Effects upon Olive and Changes of its Physiological Status under the Compound Stresses of Ferrum and Zinc

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Abstract. Based on the high value of olive oil, this experiment was carried out in order to study its growth under heavy metal stress. In the experiment, Fe and Zn were used as heavy metal stress factors. Effects of Fe-Zn combined stress on physiological indexes of malondialdehyde (MDA), soluble sugar, SOD, POD, CAT and soluble protein in olive leaves. The results of the last experiment showed that with the increase of Fe²⁺ and Zn²⁺ concentration, the activity of POD and CAT showed a trend of increasing first and then decreasing, and the activity of SOD enzyme was always decreased. Malondialdehyde (MDA), soluble sugar content and accommodating protein the content is gradually increasing.

1. Introduction

Olea europaea L is an evergreen tree of the genus Oleaceae. The fruit is rich in vegetable oil. The resin contains olargene and isoflavone. Its skin, leaves and fruit can be extracted from the olive. Ester, has high economic value and medicinal value [1]. Olive oil is mainly found in Mediterranean regions such as Greece and Italy. Nowadays, all countries in the world are introduced and cultivated. In the 1960s, China began to introduce olive oil in large scale [2], and Sichuan has large-scale planting in Guangyuan City, Kaijiang County and Xichang City [3]. In the Xichang area, olive oil cultivation is mainly located in Xixi Township and Lizhou Town. Elements such as Fe and Zn are of great significance to animals and plants. For example, severe Fe deficiency can lead to anemia; severe Zn deficiency may lead to Zn deficiency dwarfism [4, 5]. Both Fe and Zn are nutrients for plants to maintain normal growth [6]. If there is a lack of Fe in plants, it will lead to yellow leaf disease [7]; if apples lack Zn, it will lead to lobular disease [8]. However, the excessive accumulation of these metal elements can also cause damage to animals and plants. Excessive Fe in the soil can cause plant growth to be hindered [9]. With the development of modern technology, industrial mining is becoming larger and larger. Metals such as Fe and Zn are gradually accumulated in the surrounding soil during mining, transportation and smelting. This phenomenon may lead to excessive metal content in the soil.

Under normal circumstances, plants have a certain ability to absorb and transform metal elements and promote plant growth. Studies have confirmed [10, 11] that single Fe and Zn have important effects on the physiological properties of olive oil. In related research, the use of Fe and Zn for complex stress is rare. Among the compound stresses [12], used copper and Zn elements [13], used lead and Zn elements, and [14] used Fe and manganese as factors, and the three were subjected to stress experiments on bead buds, buds and geranium.
This experiment is to simulate the natural conditions, using Fe and Zn two metal elements to study the combined stress of olive seedlings. The olive oil seedlings in the Lizhou area of Xichang City, Liangshan Prefecture, Sichuan Province were selected and analyzed. The effects of different concentrations of heavy metal stress on plant growth and antioxidant enzyme activities were analyzed. Provide some basic theoretical basis for future olive planting.

2. Materials and research methods

2.1. Test materials and test drugs

In this test, an annual oil olive seedling was used as a material for experiments. The reagents required for this test are: ferrous sulfate heptahydrate (FeSO₄·7H₂O), Zn sulfate heptahydrate (ZnSO₄·7H₂O), disodium hydrogen phosphate (Na₂HPO₄·12H₂O), sodium dihydrogen phosphate (NaH₂PO₄·H₂O), Silica (SiO₂), riboflavin (C₁₇H₂₀N₄O₆), hydrogen peroxide (H₂O₂), Coomassie Brilliant Blue G-250, guaiacol (C₇H₈O₂), methionine (C₉H₁₈O₂NS), nitroblue tetrazolium (C₄₀H₃₀N₁₀O₆C₁₂), Trichloroacetic acid (C₂HCl₃O₂), thiobarbituric acid (C₄H₄N₂O₂S) and sodium hydroxide (Na₂OH).

2.2. Research methods

In this experiment, four concentration treatment groups and one control group were set, and each group was repeated three times, and the results were averaged. The control group was recorded as AI, and the treatment group was labeled as AII, AIII, AIV, and AV, respectively. The measurement of each index was carried out after 30 days of treatment under heavy metal stress under normal water supply conditions. The concentrations of Fe and Zn were set to AI: 400 mg/kg + 150 mg/kg, AII: 600 mg/kg + 250 mg/kg, AIII: 800 mg/kg + 300 mg/kg, and AV: 1000 mg/kg + 325 mg/kg.

2.3. Sample determination

2.3.1. Measurement indicators. In this test, SOD, POD, CAT, soluble protein, MDA (malondialdehyde) and soluble sugar.

2.3.2. Determination method. In this test, the determination of SOD was carried out by the tetrazolium blue method; the POD method was performed using the guaiacol method; the CAT was subjected to the hydrogen peroxide method; the MDA and soluble sugar were determined by the TBA method; and the soluble protein was determined by the staining method.

2.4. Statistical analysis

The data obtained in this experiment were initially summarized by EXCEL software, and the variance analysis was calculated using SPSS 19.0 software. The calculation results were labeled and analyzed using multiple comparison methods.

3. Results and analysis

3.1. Effects of Fe and Zn stress on the activity of POD in olive oil

It can be seen from Table 1 that the enzyme activity of POD in each treatment group was significantly higher than that in the control group after stress (P<0.05); the activity of POD in olive leaves increased with the concentration of metal stress, when the concentration of Fe²⁺ was 600 mg. When /Kg and Zn²⁺ are 250 mg/Kg, the POD value is the largest, and when the concentration continues to increase, the activity of the olive POD enzyme is gradually weakened. The results showed that when the concentration of Fe²⁺ and Zn²⁺ increased at a certain threshold, the activity of POD enzyme in olive leaves was stimulated. When the concentration exceeded the boundary value, the activity of POD enzyme in leaves was inhibited. . In Table 2, the value of the F statistic is 106.398. When the probability P value (significance value) is close to 0 and <0.05, it can be considered that different concentrations have a significant influence on the value of POD.
Table 1. Effect of combined stress of Fe and Zn on POD enzyme.

| Processing group | Stress concentration (mg/kg) Fe²⁺ + Zn²⁺ | POD value (u/g) |
|------------------|-----------------------------------------|----------------|
| AI               | 0 + 0                                   | 106.67±2.404 c |
| AII              | 400 + 150                               | 114.67±1.764 b |
| AIII             | 600 + 250                               | 122.00±3.055 a |
| AIV              | 800 + 300                               | 88.67±0.667 d  |
| AV               | 1000 + 325                              | 68.67±1.764 e  |

Table 2. POD variance analysis results.

| Analysis of variance (POD) | sum of square | df | Mean square | F    | Significant |
|-----------------------------|---------------|----|-------------|------|-------------|
| Group                       | 5561.067      | 4  | 1390.267    | 106.398 | 0.000       |
| Within the group            | 130.667       | 10 | 13.067      |       |             |
| Total                       | 5691.733      | 14 |             |       |             |

3.2. Effect of Fe and Zn stress on CAT activity of olive oil

It can be seen from Table 3 that the activity of CAT of O. oleracea L. shows a first increase and then decrease with the concentration change of Fe and Zn. The activity is achieved when the metal concentration is Fe²⁺ (600mg/Kg) and Zn²⁺ (250mg/Kg). The peak activity of CAT was significantly higher than that of the control group (P<0.05). At this time, the activity was enhanced by 52.63%. The significant difference between AV and AI, AII and AIII was significant, but not significant compared with AIV (P<0.05). It can be seen from Table 4 that the concentration of Fe-Zn combined stress has a significant effect on the CAT value.

Table 3. Effect of combined stress of Fe and Zn on CAT enzyme.

| Processing group | Stress concentration (mg/kg) Fe²⁺ + Zn²⁺ | CAT value (u/g) |
|------------------|-----------------------------------------|----------------|
| AI               | 0 + 0                                   | 9.50±0.500 b   |
| AII              | 400 + 150                               | 10.83±0.441 b  |
| AIII             | 600 + 250                               | 14.50±1.500 a  |
| AIV              | 800 + 300                               | 8.667±0.441 bc |
| AV               | 1000 + 325                              | 6.667±0.882 c  |

Table 4. CAT analysis of variance results.

| Analysis of variance (CAT) | sum of square | df | Mean square | F     | Significant |
|---------------------------|---------------|----|-------------|-------|-------------|
| Group                     | 102.233       | 4  | 25.558      | 11.617| 0.001       |
| Within the group          | 22.000        | 10 | 2.200       |       |             |
| Total                     | 124.233       | 14 |             |       |             |

3.3. Effects of Fe and Zn stress on the activity of SOD in olive oil

SOD enzyme is present in animals and plants, and its role is to protect cells exposed to oxygen, which is an important antioxidant [15]. The change of SOD activity of the olive oil after the combined stress treatment is shown in Table 5. There is a significant difference between the control group and the SOD enzyme activities in each concentration treatment group (P<0.05). The activity of SOD enzyme decreased with the increase of Fe²⁺ and Zn²⁺ concentrations. The concentration of each concentration group decreased by 32.16%, 35.01%, 37.25% and 43.99%, respectively. The decrease of vigor was
obvious, but the changes in each concentration treatment group were relatively gradual, the difference was not obvious (P>0.05). When the concentration of Fe$^{2+}$ and Zn$^{2+}$ increased from 0 to 1000 and 325, the activity of SOD in olive leaves was inhibited. From Table 5, there is a significant difference between concentration and SOD.

(Note: Vitality decline rate = (1 – $\frac{Ai}{AI}$)×100%, $i$ = II, III, IV, V.)

Table 5. Effect of combined stress of Fe and Zn on SOD enzyme.

| Processing group | Stress concentration (mg/kg) | SOD value (Fw/g) |
|------------------|-----------------------------|------------------|
| AI               | 0 + 0                       | 14.889±0.047 a   |
| AII              | 400 + 150                   | 10.100±0.367 b   |
| AIII             | 600 + 250                   | 9.800±0.633 b    |
| AIV              | 800 + 300                   | 9.342±0.926 b    |
| AV               | 1000 + 325                  | 8.338±0.930 b    |

Table 6. SOD analysis of variance results.

| Analysis of variance (SOD) | sum of square | df | Mean square | F     | Significant |
|----------------------------|---------------|----|-------------|-------|-------------|
| Group                      | 77.770        | 4  | 19.442      | 14.336| 0.000       |
| Within the group           | 13.562        | 10 | 1.356       |       |             |
| Total                      | 91.332        | 14 |             |       |             |

3.4. Effects of Fe and Zn stress on MDA content in leaves

Malondialdehyde (MDA) content is an important indicator used to indicate the level of stress in plants under adverse conditions [16]. The higher the content of MDA, the more obvious the stress of the plants.

It can be seen from Table 7 that the MDA content in the olive oil showed an increasing change with the treatment concentration after the combined action of Fe and Zn. The difference of malondialdehyde content between the control group and each concentration treatment group was significant. (P<0.05), the values of the treatment groups in each concentration were 1.75, 1.81, 1.98 and 2.45 times of the control group, respectively, indicating that the response of olive oil to the combined stress of Fe and Zn is getting stronger and stronger. In Table 8, the probability P value is 0.004, <0.005, indicating that different concentrations of Fe and Zn significantly affect the MDA content.

(Note: Change in content = $\frac{Ai}{AI}$; $i$ = II, III, IV, V)

Table 7. Effect of combined stress of Fe and Zn on MDA.

| Processing group | Stress concentration (mg/kg) | MDA(umol/L) |
|------------------|-----------------------------|-------------|
| AI               | 0 + 0                       | 0.148±0.022 c |
| AII              | 400 + 150                   | 0.260±0.036 b |
| AIII             | 600 + 250                   | 0.268±0.022 b |
| AIV              | 800 + 300                   | 0.293±0.012 ab|
| AV               | 1000 + 325                  | 0.362±0.038 a |
Table 8. MDA ANOVA data.

| Analysis of variance (MDA) | sum of square | df | Mean square | F      | Significant |
|----------------------------|--------------|----|-------------|--------|-------------|
| Group                      | 0.072        | 4  | 0.18        | 7.820  | 0.004       |
| Within the group           | 0.023        | 10 | 0.002       |        |             |
| Total                      | 0.095        | 14 |             |        |             |

3.5. Effects of Fe and Zn combined stress on soluble protein in leaves

It can be seen from Table 9 that there is no significant difference in the concentration of each concentration stress group (P>0.05), and the difference of soluble protein content in the leaves of olive leaves is small, indicating that the seeds of olive seedlings are under different concentrations of stress. The soluble protein content has less effect. If the significance test level is 0.05, the P value in Table 10 is 0.783, 0.783>0.05, the concentration does not have a significant effect on the soluble protein content.

Table 9. Effect of Fe and Zn combined stress on soluble protein.

| Processing group | Stress concentration (mg/kg) | Soluble protein (umol/L) |
|-----------------|-----------------------------|--------------------------|
|                 | Fe\textsuperscript{2+} + Zn\textsuperscript{2+} |                           |
| AI              | 0 + 0                       | 0.058±0.005 a             |
| AII             | 400 + 150                   | 0.059±0.003 a             |
| AIII            | 600 + 250                   | 0.061±0.004 a             |
| AIV             | 800 + 300                   | 0.062±0.002 a             |
| AV              | 1000 + 325                  | 0.064±0.004 a             |

Table 10. Soluble protein analysis of variance data.

| Analysis of variance (soluble protein) | sum of square | df | Mean square | F      | Significant |
|---------------------------------------|--------------|----|-------------|--------|-------------|
| Group                                 | 0.000        | 4  | 0.000       | 0.432  | 0.783       |
| Within the group                      | 0.000        | 10 | 0.000       |        |             |
| Total                                 | 0.000        | 14 |             |        |             |

3.6. Effect of Fe and Zn combined stress on soluble sugar in leaves

Studies have shown that [17, 18], when the degree of stress is increased, the content of soluble sugar will increase, and the more unfavorable the plant growth.

It can be seen from Table 11 that there is no significant difference in the soluble sugar content between the control group AI and the treated group AII, AIII, AIV and AV (P<0.05), and the soluble sugar content in the leaves of the olive seedling leaves still with the compound stress concentration. The increase and the gradual increase indicate that the growth of the olive seedlings is inhibited, which is consistent with the results exhibited by the olive leaf MDA. In Table 12, the P value was 0.610, and the concentration did not significantly affect the content of soluble sugar.

Table 11. Effect of combined stress of Fe and Zn on soluble sugar.

| Processing group | Stress concentration (mg/kg) | Soluble sugar (umol/L) |
|-----------------|-----------------------------|------------------------|
|                 | Fe\textsuperscript{2+} + Zn\textsuperscript{2+} |                         |
| AI              | 0 + 0                       | 3.603±0.285 a          |
| AII             | 400 + 150                   | 3.966±0.139 a          |
| AIII            | 600 + 250                   | 4.122±0.270 a          |
| AIV             | 800 + 300                   | 4.286±0.726 a          |
| AV              | 1000 + 325                  | 4.481±0.310 a          |
Table 12. Soluble sugar variance analysis data.

| Analysis of variance (soluble sugar value) | sum of square | df | Mean square | F    | Significant |
|------------------------------------------|---------------|----|-------------|------|-------------|
| Group                                    | 1.334         | 4  | 0.334       | 0.698| 0.610       |
| Within the group                         | 4.777         | 10 | 0.478       |      |             |
| Total                                    | 6.112         | 14 |             |      |             |

3.7. Effect of Fe and Zn stress on the growth of olive plants

After 30 days of treatment, it can be seen from Table 13 that the plant height of the olive seedlings increased, but the growth rate was different. The growth of the seedlings without stress was faster. With the increase of the concentration, the growth rate of the olive seedlings showed a downward trend. The growth rate of the control group was 60.32%, 42.06%, 31.74% and 22.22%, and under the high concentration of stress, the leaves of the seedlings showed yellowing, indicating that different treatment concentrations had a greater impact on the growth rate of the plants.

(Note: $\text{Growth rate} = \left(\frac{\text{Ai} + \text{AI}}{2}\right) \times 100\%$; $0 = \text{II, III, I}$; $V_o$

Table 13. Effect of combined stress of Fe and Zn on plant development status.

| Processing group | Before treatment | After 30d processing | Amount of change |
|------------------|------------------|----------------------|------------------|
|                  | Plant height (cm)| Plant height (cm)    | (cm)             |
| AI               | 71.0             | 83.6 -               | 12.6             |
| AII              | 71.0             | 78.6 -               | 7.6              |
| AIII             | 70.8             | 76.1 -               | 5.3              |
| AIV              | 69.4             | 73.4 -               | 4                |
| AV               | 72.1             | 74.9 -               | 2.8              |

Note: Growth status: - the plant is normal, - there is a yellowing phenomenon.

4. Conclusion

Under the combined action of low concentration of Fe and Zn, the olive oil showed some adaptability and the plants grew normally. With the increase of Fe$^{2+}$ and Zn$^{2+}$ concentration, the regulation ability of olive seedlings decreased, especially the activity of POD and CAT became weaker, the activity of SOD decreased, and the growth of plants was slow. Among them, malondialdehyde (MDA), soluble sugar and soluble protein increased in the whole complex stress, and the plants showed some resistance.

(1) Reactive oxygen free radicals (ROS) are highly active substances commonly found in animals and plants. They are easy to react with cells in the body, causing damage to cellular components and further affecting the normal growth of organisms [19, 20]. POD enzyme, CAT enzyme and SOD enzyme are collectively referred to as animal and plant antioxidant enzyme protection system to destroy active oxygen free radicals. Under normal circumstances, ROS can be effectively removed for protection purposes [21]. The decrease of SOD activity is the phenomenon that active free radicals can not be eliminated. The olive seedlings are inhibited at this time, and the normal development of seedlings is restricted. When the concentration of Fe-Zn combined stress is 600mg/kg or 250mg/kg, CAT and POD activity is enhanced to play a role in protecting olive plants. When the concentration of stress continues to increase, the ability of POD and CAT enzymes to scavenge ROS decreases, and the ability of plants to resist stress is weakened, resulting in the growth and development of plants. It can be seen that the antioxidant system can protect the olive seedlings in a relatively small concentration environment.

(2) Under normal circumstances, the content of MDA and soluble sugar and soluble protein are directly proportional to the degree of stress [16-18]. Under the condition of increasing concentration of...
Fe and Zn combined stress, both MDA and soluble sugar content showed an increasing trend, which confirmed the situation in Table 13. The greater the concentration of stress, the more unfavorable to plant growth.

3) In plants, soluble proteins can be used to express plant resistance [22]. In the experiment, the soluble protein content has been increased, which is an adaptation of the olive seedlings to the adverse environment after being stressed.

4) The different stress concentrations in this experiment have a significant effect on the results of POD, CAT, SOD and MDA, but the effects on soluble protein and soluble sugar are opposite.

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