

Effects of Bioorganic Fertilizers on the Leaf Antioxidant Enzyme Activity and the Photosynthetic Characteristics of Replanted Szechuan Pepper, *Zanthoxylum simulans*

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Abstract: Continuous cropping obstacles hamper the efficient growth and yield of Szechuan pepper, *Zanthoxylum simulans*. The current study investigated the impact of different levels of bioorganic fertilizer on the leaf physiological and photosynthetic characteristics of *Z. simulans* to provide a theoretical reference for continuous *Z. simulans* crop cultivation. A bioorganic fertilizer was used to treat seedlings growing in 25-year-old continuous cropping soil. Five fertilizer treatments were applied. The impacts of the treatments on the activity of defense enzyme and photosynthetic parameters of *Z. simulans* leaves were determined. The different concentrations of bioorganic fertilizer reduced to varying degrees the malondialdehyde (MDA) content and intercellular CO₂ concentration (Ci), and increased the activity of peroxidase (POD), superoxide dismutase (SOD), and ascorbate peroxidase (APX), as well as the chlorophyll content, net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) of *Z. simulans* leaves. The results showed that most significant increases or decreases were achieved with 100 g/L bioorganic fertilizer (Y2). Thus, the application of bioorganic fertilizer at a rate of 100 g/L can significantly improve the activity of relevant defense enzymes and photosynthetic parameters of *Z. simulans*, and reduce the MDA content, enhancing the stress resistance of the plants, promoting their growth and addressing, to some extent, obstacles associated with continuous cultivation.

Key words: Bio-organic fertilizer, continuous cropping obstacle, *Zanthoxylum simulans*, antioxidant enzyme activity, photosynthetic characteristics.

1. Introduction

Zanthoxylum simulans (Szechuan pepper) is a small deciduous tree or shrub of the Rutaceae, and is characterized by early yield, wide use and high value. It is the main economic tree species in some areas of China, as well as being an important species for soil and water conservation in mountainous areas [1, 2]. However, because of inappropriate management and perennial continuous cropping in some areas, the yield and quality of *Z. simulans* are often reduced and the survival rate of seedlings after replanting is low, significantly restricting the

development of the pepper industry [3].

Continuous cropping can result in soil-borne diseases and stunted plant growth caused by the long-term single planting of a certain crop in a specific area. Generally, continuous cropping obstacles are mostly caused by allelopathic autotoxicity, which changes the type and composition of soil microorganisms through root secretion and leaching [4, 5]. However, the specific mechanisms of continuous production obstacles are complex and not fully understood [6]. In a study of continuously cropped *Z. simulans*, Li *et al.* [7] reported that the soil fertility index and chemical properties decreased with the increase in continuous cropping time. Xu *et al.* [8] showed that long-term continuous cropping increased the acidity of melon fruits,

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decreased the photosynthetic efficiency of the plants and, thus, reduced both their growth and yield. Although continuous cropping obstacles are inevitable, the situation can be improved to some extent by adopting appropriate planting methods or rational fertilizer use. Research has shown that, in vegetables, appropriate fertilization can effectively improve the soil structure and microbial activity, maintain soil fertility and reduce the impact of continuous cropping obstacles [9]. Appropriate rotation, tillage and mulching can also improve the physical and chemical properties of soil, which can improve soil fertility and the stress resistance of the crops [10]. The application of phosphate and potash fertilizers, various mixed fertilizers and crop straw can also help improve soil fertility [11].

Biological organic fertilizers are a high-efficiency fertilizer rich in organic and inorganic nutrients. Generally, it is an organic fertilizer containing a certain amount of functional microorganisms that are processed from livestock manure, straw and some organic waste through fermentation bacteria, with a positive impact on the plant [12, 13]. Compared with other fertilizers, such as traditional fertilizers, farm fertilizers and bacterial fertilizers, biological organic fertilizers are characterized by low prices, complete nutrition, high fertilizer efficiencies and fast effects [14]. Various studies have shown that bioorganic fertilizers applied to crops and fruit trees, such as wheat and corn [15, 16], sunflower [17], cucumber [18, 19] and apple [20], helped to alleviate the obstacles associated with continuous cropping.

Although the above studies provide a theoretical basis for the current study, there are few reports on the physiological effects of biological organic fertilizers on replanted *Z. simulans*. Therefore, this study investigated the effects of different gradients of biological organic fertilizer to determine the optimal fertilizer concentration that could effectively improve the enzyme activity and photosynthetic characteristics of the leaves of replanted *Z. simulans*. These results provide a theoretical basis for alleviating the obstacles

associated with the continuous cropping of *Z. simulans*.

2. Materials and Methods

2.1 Test Materials

Two-year-old “Da Hong Pao” *Z. simulans* seedlings were selected for the study. These were acquired from Qinan county Tianshui city.

The soil used for continuous cropping was collected from an old pepper garden in Tianshui city, where *Z. simulans* had been cultivated for over 25 years. The soil type is sandy loam soil. The texture and chemical properties of the soil are shown in Table 1.

Bioorganic fertilizers were purchased by the Gansu Provincial Academy of Agricultural Sciences and produced by the Shandong Ruipu Biotechnology Co., Ltd. The effective components were as follows: organic matter $\geq 30\%$, $N + P_2O_5 + K_2O \geq 6\%$, alginic acid $\geq 15\%$, crude protein $\geq 9\%$, trace elements in a chelating state, and ≥ 200 million per gram active biological bacteria. The implementation standard number of bio-organic fertilizer is NY 884-2012, and the international standard classification number is 65_080.

2.2 Test Design

The *Z. simulans* seedlings were transferred to a $32 \times 10 \times 45$ cm (outside diameter \times inside diameter \times height) pot in May 2018 (leaf expansion stage). Each pot was filled with 7.5 kg continuous cropping soil and contained six plants. After 30 d of growth, three plants showing uniform growth were selected for further study, one plant each pot. Five treatment regimens were established, with three plants used per treatment: (i) continuous cropping soil (CK); (ii) continuous cropping soil + bioorganic fertilizer 50 g/L (Y1); (iii) continuous cropping soil + bioorganic fertilizer 100 g/L (Y2); (iv) continuous cropping soil + bioorganic fertilizer 150 g/L (Y3); (v) continuous cropping soil + bioorganic fertilizer 200 g/L (Y4). During this period, pots were watered with 750 mL water every 3 d, and dug regularly.

Table 1 Basic physical and chemical properties of the continuous cropping soil used to cultivate *Zanthoxylum simulans* in the current study.

pH	Organic matter (g/kg)	Total nitrogen (g/kg)	Total phosphorus (g/kg)	Total potassium (g/kg)	Available nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)
8.29	16.21	0.79	0.61	7.85	45	4.36	75.68

The soil has poor texture and low fertility level.

2.3 Experimental Methods

2.3.1 Determination of Photosynthetic Characteristics

The photosynthetic parameters of leaves on each plant were measured at the beginning of September 2018. From 14:00 to 16:00 on a sunny day with no wind or breeze and sufficient sunshine, the intercellular CO₂ concentration (Ci), net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) were measured by using a Li-6400XT portable photosynthetic instrument. Healthy second and third leaves from the top of each plant in each treatment group were removed for testing, and each sample was measured five times.

2.3.2 Determination of Antioxidant Enzyme Activity and Chlorophyll Content in Leaves

After determining the photosynthetic parameters, healthy leaves were selected from the east, west, south and north sides of each plant from each treatment group, and the collected leaves were quickly stored in a refrigerator at -80 °C for later use. During the measurement, each plot was measured three times, and the results were averaged. The activity of antioxidant and the chlorophyll content in leaves were determined following the methods of Gao [21] and Zhang *et al.* [22]. Malondialdehyde (MDA) was determined by using the 2-thiobarbituric acid method [21]. Superoxide dismutase (SOD) was determined by using the nitroblue tetrazolium (NBT) photoreduction method [21]. POD was determined by using the guaiacol reduction method. Chlorophyll was extracted by a rapid grinding method with 96% ethanol under dark light [21]. Ascorbate peroxidase (APX) was assessed by measuring the change in absorbance of ascorbic acid and H₂O₂ in a unit time [22].

2.4 Statistical Analysis

All data were verified to be in a normal distribution and were collated using Excel 2010. Statistical Product and Service Solutions (SPSS) 22.0 was used to conduct analysis of variance (ANOVA) and Duncan's multiple range tests. Differences were significant at $p < 0.05$.

3. Results

3.1 Effects of Different Fertilizer Treatments on Anti-oxidase Activity of *Z. simulans* Seedlings

MDA content varied significantly under the different fertilizer treatments (Fig. 1), with all fertilizer treatment groups showing reduced levels of MDA compared with the CK, with the reduction being most significant in the Y2 group, decreased by 144.7%, respectively compared with the CK ($p < 0.05$). By contrast, the levels of SOD, POD and APX were all increased compared with the CK, also being most significant in the Y2 group, increasing by 124.3%, 119.1% and 145.3%, respectively, compared with the CK ($p < 0.05$).

3.2 Effects of Different Fertilizer Treatments on Chlorophyll Content of *Z. simulans* Seedlings

The chlorophyll content in each of the treatment groups was increased compared with the CK (Fig. 2), with the increase being most significant in the Y2 group ($p < 0.05$). The increases in the Y1, Y3 and Y4 groups were lower than that in Y2. This might be because the excess amount of organic fertilizer could not be fully absorbed by the plants, causing salinization of the soil and resulting in water loss from the plant cells and hindering chlorophyll synthesis [23-25].

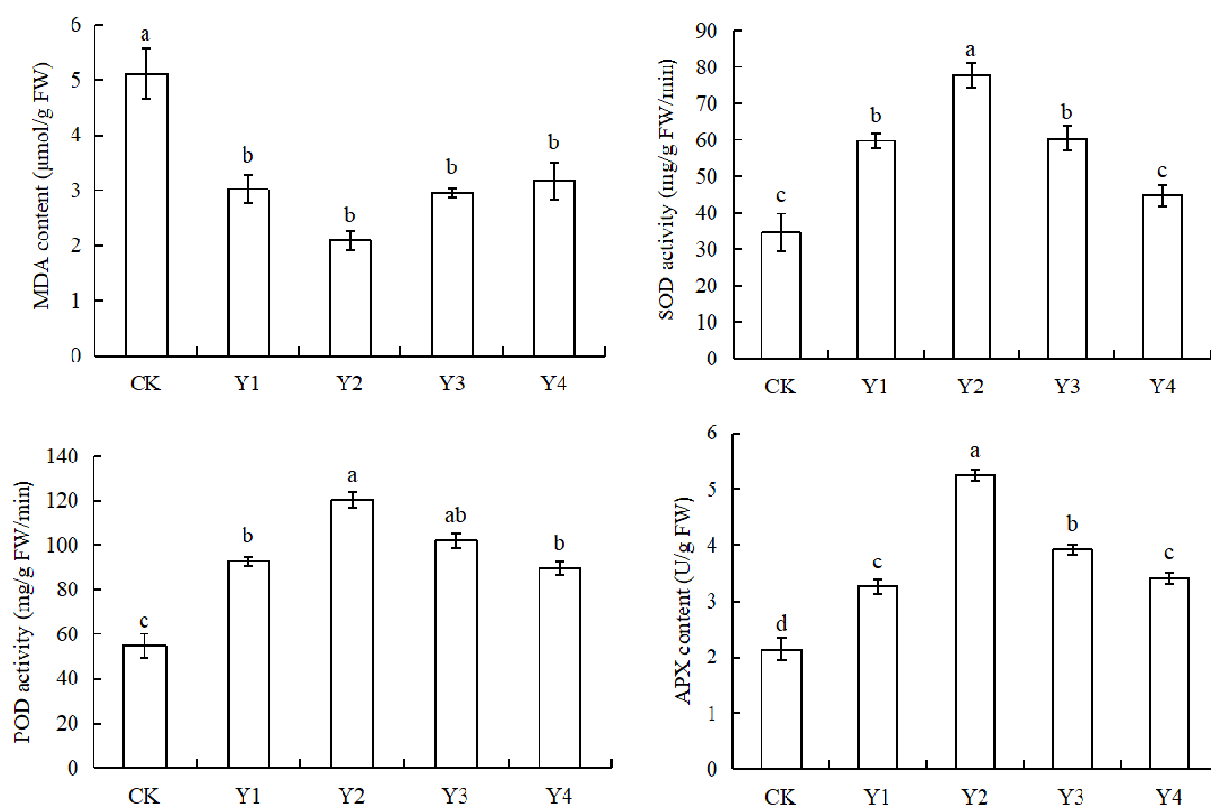


Fig. 1 Effects of different bio-organic fertilizer treatments on the levels of antioxidant activities in leaves of *Zanthoxylum simulans*.

The abscissa indicates different concentrations of bio-organic fertilizer. The ordinate shows the activity or content of various antioxidant enzymes. In the abscissa: CK = continuous cropping soil, Y1 = continuous cropping soil + biological organic fertilizer 50 g/L, Y2 = continuous cropping soil + biological organic fertilizer 100 g/L, Y3 = continuous cropping soil + biological organic fertilizer 150 g/L, Y4 = continuous cropping soil + biological organic fertilizer 200 g/L; in the ordinate: MDA = malondialdehyde, SOD = superoxide dismutase, POD = peroxidase, APX = ascorbate peroxidase; different letters indicate significant differences between means using Duncan's multiple range test, and the significance level of all data was analyzed at $p < 0.05$.

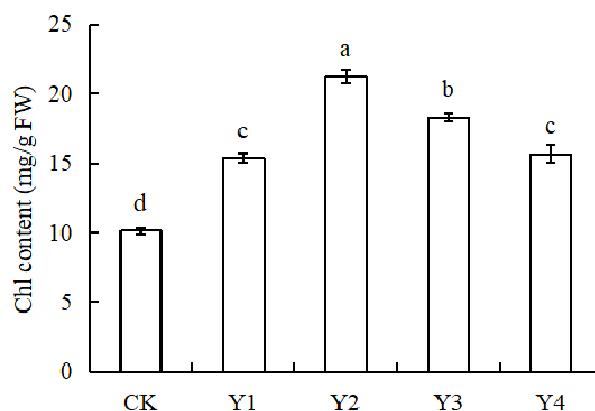


Fig. 2 Effects of different bio-organic fertilizer treatments on the chlorophyll content of leaves of *Z. simulans*.

The abscissa indicates different concentrations of bio-organic fertilizer. The ordinate indicates the chlorophyll content. In the abscissa: CK = continuous cropping soil, Y1 = continuous cropping soil + biological organic fertilizer 50 g/L, Y2 = continuous cropping soil + biological organic fertilizer 100 g/L, Y3 = continuous cropping soil + biological organic fertilizer 150 g/L, Y4 = continuous cropping soil + biological organic fertilizer 200 g/L; different letters indicate significant differences between means using Duncan's multiple range test, and the significance level of all data was analyzed at $p < 0.05$.

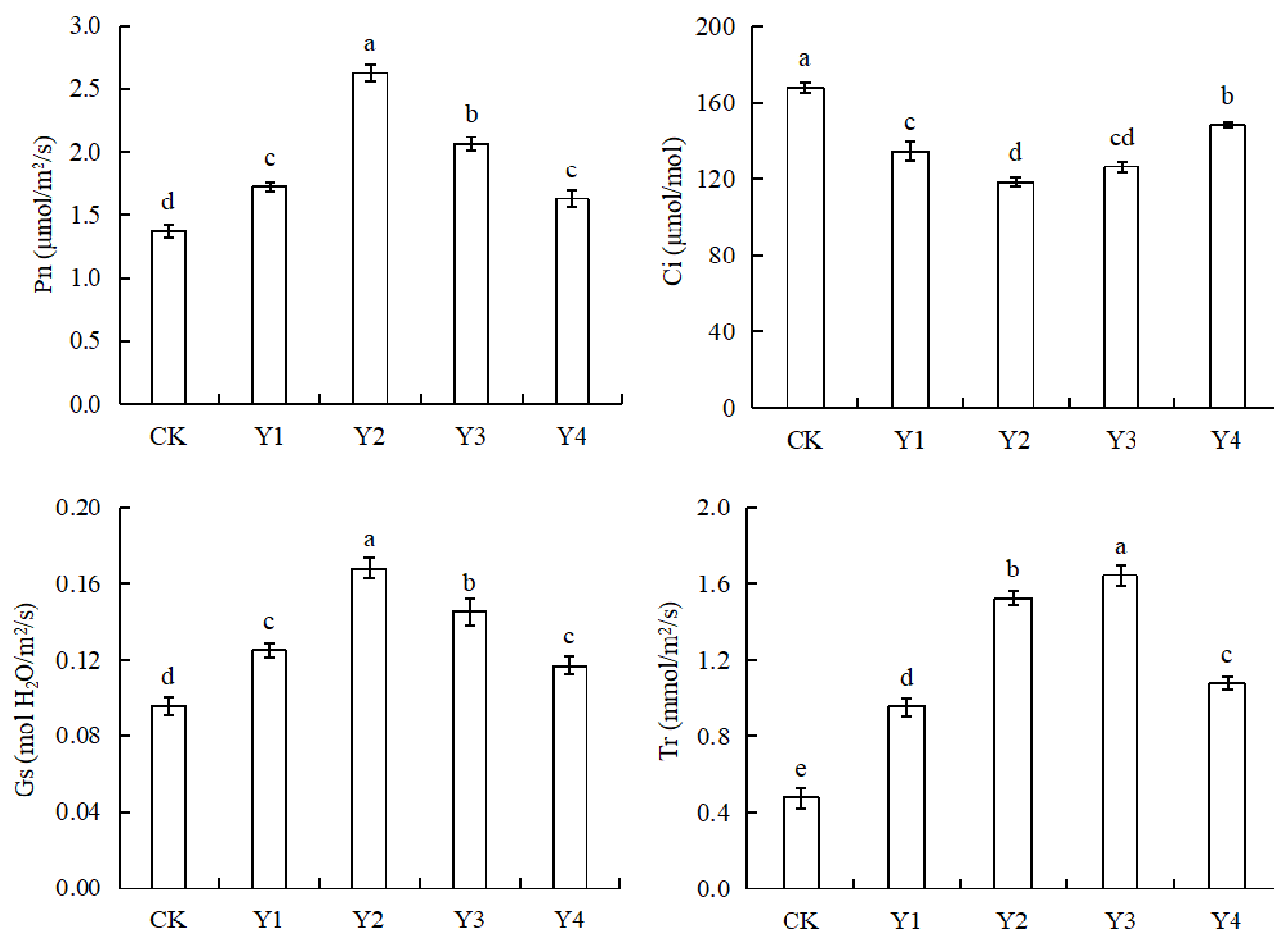


Fig. 3 Effects of different bio-organic fertilizer treatments on photosynthetic parameters of leaves of *Z. simulans*.

The abscissa indicates different concentrations of bio-organic fertilizer. The ordinate indicates the chlorophyll content. In the abscissa: CK = continuous cropping soil, Y1 = continuous cropping soil + biological organic fertilizer 50 g/L, Y2 = continuous cropping soil + biological organic fertilizer 100 g/L, Y3 = continuous cropping soil + biological organic fertilizer 150 g/L, Y4 = continuous cropping soil + biological organic fertilizer 200 g/L; in the ordinate: Pn = net photosynthetic rate, Ci = intercellular CO_2 concentration, Gs = stomatal conductance, Tr = transpiration rate; different letters indicate significant differences between means using Duncan's multiple range test, and the significance level of all data was analyzed at $p < 0.05$.

3.3 Effects of Different Fertilizer Treatments on Photosynthetic Characteristics of *Z. simulans* Seedlings

The Pn directly reflects the photosynthetic capacity of green plants. Under certain conditions, the higher the Pn, the stronger the photosynthetic capacity of a plant. Pn also determines crop yield to some extent. In each of the treatment groups, Pn was higher than in the CK (Fig. 3) (being 25.75%, 91.68%, 50.33% and 18.89% for Y1, Y2, Y3 and Y4, respectively). Tr increased by 100.63%, 200.21%, 245.89% and 126.74% for Y1, Y2, Y3 and Y4, respectively, compared with the CK, with the Y3 treatment being the

most significant different ($p < 0.05$).

The Gs reflects the degree of stomatal opening of plant leaves and is an important physiological indicator reflecting the gas exchange capacity between plant and the outside world [26]. Studies have shown that Gs can also promote Pn and Tr to some extent [27]. Gs increased by 100.0%, 233.3%, 133.3% and 118.5% for Y1, Y2, Y3 and Y4, respectively, compared with the CK.

The Ci is a commonly used index in the study of photosynthetic physiology of green plants. In general, the Ci was negatively correlated with Gs and Pn,

whereas the C_i varied among the treatment groups compared with the CK. Compared with CK treatment, the average C_i of leaves treated with Y1, Y2, Y3 and Y4 decreased by 19.99%, 29.49%, 24.79% and 11.76%, respectively.

Thus, the application of biological organic fertilizer improved the P_n , Tr and G_s of leaves of *Z. simulans* to different degrees, and reduced the C_i . All treatments reached a significant level, with the most significant improvement in the 100 g/L (Y2) treatment group and the most significantly improved Tr in the 150 g/L (Y3) treatment group.

4. Discussion

4.1 Effects of Bioorganic Fertilizer Treatment on Physiological Characteristics of Leaves of *Z. simulans* Seedlings in Continuous Cropping

Continuous cropping soil can be regarded as a specific stress experienced by crops. Over time, even normal management will cause salt accumulation, making the soil harder and affecting its permeability to water and air [24]. Long-term continuous cropping causes a soil nutrient imbalance, decreased microbial diversity, and increased number of pathogenic microorganisms [23, 28]. Thus, under such adverse conditions, seedlings are difficult to grow rapidly. The results of the current study showed that, compared with the treatment groups, the CK group without bio-organic fertilizer showed slow growth with few new branches or leaves during the late growth stage of *Z. simulans* seedlings. Therefore, long-term continuous cropping can significantly inhibit seedling growth in this species.

The antioxidant enzyme content is a main index reflecting the degree of peroxidation of cells in the plant. In the current study, the activity and content of antioxidant enzymes in leaves of *Z. simulans* seedlings were studied following the application of bio-organic fertilizer. The results showed that a certain amount of bio-organic fertilizer improved the SOD and POD activity of *Z. simulans* seedlings, reduced

the MDA content and degree of membrane lipid peroxidation, and, thus, improved the ability of the plant to resist stress. These results are similar to those of Wang *et al.* [20] and Li *et al.* [27] on maize, who reported that plants resist external adverse conditions via the synergistic action between protective enzymes [29, 30].

As a product of membrane lipid peroxidation, the MDA content directly reflects the degree of membrane lipid peroxidation. According to the current results, it may be because the plants in the Y2 group were able to fully absorb the bio-organic fertilizer and decompose and transform it in a timely manner. With the high activity of various enzymes and strong metabolic capacity, peroxidation products in plants can be removed in a timely manner to prevent their accumulation, thus increasing the plant stress resistance [31, 32]. The changes recorded in levels of SOD, POD and APX were similar across the treatment groups, being the highest in the Y2 group. This suggests that the plants were able to absorb nutrients efficiently and synthesize organic matter. However, after the treatment concentration exceeded that in the Y2, MDA content gradually increased, and the SOD, POD and APX contents showed a gradual decrease. This might be because of excessive fertilizer application, causing damage to the seedlings, inhibiting seedling growth.

In plants, APX catalyzes the H_2O_2 -dependent oxidation of ascorbic acid and has an important role in maintaining plant physiology [33, 34]. In the current study, the level of APX increased with increasing fertilizer concentration, similar to that reported for apple [35]; thus, the application of bio-organic fertilizer improved the levels of APX, promoting seedling growth.

4.2 Effects of Bio-organic Fertilizer Treatment on the Photosynthetic Characteristics of *Z. simulans* Seedlings

Photosynthesis is an important indicator of the

influence of external factors on plant growth and metabolism. Generally, plant photosynthesis is easily affected by environmental conditions [35]. Stomata regulate the photosynthesis. Plants can promote transpiration by opening their stomata, thus reducing the leaf temperature, or reduce transpiration to avoid the excessive consumption of water by closing the stomata. Thus, the Tr of plants is the result of a combination of physiological factors within each plant, the light intensity, temperature, humidity, *in vitro* CO₂ concentration, and other environmental factors, and is also closely related to the strength of plant stress resistance. The results of the current study in terms of the changes in Gs, Pn and Tr of *Z. simulans* under different treatments were similar to those of Wang *et al.* [36] and Chen *et al.* [37].

The results of the current study showed that, with the increase in bio-organic fertilizer concentration, the average Gs, Pn and Tr of pepper in continuous cultivation from 14:00 to 16:00 was higher compared with the CK group, whereas the Ci showed the opposite, which was consistent with the results of Liu *et al.* [38] on young lemon trees. This might be caused by irregular stomatal opening or closing in response to the external light intensity, temperature, or climatic conditions.

It is well known the trace element content in soil is relatively low, but has a crucial role in regulating the growth, development and metabolism of plants [39]. It can be inferred from the experimental results, the application of appropriate amounts of bio-organic fertilizer can improve the aeration and porosity of the soil, significantly increasing the content of organic matter and inorganic elements in the soil and, as a result, promoting the growth of crops to a certain extent [40]. Overall, these results suggest that the application of organic fertilizer can promote the photosynthesis of *Z. simulans*.

5. Conclusions

Continuous cropping obstacles are common

problems in agriculture, seriously restricting agroforestry production and management and its sustainable development for several crops, cash crops and artificial forests in China. The current study showed that the application of bio-organic fertilizer could improve the activity of related defense enzymes, reduce the content of MDA, and promote the photosynthesis of the replanted *Z. simulans* seedlings, with the most significant results seen with treatment with 100 g/L bio-organic fertilizer. Therefore, the application of an appropriate concentration of bio-organic fertilizer to *Z. simulans* could reduce to some extent the continuous cropping obstacles associated with this crop.

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