Effects of Different Treatments on Seed Germination of Salvia miltiorrhiza

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Abstract. To study the effects of different treatments on the germination characteristics of Salvia miltiorrhiza seed, Salvia miltiorrhiza seeds were treated by fludioxonil and carbendazim, and were studied with different thickness of covering soil. The results showed that with the increase of soil cover thickness, the germination rate of Salvia miltiorrhiza seeds significantly decreased. The thickness of covering soil was 0 cm, the germination of Salvia miltiorrhiza seeds was the best and the germination rate was 42.08%. When the thickness of covering soil was 1.0 cm, the germination rate of Salvia miltiorrhiza seeds significantly decreased, only 2.91%, which was 93.08% lower than the control. The appropriate concentration of seed coating agent can improve the germination rate of Salvia miltiorrhiza seeds. When the soil thickness was 0 cm+1.25 g·L-1 fludioxonil, the best germination effect of Salvia miltiorrhiza seeds was observed, and the germination rate was 59.17%, which increased 40.61% compared to 0 cm soil alone. The germination rate of Salvia miltiorrhiza seeds treated with 0 cm+2.5 g·L-1 fludioxonil was only 13.75%, which was 28.33% lower than the germinationg rate with the soil thickness of 0 cm alone. There was no significant difference in the germination rate of Salvia miltiorrhiza seeds treated with 0.05% carbendazim compared with the control group; The germination rate of Salvia miltiorrhiza seeds treated with 0.1% carbendazim was significantly lower than that of the control group covered with 0 cm soil alone. In summary, The best germination level of Salvia miltiorrhiza seed was the cover thickness of 0 cm soil and 1.25 g · L-1 concentration of fludioxonil, (0 cm+1.25 g·L-1). The treatment of seeds with 2.5 g·L-1 concentration of chloramphenicol showed a significant inhibitory effect on the germination of Salvia miltiorrhiza seeds. In conclusion, Salvia miltiorrhiza seeds treated with 1.25 g·L-1 concentration of fludioxonil and 0 cm covered soil, the germination rate, germination potential, and germination index are 59.17%, 51.67%, and 14.27, respectively, which increased by 40.61%, 61.07%, 77.28% compared with the soil of 0 cm alone. It is suggested that breeding of Salvia miltiorrhiza seeds should be carried out on land with the appropriate concentration of fludioxonil.

Key words: Salvia miltiorrhiza Bunge；seed; cover soil thickness; germination rate；seed dressing agent.

1. Introduction

Salvia miltiorrhiza Bunge is a perennial herb belonging to the genus Salvia in the labiaceae family and is one of important bulk medicinal plants in China[1]. It is also known as red root, mountain root, honey pot head, Dahongpao and blood ginseng root, etc. The medicinal materialsof Salvia miltiorrhiza can relieve pain, promote blood circulation and remove blood stasis, clear heart and remove dryness, nourish serum and brain, and can also treat cardiovascular diseases and clinical coronary heart disease[2-4]. In recent years, Salvia miltiorrhiza as the traditional Chinese medicine

has made new development, and has obvious effects in the treatment of psoriasis, acne and other skin diseases[5]. At present, due to the frequent occurrence of cardiovascular diseases and people's health awareness increasingly, the demand for Salvia miltiorrhiza in China is increasing. The low germination rate of Salvia miltiorrhiza seeds in the natural state has become one of the factors limiting the reproduction and production of Salvia miltiorrhiza[6]. Therefore, how to improve the germination rate of Salvia miltiorrhiza seeds becomes more important.

Seed germination and seedling growth of Salvia miltiorrhiza are the key links in the growth process. Dressing, soaking and coating the seeds before sowing are important measures to increase germination rate and promote seedling production. Seeds treated by agent can reduce the pathogenicity of seeds, improve seed viability, and increase their germination percentage and emergence rates[7,8]. Yu Hailong's research showed that cowpea seeds coated with carbendazim can improve the storage time of cowpea seeds and increase the germination rate of aged seeds [9]. Coating corn seeds with micronutrients significantly increases the emergence rate of corn seeds [10].

Due to the seeds of Salvia miltiorrhiza are relatively small, the thickness of soil cover has a great influence on seed germination and seedling emergence[11]. Pan Tianchun’s study showed that soil cover thickness had a great influence on the germination potential of maca seeds[12]. At present, seed dressing agents are used in seed germination studies of other crops, but there are few studies about Salvia miltiorrhiza seeds. Based on this, the seed germination characterics of Salvia miltiorrhiza seeds under different soil cover thickens with two medicines, fludioxonil and carbendazim was studied , the aim of our study is to promote the germination of Salvia miltiorrhiza seeds and the appropriate soil cover thickens which were sought to provide theoretical basis for improving the quality of Salvia miltiorrhiza seedlings, the yield and quality of Salvia miltiorrhiza in the later stage.

2. Materials and methods

2.1 Experimental Materials

2.1.1 Salvia miltiorrhiza seeds

The seeds of Salvia miltiorrhiza were provided by Pingyi Yuantong Traditional Chinese Medicine Technology Development Co., LTD., and the seeds of Salvia Miltiorrhiza harvested in the same year were plump, complete, uniform in size and free of diseases and pests.

2.1.2 Type of seed coating agent

The tested agent was a suspended agent in the form of carbendazim with an effective component content of 40%, purchased from Jiangsu Lanfeng Biological Chemical Co., LTD. The tested fludioxonil was a suspended seed coating agent with an active ingredient content of 25 g·L-1, purchased from Syngenta Nantong Crop Protection Co., LTD

2.2 Seed coating treatment

Fludioxonil concentration 1 (2.5 g·L-1) : Take 0.1ml of 25 g·L-1 suspension concentrate mixture and dilute it 10 times with water. Take 0.4 ml and slowly pour into 2000 seeds of Salvia miltiorrhiza, turn while pouring, until the seeds are evenly coated, and let dry. Fludioxonil concentration 2 (1.25 g·L-1) : Take 0.1 ml of 25 g·L-1 suspension concentrate and dilute it 20 times with water. Slowly pour 0.4 ml into 2000 Salvia miltiorrhiza seeds, turn while pouring, until the seeds are evenly coated, and let dry.

Carbendazim 0.05% : Take 0.1 ml of carbendazim coating with 40% active ingredient content and dilute it with 80 ml of water, dilute it 800 times, put 2000 seeds of Salvia miltiorrhiza into the solution and soak the seeds for 1 h.

Carbendazim 0.1% : Take 0.1 ml of carbendazim coating with 40% active ingredient and dilute it with 40ml of water, dilute it 400 times, put 2000 seeds of Salvia miltiorrhiza into the solution and soak the seeds for 1 h.

2.3 Experimental design

The test was conducted in September to October 2022, and 20 treatments were set up. Two treatments (fludioxonil and carbendazim) were set up to mix seeds with no chemicals as the blank control. Orthogonal design was used in the experiment, and the factors were seed coating agent (A) and soil cover thickness (B). The levels of seed coating agent factors were A1 (blank control), A2 (fludioxonil: 1.25 g·L-1), A3 (fludioxonil: 2.5g·L-1), A4 (0.05% carbendazim), A5 (0.1% carbendazim); The level of soil cover thickness factor: B1 (soil cover thickness 0 cm), B2 (soil cover thickness 0.3 cm), B3 (soil cover thickness 0.6 cm), B4 (soil cover thickness 1.0 cm). The horizontal orthogonality of the two factors constituted 20 treatments with 3 repetitions per treatment.

Then, the prepared substrate was sprayed with water, mixed, put into the seedling hole dish, press the hole, and then seeds of different treatments were evenly seeded into the hole dish, respectively, with no soil cover, soil cover 0.3 cm, soil cover 0.6 cm, and soil cover 1.0 cm. 160 seeds were planted in each treatment, and 10 seeds were planted in each hole. The substrate was kept moist before emergence, the data were observed and recorded at a fixed time every day, and the germination number of Salvia miltiorrhiza seeds under different treatments was counted. The germination potential was counted on the 7th day, and the germination rate was counted on the 15th day.

The experiment is divided into 20 treatments, which are as follows:

L1: blank control (CK) , L2: soil cover thickness: 0.3 cm
L3: soil cover thickness: 0.6 cm, L4: soil cover thickness 1.0 cm
T1: 1.25 g·L-1+0 cm(fludioxonilconcentration+ soil thickness, the same below)
T2: 1.25 g·L-1+ 0.3 cm, T3: 1.25 g·L-1+0.6 cm, T4: 1.25 g·L-1+1.0 cm
T5: 2.5 g·L-1+0 cm, T6: 2.5 g·L-1+0.3 cm, T7: 2.5 g·L-1+0.6 cm
T8: 2.5 g·L-1+1.0 cm
Y1: 0.05% +0 cm(Carbendazim concentration+ soil thickness, the same below)
Y2: 0.05% + 0.3 cm, Y3: 0.05% +0.6 cm, Y4: 0.05% +1.0 cm, Y5: 0.1% + 0 cm
Y6: 0.1% +0.3 cm, Y7: 0.1% + 0.6 cm, Y8: 0.1% +1.0 cm

2.4 Measurement items

Germination rate (GR)=(total number of germinated seeds/total number of tested seeds)×100%

Germinating potential (GE)=(total number of germinated seeds on the 7th day/total number of tested seeds)×100%

Germination index (GI)=∑(Gt/Dt), where: Gt is the number of germination seeds on the t day, Dt is the number of germination days [13].

Data Processing

Statistical software such as Excel, SPSS27.0 and GraphpadPrism5 were used for statistical analysis, plotting and tabulating of the experimental data in each process.

3. Results and analysis

3.1 Effects of different soil cover thickness on seed germination characteristics of Salvia miltiorrhiza

The germination characteristics of seeds under different treatments was shown in Table 1. There is a significant difference in the germination of Salvia miltiorrhiza seeds with different soil cover

thicknesses. The best germination was 0 cm soil cover. The germination rate of seeds under each treatment was significantly lower than that of the control, decreased by 40.59%, 72.27% and 93.06%, respectively($P<0.05$). The germination potential of the seeds of each treatment was significantly lower than that of the control, decreased by 45.45%, 74.03% and 92.21%, respectively($P<0.05$). And germination decreased significantly with the increase of soil cover thickness. Above all, the germination rate, germination potential and germination index was best with 0 cm soil cover thickness.

Table.1 Effects of different soil cover thickness treatments on seed germination characteristics of Salvia miltiorrhiza

Treatment	Germination rate（%）	Germination potential（%）	Germination index
L1	42.08±1.10a	32.08±1.50a	8.01±0.70a
L2	25.00±0.72b	17.50±0.72b	4.89±0.28b
L3	11.67±0.72c	8.33±1.10c	3.11±0.34c
L4	2.92±0.42d	2.50±0.00d	1.03±0.19d

Note: Different lowercase letters indicate significant differences between different treatments ($P<0.05$), the same below.

3.2 Effects of fludioxonil seed dressing + different soil thickness on seed germination characteristics of Salvia miltiorrhiza

The germination of each treatment was shown in Table 2. The results showed that the effect of different concentrations of fludioxonil on the germination of Salvia miltiorrhiza seeds was very different and there were obvious differences in germination among different treatments. The highest germination rate was obtained in fludioxonil treatment(1.25g·L-1) with 0 cm soil thickness(T1), while the lowest germination rate was obtained in fludioxonil treatment(2.5g·L-1) with 1.0 cm thickness of soil cover(T8). Considering the thickness of soil cover, the germination percentage of Salvia miltiorrhiza seeds decreased significantly with the increase of soil cover thickness. The germination rate, germination potential and germination index of salvia miltiorrhiza seeds treated with fludioxonil and soil thickness(1.25g·L-1+0 cm,T1) were increased by 40.61%, 61.07% and 77.28% compared with 0 cm soil thickness alone. The germination of salviae miltiorrhiza seeds was significantly decreased when treated with fludioxonil and soil thickness(2.5 g·L-1+0 cm,T5) ($P<0.05$). With the increase of fludioxonil concentration, the germination of Salvia miltiorrhiza seeds showed a trend of first increasing and then decreasing. fludioxonil treated with 1.25 g·L-1 concentration promoted the germination of Salvia miltiorrhiza seeds. While the fludioxonil concentration at 2.5 g·L-1 concentration had obvious inhibitory effect on germination of Salvia miltiorrhiza .Compared with 1.0 cm soil thickness alone, the germination rate, germination potential and germination index of T8 were increased by 42.81%, 13.20% and 18.45%(table 1 and table 2).

Table.2 Effects of different soil thickness on seed germination characteristics of Salvia miltiorrhiza

Treatment	Germination rate（%）	Germination potential（%）	Germination index
T1	59.17±1.50a	51.67±2.20a	14.27±0.65a
T2	42.50±0.72b	35.00±0.72ab	10.15±0.09b
T3	22.92±1.10c	20.00±0.72b	4.86±0.01c
T4	8.75±0.72d	7.50±0.72cd	2.23±0.23d
T5	13.75±0.72d	12.08±1.10c	3.66±0.39c
T6	10.00±0.72d	8.75±0.72cd	2.97±0.31c
T7	8.34±0.42d	7.08±0.42cd	2.57±0.18c
T8	4.17±0.42e	2.83±0.00e	1.22±0.04d

3.3 Effects of carbendazim seed dressing + different soil cover thickness on seed germination characteristics of Salvia miltiorrhiza

The germination of each treatment was shown in Table 3. The test was found that there were obvious differences in the germination among the treatments. The highest germination rate was obtained in carbendazim treatment and soil thickness(0.05%+0 cm,Y1), while the lowest germination rate was obtained in Y8. Salvia miltiorrhiza seeds with soil thickness of 0 cm+0.05% carbendazim treatment had no significant difference compared with soil thickness of 0 cm alone(table 1 and table 3). The germination of Salvia miltiorrhiza seeds with soil thickness of 0 cm+0.1% carbendazim treatment significantly decreased. The germination rate, germination potential and germination index of Y5 level decreased by 72.27%, 35.71% and 7.8% compared with that of 0 cm soil thickness alone(table 1 and table 3). The germination rate of Salvia miltiorrhiza seed decreased gradually with the increase of soil cover thickness and the carbendazim concentration. Compared with Y1 level, the germination rate, germination potential and germination index of Y5 level were decreased by 57.60%, 61.81% and 61.12%, respectively. It was concluded that 0.05% carbendazim had no obvious effect on the germination of Salvia miltiorrhiza seeds, but 0.1% carbendazim had obvious inhibitory effect on seed germination(table 1 and table 3).

Table 3 Effects of carbendazim seed mixing + different soil cover thickness on seed germination characteristics of Salvia miltiorrhiza

Treatment	Germination rate（%）	Germination potential（%）	Germination index
Y1	41.67±1.10a	32.08±1.50a	14.17±0.50a
Y2	27.08±1.10b	19.58±0.72b	10.64±0.31b
Y3	14.17±1.10c	13.34±0.83c	5.42±0.67c
Y4	4.58±0.42d	4.17±0.42d	1.49±0.18d
Y5	17.67±0.83c	12.25±0.72c	5.51±0.21c
Y6	5.42±0.42d	3.75±0.72d	1.72±0.23d
Y7	3.75±0.72d	3.34±0.42d	1.38±0.19d
Y8	1.25±0.00e	1.25±0.00e	0.38±0.12e

4. Discussion and conclusion

Salvia miltiorrhiza has high medicinal value, and the market demand is increasing. At present, the germination of Salvia miltiorrhiza seeds is inconsistent or the germination speed is irregular[14]. It is very important to adopt scientific seeding treatments to improve the germination rate of Salvia miltiorrhiza. In this study, the germination of Salvia miltiorrhiza seeds under different soil thickness and coating agent treatment was explored, and the best conditions for seed sowing were provided for the cultivation and popularization of Salvia miltiorrhiza.

Soil cover thickness is an important factor affecting seed germination and has a certain influence on seed germination characteristics[15]. Dong Aixiang's research showed that no covering or covering the seeds lightly with soil was best for seed Germination of Salvia splendens[16]. Zhang Chunmei's study showed that when the thickness of covering soil was 0 cm, the germination rate of silver spot thyme seed was the highest, up to 86.67%, which was 48% higher than that of the soil cover thickness of 2 cm[17]. Our study showed that the germination rate, germination potential and germination index of salvia miltiorrhiae seeds decreased significantly with the increase of the thickness of covering soil, which was consistent with the results of Dong Aixiang and Zhang Chunmei.

Seed-coating agent can improve the disease resistance of seeds and promote seed germination [18], but it can not be applied to all plants. In addition, different concentrations of the same seed-coating agent have different effects on seed germination of the same plant, and appropriate concentrations can promote seed germination. The improper concentration could not promote but

inhibit the germination of seeds. Tan Fangjun's study showed that the germination rate of pepper seeds was improved when pepper seed was treated by suitable fludioxonil suspension seed coating agent[19]. Yang Guosheng's study showed that carbendazim was used to treat peanut seeds, the germination rate of peanut was lower than that of the control[20].Wang's study showed that the rate of seedling emergence was higher than that of white seed without coating with different concentrations of fludioxinil suspension seed coating agent and the seed emergence rate of the treatment with fludioxinil suspension seed coat agent 8.33 mL constant volume to 50.00 mL seed mixing 1 kg was the highest, reaching 95.71%[21], this result was consistent with the results of our study.

In conclusion, the optimal level for germination of Salvia miltiorrhiza was the soil covering thickness of 0 cm+1.25 g·L-1 concentration of fludioxinil(T1). The germination rate, germination potential and germination index of combined Salvia Miltiorrhiza seeds at T1 level were 59.17%, 51.67% and 14.27, respectively, which were 40.61%, 61.07% and 77.28% higher than that of single soil cover thickness of 0 cm.While the 2.5 g·L-1 concentration of fludioxinil would the germination of alvia miltiorrhiza seeds. However, the specific parameters of seed coating agent such as the concentration of carbendazim and soaking time need further research.

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