forests

MDPI

*Article*

# Changes in the Profiles of Yield, Yield Component, Oil Content, and Citral Content in *Litsea cubeba* (Lour.) Persoon Following Foliar Fertilization with Zinc and Boron

**Ming Gao [1,2], Yicun Chen [1,2], Liwen Wu [1,2] and Yangdong Wang [1,2,*]**

[1] State Key Laboratory of Tree Genetics and Breeding, Chinese Academy of Forestry, Beijing 10091, China; minggao1984@caf.ac.cn (M.G.); yicun_chen@163.com (Y.C.); wuliwenhappy@caf.ac.cn (L.W.)

[2] Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou 311400, China

* Correspondence: wangyangdong@caf.ac.cn; Tel.: +86-571-6332-7982

Received: 25 November 2018; Accepted: 12 January 2019; Published: 13 January 2019

check for updates

**Abstract:** Mountain pepper (*Litsea cubeba* (Lour.) Persoon) is an important oil plant used as an ingredient in edible oil, cooking condiments, cosmetics, pesticides, and potential biofuels. Zinc and boron are essential micronutrients for plant growth. However, the effects of zinc and boron on the yield, yield component, oil content, and citral content in *L. cubeba* have not been determined. This study was conducted to evaluate the efficacy of the foliar application of zinc, boron, and multiple micronutrients (zinc + boron) on the yield, yield component, oil content, and citral content of three varieties (Fuyang 1 (FY1), Jianou 2 (JO2), and Jianou 3 (JO3)) of *L. cubeba*. Zinc sulfate (0.25%), boric acid (0.25%), and zinc sulfate (0.25%) + boric acid (0.25%) were sprayed on selected trees at five different times at full bloom and 28 days before harvest, once every seven days. The results indicated that Zn had a negative effect on the yield, yield component, oil content, and citral content of the FY1, JO2, and JO3 varieties compared to the untreated trees. B had positive effects on the yield, yield component, oil content, and citral content of the JO2 and JO3 varieties but not on those of the FY1 variety when compared to the untreated trees. The highest levels of yield, yield component, oil content, and citral content for all three varieties were obtained with the combined application of zinc sulfate + boric acid. Hence, the foliar application of multiple micronutrients (zinc + boron) is an effective method to improve the yield, oil content, and citral content in *L. cubeba*. In addition, the 100-fruit weight (HFW) was positively correlated with the yield, oil content, and citral content and could be used as a tool to select new cultivars with high yield, high oil content, and high citral content under zinc sulfate, boric acid, and zinc sulfate + boric acid applications in *L. cubeba*.

**Keywords:** *Litsea cubeba* (Lour.) Persoon; zinc; boron; yield; oil content

## 1. Introduction

Mountain pepper (*Litsea cubeba* (Lour.) Persoon) is an important natural aromatic plant of the Lauraceae family, which is native to southern China and is widely distributed in Southeast Asia, Japan, and Taiwan [1]. All parts of this plant are rich in aromatic essential oil, and the highest essential oil content is in the fruit [2]. The essential oil has been widely used as a raw material for cosmetics, pesticides, food additives, and biodiesel fuel. Meanwhile, the essential oil has various biological properties including antioxidative [3], acaricidal [4], anti-inflammatory [5], anticancer [6,7], immunosuppressive [8], fungicidal [9,10], antibacterial [10], and insecticidal [3,11,12] activities that enable its use as medicines, botanical insecticides, preservatives, and citral (neral and geranial). In addition, fruits of *L. cubeba* are used as a cooking condiment, which is frequently used in the aboriginal cuisine of southwest China and Taiwan. China is the greatest producer and exporter of *L. cubeba* essential oil [13].

Several studies have shown that yield and oil content are influenced by management measures, such as the fertilization process, and by the harvesting period [14–16]. Fertilization is an important and controllable method to promote fruit yield and oil content in terms of quantity and quality, and to ensure the economic benefit of the orchard and orchard workers as long as possible [15]. The type and supply patterns of the fertilizer influence the plant yield [16]. Both zinc (Zn) and boron (B) are essential micronutrients for all plants and are necessary for the growth and development of higher plants. Zinc (Zn) and boron (B) are not only known to be involved in cell wall synthesis, cell wall structure integrity, photosynthesis, respiration, carbohydrate metabolism, RNA metabolism, and other biochemical activities [17,18], but also participate in the catalytic and regulatory activities of more than 300 enzymes [19]. Hence, Zn or B deficiency directly influence plant growth and development, reducing yield, or in severe cases, causing plant death, and indirectly affect the biosynthesis of primary and secondary compounds in plants [17,20–26]. Zinc or boron fertilizer are widely used to improve crop yield and product quality and quantity [27–30]. Foliar application is one of the primary methods of Zn or B fertilization and has the advantages of avoiding leaching through the soil profile, a uniform distribution, and quick plant responses to the nutrients applied [24,31,32].

For the particular case of *L. cubeba*, the yield, essential oil content, and chemical composition are influenced by some aspects such as different distribution areas and varieties [2,33] and different ripening stages [34]. However, to our knowledge, no published data are available regarding the influence of fertilization on the yield, essential oil, and citral contents of *L. cubeba*. In addition, the essential oil of *L. cubeba* is comprised mainly of monoterpenes, sesquiterpenes, and non-terpene compounds [33]. A large number of enzymes are involved in the biosynthesis of compounds [35]. Zn or B deficiency may affect the biosynthesis of primary and secondary compounds. With this in mind, experiments should be conducted to study the oil composition of *L. cubeba* in relation to Zn and B fertilization. Previous studies have reported that the effect of foliar application of multiple micronutrients is superior to that of single micronutrients to increase grain yield, oil content, and nutrient concentration [29,36]. Therefore, to promote greater yield, oil content, and citral contents, it is also important to evaluate the applicability of foliar-applied multiple micronutrients on *L. cubeba*.

The primary research question of this study was whether zinc and boron foliar application could significantly affect the yield, yield component, essential oil content, and citral content of the fruits of *L. cubeba*. We also aimed to determine the effect of the applicability of foliar-applied multiple micronutrients on the yield, yield component, essential oil content, and citral content of the fruits of *L. cubeba*.

## 2. Materials and Methods

### *2.1. Experimental Site*

This study was conducted in a forest of the Research Institute of Subtropical Forestry, Chinese Academy of Forestry, China in 2017. The forest is located in Fuyang District, Hangzhou City in Zhejiang Province (30°04′ N, 119°99′ E). This site has an annual average precipitation of 1477.9 mm with an annual average mean temperature of 16.7 °C (ranging from 6.9 °C in January to 31.2 °C in July), an accumulated temperature above 0 °C of 6617.4 °C, and a frost-free period of 295 days. The annual sunshine duration and average relative humidity are 1816 h and 77%, respectively. The soil type where the experiment took place was sandy loam. A soil sample (0–30 cm depth and 30–60 cm depth) was analyzed in 2017, and some chemical properties are shown in Table 1.

**Table 1.** Some chemical characteristics of the experimental soil of the *L. cubeba* (Lour.) Persoon forest.

| Characteristics | Soil Depth | |
|---|---|---|
| | 0–30 cm | 30–60 cm |
| pH (1:1 $H_2O$) | 5.69 | 5.82 |
| Organic matter (g/kg) | 7.74 | 5.75 |
| Total N (g/kg) | 0.60 | 0.44 |
| Total P (g/kg) | 0.35 | 0.34 |
| Total K (g/kg) | 9.01 | 6.84 |
| POlsen (mg/kg) | 13.43 | 19.50 |
| K (exchangeable mg/kg) | 44.63 | 40.00 |
| Ca (g/kg) | 2.49 | 2.47 |
| Mg (g/kg) | 6.15 | 5.57 |
| S (mg/kg) | 398.67 | 213.33 |
| Cu (mg/kg) | 17.20 | 12.73 |
| Fe (g/kg) | 25.87 | 28.63 |
| Zn (mg/kg) | 92.83 | 74.07 |
| Available Zn(mg/kg) | 6.10 | 2.61 |
| B (mg/kg) | 22.10 | 21.40 |
| Available B (mg/kg) | 0.08 | 0.04 |
| Mn (g/kg) | 0.18 | 0.35 |

### 2.2. Plant Material and Treatment

Three four-year old varieties of *L. cubeba,* including Jianou 2 (JO2), Jianou 3 (JO3), and Fuyang 1 (FY1), were used in this experiment (Table 2). The trees were planted at 3 m × 3 m distances apart and received the same horticultural management, except in regard to fertilization with foliar treatments. Foliar fertilizer treatments were C (control, distilled water), A1 (applied $ZnSO_4$ 0.25%), A2 (applied $H_3BO_3$ 0.25%), and A3 (applied $ZnSO_4$ 0.25% + $H_3BO_3$ 0.25%). The trees were arranged randomly in blocks according to a factorial design Three replicates were used and each replicate contained 12 trees. The treatments were applied at five different times: at full bloom and 28 days before harvest, once every seven days. The trees were sprayed using a spraying motor (3 L capacity) until the runoff stage. The experiment was performed from March 20 to July 30 in 2017.

**Table 2.** The nutritional levels in leaves of Jianou 2 (JO2), Jianou 3 (JO3), and Fuyang 1 (FY1) varieties.

| Nutrition | FY1 | JO2 | JO3 |
|---|---|---|---|
| N (g/kg) | 24.6 | 27.2 | 26.5 |
| P (g/kg) | 5.05 | 2.55 | 2.46 |
| K (g/kg) | 10.7 | 16.9 | 15.8 |
| Ca (g/kg) | 7.86 | 8.94 | 8.47 |
| Mg (g/kg) | 2.29 | 2.25 | 2.27 |
| S (g/kg) | 1.85 | 2.13 | 2.01 |
| Cu (mg/kg) | 7.75 | 8.34 | 8.16 |
| Fe (g/kg) | 0.155 | 0.188 | 0.16 |
| Zn (mg/kg) | 32.9 | 42.6 | 39.4 |
| B (mg/kg) | 17.9 | 28.4 | 27.9 |

### 2.3. Plant Measurements

#### 2.3.1. Preparation of Fruit Sampling

A random sample of fruits from each experimental tree was handpicked and immediately transported to the laboratory. Only fresh and healthy fruits at a technologically mature stage (this differs from the physiological maturity stage, as seeds in this stage can be used to extract oil) were used.

2.3.2. Determination of the Fruit Essential Oil Percentage

The oil content was extracted from the fruit samples using hydrodistillation, as described by Gao et al. [34]. All samples were stored at 4 °C until analysis.

2.3.3. Volatile Compounds Using GC-MS Analysis

This process was almost identical to the method of Gao et al [33]. The qualitative and quantitative analyses of the volatile compounds of the fruit essential oil were conducted on an Agilent 6890N/5975B gas chromatograph-mass spectrometer (GC-MS) (Agilent Technologies Co., Ltd, Palo Alto, CA, USA) using a HP-5MS fused silica capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). The oven temperature was initially set at 50 °C for 2 min, then increased to 120 °C for 2 min at a rate of 3 °C/min, and finally increased to 250 °C for 2 min at 15 °C/min. The carrier gas was $N_2$ at a flow rate of 1 mL/min; the injected volume was 1.0 µL (1:10 in $Et_2O$), and the injector temperature was 220 °C. The other GC-MS conditions are as follows: interface temperature, 250 °C; ion source temperature, 230 °C; quadrupole temperature, 150 °C; ionization mode, EI; and ionization energy, 70 eV. The compound identification was confirmed by comparing the retention indices (RIs) of the samples with those reported in the literature. The RIs were calculated using a series of n-alkanes (C7–C30) under identical operating conditions. The relative amounts of the individual components were calculated using peak area normalization. In total, 47 compounds were identified from all samples.

### *2.4. Statistical Analysis*

The data were analyzed using IBM SPSS Statistics 19 software (SPSS Inc., Chicago, IL USA). Two-way ANOVA was used to evaluate the effects of variety, fertilization, and their interaction. A Duncan multiple-comparison test was used to detect the differences between the means. A $p$ value < 0.05 was considered significant. Pearson correlation analysis was used to analyze correlation patterns between the analyzed traits. SigmaPlot 12.0 and R3.5 (Systat Software Inc., San Jose, CA, USA) were used to draw the figures.

## 3. Results

### *3.1. The Change in Yield and Yield Components during the Different Foliar Applications of Micronutrient Solutions*

Analysis of variance of yield, yield components, fruit oil content, and citral contents is shown in Table 3. Different micronutrient fertilization treatments had different effects on the yield of *L. cubeba* (Figure 1). Zinc had a negative effect on the yield of *L. cubeba* varieties. The yields of the FY1, JO2, and JO3 varieties all decreased by 11.30%, 10.00%, and 17.78%, respectively, when compared to the untreated fruit. Under boric acid application, the yields in the JO2 and JO3 varieties increased by 6.67% and 22.22%, respectively, when compared to the untreated fruits. However, the yield of FY1 variety declined 3.22%. Zinc sulfate + boric acid foliar application had a positive effect on the yields of the FY1, JO2, and JO3 varieties, which were significantly increased by 45.16%, 33.33, and 33.33%, respectively, when compared to the untreated fruits.

The change in yield components during the different foliar applications of micronutrient solutions is shown in Figure 2. The same variation trend occurred between the 100-fruit weight (HFW) and yields under different micronutrient applications. The HFW of the FY1, JO2, and JO3 varieties all significantly increased (by 20.41%, 19.80%, and 41.27%, respectively) using the zinc sulfate + boric acid foliar application compared to the untreated fruits. Zn had a negative effect on the HFW of the FY1, JO2, and JO3 varieties, while B had a positive effect on the HFW of the JO2 and JO3 varieties. However, the response of the fruit longitudinal diameter (FLD), fruit transverse diameter (FTD), and fruit shape index (FSI) to the different micronutrient applications was different from those of the HFW and yield. For instance, the FLDs were all decreased with the three kinds of applications (Zn, B, Zn + B) in the

FY1 variety. Zn had a larger positive effect on the FLD and FTD of the JO2 variety than the other two kinds of applications (B, Zn + B).

**Table 3.** Analysis of variance of yield, yield components, fruit oil content, and citral contents.

| Variation | Yield | HFW | Oil Content | Citral | Neral | Geranial | FLD | FTD | FSI |
|---|---|---|---|---|---|---|---|---|---|
| variety (V) | 38.63 ** | 42.60 ** | 416.19 ** | 1783.70 ** | 260.74 ** | 680.65 ** | 39.09 ** | 28.38 | 35.08 ** |
| fertilization (F) | 31.09 ** | 32.07 ** | 449.92 ** | 3253.98 ** | 602.73 ** | 1086.04 ** | 21.57 ** | 25.43 | 30.05 ** |
| V × F | 1.35 ns | 1.86 ns | 185.37 ** | 1482.95 ** | 249.10 ** | 538.89 ** | 1.05 ns | 1.15 ns | 1.34 ns |

ns: not significant at 5% probability level; *: significant at 5% probability level. **: significant at 1% probability level. HFW: 100-fruit weight; FLD: fruit longitudinal diameter; FTD: fruit transverse diameter; FSI: fruit shape index.

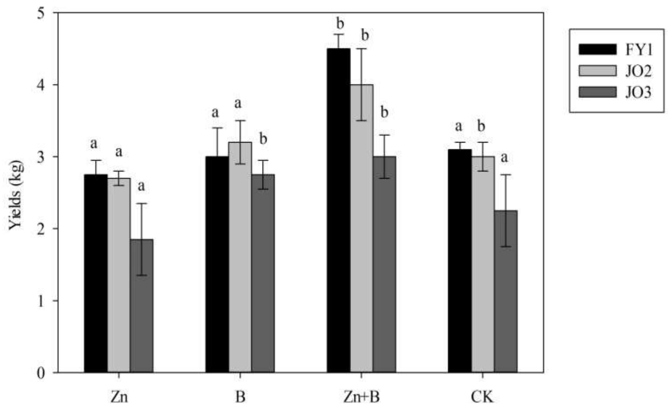
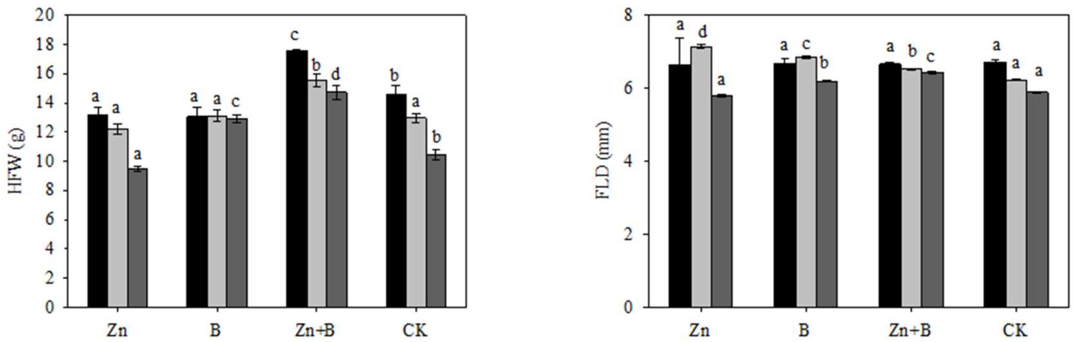



**Figure 1.** Effects of the foliar applications of the micronutrient solutions on the yield in *L. cubeba* (Lour.) Persoon. The different lowercase letters indicate significant differences ($p < 0.05$) from the different foliar applications of the nutrient solutions. Identical letters indicate the absence of significant differences. The abbreviations of "Zn", "B", "Zn + B", and "CK" represent $ZnSO_4$, $H_3BO_4$, and $ZnSO_4$ + $H_3BO_4$, and the control. Error bars are standard errors. The same is as below.




**(a)** **(b)**

**Figure 2.** *Cont.*

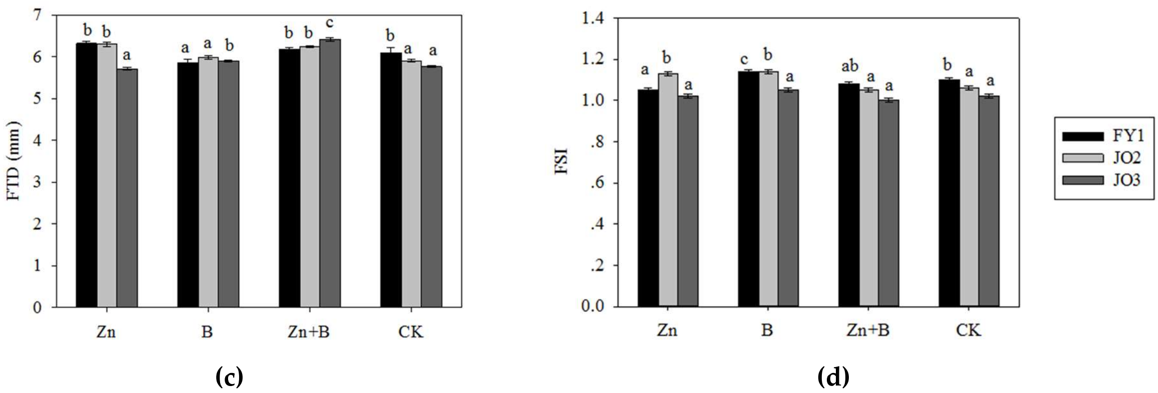



**Figure 2.** Effects of the foliar applications of the micronutrient solutions on the yield components in *L. cubeba*. (**a**) HFW: 100-fruit weight; (**b**) FLD: fruit longitudinal diameter; (**c**) FTD: fruit transverse diameter; (**d**) FSI: fruit shape index.

### 3.2. The Change in Fruit Oil Content during the Different Foliar Applications of Micronutrient Solutions

The measurement of the fruit oil content during the different foliar applications of the micronutrient solutions is shown in Figure 3. The variation trend of the fruit oil content with different micronutrients application was consistent with that of the yields. Boric acid also had a positive impact on the fruit oil content in the JO2 and JO3 varieties, which increased 25.63% and 67.03%, respectively, when compared to the untreated fruit, except for the FY1 variety. However, the zinc sulfate treatment tended to reduce the oil content in the fruits of all three varieties when compared to the untreated fruits, and the oil content of the FY1, JO2, and JO3 varieties decreased by 25.12%, 8.00%, and 1.62%, respectively. The fruit oil content increased significantly with the Zn + B application in the FY1, JO2, and JO3 varieties and was 16.28%, 26.67%, and 103.24% higher than the untreated fruits, respectively.

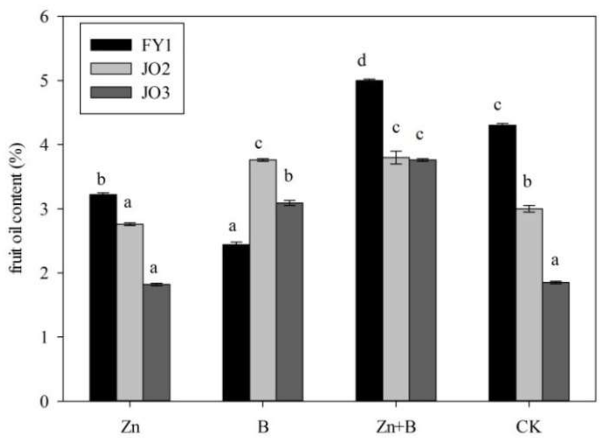



**Figure 3.** Effects of the foliar applications of the micronutrient solutions on the oil content in *L. cubeba*.

### 3.3. The Change in Citral Contents in the Oil during the Different Foliar Applications of Micronutrient Solutions

The most dominant chemical constituent of the fruit oil in *L. cubeba* is citral, which constitutes approximately 80% of the chemical content of the essential oils [34]. $\alpha$-citral (geranial) and $\beta$-citral (neral) are cis-trans isomers of citral [37]. The foliar applications of the micronutrient solutions had different effects on the chemical constituents of the different *L. cubeba* varieties (Figure 4). In the FY1 variety, both the Zn and B foliar sprays had negative effects on the content of neral, geranial, and citral. The zinc treatment decreased these three chemical constitutions when compared to the untreated fruit by 3.84%, 2.64%, and 3.17%, respectively, and the boric acid treatment decreased these three chemical constitutions from 38.31% to 36.62%, 48.03% to 45.91%, and 86.34% to 82.53%, respectively. However, the contents of neral, geranial, and citral significantly increased with the B foliar spray in the JO2 and JO3 varieties, respectively. For instance, the citral contents following the boric acid treatment increased significantly from 79.33% to 82.01% and 72.31% to 85.91% in the JO2 and JO3 varieties. However, the foliar application of the compound nutrients (Zn + B) increased three chemical constituent contents in all three varieties. For example, the citral content increased by 0.27%, 7.37%, and 23.58%, respectively, when compared to that of the untreated fruits.

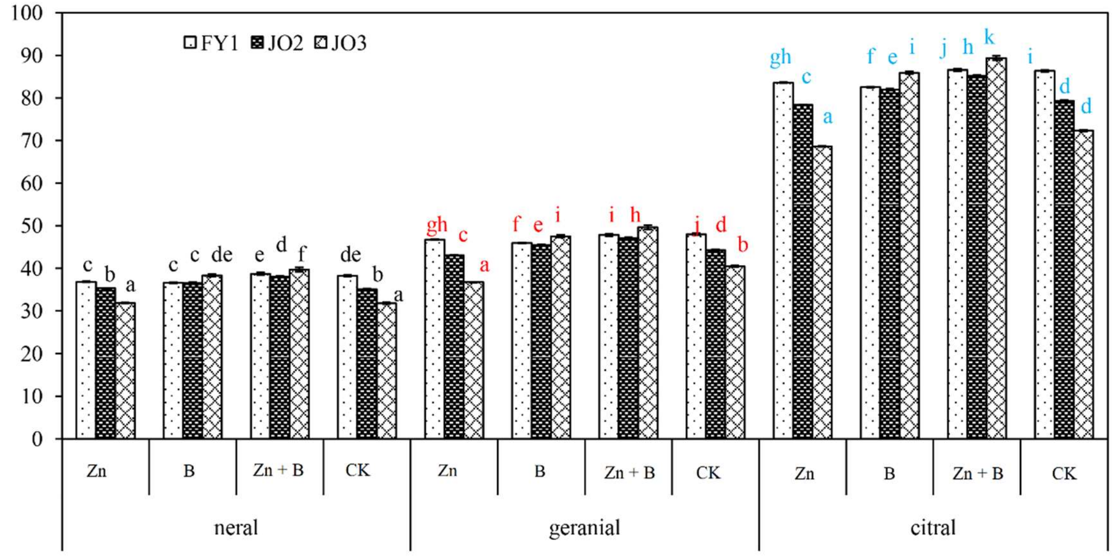



**Figure 4.** Effects of the foliar applications of micronutrient solutions on the neral, geranial, and citral content in *L. cubeba*. The letters in black indicate significant differences of neral from the different foliar applications of the nutrient solutions. The letters in red indicate significant differences of geranial content from the different foliar applications of the nutrient solutions, and the letters in blue mean indicate differences of citral content from the different foliar applications of the nutrient solutions.

### *3.4. Correlations*

Under the zinc sulfate foliar application, the yield was positively correlated with the HFW, oil content, neral, geranial, and citral (Figure 5a). In addition, there were positive correlations between the HFW, oil content, neral, geranial, and citral. Under boric acid foliar application, the yield was positively correlated with the FLD, FSI, and HFW (Figure 5b). In addition, the oil content was positively correlated with the FTD and HFW. The four indicators of HFW, neral, geranial, and citral were positively correlated with each other. Under the zinc sulfate + boric acid foliar applications, the yield was positively correlated with the HFW and oil content (Figure 5c). In addition, the five indicators of HFW, oil content, neral, geranial, and citral had positive correlations with each other.

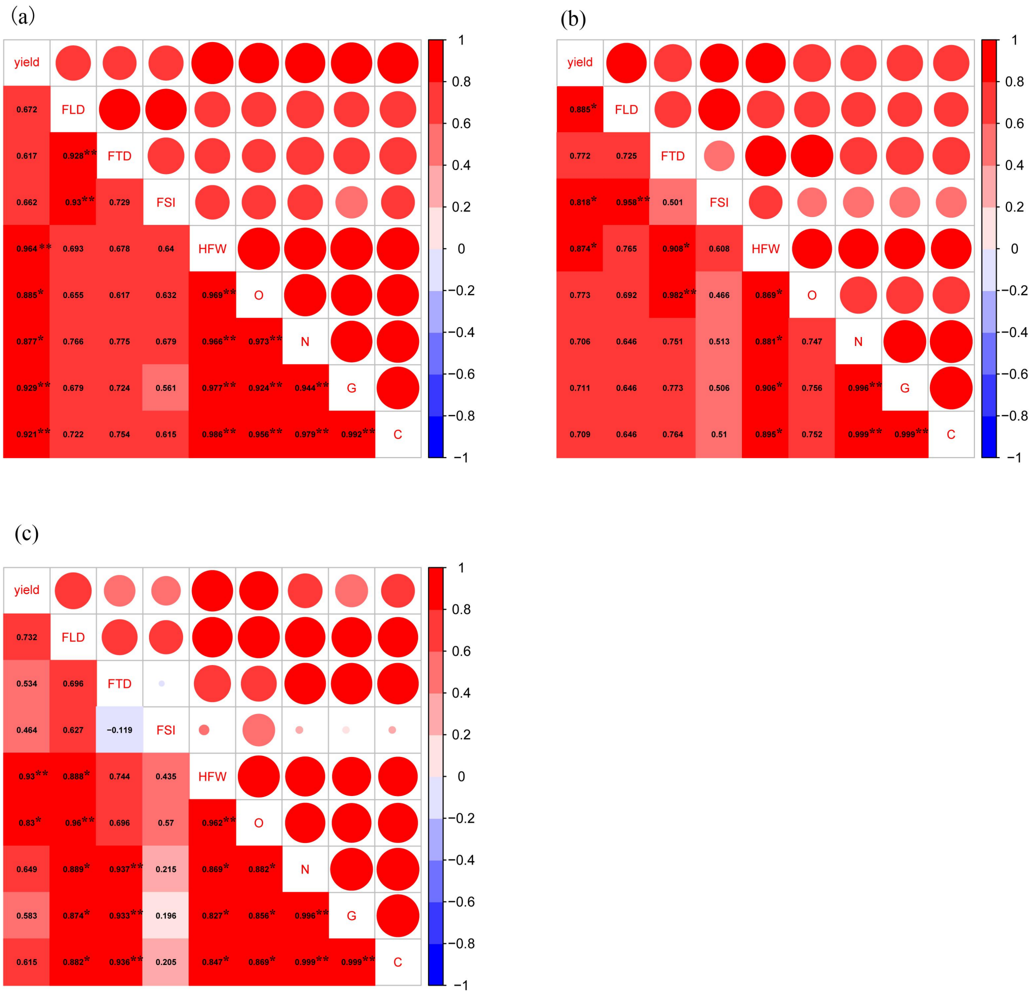



**Figure 5.** Analysis of the yields, yield components, oil contents, and citral contents under zinc sulfate (**a**), boric acid (**b**), and zinc sulfate + boric acid (**c**) foliar applications in *L. cubeba*. The cells of the lower left triangle and the circles of the upper right triangle mean the same. The color in red indicates a positive correlation and blue indicates a negative correlation. The darker the color, the larger the correlation value. The correlation factors are labeled on the middle diagonal. The abbreviations "O", "N", "G," and "C" represent the oil content, neral, geranial, and citral, respectively. The symbol "*" indicates a significant correlation at the 0.05 level, and "**" indicates an extremely significant correlation at the 0.01 level.

## 4. Discussion

### *4.1. The Effect of Zn and B Foliar Applications on the Yield and Yield Components in L. cubeba*

The yields of the FY1, JO2, and JO3 varieties all decreased under zinc application, which suggested that the zinc fertilizer application had a negative effect on the yield of *L. cubeba* with 6.1 mg/kg of available zinc content in the soil at a depth of 0–30 cm in our experiment. This finding was consistent with those of Wang et al. [28], Zhang et al. [38], and Wang et al. [32]. The capacity of the soil zinc supply was divided into five levels according to the available Zn content in China, and the available Zn content of 1.5 mg/kg was used for the threshold of the soil Zn deficiency [39]. This indicates that the soil in this experiment cannot be classified as deficient in Zn, and therefore, the available Zn in the soil is not low enough to show a yield increase by applying additional Zn [28]. In addition, the fact that the yield did not increase with the Zn application could be due to the application of the treatments during the reproductive vegetative stage and not during the seedling stage. Some previous studies demonstrated that plant growth and yield did not increase with late Zn application [28,40,41]. The root and shoot growths and the yield of the plants from high-Zn seeds were markedly greater than those from low-Zn seeds [42,43]. For the particular case of *L. cubeba*, the effect of foliar fertilization of zinc in the growing period is unknown, which should be determined in future research.

In China, soil available boron content of 0.5 mg/kg is the critical threshold of soil boron deficiency, and soil available boron content between 0.5 and 1.0 mg/kg is a medium level [44]. In this case, the yield would increase with boron application. In our research, the available boron content of the soil at a depth of 0–30 cm was 0.8 mg/kg and 0.4 mg/kg at a depth of 30–60 cm. *L. cubeba* is a shallow-rooted plant, and the depth of the root is above 25 cm. Hence, the boron application was available to the trees in our study. However, the yield of the FY1 variety was not affected by boron application, which is likely because the effect of the boron application also depends on a different genotype. Therefore, breeders should use available genetic diversity to enhance yield [45]. In addition, foliar sprays of B may be used to address a temporary deficiency, but to achieve full growth and development, B must be applied throughout the plant cycle [24].

In our experiment, the highest yields of all three varieties were obtained by the zinc sulfate + boric acid foliar application. This is likely because the effect of foliar application of compound nutrients was better than that of single micronutrients to increase the oil content of *L. cubeba*.

Yield components are very important because they are related to the fruit yield [14]. In *L. cubeba*, one of the most important yield components is the HFW, which is significantly positively correlated to the fruit yield, but there was no relation among the FLD, FTD, FSI, and yield [33]. Our results showed that the effect of micronutrient application on HFW was more than the effect on FLD, FTD, and FSI.

### *4.2. The Effect of Zn and B Foliar Applications on the Oil Content and Citral Contents in L. cubeba*

In our study, the oil contents of all three varieties were decreased under zinc sulfate treatment. However, the reports of Saadati et al. and Ramezani et al. reported higher oil contents in olive cultivar fruits following foliar zinc application [29,46]. This might indicate that different plants have different sensitivities to micronutrients [40]. Maize, sorghum, and soybean are very sensitive to zinc, but wheat, barley, and carrot are not. At the same time, several factors also limit the efficiency of the micronutrients on oil content, including soil condition, environment, time of application, and the background nutrient status of the soil and plants [32].

These findings were consistent with those of Desouky et al. and Saadati et al. [27,29]. Desouky et al. reported that the foliar application of different micronutrients on olive cultivars could manipulate the compositions of fatty acids differently. In addition, variations in the oil chemical constitution during the foliar application of the nutrient solutions observed in the *L. cubeba* varieties could be related to both genetic factors and environmental conditions [47].

### *4.3. The Tool to Select New Cultivars with High Yield, High Oil Content, and High Citral Content under Zn and B Foliar Applications*

*L. cubeba* is a species that has not been studied extensively, and its responses to different micronutrient treatments (e.g., zinc sulfate and boric acid) are unknown. This is the first report on the effect of zinc sulfate and boric acid supply on the yields, yield components, oil contents, and citral contents, and their relationship with each other. Obviously, the HFW can be used as a tool to select new cultivars with high yield, high oil content, and high citral content under zinc sulfate, boric acid, and zinc sulfate + boric acid supplies. However, more research is needed to explore these tools for *L. cubeba* breeding and for better *L. cubeba* management, especially under other nutrient conditions.

## 5. Conclusions

Zinc and boron are essential micronutrients for all plants and are necessary for the growth and development of higher plants. We studied whether Zn and B foliar applications could affect the yield, yield component, oil content, and citral content in *L. cubeba*. Zn had a negative effect on the yield, yield component, oil content, and citral content of the FY1, JO2, and JO3 varieties when compared to the untreated trees. B had a positive effect on the yield, yield component, oil content, and citral content of the JO2 and JO3 varieties, when compared to untreated trees. This indicated that the effect of the boron application also depended on different genotypes in *L. cubeba*. The combination of the zinc sulfate + boric acid application could significantly increase the levels of the yield, yield component, oil content, and citral content of all three varieties. Hence, the foliar application of multiple micronutrients (zinc + boron) is an effective method to improve the yield, oil content, and citral content in *L. cubeba*. Meanwhile, the HFW was positively correlated with the yield, oil content, and citral content and can be used as a tool to select new cultivars with high yield, high oil content, and high citral content under zinc sulfate, boric acid, and zinc sulfate + boric acid supplies in *L. cubeba*.

**Author Contributions:** Conceptualization: M.G. and Y.W.; Methodology: M.G. and Y.C.; Software: M.G., Y.C., and L.W.; Validation: M.G.; Formal Analysis: M.G.; Writing—Original Draft Preparation: M.G.; Writing—Review and Editing: M.G. and Y.W.; Funding Acquisition: M.G.

**Funding:** This research was funded by the National Key R&D Program of China, grant number 2017YFD0600704, the National Natural Science Foundation of China, grant number 31700523.

**Conflicts of Interest:** The authors declare no conflict of interests.

## References

1. Li, X.W. Angiospermae, Dicotyledoneae, Lauraceae, Hernandiaceae. In *Florae Reipublicae Popularis Sinicae*; Wu, Z.Y., Ed.; Science Press: Beijing, China, 2004; pp. 271–272.
2. Si, L.L.; Chen, Y.C.; Han, X.J.; Zhan, Z.Y.; Tian, S.P.; Cui, Q.Q.; Wang, Y.D. Chemical composition of essential oils of *Litsea cubeba* harvested from its distribution areas in China. *Molecules* **2012**, *17*, 7057–7066. [CrossRef] [PubMed]
3. Hwang, J.K.; Choi, E.M.; Lee, J.H. Antioxidant activity of *Litsea cubeba*. *Fitoterapia* **2005**, *76*, 684–686. [CrossRef] [PubMed]
4. Pumnuan, J.; Chandrapatya, A.; Insung, A. Acaricidal activities of plant essential oils from three plants on the mushroom mite, *Luciaphorus perniciosus* Rack (Acari: Pygmephoridae). *Pak. J. Zool.* **2010**, *42*, 247–252.
5. Liao, P.C.; Yang, T.S.; Chou, J.C.; Chen, J.; Lee, S.C.; Kuo, Y.H.; Ho, C.L.; Chao, L.K.P. Anti-inflammatory activity of neral and geranial isolated from fruits of *Litsea cubeba* Lour. *J. Funct. Foods* **2015**, *19*, 248–258. [CrossRef]
6. Ho, C.L.; Jie-Pinge, O.; Liu, Y.C.; Hung, C.P.; Tsai, M.C.; Liao, P.C.; Wang, E.I.; Chen, Y.L.; Su, Y.C. Compositions and in vitro anticancer activities of the leaf and fruit oils of *Litsea cubeba* from Taiwan. *Nat. Prod.* **2010**, *5*, 617–620.
7. Seal, S.; Chatterjee, P.; Bhattacharya, S.; Pal, D.; Dasgupta, S.; Kundu, R.; Mukherjee, S.; Bhattacharya, S.; Bhuyan, M.; Bhattacharyya, P.R. Vapor of volatile oils from *Litsea cubeba* seed induces apoptosis and causes cell cycle arrest in lung cancer cells. *PLoS ONE* **2012**, *7*, e47014. [CrossRef]
8. Chen, H.C.; Chang, W.T.; Hseu, Y.C.; Chen, H.Y.; Chuang, C.; Lin, C.C.; Lee, M.S.; Lin, M.K. Immunosuppressive effect of *Litsea cubeba* L. essential oil on dendritic cell and contact hypersensitivity responses. *Int. J. Mol. Sci.* **2016**, *17*, 1319. [CrossRef]
9. Yang, Y.; Jiang, J.; Qimei, L.; Yan, X.; Zhao, J.; Yuan, H.; Qin, Z.; Wang, M. The fungicidal terpenoids and essential oil from *Litsea cubeba* in Tibet. *Molecules* **2010**, *15*, 7075–7082. [CrossRef]
10. Zhang, W.; Hu, J.; Lv, W.; Zhao, Q.; Shi, G. Antibacterial, antifungal and cytotoxic isoquinoline alkaloids from *Litsea cubeba*. *Molecules* **2012**, *17*, 12950–12960. [CrossRef]
11. Amer, A.; Mehlhorn, H. Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex mosquitoes*. *Parasitol. Res.* **2006**, *99*, 478–490. [CrossRef]
12. Seo, S.M.; Kim, J.; Lee, S.G.; Shin, C.H.; Shin, S.C.; Park, I.K. Fumigant antitermitic activity of plant essential oils and components from Ajowan (*Trachyspermum ammi*), Allspice (*Pimenta dioica*), caraway (*Carum carvi*), dill (*Anethum graveolens*), Geranium (*Pelargonium graveolens*), and Litsea (*Litsea cubeba*) oils against Japanese termite (*Reticulitermes speratus Kolbe*). *J. Agric. Food Chem.* **2009**, *57*, 6596–6602. [PubMed]
13. Chen, Y.C.; Wang, Y.D.; Han, X.J.; Si, L.L.; Wu, Q.K.; Lin, L.L. Biology and chemistry of *Litsea cubeba*, a promising industrial tree in China. *J. Essent. Oil Res.* **2013**, *25*, 103–111. [CrossRef]

14. Dordas, C.A.; Sioulas, C. Safflower yield, chlorophyll content, photosynthesis, and water use efficiency response to nitrogen fertilization under rainfed conditions. *Ind. Crops Prod.* **2008**, *27*, 75–85. [CrossRef]
15. Santoni, F.; Paolini, J.; Barboni, T.; Costa, J. Relationships between the leaf and fruit mineral compositions of *Actinidia deliciosa* var. Hayward according to nitrogen and potassium fertilization. *Food Chem.* **2014**, *147*, 269–271. [CrossRef]
16. Tekaya, M.; Mechri, B.; Cheheb, H.; Attia, F.; Chraief, I.; Ayachi, M.; Boujneh, D.; Hammami, M. Changes in the profiles of mineral elements, phenols, tocopherols and soluble carbohydrates of olive fruit following foliar nutrient fertilization. *LWT Food Sci. Technol.* **2014**, *59*, 1047–1053. [CrossRef]
17. Ahmad, W.; Zia, M.H.; Malhi, S.S.; Niaz, A.; Saifullah. Boron deficiency in soils and crops: A review. In *Crop Plant*; Aakash, G., Ed.; InTech: Rijeka, Croatia, 2012; pp. 77–114.
18. Chen, X.; Ludewig, U. Biomass increase under zinc deficiency caused by delay of early flowering in *Arabidopsis*. *J. Exp. Bot.* **2018**, *69*, 1269–1279. [CrossRef] [PubMed]
19. Jose Brandao-Neto, M.D.; Vivian Stefan, M.D.; Berenice, B.; Mendonca, M.D.; Walter Bloise, M.D.; Ana Valeria, B.; Castro, M.D. The essential role of zinc in growth. *Nutr. Res.* **1995**, *15*, 335–358. [CrossRef]
20. Yoshida, S.; Tanaka, A. Zinc deficiency of the rice plant in calcareous soils. *Soil Sci. Plant Nutr.* **1969**, *15*, 75–80. [CrossRef]
21. Han, S.; Chen, L.S.; Jiang, H.X.; Smith, B.R.; Yang, L.T.; Xie, C.Y. Boron deficiency decreases growth and photosynthesis, and increases starch and hexoses in leaves of citrus seedlings. *J. Plant Physiol.* **2008**, *165*, 1331–1341. [CrossRef]
22. Widodo; Broadley, M.R.; Rose, T.; Frei, M.; Pariasca-Tanaka, J.; Yoshihashi, T.; Thomson, M.; Hammond, J.P.; Aprile, A.; Close, T.J.; et al. Response to zinc deficiency of two rice lines with contrasting tolerance is determined by root growth maintenance and organic acid exudation rates, and not by zinc-transporter activity. *New Phytol.* **2010**, *186*, 400–414. [CrossRef]
23. Rosolem, C.A.; Bogiani, J.C. Physiology of boron stress in cotton. In *Stress Physiology in Cotton*; Oosterhuis, D.M., Ed.; The Cotton Foundation: Cordova, TN, USA, 2011; pp. 113–124.
24. Bogiani, J.C.; Sampaio, T.F.; Abreu-Junior, C.H.; Rosolem, C.A. Boron uptake and translocation in some cotton cultivars. *Plant Soil* **2014**, *375*, 241–253. [CrossRef]
25. Nanda, A.K.; Pujol, V.; Wissuwa, M. Patterns of stress response and tolerance based on transcriptome profiling of rice crown tissue under zinc deficiency. *J. Exp. Bot.* **2017**, *68*, 1715–1729. [CrossRef] [PubMed]
26. Hamza, S.; Leela, N.K.; Srinivasan, V.; Nileena, C.R.; Dinesh, R. Influence of zinc on yield and quality profile of ginger (*Zingiber officinale* Rosc.). *J. Spices Aromat. Crops* **2013**, *22*, 91–94.
27. Desouky, I.M.; Haggag, L.F.; Elmigeed, M.M.M.A.; Kishk, Y.F.M.; Elhady, E.S. Effect of boron and calcium nutrients sprays on fruit set, oil content and oil quality of some olive oil cultivars. *World J. Agric. Sci.* **2009**, *5*, 180–185.
28. Wang, J.W.; Hui, M.; Zhao, H.B.; Huang, D.L.; Wang, Z.H. Different increases in maize and wheat grain zinc concentrations caused by soil and foliar applications of zinc in Loess Plateau, China. *Field Crops Res.* **2012**, *135*, 89–96. [CrossRef]
29. Saadati, S.; Moallemi, N.; Mortazavi, S.M.H.; Seyyednejad, S.M. Effects of zinc and boron foliar application on soluble carbohydrate and oil contents of three olive cultivars during fruit ripening. *Sci. Hortic.* **2013**, *164*, 30–34. [CrossRef]
30. Mei, L.; Li, Q.H.; Wang, H.; Sheng, O.; Peng, S.A. Boron deficiency affects root vessel anatomy and mineral nutrient allocation of *Poncirus trifoliata* (L.) Raf. *Acta Physiol. Plant.* **2016**, *38*, 86. [CrossRef]
31. Kutman, U.B.; Kutman, B.Y.; Ceylan, Y.; Ova, E.A.; Cakmak, I. Contributions of root uptake and remobilization to grain zinc accumulation in wheat depending on post-anthesis zinc availability and nitrogen nutrition. *Plant Soil* **2012**, *361*, 177–187. [CrossRef]
32. Wang, S.; Meng, L.; Tian, X.; Jin, L.; Li, H.; Ni, Y.; Zhao, J.; Chen, Y.; Guo, C.; Zhao, A. Foliar Zinc, nitrogen, and phosphorus application effects on micronutrient concentrations in winter wheat. *Agron. J.* **2015**, *107*, 61. [CrossRef]
33. Gao, M.; Chen, Y.; Wang, Y. Evaluation of the yields and chemical compositions of the essential oils of different *Litsea cubeba* varieties. *J. Essent. Oil Bear. Plants* **2016**, *19*, 1888–1902. [CrossRef]
34. Gao, M.; Lin, L.Y.; Chen, Y.C.; Wang, Y.D. Digital gene expression profiling to explore differentially expressed genes associated with terpenoid biosynthesis during fruit development in *Litsea cubeba*. *Molecules* **2016**, *21*, 1251. [CrossRef] [PubMed]

35. Han, X.J.; Wang, Y.D.; Chen, Y.C.; Lin, L.Y.; Wu, Q.K. Transcriptome Sequencing and Expression Analysis of Terpenoid Biosynthesis Genes in *Litsea cubeba*. *PLoS ONE* **2013**, *8*, e76890. [CrossRef] [PubMed]
36. Zhang, Y.; Shi, R.; Rezaul, K.M.; Zhang, F.; Zou, C. Iron and zinc concentrations in grain and flour of winter wheat as affected by foliar application. *J. Agric. Food Chem.* **2010**, *58*, 12268–12274. [CrossRef] [PubMed]
37. Penfold, A.R.; Morrison, F.R.; Willis, J.L.; McKern, H.H.G.; Spies, M.C. The essential oil of a physiological form of *Eucalyptus citriodora* Hook. *J. Proc. R. Soc. New South Wales* **1951**, *85*, 120–122.
38. Zhang, Y.Q.; Sun, Y.X.; Ye, Y.L.; Karim, M.R.; Xue, Y.F.; Yan, P.; Meng, Q.F.; Cui, Z.L.; Cakmak, I.; Zhang, F.S. Zinc biofortification of wheat through fertilizer applications in different locations of China. *Field Crops Res.* **2012**, *125*, 1–7. [CrossRef]
39. Liu, Z.; Zhu, Q.; Tang, L. Geographical distribution of trace elements-deficient soils in China. *Acta Pedol. Sin.* **1982**, *19*, 209–223.
40. Cakmak, I.M.; Kalayci, Y.; Kaya, A.A.; Torun, N.; Aydin, Y.; Wang, Z.; Arisoy, H.; Erdem, A.; Yazici, O.L.O.; et al. Biofortification and localization of zinc in wheat grain. *J. Agric. Food Chem.* **2010**, *58*, 9092–9102. [CrossRef]
41. Cakmak, I.; Pfeiffer, W.H.; McClafferty, B. Biofortification of durum wheat with zinc and iron. *Cereal Chem.* **2010**, *87*, 10–20. [CrossRef]
42. Rengel, Z.; Graham, R.D. Importance of seed Zn content for wheat growth on Zn-deficient soil. *Plant Soil* **1995**, *173*, 259–266. [CrossRef]
43. Yilmaz, A.; Ekiz, H.; Gültekin, I.; Torun, B.; Barut, H.; Karanlik, S.; Cakmak, I. Effect of seed zinc content on grain yield and zinc concentration of wheat grown in zinc-deficient calcareous soils. *J. Plant Nutr.* **1998**, *21*, 2257–2264. [CrossRef]
44. Liu, Z.; Zhu, Q.Q.; Tong, L.H. Boron-deficient soils and their distribution in China. *Acta Pedol. Sin.* **1980**, *17*, 228–239.
45. Pallotta, M.; Schnurbusch, T.; Hayes, J.; Hay, A.; Baumann, U.; Paull, J.; Langridge, P.; Sutton, T. Molecular basis of adaptation to high soil boron in wheat landraces and elite cultivars. *Nature* **2014**, *514*, 88–91. [CrossRef] [PubMed]
46. Ramezani, S.; Shekafandeh, A.; Taslimpour, M.R. Effect of GA3 and zinc sulfate on fruit yield and oil percentage of 'Shengeh' olive trees. *Int. J. Fruit Sci.* **2010**, *10*, 228–234. [CrossRef]
47. Connor, D.J.; Fereres, E. The physiology of adaptation and yield expression in olive. *Hortic. Rev.* **2005**, *31*, 155–229.