Effects of Different Pesticide Treatments on Soft Rot Control and Yield of Konjac

Xiong Jiang, Zhengxiang Wang, Fuqing Yang, Zhizhou Chen, Sumian Yang, Zesheng Yan and Yaoguo Qin*

College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China

*Corresponding author’s e-mail: qinyaoguo@sicau.edu.cn

Abstract. To screen pesticides with improved disease control and increase the yield of konjac plant, we grew one-year-old konjac corms, determined the field control effect, growth indicators, photosynthetic indicators, and yield of konjac plants, and compared the results with those obtained after treatments with three kinds of antibiotic pesticides, three kinds of copper fungicides, chloroisobromine cyanuric acid, bismethiazol, and water (as control). The results showed that the soft rot incidences in konjac plants after treatment with several pesticides were significantly lower than that of the control (CK). The control effects of tetramycin, copper succinate, and chloroisobromine cyanuric acid were high, the growth status of the plants treated with cuprous oxide, tetramycin, and copper succinate improved; the net photosynthetic rates of each pesticide treatment showed no significant difference from that of the CK, and the yield was the highest with copper succinate and tetramycin. This study demonstrated that certain copper fungicides or antibiotic pesticides can be used for the control of konjac soft rot, but their effects are different. The comprehensive comparison suggests that the effects of copper succinate and tetramycin are the best for the disease control and yield increase of konjac.

1. Introduction
Konjac (Amorphophallus konjac) belongs to the perennial herbs of genus Amorphophallus Blume, Family Araceae. This plant is widely distributed in Southern China, to the Himalaya Range in the west, Indochina Peninsula in the south, and Japan in the east[1]. Konjac is an important economic crop rich in glucomannan, which has unique physical and chemical properties and physiological functions and is widely used in food, medicine, and chemical industries[2-3]. As the demand for konjac and its processed products in markets continually increased, the planting industry has become a bottleneck in the konjac industrial development, and how to increase the yield of konjac considerably has become an urgent problem to be solved.

With the continuous growth of konjac large-scale planting areas and the changes in cultivation environment, konjac diseases often occur. The most serious disease that harms konjac is soft rot, and the output loss often reaches 30% to 50%. The loss amounts to more than 80% in serious cases or results in the lack of harvest[4]. The disease has become the major factor restricting the development of konjac industry. The pathogens of konjac soft rot are members of Erwinia[5], including Erwinia carotovora subsp. carotovora and Erwinia chrysanthemi[6-10]. However, other pathogenic bacteria need to be determined. The pathogens invade mainly from the young organs or wounds of the plant and spread through the seed corm, soil, rainwater, and disease residue[11]. Compared with other crops
that can be infected with bacterial soft rot, konjac is particularly seriously damaged, probably because of the preference of pathogenic bacteria for the glucomannan contained in corms or the easy infection and transmission route. The exact reason is still difficult to explain[1]. Therefore, effective control methods for this disease must be studied.

Previous studies of konjac soft rot typically concentrated on chemical and biological control[12-14], but their practical application effects often differ in various regions. The disease-resistant gene is transferred to konjac corn tissues through genetic engineering to obtain disease-resistant materials[15-16]. The disease-resistant *Amorphophallus bulbifer* is now being grown[17]. In addition, suitable areas are selected to grow konjac[18]. As konjac with the largest cultivation area has no soft rot resistant cultivars, to date, however, a particularly effective method to control the disease has not been found, and achieving control in many areas, especially in low-altitude planting locations, is still difficult. In this experiment, eight pesticides, including antibiotics, copper fungicides, chloroisobromine cyanuric acid, and bismethiazol, were used to soak the seeds of konjac. The pesticides were sprayed and poured on plants in the field to select those with good control effects. Their effects on plant growth, photosynthesis, and yield were studied, providing a theoretical basis for the control of konjac diseases and high yield cultivation.

2. Materials and methods

2.1. Materials

One-year-old seed corms of *Amorphophallus konjac* cv. “Chumohua Yihao” were obtained from Chengdu Yucai Konjac Technology Co., Ltd.

The tested pesticides were as follows: tetramycin (0.3% aqueous solution; Liaoning Wkioc Bioengineering Co., Ltd.); zhongshengmycin (2% wettable powder; Shandong Yijia Agrochemical Co., Ltd.); streptomycin (72% wettable powder; Chongqing Shuangqiao Agricultural Chemical Factory); copper succinate (30% wettable powder; Beijing Beinong Luheng Technology Development Co., Ltd.); cuprous oxide (86.2% wettable powder; Tongchuang Chemical Technology Co., Ltd.); copper calcium sulfate (77% wet powder; Tongchuang Chemical Technology Co., Ltd.); chloroisobromine cyanuric acid (50% water soluble powder; Zhengzhou Deminxin Agricultural Biotechnology Co., Ltd.); bismethiazol (20% wettable powder; Zhejiang Longwan Chemical Co., Ltd.).

2.2. Methods

2.2.1. Disinfection of seed corms.

Non-invasive konjac seed corms of the same size were selected. After exposed to the sun for 2 days, the seed corms were soaked in different pesticides for 4 h, and water served as the control (CK). Then, the seed corms were taken out for drying. These pesticides used were prepared according to their commonly used concentrations, respectively: tetramycin (0.3% AS) 600 times solution, zhongshengmycin (2% WP) 1200 times solution, streptomycin (72% WP) 1500 times solution, copper succinate (30% WP) 300 times solution, cuprous oxide (86.2% WP) 800 times solution, copper calcium sulfate (77% WP) 1200 times solution, chloroisobromine cyanuric acid (50% SP) 1000 times solution, bismethiazol (20% WP) 600 times solution.

2.2.2. Soil preparation and sowing.

The experiment was conducted on Chengdu Plain (30°70‘N, 103°85‘E; altitude: 633 m) in Sichuan Province from April 2017 to December 2017. In late April, the seed corms were sowed when the average ground temperature at 10 cm depth within 5 days reached 10 °C or higher. Prior to sowing, soil preparation and fertilization were performed with 3000 kg organic fertilizer and 50 kg konjac special fertilizer per 667 m2 as the base fertilizer. Ditches with 10 cm depth were dug, and the seed corms were then sowed and covered with about 8 cm-thick soil. Sowing distances were 40 cm×15 cm.
The plot area was 2 m², with 40 plants in each plot arranged in a randomized block design with three replications.

2.2.3. Pesticide treatment.
In each plot, the same pesticides and their concentrations were used for foliar spraying and pouring root in late June and early and late July.

2.2.4. Measurement indicators and methods.
In early September, the incidence of konjac soft rot in every plot was investigated, and the incidence rate and control effect were calculated in accordance with the following formula. Incidence rate of soft rot (%)= number of diseased plants/total number of plants × 100%. Control effect (%) = (incidence rate of control - incidence rate of treatment)/incidence rate of control × 100%.

2.2.5. Determination of growth indicators.
During the expanding stage of corms, 10 plants were randomly selected from each plot to measure the plant height, plant width, petiole diameter, and leaf area.

2.2.6. Determination of photosynthetic indicators.
During the expanding stage of corms, starting from 9:00 in a clear day, the top leaflets of four plants were randomly selected in each plot. The net photosynthetic rate ($P_n$), stomatal conductance ($G_s$), intercellular CO2 concentration ($C_i$), and transpiration rate ($T_r$) were determined using a LI-6400XT photosynthesis system (Li-Cor, USA).

2.2.7. Determination of yield indicators.
After the corms were harvested, 10 corms were randomly selected from each plot to measure the corm weight, and plot yield was recorded.

2.2.8. Statistical analysis.
Statistical analysis was performed using SPSS software version 19.0 (IBM Corporation, USA). The differences between the means were compared using Duncan’s multiple range test, and significance was determined by $p<0.05$.

3. Results & Discussion

3.1. Effects of different pesticide treatments on the incidence of soft rot disease and control effect of konjac plants
Figure 1 shows that pesticide treatments had significant effects on the incidence of konjac soft rot and its control effect. The incidence rates of plants treated with tetramycin, copper succinate, chloroisobromine cyanuric acid, zhongshengmycin, and cuprous oxide, which were reduced by 58.2%, 52.7%, 50.0%, 41.6%, and 33.3%, respectively, were significantly lower than that of CK, whereas the difference between other treatments and CK was not significant. Compared with the other pesticides, the control effects of tetramycin, copper succinate, and chloroisobromine cyanuric acid on soft rot were better (reaching more than 50%), and no significant difference was observed among these treatments.
Figure 1. Effects of different pesticide treatments on the incidence of soft rot and control effect of konjac plant. The bars represent mean ± standard error of the mean, and labeled with different lowercase letters are significantly different ($p < 0.05$).

Prior studies have noted the importance of pesticides in the control of soft rot. Zhang et al. reported that seedling rate was the highest under the condition of seeds soaked in 250 mg/kg agricultural streptomycin for 5 h compared with other fungicides; streptomycin can control the occurrence of konjac soft rot in the seedling stage[19]. Lu et al. investigated the effects of several pesticides on soft rot, showing that the best control effect was obtained with thiediazole copper, bismethiazol, and copper hydroxide[20]. Most of the pesticide treatments in this experiment can effectively reduce the incidence rate of konjac soft rot. The most effective ones were tetramycin, copper succinate, and chloroisobromine cyanuric acid. Different pesticides have a certain bactericidal mechanism. Tetramycin has systemic antibacterial activity that can prevent the invasion and expansion of bacteria and has a strong bacterial killing effect (Gram-negative and Gram-positive) [21]. Copper succinate is a protective fungicide that can coagulate the surface protein of pathogen cell membrane and affect the activity of intracellular enzyme[22]. Chloroisobromine cyanuric acid can inactivate the protein of pathogenic bacteria, affecting the physiological and biochemical process and DNA synthesis[23]. The three pesticides have significant effects on the prevention and control of bacterial diseases in practical applications.

3.2. Effects of different pesticide treatments on the growth indicators of konjac plants
Table 1 compares the growth indicators of konjac plants treated with different pesticides, indicating that significant effects were produced for plant height, petiole diameter, leaf width, and leaf area of plants. The growth indicators of plants treated with cuprous oxide, tetramycin, and copper succinate showed good results, achieving high plant height, thick petiole diameter, and large leaf width and area. No significant difference was observed among these pesticide treatments, and the plants exhibited vigorous growth.
Table 1. Effects of different pesticide treatments on growth indicators of konjac plant.

| Treatment              | Plant height (cm) | Petiole diameter (mm) | Leaf width (cm) | Leaf area (cm²) |
|------------------------|-------------------|-----------------------|-----------------|-----------------|
| Tetramycin             | 51.20±2.17ab      | 16.71±0.81ab          | 35.73±2.79ab    | 497.16±13.37a   |
| Zhongshengmycin        | 42.90±2.27abc     | 14.56±0.40b           | 29.52±0.83b     | 343.08±24.92bc  |
| Streptomycin           | 41.36±2.79bc      | 15.37±1.40ab          | 32.20±2.47ab    | 321.44±47.67c   |
| Copper succinate       | 47.44±1.85ab      | 16.51±0.45ab          | 34.16±0.63ab    | 505.22±43.03a   |
| Cuprous oxide          | 51.94±1.72a       | 17.97±1.09a           | 36.66±2.33a     | 563.67±37.45a   |
| Copper calcium sulfate | 46.70±1.50b       | 14.27±0.50b           | 36.14±1.30a     | 454.59±47.86ab  |
| Chloroisobromine cyanuric acid | 40.82±2.43bc | 16.30±0.67ab          | 33.76±1.96ab    | 339.23±35.07bc  |
| Bismuthiazol           | 38.44±0.91c       | 15.32±0.58ab          | 32.88±2.07ab    | 357.98±22.42bc  |
| CK                     | 43.52±3.65bc      | 16.19±0.92ab          | 29.75±1.82b     | 355.46±27.35bc  |

Data are presented as mean ± standard error of the mean. Values with different lowercase letters are significantly different at p<0.05 (same below in all tables).

3.3. Effects of different pesticides on the photosynthetic indicators of konjac plants

As shown in Table 2, pesticide treatments had significant effects on $P_n$, $G_s$, and $T_r$. In terms of $P_n$, pesticide treatments revealed significant differences. The $P_n$ level was high in cuprous oxide treatment, whereas no significant differences were found between any of the pesticide treatments and CK. As regards $G_s$, high levels were observed with zhongshengmycin and CK, with no significant difference between the two treatments. Regarding $C_i$, no significant differences were observed between treatments. With regard to $T_r$, the difference between any pesticide treatment and CK was not significant. The results showed that pesticide treatments had little effect on $P_n$ compared with CK.

Table 2. Effects of different pesticides on photosynthesis indicators of konjac plant.

| Treatment              | $P_n$ (µmol·m⁻²·s⁻¹) | $G_s$ (mol·m⁻²·s⁻¹) | $C_i$ (µmol·mol⁻¹) | $T_r$ (mmol·m⁻²·s⁻¹) |
|------------------------|----------------------|---------------------|-------------------|---------------------|
| Tetramycin             | 8.09±0.40ab          | 0.11±0.01b          | 275.43±26.12a     | 4.64±0.45a          |
| Zhongshengmycin        | 10.73±0.63ab         | 0.16±0.00b          | 285.53±30.86a     | 2.18±0.19b          |
| Streptomycin           | 8.14±0.55ab          | 0.14±0.01ab         | 286.37±21.32a     | 3.38±0.19ab         |
| Copper succinate       | 8.99±0.36ab          | 0.14±0.01ab         | 295.43±15.40a     | 2.20±0.04b          |
| Cuprous oxide          | 13.87±0.37a          | 0.14±0.01ab         | 267.06±25.56a     | 2.27±0.20b          |
| Copper calcium sulfate | 6.17±0.45b           | 0.10±0.00b          | 271.39±28.89a     | 3.49±0.34ab         |
| Chloroisobromine cyanuric acid | 7.99±0.49ab | 0.11±0.01b          | 279.89±21.09b     | 2.18±0.23b          |
| Bismuthiazol           | 7.14±0.38ab          | 0.12±0.01b          | 281.67±25.56a     | 4.73±0.41a          |
| CK                     | 9.89±0.13ab          | 0.16±0.02b          | 267.18±30.05a     | 3.41±0.38ab         |

Pesticide treatment had certain killing and inhibiting effects on pathogenic bacteria and had significant effects on growth indicators and specific physiological indicators of konjac plants. The results showed that the plants treated with cuprous oxide, tetramycin, and copper succinate had strong growth vigor, and those treated with cuprous oxide had strong photosynthetic capacity, thus laying a good foundation for yield formation.

3.4. Effects of different pesticide treatments on the yield of konjac plants

Table 3 shows that pesticide treatments had significant effects on the yield indicators. In terms of corm weight, the value obtained under copper succinate treatment was significantly higher than that of most treatments, including CK, followed by tetramycin treatment. The plot yields of all pesticide treatments...
were significantly higher than that of CK, which indicates that the selected pesticide treatments had a promoting effect on konjac yield. The yields of copper succinate and tetramycin were the highest, and the yield increase effect was the most significant, which was consistent with the results of low-incidence soft rot and high control effect of these pesticide treatments.

Table 3. Effects of different pesticide treatments on yield of konjac corm.

| Treatment               | Corm weight (g) | Plot yield (kg) |
|-------------------------|-----------------|-----------------|
| Tetramycin              | 106.3±9.86a     | 2.31±0.12a      |
| Zhongshengmycin         | 81.62±8.24bc    | 1.77±0.3ab      |
| Streptomycin            | 58.73±6.66ad    | 1.30±0.33bc     |
| Copper succinate        | 111.27±9.19a    | 2.53±0.36a      |
| Cuprous oxide           | 87.25±7.08ab    | 1.31±0.10bc     |
| Copper calcium sulfate  | 67.24±6.87bcd   | 1.03±0.32bc     |
| Chloroisobromine cyanuric acid | 53.16±4.52d | 1.02±0.19bc |
| Bisperthiazol           | 71.05±5.35bcd   | 1.07±0.01bc     |
| CK                      | 69.39±9.94bcd   | 0.71±0.13c      |

4. Conclusion
From the comprehensive comparison of the control effect of konjac soft rot, growth, photosynthesis, and yield, we conclude that copper fungicides and antibiotics at a certain amount can be used for the control of konjac soft rot. However, the effects of such treatments are different. Copper succinate and tetramycin show excellent performance in disease control and yield increase. In actual production and application, these chemicals can be used alternately with other pesticides to delay the emergence and development of pesticide resistance. Only by adopting integrated management, including chemical and biological control, can we effectively and economically control konjac soft rot and ensure the sustainable development of the konjac industry.

Acknowledgments
This study was supported by the Science and Technology Plan Project of Sichuan (2014ZZ0031).

References
[1] Liu, P. Y. (2004) Konjac. China Agriculture Press, Beijing.
[2] Zhang, Y., Xie, B., Gan, X. (2005) Advance in the applications of konjac glucomannan and its derivatives. Carbohydrate Polymers, 60: 27-31.
[3] Chua, M., Baldwin, T.C., Hocking, T.J., et al. (2010) Traditional uses and potential health benefits of Amorphophallus konjac K. Koch ex NE Br. Journal of ethnopharmacology, 128: 268-278.
[4] Gu, H.H., Wang, Z.X., Jiang, X., et al. (2018) Soft rot of Amorphophallus and its control research progress. Journal of Agriculture, 8: 15-19.
[5] Hayashi, N. (1988) The seed corm transmission of konnyaku's (Amorphophallus konjac) soft rot caused by Erwinia carotovora subsp. carotovora. Gunma Journal of Agricultural Research, 5: 25-34.
[6] Xiu, J.H, Ji, G.H., Wang, M., et al. (2006) Molecular identification and genetic diversity in Konnyaku's soft rot bacteria. Acta Microbiologica Sinica, 46: 522-525.
[7] Wu, J.P., Ding, Z.L., Diao, Y., et al. (2011) First report on Enterobacter sp. causing soft rot of Amorphophallus konjac in China. Journal of General Plant Pathology, 77: 312-314.
[8] Wu, J., Diao, Y., Gu, Y., et al. (2011) Molecular detection of Pectobacterium species causing soft rot of Amorphophallus konjac. World Journal of Microbiology and Biotechnology, 27: 613-618.
[9] He, F., Duan, J.L., Luo, B.F., et al. (2013) Pathogenic bacteria biodiversity of *Amorphophallus* infected with soft rot disease in Langao, Shaanxi. Journal of Northwest A&F University (Nat. Sci. Ed.), 41: 91-98.

[10] Wei, H., Yang, M., Pei, W., et al. (2020) First Report of Pectobacterium aroidearum Causing Soft Rot of *Amorphophallus konjac* in China. Plant Disease, 104: 969.

[11] Wu, J.P., Ying, D., Gu, Y.C., et al. (2010) Infection pathways of soft rot pathogens on *Amorphophallus konjac*. African Journal of Microbiology Research, 4: 1495-1499.

[12] Ding, Z.L., Wan, Z.Y., Jiao, Z.B., et al. (2014) Progress and countermeasures for soft rot disease of *Amorphophallus konjac*. Chinese agricultural science bulletin, 30: 238-241.

[13] Wu, J., Liu, X., Diao, Y., et al. (2012) Authentication and characterization of a candidate antagonistic bacterium against soft rot of *Amorphophallus konjac*. Crop protection, 34: 83-87.

[14] He, F., Zhang, Z.L., Cui, M., et al. (2015) Disease prevention and growth promotion effects of actinomycete strain D74 on *Amorphophallus konjac*. Acta Horticulturae Sinica, 42: 367-376.

[15] Chen, L., Liao, T.T., Guo, Z.H., et al. (2014) Tissue specific expression of soft rot disease resistant gene in *Amorphophallus konjac*. Molecular Plant Breeding, 12: 1230-1234.

[16] Ban, H., Chai, X., Lin, Y., et al. (2009) Transgenic *Amorphophallus konjac* expressing synthesized acyl-homoserine lactonase (aiiA) gene exhibit enhanced resistance to soft rot disease. Plant Cell Reports, 28: 1847-1855.

[17] Zhang, F.J., Liu, H.L., Zhang, J., et al. (2013) Research progress of *Amorphophallus bulbifer* resources. South China Agriculture, 7: 64-67.

[18] Yin, P., Guan, Q.L., Hu, X., et al. (2018) Effects of altitude and growth stage on *Amorphophallus konjac* microecosystem in mount emei. International Journal of Agriculture and Biology, 20: 2265-2270.

[19] Zhang, X.P., Li, A.L., Wang, S.B., et al. (2002) A study on occurrence rule and control technology of soft rot of *Amorphophallus*. Acta Agriculturae Boreali-occidentalis Sinica, 11: 78-81.

[20] Lu, H.M., Wang, C.J., Zhou, Z.P. (2012) Effects of some pesticides for control of konjac soft rot. Pesticide Science and Administration, 33: 59-60.

[21] Chen, L.L., Guo, B.B., Li, B.X., et al. (2017) Toxicity and control efficacy of tetramycin against *Passalora fulva*. Chinese Journal of Pesticide Science, 19: 324-330.

[22] Ma, C., Zhang, L.B., Li, J.H., et al. (2019) Preparation and physical stability analysis of copper (succinate+glutarate+adipate) 30% SC. Agrochemicals, 58: 262-265.

[23] Wang, X.G., Li, Y.Q., Sun, H.Q., et al. (2012) Residues and decline dynamics of chloroisobromine cyanuric acid in tobacco and soil. Chinese Journal of Pesticide Science, 14: 191-197.