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

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Abstract

The aim of this study was to compare the effects of four fertilizer applications—control (C), chemical fertilizer (F), compost (O), and in situ earthworm breeding (E)—on the growth, quality and yield of papaya (*Carica papaya L.*). In this study, 5 g plant^{-1} urea (CH_4N_2O, %N = 46.3%) and 100 g plant^{-1} microelement fertilizer was applied to each treatment. Then the fertilizer applications of these four treatments are different from each other. The results showed that the E treatment had the highest growth parameters over the whole growth period. At 127 days after transplantation, the plant heights were ordered E > F > O > C, and the stem diameters were E > F > O > C, with significant differences between all treatments. Soluble-solid, sugar, vitamin C, and protein content significantly increased in the E treatment. In addition, the total acid and the electrical conductivity of the fruit significantly decreased in the E treatment. Fruit firmness clearly increased in the O treatment, and it decreased in the F treatment. The fresh individual fruit weights, fruit numbers, and total yields were greatly improved in the F and E treatments, and the total yield of the E treatment was higher than that in the F 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

30 **1. Introduction**

31   Papaya (*Carica papaya L.*) is one of the most important fruit crops which is widely cultivated in tropical and

32 subtropical areas. It is rich in nutrition, sugar, vitamin C, protein, and amino acids, and it is the primary raw

33 material that contains papain. Papaya is also widely planted in southern China, especially in Guangdong,

34 Yunnan, and Hainan Provinces. However, papaya production is frequently low and unreliable. Although

35 chemical fertilizer application is a common method for improving papaya yields, it is unfriendly to the

36 environment . It can impair soil structure, and decrease soil fertility by reducing the carbon and nitrogen

37 content (Ngo et al., 2012). Moreover, chemical fertilizer application can also affect animal and human health

38 (Vu et al., 2007), such as some fish in the rice paddy were killed, and also it is bad for human health through

39 the food chain due to the content of heavy metals in chemical fertilizer. For these reasons, the importance of

40 organic fertilization has been increasing in recent years, and suitable organic amendments including

41 composting, vermicomposting and in situ earthworm breeding have become promising biological ways to

42 improve the growth, fruit quality and yield of papayas.

43   The addition of compost to soil has been described as an ideal alternative method for improving soil

44 fertility and plant nutrition (Cantanazaro et al., 1998; Caravaca et al., 2002), and this method is especially

45 appropriate for sustainable agriculture. While among the soil organisms favored by organic fertilization,

46 earthworms have been identified as a key functional group (Jouquet et al., 2006). Earthworms have a great

47 ability to consume organic wastes, reducing the volume by approximately 50% and expelling the digested

48 materials as castings, which are useful for soil amendments and may be easily stored for agricultural use

49 (Tomati et al., 1985). In situ earthworm breeding in orchards usually has three important advantages. First, this

50 method can be used to manage a large amount of organic wastes from agriculture. At present, earthworms have

51   attracted a great deal of attention as an efficient and low-cost means of composting organic wastes such as

52   animal wastes and crop residues (Ndegwa and Thompson, 2001; Singh et al., 2008). They not only reduce

53   organic waste pollution but also improve the environment of rural areas. Second, this method produces a large

54   amount of high-quality compost, known as "vermicompost," which comes from the biological degradation of

55   organic wastes by earthworms (Chaoui et al., 2003). Third, earthworm activities improve the soil structure,

56   microbial activity and biodiversity, and soil OM dynamics (Jongmans et al., 2001; Pulleman et al., 2005;

57   Jouquet et al., 2007; Bottinelli et al.,   2010; Bernard et al., 2011). Furthermore, earthworm activity is also an

58   important factor that controls vegetation dynamics and has a positive influence on plant growth (Doan et al.,

59   2013). However, there is still a lack of knowledge about the effects of in situ earthworm breeding in orchards

60   on the growth, quality, and yield of fruits.

61   Thus, the aim of our study was to evaluate the effects of chemical fertilizer, compost, and in situ earthworm

62   breeding in orchards on the growth, fruit quality, and yield of papayas and to explore a potential application of

63   organic fertilizer that can not only be used as a substitute for chemical fertilization but also improve papaya

64   yield and quality.

65   **2 Materials and methods**

66   2.1. Site description

67   This study was conducted at Yinghuwan reclamation land, Xinhui district, Jiangmen city (23°   N, 113°E),

68   which is located in the southwestern Pearl River Delta in Guangdong Province, China. The area is

69   characterized by a typical subtropical monsoon climate. The average annual precipitation is 1,763 mm, of

70   which approximately 80% falls during the wet season between May and September. The annual effective

71   accumulated temperature is 7,693 °C. The average mean temperature is 23.8 °C, with the lowest and highest

72   monthly mean temperatures in January and July, respectively. The annual solar radiation is 110 kcal cm^{-2}. The

73   background values for the soil pH, soil organic matter, total nitrogen (N), total phosphorus (P), total potassium,

74   available N, available P, and available potassium are 6.72, 24.26 g kg^{-1}, 1.21 g kg^{-1}, 0.72 g kg^{-1}, 22.29 g kg^{-1},

75   80.92 mg kg^{-1}, 62.80 mg kg^{-1}, and 286.42 mg kg^{-1}, respectively.

76   2.2. Experimental design and treatments

77   This experiment was conducted in a Hawaiian papaya orchard from March to December of 2008. Hawaiian

78   papaya plants were transplanted on March 31, with a planting space of 3.1 m × 2.7 m. Four treatments were

79   used in our study. These treatments consisted of a control (C), chemical fertilizer (F), compost (O), and in situ

80   earthworm breeding (E). All treatments were repeated three times during the experiment. 5 g plant^{-1} urea

81   (CH$_4$N$_2$O, %N = 46.3%) and 100 g plant^{-1} microelement fertilizer was applied to each treatment. Since then,

82   no chemical or fertilizer were applied to C. But 45 g plant^{-1} urea (CH$_4$N$_2$O, %N = 46.3%), 100 g plant^{-1}

83   phosphate (%P$_2$O$_5$, P = 12%), 500 g plant^{-1} compound fertilizer was applied in the F treatment. The O was

84   prepared by using cow manure. 10 kg plant^{-1} cow manure was applied to the O treatment. The E field pattern

85   can be found in Fig. 1. An earthworm bed (length: 16 m, above width: 40 cm, below width: 60 cm, and height:

86   30 cm) was prepared approximately 50 cm from the papaya plant in each plot. We added 4.858 kg m^{-3}

87   organic wastes which produced in the process of producing beer to the bottom of the bed, and then we

88   put earthworms (*Eisenia fetida*) into it at a density of 8 g per m^2. Next, we put rice straw and sun shading net

89   on the bed, and water and organic wastes were added regularly so that we could provide a better environment

90   for the earthworms' growth and reproduction. The providing nutrients of O and E were listed in Table 1.

91   On April 28, 5 g plant^{-1} urea (CH$_4$N$_2$O, %N = 46.3%) was added to each plot. On May 24, 100 g plant^{-1}

92   compound fertilizer (%N-%P-%K = 15%-15%-15%) was applied to the F treatment, and 1 kg plant^{-1} cow

93   manure was applied to the O treatment. The chemical fertilizer and compost was applied at the base of the

94   papaya plants. On June 12, the application of F and O was the same as that on May 24. On July 4, 40 g plant^{-1}

95   urea and 100 g plant^{-1} phosphate (%P_2O_5, P = 12%) were applied to the F treatment, and 2 kg plant^{-1} cow

96   manure was applied to the O treatment. On August 25, 100 g plant^{-1} compound fertilizer was applied to the F,

97   and 3 kg plant^{-1} cow manure was applied to the O. Simultaneously, 100 g plant^{-1} microelement fertilizer was

98   applied to each treatment. On September 12, 200 g plant^{-1} compound fertilizer was distributed over the F, and

99   3 kg plant^{-1} cow manure was distributed over the O. The total amounts of N, P, K, organic matter and

100 microelements that were included in the fertilizer application for each treatment were analyzed (Table 2).

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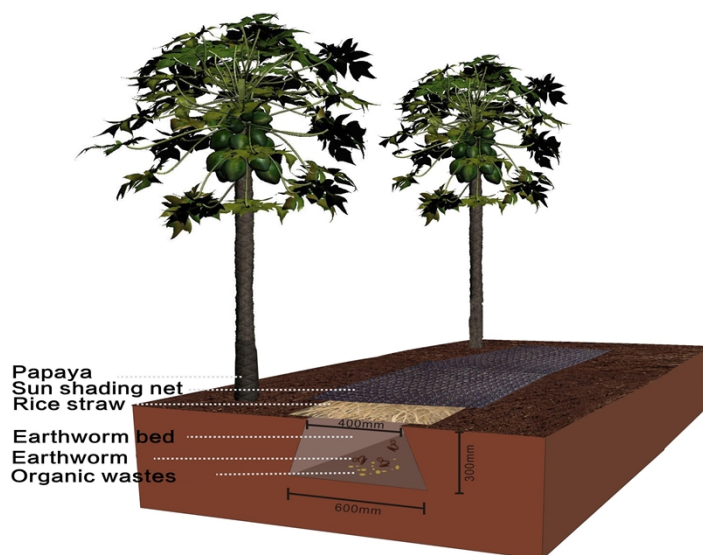
103 **Figure 1:** The E field pattern in a papaya orchard. E: in situ earthworm breeding.

104

105 **Table 1** The providing nutrient of compost and in situ earthworm breeding. O-compost, E-in situ earthworm
106 breeding.

Treatment	pH	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	Organic matter (g/kg)
O	6.12	9.58	4.23	4.03	193.22
E	5.98	11.64	6.64	7.60	179.70

107

108 **Table 2** The total amounts of N, P, K, organic matter and microelements in the fertilizer application by human for
109 each treatment. C-control, F-chemical fertilizer, O-compost, and E-in situ earthworm breeding.

Treatment	Total N (g)	Total P (g)	Total K (g)	Organic matter (kg)	Microelements (g)
C	2.32	0.00	0.00	0.00	50.00
F	110.88	102.00	90.00	0.00	50.00
O	98.12	42.30	40.30	1.93	50.00
E	2.32	0.00	0.00	0.00	50.00

110

111 ## 2.3. Measurements

112   Measurements of the plant height (cm) and stem diameter (cm) were recorded for seven plants from each

113 replication at 38, 55, 76, 94, and 127 days after transplanting the papayas plants. The ripen fruits of five plants

114 from each replication that growing trend consistent were chosen to observation the fruit quality. Quality

115 parameters such as the total acid and soluble-solid content were determined in accordance with the AOAC

116 (1989). The vitamin C content was assessed as described by Bessey and King (1933). Fifty grams of papaya

117 flesh was well homogenized with 50 mL of 2% (w/v) oxalic acid by using a kitchen blender, 20 mL of

118 homogenate was diluted to 50 mL with 2% oxalic acid, and 10 mL of the solution was titrated with 2,6-

119 dichlorophenolindophenol solution until it appeared pink in color.

120   The total sugar content was measured as follows: 1 g of fleshy tissue was ground in 5 mL of ethanol, and the

121 mixture was then centrifuged at $12,000 \times g$ for 10 min at 4 °C. After that, 0.1 ml of ethanol extract was mixed

122 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

123 in a water bath, and the total sugar content was determined at 625 nm. The protein content was measured

124 according to a method described by Bradford (1976) with bovine serum protein as the standard, and the results

125 were expressed in mg g^{-1}. Electrical conductivity was closely related with fruit storing time, and it was

126 measured by using an Orion Star Plus pH meter (Thermo Fisher Scientific Inc., Singapore). Firmness of 25

127 fruit samples from each replicate was determined with a texture analyzer (KM-1, Stable Micro Systems,

128   Japanese) with a 2 mm diameter stainless steel probe. The fruits were tested equatorially at their maximum

129   diameter with a cross-head speed of 50 cm min^{-1}. The force was expressed in Newtons (N).   The fruit

130   numbers were counted at harvest. The individual fruit weights and total yields were measured from the fresh

131   weights of the fruits.

132   2.4. Statistical Analysis

133   Experimental data were evaluated by analysis of variance (ANOVA), and significant differences between

134   the means of three replicates ($p \leq 0.05$) were determined by Duncan's multiple range tests with SPSS 13.0 for

135   Windows. All figures were created in Origin version 8.

136   **3. Results**

137   3.1. Plant growth

138   Different fertilizer applications significantly increased the plant height and stem diameter growth

139   parameters, and these positive effects were strengthened over time (Fig. 2). For the plant height, a significant

140   difference was found only between the E and the C treatments on June   10 (55 days after planting), and on

141   July 3 (76 days after planting), the plant heights of the F and E treatments were significant higher than that of

142   the C treatment from July 3 ($p \leq 0.05$) (Fig. 2b). On August, the plant heights of the E, F, and O treatments

143   were 195.70, 188.70, and 172.60 cm, respectively, which were 24.70%, 20.23%, and 9.98% higher than the C

144   treatments, respectively (Fig. 2b). The stem diameters showed similar increasing trends under different

145   fertilizer applications (Fig. 2a). On Aug 24 (127 days after planting), the five treatments were also ordered E >

146   F > O > C, and there was a significant difference between all treatments ($p \leq 0.05$).

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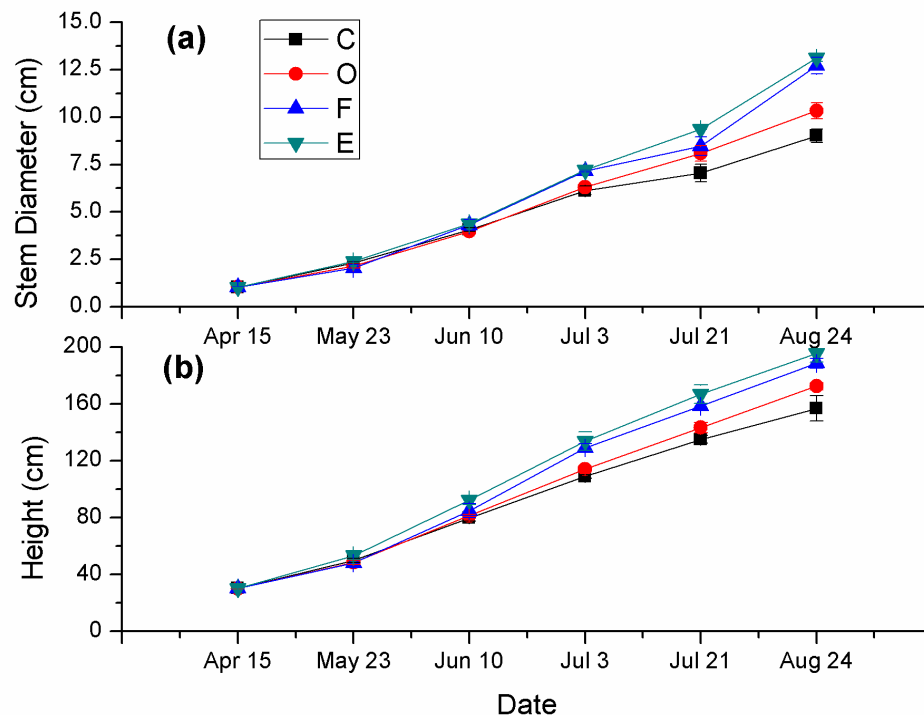
Figure 2: Plant heights and stem diameters under different fertilizer applications (mean ± standard error, n=3). C: control; O: compost, F: chemical fertilizer, and E: in situ earthworm breeding.

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3.2. Fruit quality

There were significant differences of different fertilizer applications on fruit quality parameters such as the soluble-solid, sugar, vitamin C, and protein content ($p \leq 0.05$). The soluble-solid content in each of the four treatments was ordered E > F > O > C, and this parameter was markedly improved in the E treatment ($p \leq 0.05$) (Fig. 3a). The soluble-solid content in the E treatment was 12.96%, 18.22%, and 28.22% higher than the content of the F, O, and C treatments, respectively ($p \leq 0.05$). The sugar content in the E (8.18%) treatment was also clearly increased, at 5.68%, 31.09% and 19.21% higher than the sugar in the F, O, and C treatments, respectively (Fig. 3b). The vitamin C content in the E treatment was 132.95 mg kg^{-1}, which was slightly higher than that of the C treatment ($p \leq 0.05$) (Fig. 3c). The protein content in the E (2.45 g kg^{-1}) was also increased; it

161 was 11.36% and 21.89% higher than the content of the F and C treatments (significantly different with $p \le$

162 0.05) (Fig. 3d).

163 The total acid, electrical conductivity and firmness were also affected by the different treatments. The total

164 acid was dramatically decreased in the E treatment ($p \le 0.05$) (Fig. 4a) and was reduced by 44.28%, 46.86%

165 and 65.31% compared with that of the C, O and F treatments, respectively. The electrical conductivity in the O

166 and E treatments was obviously lower than that of the C ($p \le 0.05$); they dropped by 31.88% and 28.26%

167 relative to the C (Fig. 4b). The fruit firmness was significantly enhanced in the O, but it decreased in the F

168 treatment ($p \le 0.05$) (Fig. 4c).

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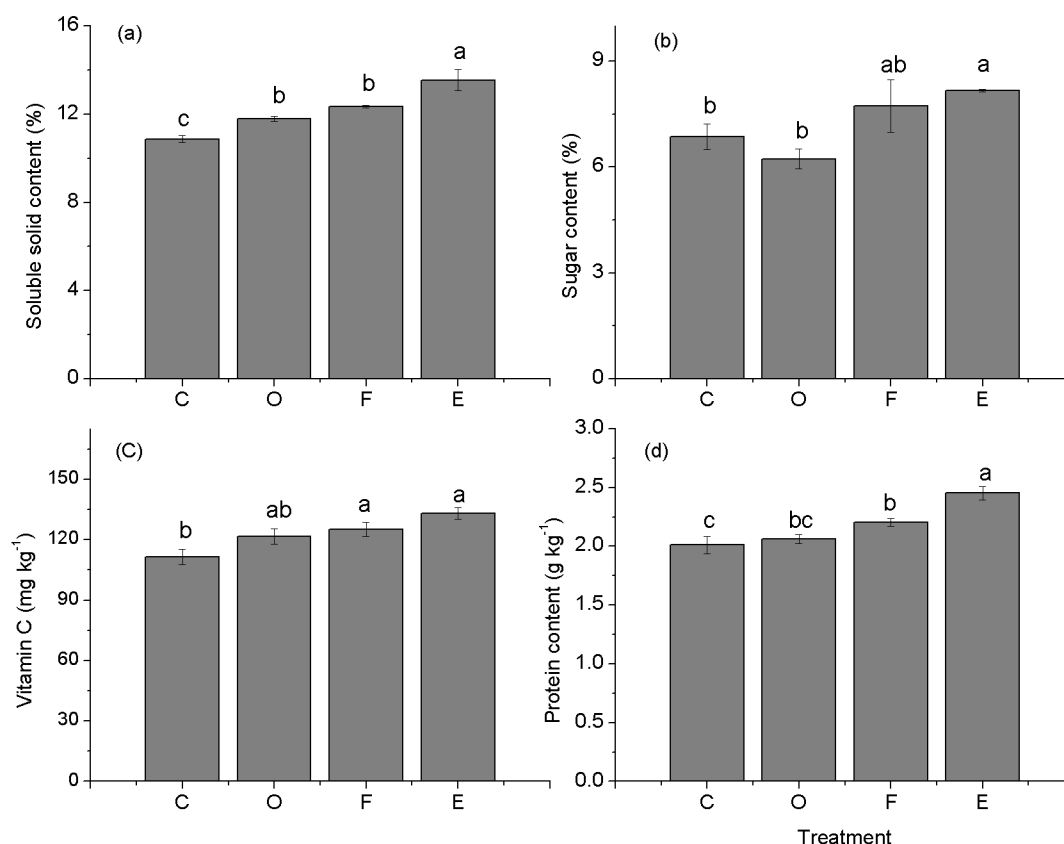
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171 **Figure 3:** Soluble-solid, sugar, vitamin C and protein content of papaya fruit under different fertilizer applications (mean ±

172   standard error, n=3). Different letters indicate significant differences between treatments at $p \leq 0.05$. C: control; O: compost, F:

173   chemical fertilizer, and E: in situ earthworm breeding.

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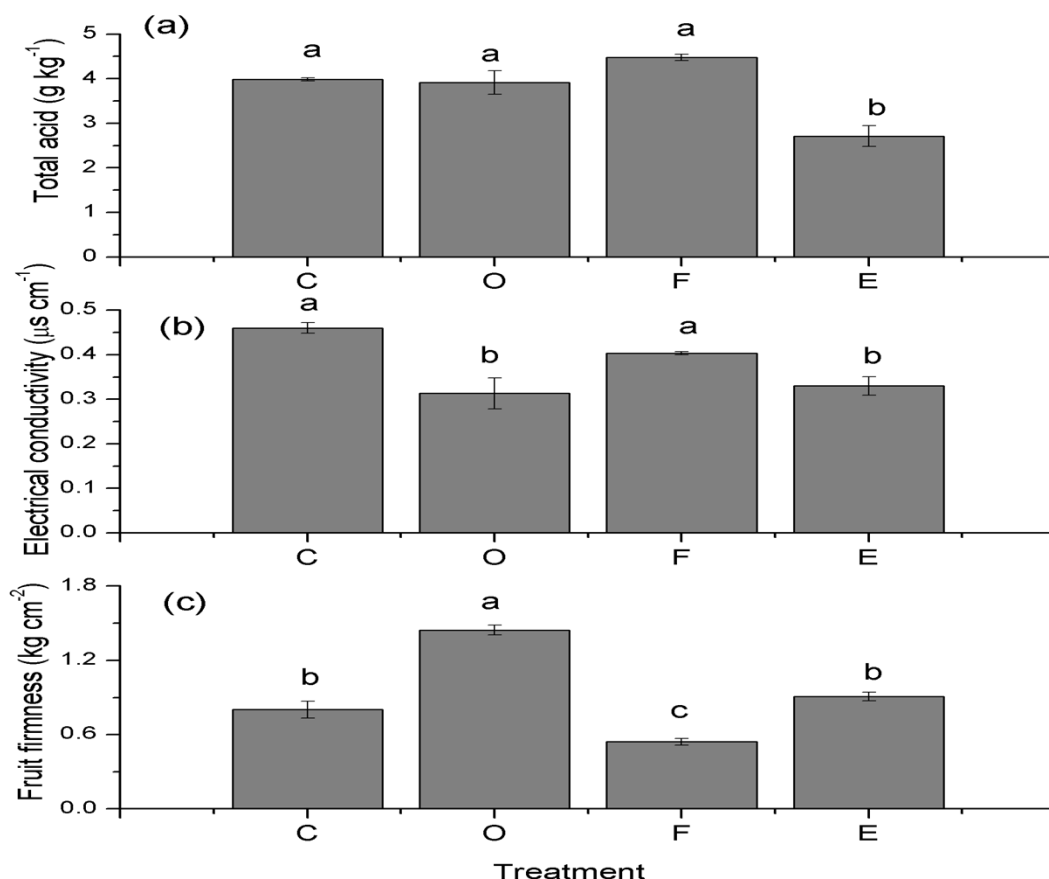
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176   **Figure 4:** Total acid, electrical conductivity and firmness of papaya fruit under different fertilizer applications (mean ± standard

177   error, n=3). Different letters indicate significant differences between treatments at $p \leq 0.05$. C: control; O: compost, F: chemical

178   fertilizer, and E: in situ earthworm breeding.

179

180   3.3. Fruit yield

181   The E treatment significantly enhanced the fresh weight per fruit, the fruit number, and the total yield (Table

182   3). The individual fruit weights for the four treatments were 373.48 (F), 359.17 (E), 299.47 (O), and 241.92 g

183   (C). Compared with the C treatment, the F and E treatments were increased by 54.38% and 48.47%,

184   respectively. In addition, the fruit numbers and total yields were also significantly increased in the F and E

185   treatments, and the E was higher than the F. The quantities of fruits in the F and E treatments were 39.86% and

186   47.59% higher than that of the C treatment. The total yields of the F and E treatments were 116.60% and

187   120.62% higher than that of the C treatment, and the E yield was improved by 1.85% relative to the F

188   treatment.

189

190   **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})
C	241.92c	18.67b	4835.51c
O	299.47b	21.44b	6927.87b
F	373.48a	26.11a	10477.19a
E	359.17a	27.56a	10671.04a

191   The means within the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$. C: control;

192   O: compost, F: chemical fertilizer, and E: in situ earthworm breeding.

193

194   4. **Discussion**

195   4.1 Effects of different fertilizer applications on plant growth

196   In this study, the E treatment significantly promoted papaya plant growth than the treatment of C and O, and

197   this treatment exhibited the highest plant heights and stem diameters of the four treatments over the whole

198   growth period. Our result is consistent with other studies on earthworms in aboveground plant communities

199   (Piearce et al., 1994; Wurst et al., 2005). The following mechanisms may be related to the results. First, the

200   earthworm activities in the E treatment can improve the soil structure (such as the porosity) and increase the

201   soil nutrients, and thus they provide a better root growth medium. Derouard et al. (1997) found that

202   earthworms significantly affect soil aggregation and water infiltration. Lee (1985) also noted that earthworms

203   alter the physical, chemical, and biological properties of soil, which can in turn modify the plant growth. In

204   this study, we found that the nutrient content such as total N, P, and K in E treatment was higher than these in

205   O treatment (table 1). This suggested that E treatment provided a better soil nutrient for the papaya plant.

206 Second, the plant-growth hormones included in the fresh earthworm casts stimulated papaya growth.

207 Numerous studies showed that earthworm casts contain plant-growth-regulating materials such as humic acids

208 (Senesi et al., 1992; Masciandaro et al., 1997; Atiyeh et al., 2002) and plant-growth regulators such as auxins,

209 gibberellins, and cytokinins (Krishnamoorthy and Vajrabhiah, 1986; Grappelli et al., 1987; Tomati et al., 1990),

210 which contribute to increases in plant growth for   many crops (Atiyeh et al., 2002). Thus, our study suggested

211 that in situ earthworm breeding in orchards can result in better plant growth.

212 4.2 Effects of different fertilizer applications on the fruit quality

213   Soluble-solid, sugar, vitamin C, and protein content are very important parameters of fruit nutrition.

214 Increases in the content of these indices can indicate the enhancement of fruit quality. During this field

215 experiment, the E treatment significantly improved the fruit quality because it increased the soluble-solid,

216 sugar, vitamin C, and protein content. The primary reason may be the presence of earthworm casts, which are

217 also known as vermicompost, in the E treatment. Vermicompost could improve the fruit quality, and our

218 results are consistent with previous studies. For example, Premuzic et al. (1998) reported that the fruits of

219 tomatoes grown on organic vermicompost substrates contained significantly higher vitamin C than those

220 grown in hydroponic media. Gutiérrez-Miceli et al. (2007) suggested that the addition of sheep manure to

221 vermicompost decreased the titratable acidity and increased the soluble and insoluble solids in tomato fruits,

222 compared with those harvested from plants cultivated in unamended soil. The beneficial effects of

223 vermicompost utilization for improving the fruit quality in other horticulture settings have also been reported

224 (Tomati et al., 1987; Hidalgo, 1999; Saciragic and Dzelilovic, 1986).

225   Moreover, the total acid, electrical conductivity, and fruit firmness were another three   important indicators

226 of fruit quality. The decreasing total acid content denoted an improvement   in fruit flavor, the lower electrical

227   conductivity indicates a longer period of fruit storage, and the higher fruit firmness represents easier storage. In

228   this study, the total acid and electrical conductivity decreased, but the fruit firmness was increased in the E

229   treatment. Therefore, the E treatment that was incorporated into soil could effectively improve the fruit quality.

230   4.3 Effects of different fertilizer applications on the fruit yield

231   Our results suggested that the F and E treatments significantly improved the papaya yield. Several field

232   studies have also found significant increases in fruit yields under earthworm inoculation and vermicompost

233   application (Goswami et al., 2001; Gutiérrez-Miceli et al., 2007; Fragoso et al. 1997). Goswami et al. (2001)

234   observed that vermicompost addition rates of 0, 20,   30, and 40 t ha^{-1} produced tomato yields of 114, 138, 163,

235   and 192 t ha^{-1} in comparison with the inorganically fertilized tomatoes that received 56 t ha^{-1}. Pashanasi et al.

236   (1996) found that plant production was significantly increased by 36% following earthworm inoculation into a

237   traditional low-input rotation. The role of earthworms in enhancing plant production depends on the

238   synlocalization and the synchronization of their activities with the period and sphere of active root growth and

239   nutrient demand. Most earthworm species release significant amounts of assimilable nutrients that can be

240   supplied to the plants that grow in their casts (Syers et al., 1979; Lavelle et al., 1992). Earthworm activities

241   promote the intense mineralization of soil, releasing considerable quantities of mineral N, P, and K. This

242   process may stimulate the plant growth and improve the fruit crop yield correspondingly. As the organic

243   material is processed by the   digestive systems of earthworms, vermicomposting differs from conventional

244   composting. The higher N, C, P, K, Ca, and Mg availability in vermicompost implies that it has a function as a

245   slow release source of plant nutrients (Chaoui et al. 2003).

246   However, can the E treatment perform better than the F treatment in improving the total yield? In a previous

247   study, 7.5 t ha^{-1} vermicompost was added to a treatment that increased the marketable fruit yield up to 58.6%

248   relative to that of the inorganic fertilizer treatment (Singh et   al., 2008). By contrast, the earthworms in the E

249   treatment in our study were added only once, and the density of earthworms was only 8 g m^{-2}. Therefore, we

250   extrapolated that if the density   of earthworms was increased in a proper range,, and it reached an appropriate

251   standard, the effect of the E treatment in terms of improving the fruit yield would be much higher than that of

252   the F treatment. Meanwhile, the price of buying the earthworms are low, it decreased the cost of agriculture.

253   For example, in our study, the cost of F and E treatment was 22.19 and 15.29 USD ha^{-1}. The cost of E was

254   lower than this in the F treatment. Though, the preparation of earthworm bed in E treatment was complicated.

255   It can be seen one of the limitations of earthworm breeding. And also we could not determine if the E

256   treatment would be suit for any type of papaya fields. This issue need be to studied further.

257   **5. Conclusion**

258   This study evaluated the effects of C, O, F, and E treatments on the plant growth, fruit quality, and yield of

259   papayas. Of the four treatments, the E treatment provided the bestmedia for plant growth. The present study

260   revealed that the E treatment was quite useful in field-grown papaya for conferring higher fruit quality and

261   total yield. Generally, the incorporation of the E treatment with soil could significantly improve the growth,

262   fruit quality, and yield of papaya in comparison with the C andO treatments. The E treatment could be used as

263   an effective substitute for chemical fertilizer. Furthermore, the E treatment could be a potential organic

264   fertilizer application because it not only improved the fruit's quality and yield but also reduced the amount of

265   organic waste in agriculture as a result of the earthworm activities.

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