Physiological Effects of Combined Stress of Cd, Pb and Ti on Olive

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Abstract. The physiological effects of Cd, Pb and Ti combined stresses on 3-year-old Olive seedlings were studied in this experiment and the change of heavy metal content in the soil after stress. The results showed that soluble protein, SOD activity and soluble sugar increased first and then decreased with the increase of Cd and Pb combined stress concentration, and then increased again with the addition of 2% and 4% Ti. POD activity decreased first then increased then decreased. The change of heavy metal content in the soil after stress first increased then decreased. Therefore, when the content of heavy metals in soil is low, olive can defend and repair itself to reduce the harm of growing environment. On the contrary, when the metal content in soil is too high, the decrease of plant stress resistance will be detrimental to its growth.

Key words: Three-year-old olive; Cd; Pb; Ti; Combined stress; Physiological effects.

1. Introduction
Olive oil is an oil plant belonging to the olean in the genus Oleaceae. These crops are mainly distributed along the Mediterranean coast, such as: Israel, Italy, Croatia and other countries, and are economic fruits that are indispensable in the subtropical region [1]. In today's domestic and international markets, olive oil occupies a large share, which covers a wide range of fields, such as processing, research, and consumption.

Studies have shown that the temperature difference between day and night in Liangshan Prefecture is large, the sun is abundant, and the dry and wet seasons are distinct. It is one of the best growing places for olive oil. Under this climatic condition, it is beneficial to the accumulation of nutrients in olive oil [1]. However, due to its rich mineral resources, most of the related enterprises in Liangshan Prefecture have distributed a large amount of harmful substances to nature, which has aggravated the heavy metal content in the soil, causing pollution to the olive oil growth environment, resulting in a decline in the quality of olive oil.

This experiment is a physiological effect test of Cd, Pb and Ti combined stress on olive oil. Cd has the characteristics of fast moving speed, high pollution degree and high toxicity. It is a "representative...
of heavy metal pollution", and low concentration can cause uncontrollable damage [2]. Pb has the characteristics of strong biological toxicity and high environmental pollution. It is one of the “five poisons” of heavy metal pollution [3]. The damage caused by excessive Cd and Pb in soil has exceeded the controllable range. Ti is a rare metal element. In the early years, there have been reports on the application of Ti in agriculture [4]. Rational application of Ti can enhance photosynthesis, resistance and stress resistance of plants [4]. In this study, the physiological changes of the olive seedlings under different stress concentrations of Cd, Pb and Ti and the changes of heavy metal contents in the soil were studied to explore the reasonable growth range and resistance mechanism of the olive seedlings under heavy metal pollution.

2. Test materials and methods

2.1. Test materials
In this experiment, three-year-old olive seedlings with the same plant type and small differences (about 75 cm in height and about 46 cm in crown width) and no disease and mechanical damage were selected as test materials, and the strain was soybean fruit, and the number of plants was 21. Purchased in Lizhou Town, Xichang City in March 2018. After being transported back to the school campus, the natural soil was taken for pot experiment (pot height 19.7 cm, outer diameter 29.5 cm, inner diameter 25.5 cm), and the seedling pot weight was about 6.5 kg. For a period of 2 months, the seedling treatment should be carried out. During the slow seedling period, attention should be paid to ventilation, heat preservation and moisturization, disease prevention, and plant integrity to carry out relevant tests.

2.2. Test design
This test is a multi-factor stress test with a total of 6 treatments and a blank control CK, each treatment repeated 3 times (see Table 1):

| Test number | Cd (mg/kg) | Pb (mg/kg) | Ti (%) |
|-------------|------------|------------|--------|
| T1          | 50         | 300        | 0      |
| T2          | 50         | 600        | 0      |
| T3          | 100        | 300        | 0      |
| T4          | 100        | 600        | 0      |
| T5          | 100        | 600        | 2%     |
| T6          | 100        | 600        | 4%     |
| CK          | 0          | 0          | 0      |

2.3. Test drugs and instruments
Test drugs: thiobarbituric acid, Cd chloride, Pb nitrate, Ti gypsum, quartz sand, distilled water, nitroblue tetrazolium, L-methionine, phosphoric acid, Coomassie Brilliant Blue G-250, guaiacol, bovine serum Protein and so on.
Test equipment: bench-top refrigerated centrifuge (Universal-320R), visible spectrophotometer (722NX), three water tank (600), electric constant temperature drying oven (202-3A).

2.4. Measurement indicators and methods

2.4.1. Extracting enzyme solution. 8 to 9 leaves of each olive oil were taken, placed in an ice box and taken back to the laboratory, the veins were °cut, and the leaves were cut, each weighing 0.3g. 2ml of pH7.8 phosphate buffer solution and an appropriate amount of quartz sand were placed in a pre-cooled mortar to grind the leaves into a slurry, and then the mortar was rinsed with a buffer solution to a final volume of 6 ml. Next, the slurry was placed in a 15000 g centrifuge at 4 °C for 10 min (TGL-16) for the supernatant, and finally stored at a low temperature of 4 °C to measure the desired index.
2.4.2. **Measurement indicators.** Soluble protein: Coomassie Brilliant Blue G-250 staining method [5]; POD activity: guaiacol method [6]; MDA: thiobarbituric acid (TBA) [7]; Soluble sugar: thiobarbituric acid (TBA) [8]; SOD activity: nitroblue tetrazolium photoreduction method [9]; Heavy metal content in soil after stress: atomic absorption spectrometry (AAS) [10].

2.4.3. **Processing and analysis of data.** The experiment was repeated 3 times, the final data was taken as the average value, and statistical analysis was performed by SPSS 20.0 software (F=0.05).

3. **Results analysis**

3.1. **Effects of combined stress of Cd, Pb and Ti on POD activity of olive oil**

Peroxidase (POD) is an antioxidant enzyme in plants [11], which can reduce damage to plants caused by adverse changes in the external environment and maintain the balance of free radicals in plants [12].

![Figure 1. Effect of combined stress of Cd, Pb and Ti on POD activity of olive oil](image)

It can be seen from Fig.1 that the overall difference was significant (p<0.05) when Cd, Pb, and Ti were combined. When the concentration of Cd was the same and the concentration of Pb was different, the activity of T2 and T1, T4 and T3 decreased by 3.10% and 19.6%, and the difference between T4 and T3 was significant (p<0.05). When the concentration of Pb was the same and the concentration of Cd was different. The T3 and T1, T4 and T2 activities were decreased by 16.9% and 31%, respectively, and the difference between T4 and T2 was extremely significant (p<0.01). It can be seen that the binding of heavy metal ions to the nucleic acid in the olive seedlings increases with the increase of the treatment concentration, and finally destroys the enzymes of the plant system, resulting in a decrease in POD activity [13]. When 2% and 4% Ti were added on the basis of T4, the ratio of T5, T6 and T4 increased by 37.8% and 53.9%, and the difference was extremely significant (p<0.01). It can be seen that the addition of Ti to the soil can reduce the absorption of Cd and Pb by the olive seedlings and reduce the damage of heavy metals to the olive seedlings [14].

3.2. **Effect of combined stress of Cd, Pb and Ti on MDA of olive oil**

Malondialdehyde (MDA) is a decomposition product after membrane lipid peroxidation and belongs to the decomposition of unsaturated fatty acids [15]. Heavy metal stress induces an increase in free radical content in plants, Pbing to an increase in MDA, so changes in MDA content are often used to reflect the extent of oxidative damage in plants [15].
Figure 2. Effect of combined stress of Cd, Pb and Ti on MDA of olive oil

It can be seen from Fig.2 that the overall difference was significant (p<0.05) when Cd, Pb, and Ti were combined. When the concentration of Cd was the same and the concentration of Pb was different, the content of T2 and T1, T4 and T3 increased by 4.1% and 13.8%, and the difference between T4 and T3 was significant (p<0.05). Within a certain range, olive oil could transform heavy metals through its own function. To promote the growth of the substance; when the concentration of Cb is the same, when the concentration of Cd is different, the MDA content of T3 is increased by 20.4% and 29.4% compared with T1, T4 and T2, and the difference between T3 and T1 is significant (p<0.05), T4 The difference between T2 and T2 was extremely significant (p<0.01). Peng Ling [16] and other research results show that under the combined stress of Cd and Pb, the damage of Cd is greater than that of Pb. When the treatment concentration is low, the plant antioxidant system can respond in time to clean up the reactive oxygen species in plants and reduce MDA. The production causes MDA to discharge the plant body; when the treatment concentration is high, the antioxidant system is blocked, the cells are damaged, and the MDA content is increased. It can be seen that the test results are consistent with the conclusions. When 2% and 4% Ti were added on the basis of T4, the degree of membrane lipid peroxidation of the olive seedlings decreased, the anti-aging and stress resistance increased, so the MDA content decreased.

3.3. Effect of combined stress of Cd, Pb and Ti on SOD activity of olive oil

Superoxide dismutase (SOD) is an enzyme that scavenges superoxide radical metals in the body and plays a vital role in preventing cells from being poisoned by oxygen free radicals [16].

Figure 3. Effect of combined stress of Cd, Pb and Ti on SOD activity of olive oil

It can be seen from Fig.3 that the overall difference was significant when Cd, Pb, and Ti were combined (p<0.05). When the concentration of Cd was the same and the concentration of Pb was different, the activity of T2 and T1, T4 and T3 decreased by 7.35% and 12.6%, and the difference between T4 and T3 was significant (p<0.05). When the concentration of Pb was the same, the concentration of Cd was different. T3 and T1, T4 and T2 were 27% and 31.2% lower than SOD activity, and the difference between T4 and T2 was extremely significant (p<0.01). When the concentration is
low, the olive seedlings can exert their own regulatory functions to resist the change of the environment; when the concentration is higher, the compound stress exceeds the normal tolerance range of the olive seedlings, and the cell membrane is damaged, which will inhibit the growth of the olive seedlings. When 2% and 4% Ti were added on the basis of T4, the SOD activity of T5 and T6 rose again, and T6 rose by 15%, and the difference was significant (p<0.05). It can be seen that the addition of Ti helps to enhance the production of active oxygen.

3.4. Effects of combined stress of Cd, Pb and Ti on soluble sugar and soluble protein in olive oil

An important osmotic regulator in soluble sugar genus, which is inextricably linked to stress resistance [17]. Soluble proteins play an extremely important role in the growth of plants and are important physiological and biochemical markers [12]. Its content is related to the permeability of functional proteins and cells [13].

![Figure 4. Effect of combined stress of Cd, Pb and Ti on soluble sugar in olive oil](image)

As can be seen from Fig. 4, when Cd, Pb, and Ti were combined, the overall difference was significant (p < 0.05). When the concentration of Cd was the same and the concentration of Pb was different, the soluble sugar content of T2 and T1, T4 and T3 decreased by 3.2% and 18.6%, and the difference between T4 and T3 was significant (p<0.05). When the concentration of Pb was the same, the concentration of Cd was not. At the same time, T3 and T1, T4 and T2 decreased the soluble sugar content by 19.4% and 36.5%, and the difference between T4 and T2 was extremely significant (p<0.01). Huang Kaifeng [13] and other research results show that: Cd, Pb combined stress treatment concentration is low, can maintain cell osmotic balance, induce increased soluble sugar content, it can be seen that the results of this test are consistent with its conclusion, the plant response to heavy metals is similar. After the addition of Ti on the T4 treatment, the soluble sugar content rose again, and T5 and T6 increased by 26% and 37.3%, respectively, and the difference with the T4 ratio was significant (p<0.05). It can be seen that the increase of chlorophyll content, the increase of photosynthesis intensity and the increase of soluble sugar content in leaves after application of Ti have laid a foundation for high yield [18].
Figure 5. Effect of combined stress of Cd, Pb and Ti on soluble protein of olive oil

As can be seen from Fig.5, when Cd, Pb, and Ti were combined, the overall difference was significant (p < 0.05). When the concentration of Cd was the same and the concentration of Pb was different, the soluble protein content of T2 and T1, T4 and T3 decreased by 2.0% and 16.8%, and the difference between T4 and T3 was significant (p<0.05). When the concentration of Pb was the same, the concentration of Cd was not. At the same time, T3 and T1, T4 and T2 decreased the soluble sugar content by 19.1% and 35.3%, and the difference between T4 and T2 was extremely significant (p<0.01). Shi Yatian[19] and other results showed that when Cd and Pb were combined, low concentration of heavy metal stress promoted soluble protein, and soluble protein content decreased with increasing stress. The results of this experiment are consistent with their conclusions. After applying 2% and 4% Ti on the basis of T4, the growth and metabolism of the olive seedlings were enhanced, and the soluble protein content and cell number were increased [14].

3.5. Effect of Cd, Pb and Ti on the content of heavy metals in soil after combined stress

Figure 6. Effect of Cd, Pb and Ti on the content of heavy metals in soil after combined stress

It can be seen from Fig.6 that the concentration of heavy metals in the olive planting soil is proportional to the concentration of T1, T2, T3 and T4 after Cd and Pb combined stress, and the overall difference is significant (p<0.05). With the increase of treatment concentration, the decomposition rate increased, and the content of heavy metals in the soil increased: the decomposition rate of T1 and T2 was significantly different from that of CK (p<0.05); the difference between T3, T4 and CK was extremely significant (p<0.01). When Ti was added on the basis of T3 and T4, the content of heavy metals in the soil decreased obviously. It can be seen that Ti can inhibit the decomposition of heavy metals, and the inhibition effect is more obvious with the increase of Ti concentration [14].
4. Conclusions and discussion

The physiological effects of three-year-old olive seedlings and the content of heavy metals in soil after stress were studied. The changes of physiological indexes and their own response mechanisms at different concentrations were briefly described. The following conclusions are drawn:

1. When Cd, Pb and Ti were combined, when the treatment concentration was Cd 50mg/Kg+Pb 300mg/Kg, Cd 50mg/Kg+Pb 600mg/Kg, the activity of olive antioxidant enzyme was induced, soluble protein and soluble sugar. Increased SOD activity, decreased MDA, and promoted olive growth; soluble protein, soluble sugar, POD activity, SOD activity when treated at Cd 100mg/Kg+Pb 300mg/Kg, Cd 100mg/Kg+Pb 600mg/Kg When the content of MDA increased, the change of POD activity was the largest, and the effect of olive seedlings was inhibited. The content of heavy metals in soil increased with the increase of treatment concentration after stress, and decreased after using 2% and 4%. In summary, when the treatment concentration was Cd 100mg/Kg+Pb 600mg/Kg, cell membrane permeability and peroxidase activity were the most harmful, and the inhibition was extremely significant (P<0.01); the treatment concentration was Cd 50mg/Kg+ Pb 300mg/Kg is the best tolerance range for olive seedlings, Cd 50mg/Kg+Pb 600mg/Kg, and the change of Cd concentration Pbs to the difference between Pb concentration. Cd is more toxic to olive seedlings than Pb[2]. In this test, Ti did not exceed the standard and did not cause adverse effects. On the contrary, using 2% and 4% of Ti on the basis of T4 increased the activity and resistance of the enzyme in the plant, and activated the activity of different enzymes in the olive oil [4], MDA. After content and stress, the content of heavy metals in soil decreased, POD activity, SOD activity, soluble sugar and soluble protein increased, and the damage of heavy metals to olive seedlings was inhibited.

2. In this test, the health of olive seedlings showed: CK>T1>T2>T5>T6>T3>T4. When the content of heavy metals in the soil is high, the olives will have fruit rot, severe defoliation, dead plants, slow plant growth (normal plant height of about 120cm, damaged plant height of about 80cm).

According to this study, when the concentration of Cd and Pb complex stress is low, the olive seedlings themselves have a stress mechanism, which can protect the olive seedlings from promoting their growth, such as: osmotic adjustment system, degradation of toxicity of heavy metals, and maintenance of balance in vitro and in vivo of plants. When the concentration of Cd and Pb complex stress is high, the reactive oxygen species free radicals can damage the cell membrane and affect the metabolism of the cells, resulting in the physiological function of the cells not working properly, which will destroy the physiological system, defense function and reversal of the olive seedlings. Sexually, even the beneficial substances in the olive seedlings are excreted, and in severe cases, the olive seedlings may die. In this test, 2% and 4% of Ti inhibited the decomposition of heavy metals to a certain extent. Ti is a heavy metal pollution improver. Adding an appropriate amount of Ti in the soil can reduce the adsorption of heavy metals on the soil [20], and reduce the heavy metals on the olives. Absorb and reduce the harm caused by heavy metals. It has been reported that the application of Ti to reduce the damage caused by heavy metals is rare, so it is necessary to use Ti carefully, pay attention to the amount and land used during application to prevent damage to the environment. In this test, explanations are given only based on the experimental results. For the changes in physiological indicators, the advantages and disadvantages of applying Ti, further research efforts are needed.

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