163, and 192 t ha^{-1} in comparison with the inorganically fertilized tomatoes that received 56 t ha^{-1}. Pashanasi et al. (1996) found that plant production was significantly increased by 36% following earthworm inoculation into a traditional low-input rotation. The role of earthworms in enhancing plant production depends on the synlocalization and the synchronization of their activities with the period and sphere of active root growth and nutrient demand. Most earthworm species release significant amounts of assimilable nutrients that can be supplied to the plants that grow in their casts (Syers et al., 1979; Lavelle et al., 1992). Earthworm activities promote the intense mineralization of soil, releasing considerable quantities of mineral N, P, and K. This process may stimulate the plant growth and improve the fruit crop yield correspondingly. As the organic material is processed by the digestive systems of earthworms, vermicomposting differs from conventional composting. The higher N, C, P, K, Ca, and Mg availability in vermicompost implies that it has a function as a slow release source of plant nutrients (Chaoui et al. 2003).

However, can the EB treatment perform better than the FL treatment in improving the total yield? In a previous study, 7.5 t ha^{-1} vermicompost was added to a treatment that increased the marketable fruit yield up to 58.6% relative to that of the inorganic fertilizer treatment (Singh et al., 2008). By contrast, the earthworms in the EB treatment in our study were added only once, and the density of earthworms was only 8 g cm^{-2}. Therefore, we extrapolated that if the density of earthworms was increased and eventually reached an appropriate standard, the effect of the EB treatment in terms of improving the fruit yield would be much higher than that of the FL treatment. However, this issue must be studied further.

5. Conclusion

This study evaluated the effects of CK, CM, FL, and EB treatments on the plant growth, fruit quality, and yield of papayas. Of the four treatments, the EB treatment provided the best media for plant growth. The present study revealed that the EB treatment was quite useful in field-grown papaya for conferring higher fruit quality and total yield. Generally, the incorporation of the EB treatment with soil could significantly improve the growth, fruit quality, and yield of papaya in comparison with the CK, CM and FL treatments. The EB treatment could be used as an effective substitute for chemical fertilizer. Furthermore, the EB treatment could be a potential organic fertilizer application because it not only improved the fruit's quality and yield but also reduced the amount of organic waste in agriculture as a result of the earthworm activities.

Acknowledgements

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