The effects of four cadmium tolerant plant straws on the growth and cadmium content of jujube seedlings

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Abstract: To study the effects of four cadmium-tolerant plant straws on the growth and cadmium content of Zizyphus acidojujuba seedlings, Zizyphus acidojujuba seedlings were planted in cadmium-contaminated soil (5mg · kg⁻¹) with cadmium-tolerant plants straws such as Ranunculus sieboldii, Clinopodium confine, Plantago asistica and Mazus pumilus through pot experiments. Results showed that the biomass, photosynthetic pigment content, peroxidase (POD) activity and soluble protein content of Zizyphus acidojujuba seedlings could be improved by applying four kinds of plant straws with cadmium tolerance to cadmium-contaminated soil. At the same time, the catalase (CAT) activity in cadmium-contaminated soil was increased, the growth of Zizyphus acidojujuba seedlings was promoted, and the cadmium content in root and overground parts of Zizyphus acidojujuba seedlings was reduced. The cadmium transfer coefficient of Zizyphus acidojujuba seedlings could be increased by only applying the straw of the Clinopodium confine, while the cadmium transfer coefficient of Zizyphus acidojujuba seedlings could be reduced by all the other straw treatments Therefore, the four kinds of cadmium-tolerant plant straws are all suitable for the restoration of cadmium-polluted Zizyphus acidojujuba gardens, among which the Ranunculus sieboldii, Plantago asistica and Mazus pumilus straws are better.

1. Introduction
Due to the acceleration of urbanization and industrialization in recent years, as well as the abuse of pesticides and fertilizers and the increase of domestic sewage discharge, the content of heavy metals in soil has increased dramatically[1]. Heavy metal cadmium is widely distributed and has high biological toxicity. It cannot be degraded by organisms and is easy to be accumulated by crops, after excessive accumulation in human body through food chain, it will produce serious toxic effects[2-3]. In view of the increasingly serious problem of cadmium pollution in soil, soil remediation technologies such as physical, chemical and biological technologies are widely used to reduce the cadmium content in soil or change the existing forms of cadmium, thus reducing the absorption of cadmium by plants and reducing its inflow into the biological chain[4], but most of these methods have unstable effects, long cycles and high costs[5].

In the current agricultural production practice, the method of returning straw to the field has been applied to pest control, weed control, promotion of nutrient recycling and improvement of soil fertility[6]. Straw returning to the field can form a good soil ecological system. In addition to providing
a certain amount of nutrients for the soil, allelochemicals released during its decay and decomposition can also affect the crop growth[7-8]. In addition, after returning straw to the field, cadmium in the soil can be fixed through adsorption, chelation and other actions. At the same time, oxygen is consumed in the decomposition process, so that the soil is in a reduced state and the formation of cadmium sulfide precipitation is promoted, thus reducing the effectiveness of cadmium in the soil and reducing the absorption of cadmium in the soil by plants[9-10]. Studies have shown that soil application of enriched plants such as Trifolium repens, Eclipta alba and Conyza canadensis straw can reduce cadmium content in stems and leaves of watercress[11]; Covering Plantago asistica straw on cadmium contaminated soil can increase the biomass of tree tomato seedlings and reduce the cadmium content in the overground part[12]. Fuyi Tang[13] and others pointed out that the application of Nasturtium officinale straw can increase the biomass of Galinsoga parviflora and reduce the cadmium content in the overground part. Therefore, under cadmium pollution conditions, straw returning to the field will have different effects on the growth of different plants and cadmium accumulation. 

Ziziphus acidojujuba is a jujube of Rhamnaceae, which is a shrub or small tree[14]. Zizyphus acidojujuba fruit has high medicinal and edible value due to its rich ascorbic acid[15]. Zizyphus acidojujuba has good disease resistance, drought resistance, barren resistance and strong adaptability. In recent years, it has been widely used as rootstock of jujube[16]. Therefore, Zizyphus acidojujuba has high economic cultivation value. However, the excessive heavy metals in the soil can obviously inhibit the growth of wild jujube after being absorbed by the root system of Zizyphus acidojujuba[17].

In view of this, the cadmium tolerant plants Ranunculus sieboldii[18], Clinopodium confirmed[18], Plantago asistica[19] and Mazus pumilus[18] were applied to cadmium contaminated soil in this experiment. The effects of cadmium-tolerant plant straws on physiological and biochemical characteristics and cadmium content of Zizyphus jujuba seedlings were studied in order to screen cadmium-tolerant plant straws which can significantly reduce cadmium content of Zizyphus jujuba seedlings under the condition of ensuring normal growth of Zizyphus jujuba seedlings, and provide reference for the repair and safe production of cadmium-polluted jujube orchards.

2. Materials and Methods

2.1. Test Materials

2.1.1. Plant Materials.
The Ranunculus sieboldii, Clinopodium confirmed, Plantago asistica and Mazus pumilus were collected from farmland near Chengdu campus of Sichuan agricultural university (30°70' N, 103°86' E) without heavy metal pollution. Cleaning the collected cadmium tolerant plant straws, deactivating enzymes at 110℃ for 15 min, drying to constant weight at 75℃, pulverizing respectively, and sieving with a 5 mm sieve for later use. Zizyphus jujuba seeds were purchased from Xingtai county, Hebei province. plump Zizyphus jujuba seeds were selected, soaked in seed after disinfection, and seedlings were raised. when the seedlings of plump Zizyphus jujuba grew to 10 cm high, the seedlings of plump Zizyphus jujuba with the same root and overground growth were selected as cadmium stress plants.

2.1.2. Soil.
The test soil was sandy loam soil and was taken from farmland near Chengdu campus of Sichuan Agricultural University (30°70' N, 103°86' E) without heavy metal pollution. Sandy loam is dried and crushed, and then CdCl2·2.5H2O analytically pure solution is added to the sandy loam, and is fully mixed evenly, so that the cadmium content in the soil is 5 mg · kg-1. Place it in a place with ventilation and shelter from rain and put it in a basin (2.5 kg/basin) after balancing for one month for later use.
2.2. Test Methods

2.2.1. Test Treatment.
The application amount of cadmium tolerant plant straw is 4 g/basin (1.6 g · kg⁻¹). After it is fully mixed with cadmium contaminated soil, appropriate amount of water is poured into the flowerpot to keep it moist. After balancing for one week, Ziziphus jujuba seedlings are uniformly transplanted into the flowerpot with 3 plants in each pot. The experiment was divided into five treatments: no cadmium tolerant plant straw (CK), Ranunculus sieboldii straw, Clinopodium confirmed straw, Plantago asistica straw and Mazus pumilus straw. 2 pots are treated as one and repeated 3 times. Potted Zizyphus jujuba seedlings were placed in a rain-proof and transparent greenhouse, and the soil moisture was kept at about 70% of the field capacity.

2.2.2. Determination Index and Method.
After 60 days of experimental treatment, some fresh leaves of Zizyphus jujuba seedlings were picked to measure the content of photosynthetic pigment (chlorophyll a, chlorophyll b and carotenoid)[20], antioxidant enzyme activity (SOD and POD)[21] and soluble protein[20]. Then the whole plant of Zizyphus jujuba seedlings is harvested, cleaned and dried with clear water, and the plant height is measured. Then the roots, stems and leaves of Zizyphus jujuba seedlings are cut off respectively, dried to constant weight after deactivation of enzymes, weighed, and the root-shoot ratio is calculated[22], and then crushed and sieved through a 3 mm sieve. Weigh 1.00 g of dried roots, stems and leaves of Zizyphus jujuba seedlings, add nitric acid and perchloric acid for digestion (volume ratio 4: 1), and determine the cadmium content by spectrometer method[23]. The soil near the roots of Zizyphus jujuba seedlings was collected, air dried, ground and screened, and the catalase activity[24] and soil urease activity[24] in the soil were determined.

2.3. Data Statistics and Analysis
SPSS software was used to analyze the variance of the experimental data. The significant difference among the treatments was expressed by different lowercase letters (P < 0.05). Transport coefficient (TF) = cadmium content in aerial parts of plants / cadmium content in roots[25].

3. Results and analysis

3.1. The Effects of Soil Application of Four Cadmium Tolerant Plant Straw on Biomass and Plant Height of Zizyphus jujuba Seedlings
From Table 1, it can be seen that the root, stem, leaf, aboveground biomass, plant height and root-shoot ratio of Zizyphus jujuba seedlings after applying cadmium-tolerant plant straws are higher than those without application. The root biomass of Zizyphus jujuba seedlings significantly increased with the application of adjacent Clinopodium confirmed and Plantago asistica straw compared with that without straw application, which were 66.67% (p < 0.05) and 73.50% (p < 0.05), respectively, while the root biomass of Zizyphus jujuba seedlings after the application of Ranunculus sieboldii and Mazus pumilus straw had no significant difference compared with that without straw application. Compared with no straw application, the plant height of Zizyphus jujuba seedlings was significantly increased by application of Ranunculus sieboldii straw, adjacent Clinopodium confine straw, Plantago asiatica straw and Mazus pumilus straw, which were 31.12% (p < 0.05), 28.43% (p < 0.05), 27.18% (p < 0.05) and 28.58% (p < 0.05), respectively. Compared with non-application of straw, application of cadmium tolerant plant straw increased the root-shoot ratio of Zizyphus jujuba seedlings in the order of Plantago asiatica straw > Ranunculus sieboldii straw > adjacent Clinopodium confine straw > Mazus pumilus straw > non-application of straw.
Table 1 Effects of Soil Application of Four Cadmium Tolerant Plant Straw on Biomass and Plant Height of Zizyphus jujuba Seedlings

| Tolerant plant        | Root/strain g$^{-1}$ | Stem/strain g$^{-1}$ | Leaves/strain g$^{-1}$ | Above ground part/strain g$^{-1}$ | Root-shoot ratio | Plant height/cm |
|-----------------------|----------------------|----------------------|------------------------|-----------------------------------|------------------|----------------|
| Not applied (CK)      | 2.34 ± 0.10c         | 0.89 ± 0.17b         | 0.73 ± 0.46b           | 1.62 ± 0.22b                      | 1.47 ± 0.20b     | 20.05 ± 0.90b  |
| Ranunculus sieboldii  | 2.64 ± 0.32c         | 0.86 ± 0.04b         | 0.60 ± 0.08b           | 1.46 ± 0.04b                      | 1.81 ± 0.26a     | 26.29 ± 0.70a  |
| Clinopodium confine   | 3.90 ± 0.25a         | 1.21 ± 0.58a         | 0.94 ± 0.74a           | 2.15 ± 0.07a                      | 1.80 ± 0.06a     | 25.75 ± 0.90a  |
| Plantago asistica     | 4.06 ± 0.35a         | 1.17 ± 0.53a         | 0.97 ± 0.74a           | 2.14 ± 0.02a                      | 1.90 ± 0.18a     | 25.50 ± 1.30a  |
| Mazus pumilus         | 2.81 ± 0.24b         | 1.07 ± 0.25b         | 0.77 ± 0.12b           | 1.84 ± 0.37b                      | 1.61 ± 0.46b     | 25.78 ± 0.58a  |

Note: English lowercase letters after the same column of data indicate that there are significant differences in some indexes among different treatments (p < 0.05). The same below.

3.2. The Effects of Soil Application of Four Cadmium Tolerant Plant Straw on Photosynthetic Pigment Content of Zizyphus jujuba Seedlings

From Table 2, it can be seen that the application of cadmium tolerant plant straws has increased the chlorophyll a content, chlorophyll b content and total chlorophyll content of Zizyphus jujuba seedlings. Compared with no straw application, the total chlorophyll of Zizyphus jujuba seedlings was significantly increased by application of Ranunculus sieboldii straw, adjacent Clinopodium confine straw, Plantago asistica straw and Mazus pumilus straw, which were 42.86% (p < 0.05), 35.71% (p < 0.05), 20.00% (p < 0.05) and 42.86% (p < 0.05), respectively. Chlorophyll a/b of Zizyphus jujuba seedlings treated with cadmium-tolerant plant straw decreased as compared with those treated with no straw. Compared with no straw application, the carotenoid content of Zizyphus jujuba seedlings treated with only the stalk of Clinopodium polycephyllum did not change, while the carotenoid content of Zizyphus jujuba seedlings treated with the other treatments increased in the order of: Mazus pumilus straw > Ranunculus sieboldii straw > Plantago asistica straw > Clinopodium confine straw = no straw application, among which the carotenoid content of Zizyphus jujuba seedlings treated with the stalk of Mazus pumilus straw rose by 21.88% (p < 0.05), which was the most significant.

Table 2 The Effects of Soil Application of Four Cadmium Tolerant Plant Straw on Photosynthetic Pigments of Zizyphus jujuba Seedlings

| Tolerant plant        | Chlorophyll a/ (mg · g$^{-1}$) | Chlorophyll b/ (mg · g$^{-1}$) | Total chlorophyll/ (mg · g$^{-1}$) | Chlorophyll a/b | Carotenoids/ (mg · g$^{-1}$) |
|-----------------------|--------------------------------|--------------------------------|------------------------------------|-----------------|-------------------------------|
| Not applied (CK)      | 1.07 ± 0.01c                    | 0.33 ± 0.01c                    | 1.40 ± 0.02c                       | 3.22 ± 0.07a    | 0.25 ± 0.00b                  |
| Ranunculus sieboldii  | 1.47 ± 0.04a                    | 0.53 ± 0.04a                    | 2.00 ± 0.08a                       | 2.71 ± 0.11b    | 0.27 ± 0.00b                  |
| Clinopodium           | 1.35 ± 0.01b                    | 0.55 ± 0.02a                    | 1.90 ± 0.03a                       | 2.47 ± 0.08c    | 0.25 ± 0.01a                  |
3.3. The Effects of Soil Application of Four Cadmium Tolerant Plant Straw on Antioxidant Enzyme Activities and Soluble Protein Content of Zizyphus jujuba Seedlings

From Table 3, it can be seen that the POD activity and soluble protein content of Zizyphus jujuba seedlings after applying cadmium tolerant plant straw are higher than those without applying straw. Compared with no straw application, SOD activity of Zizyphus jujuba seedlings treated with only Mazus pumilus straw decreased by 14.57% (p < 0.05). Compared with no straw application, the POD activity of Zizyphus jujuba seedlings was significantly increased by application of Ranunculus sieboldii straw, adjacent Clinopodium confine straw, Plantago asistica straw and Mazus pumilus straw, which were 40.02% (p < 0.05), 21.41% (p < 0.05), 40.53% (p < 0.05) and 77.86% (p < 0.05), respectively. At the same time, the soluble protein content of Zizyphus jujuba seedlings was significantly increased by application of Ranunculus sieboldii straw, adjacent Clinopodium confine straw, Plantago asistica straw and Mazus pumilus straw, which were 21.05% (p < 0.05), 81.58% (p < 0.05), 44.74% (p < 0.05), and 68.42% (p < 0.05).

| Tolerant plant  | SOD activity/ (U · g⁻¹) | POD activity/ (U · g⁻¹) | Soluble protein/ (mg g⁻¹) |
|-----------------|--------------------------|--------------------------|---------------------------|
| Not applied (CK)| 128.97 ± 8.17bc          | 549.07 ± 6.20c           | 0.38 ± 0.00c              |
| Ranunculus sieboldii | 158.32 ± 3.15a          | 768.80 ± 0.80b           | 0.46 ± 0.01b              |
| Clinopodium confine | 144.71 ± 2.70a         | 666.60 ± 1.51c           | 0.69 ± 0.02a              |
| Plantago asistica | 160.83 ± 3.64a          | 771.60 ± 6.16b           | 0.55 ± 0.01b              |
| Mazus pumilus   | 110.18 ± 13.00c         | 976.60 ± 1.31a           | 0.64 ± 0.03a              |

3.4. The Effects of Soil Application of Four Cadmium Tolerant Plant Straw on Cadmium Content of Zizyphus jujuba Seedlings

It can be seen from Table 4 that compared with no straw application, cadmium content in roots and overground parts of Zizyphus jujuba seedlings was significantly reduced after applying cadmium tolerant plant straw. The application of Ranunculus sieboldii straw, the straw from the Clinopodium confine, the straw from the Plantago asistica and the straw from Mazus pumilus significantly reduced the cadmium content in the roots of Zizyphus jujuba seedlings by 51.51% (p < 0.05), 51.27% (p < 0.05), 25.81% (p < 0.05) and 48.15% (p < 0.05), respectively, compared with the non-application of straw. After soil application of four kinds of cadmium tolerant plant straws, the cadmium content in the overground part of Zizyphus jujuba seedlings was significantly reduced compared with that without straw application. In terms of transport coefficient, compared with no straw application, except for the application of straw from the Clinopodium confine, the application of Ranunculus sieboldii, Plantago asistica and Mazus pumilus straw all reduced the cadmium transport coefficient of Zizyphus jujuba seedlings. The order of size is as follows: Clinopodium confine straw > non-applied straw > Ranunculus sieboldii straw > Mazus pumilus straw > Plantago asistica straw.
Table 4 Effects of Soil Application of Four Cadmium Tolerant Plant Straw on Cadmium Content of Zizyphus jujuba Seedlings

| Tolerant plant       | Root/ (mg · kg⁻¹) | Stem/ (mg · kg⁻¹) | Leaves/ (mg · kg⁻¹) | Above ground-part/ (mg · kg⁻¹) | Transfer coefficient (TF) |
|----------------------|-------------------|-------------------|--------------------|-------------------------------|--------------------------|
| Not applied (CK)     | 8.64 ± 0.03a      | 0.72 ± 0.01a      | 0.51 ± 0.00a       | 1.23 ± 0.01a                  | 0.14 ± 0.00a             |
| Ranunculus sieboldii | 4.19 ± 0.65b      | 0.44 ± 0.01b      | 0.12 ± 0.01c       | 0.56 ± 0.02c                  | 0.13 ± 0.01a             |
| Clinopodium confine  | 4.21 ± 0.01b      | 0.47 ± 0.01b      | 0.14 ± 0.01c       | 0.61 ± 0.01c                  | 0.15 ± 0.01a             |
| Plantago asistica    | 6.42 ± 0.20a      | 0.54 ± 0.01b      | 0.12 ± 0.02c       | 0.66 ± 0.01c                  | 0.10 ± 0.01a             |
| Mazus pumilus        | 4.48 ± 0.04b      | 0.46 ± 0.01b      | 0.09 ± 0.01c       | 0.55 ± 0.00c                  | 0.12 ± 0.00a             |

3.5. The Effects of Soil Application of Four Cadmium Tolerant Plant Straw on Soil Enzyme Activities

From Table 5, it can be seen that the application of Ranunculus sieboldii straw, the straw from Clinopodium confine, the straw from Plantago asistica, and the straw from Mazus pumilus significantly increased the CAT enzyme activity in the soil compared with the non-application of straw, with the increase rates of 100.00% (p < 0.05), 43.33% (p < 0.05), 83.33% (p < 0.05), and 38.33% (p < 0.05), respectively. As far as urease activity is concerned, the application of only the straw from Clinopodium confine increased the urease activity in the soil by 10% (p < 0.05) compared with the non-application of straw.

Table 5 Effects of Soil Application of Four Cadmium Tolerant Plant Straw on Soil Enzyme Activities

| Tolerant plant       | CAT activity/ (ml · g⁻¹) | Urease activity/ (mg · g⁻¹) |
|----------------------|--------------------------|-----------------------------|
| Not applied (CK)     | 0.60 ± 0.01c             | 0.18 ± 0.01a                |
| Ranunculus sieboldii | 1.20 ± 0.00a             | 0.07 ± 0.00c                |
| Clinopodium confine  | 0.86 ± 0.01b             | 0.20 ± 0.00a                |
| Plantago asistica    | 1.13 ± 0.02a             | 0.06 ± 0.01c                |
| Mazus pumilus        | 0.83 ± 0.01b             | 0.16 ± 0.00b                |

4. Discussion

After straw is returned to the field, the release of organic matter and trace elements in the decomposition process can increase soil nutrient content, the number of various soil microorganisms and soil enzyme activity, thus affecting the growth and development of crops and promoting the improvement of crop yield and quality[26]. Lin Lijin[27] et al. Applying Eclipta alba and Conyza canadensis straw to cadmium contaminated soil can increase the biomass of Capsella bursa-pastoris. Studies have shown that when plants are stressed by heavy metals, the efficiency of chlorophyll synthesis by chloroplasts in cells decreases and photosynthesis of plants is inhibited[28]. In this experiment, the biomass and plant height of roots, stems, leaves and overground parts of Ziziphus jujuba seedlings were increased after soil application of four cadmium tolerant plant straws. At the same time, soil application of four cadmium tolerant plant straws increased chlorophyll a content and chlorophyll b content of Zizyphus jujuba seedlings compared with no application of straws. This
shows that under cadmium pollution conditions, the application of cadmium tolerant plant straw can improve the soil fertility, increase the photosynthetic efficiency of Zizyphus jujuba seedlings, promote the formation of dry matter, and further promote the growth and development of Zizyphus jujuba seedlings.

Antioxidant system is an important protection mechanism of plants. When plants are under stress, the activity of antioxidant enzymes in their bodies increases, eliminating excessive reactive oxygen radicals in time, preventing lipid peroxidation, and thus maintaining normal growth of plants[29]. Soluble proteins can maintain the osmotic potential of cells and the number of functional proteins, thus slowing down the damage caused by stress to plants[30]. The experimental study showed that the POD activity and soluble protein content of Zizyphus jujuba seedlings were significantly increased after the application of four cadmium tolerant plant straws. This shows that soil application of cadmium tolerant plant straw is helpful to reduce the toxic effect of cadmium contaminated soil on Zizyphus jujuba seedlings. However, among the four cadmium tolerant plant straws, the SOD activity of Zizyphus jujuba seedlings after only application of Mazus pumilus straw was lower than that without application of straw. Some studies have shown that covering Plantago asistica, adjacent Clinopodium confine and Ranunculus sieboldii straw on cadmium contaminated soil can inhibit the antioxidant enzyme activity of Achyranthes bidentata, while covering Mazus pumilus grass straw can improve the antioxidant enzyme activity of Achyranthes bidentata[31], which may be due to the different effects of different plant straw on plant stress tolerance system.

After returning straw to the field, it absorbs heavy metals in soil during the process of humification, reducing the availability of heavy metals, thus reducing the absorption of heavy metals by crops[10]. The experimental study showed that the application of four kinds of cadmium tolerant plant straw significantly reduced the cadmium content in roots and overground parts of Zizyphus jujuba seedlings compared with the application of no straw. At the same time, compared with no straw application, the application of Ranunculus sieboldii, Plantago asistica and Mazus pumilus straw can reduce the cadmium transport coefficient of Zizyphus jujuba seedlings, while the application of adjacent Clinopodium confine straw can improve the cadmium transport coefficient of Zizyphus jujuba seedlings. This shows that soil application of cadmium tolerant plant straw can reduce the cadmium content of Zizyphus jujuba seedlings, reduce the cadmium transport coefficient of Zizyphus jujuba seedlings, and inhibit the transport of cadmium from the roots of Zizyphus jujuba seedlings to the overground part.

Soil enzyme activity is an important index to evaluate soil fertility. Soil urease and CAT enzyme mainly decompose soil nutrients and promote plant absorption and utilization[24]. In this experiment, the application of four cadmium tolerant plant straws significantly increased CAT activity in soil. In addition, the urease activity in the soil was improved by applying only the straw of the adjacent Clinopodium confine album after applying the four cadmium tolerant plant straws. This shows that soil application of cadmium tolerant plant straw is helpful to improve the enzyme activity in cadmium contaminated soil and promote the absorption and growth of soil nutrients by Zizyphus jujuba seedlings.

5. Conclusion
To sum up, the application of cadmium-tolerant plants Ranunculus sieboldii, adjacent Clinopodium confine, Plantago asistica and Mazus pumilus straw in cadmium contaminated soil, compared with no application of straw, has increased the biomass, photosynthetic pigment content, POD activity and soluble protein content of Zizyphus jujuba seedlings, as well as the CAT activity in the soil, thus improving the photosynthetic efficiency of Zizyphus jujuba seedlings under cadmium contaminated conditions, reducing the toxic effect of cadmium on Zizyphus jujuba seedlings and promoting the growth of Zizyphus jujuba seedlings. At the same time, soil application of four cadmium tolerant plant straws reduced the cadmium content in roots and overground parts of Zizyphus jujuba seedlings. In addition, the application of Ranunculus sieboldii, Plantago asistica and Mazus pumilus straw all reduced the cadmium transport coefficient of Zizyphus jujuba seedlings, while the application of
adjacent Clinopodium confine straw increased the transport coefficient. Therefore, the application of
four kinds of cadmium-tolerant plant straw is beneficial to the growth and safe production of Zizyphus
jujuba seedlings, among which Ranunculus sieboldii, Plantago asistica and Mazus pumilus straw have
better effects and can be applied in the process of repairing cadmium-polluted jujube orchards.

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