Effects of priming, fungicide and biological treatments on onion (*Allium cepa* L.) seeds*

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[**Abstract**] Health tests were performed at 10 ℃ for untreated onion "Wolska" seeds, surface sterilized seeds, treated with fungicides and biological preparations (Biozym, Promot), osmoprimed and hydroprimed onion seeds. These tests were also carried out on seeds treated with fungicides and biological preparations in combination with osmo- and hydropriming. Untreated seeds were infested mainly with *Penicillium* spp. Fungicides alone reduced the incidence of *Penicillium* spp. on seeds to the large extent, whereas Biozym and Promot did not control these fungi. The percentage of seeds colonised by *Penicillium* spp. after hydropriming was significantly lower than that of untreated seeds, while after osmopriming was slightly higher. The considerable increase in inner infection by *Penicillium* spp. was observed after osmopriming. Among combined treatments the highest percentages of healthy seeds were observed when fungicides were applied during osmopriming or before hydropriming.

[**Key words**] onion seed; priming; fungicide; biological agent

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Introduction

Onion (*Allium cepa* L.) is one of the most important vegetable crops utilized in the production of seeds because of its unique flavor and odor that make it an excellent food source in the world. However, onion seeds usually have a poor quality. Various seed treatments are used widely to improve seed quality. Out of them, seed priming, very common and effective, has been devised to improve the rate and uniformity of seed germination as well as seed viability. Preplant priming improvements were greater in poor seeds than in more vigorous seeds[1]. The effects of priming include increasing germination rate; more uniform emergence; germination under a broader range of environments; improving seedling vigor and growth[2].

The treatment of seeds with microorganisms that are beneficial to plant growth has been the subject of considerable investigation for many years, often with mixed results. Many fungi and bacteria have been tested as seed treatments to provide short-term protection against seed rots and damping-off fungi (*Pythium* spp.) in the soil[3]. In this function, biological seed treatments largely have been effective because the pathogens are limited in time and space and the area of host tissue available for infection is relatively small and can be effectively covered with antagonists[4]. Such microorganisms have been collectively called bio-protectants. Fungi (*Trichoderma* spp.) and bacteria (*Enterobacter* and *Pseudomonas* spp.) have been tested for this purpose. Two commercial biological growth promoters i.e. PROMOT & BIOZYM were used in this experiment. Penncozeb 80WP was applied for treating seeds in this experiment. Maneb is one of the organic sulfur fungicides which is sometimes mixed with zinc ion and results in the formulations known as zinc ion maneb, called man-

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cozeb. The addition of zinc reduces the phytotoxicity of maneb and improves its fungicidal properties[5].

The main objectives of this research were to investigate the effects of priming, fungicide and biological treatments against seed-borne fungi on onion seed health.

1 Materials and methods

1.1 Seeds

Onion 'Wolska' seeds supplied by CNOS Seed Company in Poznan were used in the experiment.

1.2 Fungicides

Penncozeb 80WP (a. i 800 mg/g mancozeb); Apron 35 SD (a. i 350 mg/g metalaxyl).

The Institute of Plant Protection in Poland recommended both of the fungicides.

1.3 Microbial preparations

Two microbial preparations were used for treating seeds:

PROMOT —— Microbiological plant growth promoter. It can be used as a seed coating or by direct application to seeds at planting. It may also be mixed with potting soil to grow seedlings in greenhouse to promote plant growth by increasing beneficial microbial activities in the rhizosphere. It contains high concentration of spores selected beneficial fungi—*Trichoderma koningii* (3×10^7/gram) and *Trichoderma harzianum* (2×10^7/gram).

BIOZYM —— Biological growth factor. It's a natural growth factor produced by a unique fermentation process. It contains a group of beneficial microorganisms and selects enzyme complexes:

1. Bacteria: *Azotobacter* spp., *Bacillus circulans*, *B. megaterium*, *B. subtilis*, *B. thuringensis*, *Escherichia* spp., *Pseudomonas flourescens*, *P. putida* (Bacterial count is not less than 2×10^9/gram);

2. Fungi: *Aspergillus oryzae*, *Chaetomium globosums*, *Trichoderma hamatum*, *T. harzianum*, *T. koningii*, etc. (*Trichoderma* count is not less than 2×10^6/gram);

3. Enzyme complexes: Protease, Amylase, Cellulase and Lipase.

Both microbiological products were produced by JH BIOTECH, INC USA.

1.4 Osmopriming

Seeds were primed for 7 days in darkness at 15 ℃ by placing 50 seeds in 9 cm diameter Petri dishes on 4 blotters moistened with 5 mL of Polyethylene glycol (PEG 8000, Sigma Chemical Co.) solution of the osmotic potential of - 1.5 MPa. The Petri dishes were sealed with Parafilm. After priming the seeds from each replicate were washed separately under the running tap water for 5 min and next rinsed three times in sterile distilled water to remove PEG. Then, they were surface dried with blotting paper. Afterwards, the seeds were placed in semi-open Petri dishes and dried back at 20 ℃ and 45% R. H. for 48 h to an equilibrium moisture content.

1.5 Hydropriming

Seeds were placed in 100 mL flasks and 500 μL of distilled water per 1 g of seeds was added. Then flasks were sealed with Parafilm and incubated in darkness at 20 ℃ for 2 days. Afterwards, the seeds were surface dried with blotting paper, placed in semi-open Petri dishes and dried back at 20 ℃ and 45 % relative humidity for 48 h to an equilibrium moisture content.

1.6 Seed health test

Mycological analysis was performed for the following 24 treatments. Abbreviations were placed in the parenthesis. 200 seeds at each treatment were placed on the surface of the potato dextrose agar (PDA) in 9 cm diameter Petri dishes, 10 seeds per dish, and then incubated at 10 ℃ in darkness for 10 days. Streptomycine 100 μg/g was added to PDA. Determination of fungi on onion seeds was based on the appearance of their colonies and sporulation.

1. Control 1: Untreated seeds (C1);

2. Control 2: Seeds surface sterilised in 1 g/L NaOCl solution for 10 min and then rinsed 3 times in sterile distilled water (C2);

3. Fungicide treatment: Seeds were treated with Penncozeb 80WP 5 g/kg seed and Apron 35 SD 1 g/kg seed (F);

4. Biozym treatment: Seeds were treated with

Biozym 30 g/kg seed (B);

5. Promot treatment: Seeds were treated with Promot 30 g/kg seed (P);

6. Osmopriming: Seeds were osmoprimed in the way described in 1.4 (OP1);

7. Osmopriming + NaOCl: After osmopriming and drying, seeds were surface sterilised in 1 g/L NaOCl solution for 10 min and then rinsed 3 times in sterile distilled water (OP2);

8. Fungicide before osmopriming: Firstly seeds were treated with Penncozeb 80 WP 5 g/kg seed and Apron 35 SD 1 g/kg seed, then osmoprimed, rinsed and dried (FOP1);

9. Fungicide during osmopriming: Penncozeb 80 WP was added to -1.5 MPa PEG solution in concentration 5 g per 1L and Apron 35 SD in concentration 1 g per 1 L. After treatment, seeds were washed and dried at 20 ℃ and 45% R. H. for 48 h (FOP2);

10. Fungicide after osmopriming: After osmopriming, rinsing and drying, seeds were treated with Penncozeb 80 WP 5 g/kg seed and Apron 35 SD 1 g/kg seed (FOP3);

11. Biozym before osmopriming: Firstly seeds were treated with Biozym 30 g/kg seed, and then osmoprimed, rinsed and dried (BOP1);

12. Biozym during osmopriming: Biozym was added to the -1.5 MPa PEG solution at concentration 30 g per 1 L. After treatment, seeds were washed and dried at 20 ℃ and 45% R. H. for 48 h (BOP2);

13. Biozym after osmopriming: After osmopriming, rinsing and drying, seeds were treated with Biozym 30 g/kg seed (BOP3);

14. Promot before osmopriming: Firstly seeds were treated with Promot 30 g/kg seed, and then osmoprimed, rinsed and dried (POP1);

15. Promot during osmopriming: Promot was added to the -1.5 MPa PEG solution at concentration 30 g per 1 L. After treatment, seeds were washed and dried at 20 ℃ and 45% R. H. for 48 h (POP2);

16. Promot after osmopriming: After osmopriming, rinsing and drying, seeds were treated with Promot 30 g/kg seed (POP3);

17. Hydropriming: Seeds were hydroprimed in the way described in 1.5 (HP1);

18. Hydropriming + NaOCl: After hydropriming and drying, seeds were surface sterilised in 1 g/L NaOCl solution for 10 min and then rinsed 3 times in sterile distilled water (HP2);

19. Fungicide before hydropriming: Firstly seeds were treated with Penncozeb 80 WP 5 g/kg seed and Apron 35 SD 1 g/kg seed, then hydroprimed and dried (FHP1);

20. Fungicide after hydropriming: After hydropriming and drying, seeds were treated with Penncozeb 80 WP 5 g/kg seed and Apron 35 SD 1 g/kg seed (FHP2);

21. Biozym before hydropriming: Firstly seeds were treated with Biozym 30 g/kg seed, then hydroprimed and dried (BHP1);

22. Biozym after hydropriming: After hydropriming and drying, seeds were treated with Biozym 30 g/kg seed (BHP2);

23. Promot before hydropriming: Firstly seeds were treated with Promot 30 g/kg seed, then hydroprimed and dried (PHP1);

24. Promot after hydropriming: After hydropriming and drying, seeds were treated with Promot 30 g/kg seed (PHP2).

1.7 Data analysis

Seed Calculator (version 2.1) software[6] was applied to analyse the data. All statistical results were evaluated by means of variance analysis (ANOVA) followed by Duncan's multiple range tests

2 Results and analysis

In control 1, the percentage of seeds infested with *Penicillium* spp. was 92.5% (Fig. 1). Other saprophytic fungi did not occur. None of the onion seed-borne pathogenic fungi was detected (Tab. 1). After sterilisation with 1 g/L NaOCl, i.e. in C2 treatment, the incidence of *Penicillium* spp. was reduced to 1.5%. The incidences of *Alternaria alternata* (Fr.) and *Rhizopus nigricans* Ehrenberg were 2.5% and 0.5% respectively. After sterilisation

(C2 treatment), healthy seeds were at higher level (95.5%) compared with control 1 (Tab. 2).

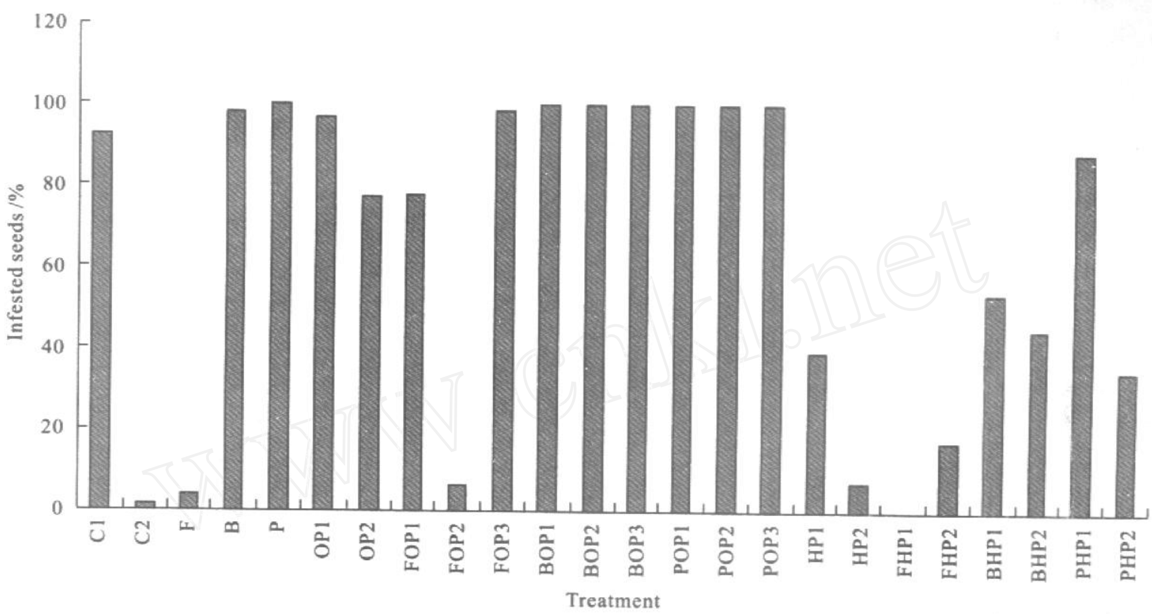

Fig. 1 Incidence of *Penicillium* spp. on onion seeds at 10 ℃

Table 1 Incidence of microorganisms in onion seeds %

Treatment	*Alternaria alternata*	*Botrytis cinerea*	*Cladosporium* sp.	*Epicoccum purpurascens*
C1	0.0 b	0.0 b	0.0 f	0.0 b
C2	2.5 a	0.0 b	0.0 f	0.0 b
F	0.0 b	0.0 b	0.0 f	0.0 b
B	0.0 b	0.0 b	0.0 f	0.0 b
P	0.0 b	0.0 b	0.0 f	0.0 b
OP1	0.0 b	0.0 b	0.0 f	0.0 b
OP2	0.0 b	1.5 ab	0.0 f	0.0 b
FOP1	0.0 b	0.0 b	0.0 f	0.0 b
FOP2	0.0 b	2 a	0.0 f	0.0 b
FOP3	0.0 b	0.0 b	0.0 f	0.0 b
BOP1	0.0 b	0.0 b	0.0 f	0.0 b
BOP2	0.0 b	0.0 b	0.0 f	0.0 b
BOP3	0.0 b	0.0 b	0.0 f	0.0 b
POP1	0.0 b	0.0 b	0.0 f	0.0 b
POP2	0.0 b	0.0 b	0.0 f	0.0 b
POP3	0.0 b	0.0 b	0.0 f	0.0 b
HP1	0.5 b	1.0 ab	6.5 d	0.0 b
HP2	3.5 a	1.0 ab	0.0 f	0.0 b
FHP1	0.0 b	0.0 b	0.0 f	0.0 b
FHP2	0.0 b	3.0 a	0.0 f	0.0 b
BHP1	0.0 b	0.0 b	15.0 b	0.0 b
BHP2	0.5 b	0.5 ab	29.0 a	1.5 a
PHP1	0.5 b	0.0 b	5.0 e	0.5 b
PHP2	0.0 b	0.0 b	11.0 c	0.0 b

Note: The data showed the percentage of infested seeds. Means in columns followed by the same letters are not significantly different at $\alpha = 0.05$ level according to Duncan's multiple range test. The following table is the same.

Fungicide treatment (F) was quite successful. Considerably higher percentage (94.5%) of healthy seeds than in control 1 and significantly lower incidence of *Penicillium* spp. (4.0%) were observed (Fig. 1, Table 2). Whereas treating seeds with Biozym and Promot (B, P) did not control *Penicillium* spp. Healthy seeds were not observed.

Table 2 Incidence of microorganisms and healthy seeds %

Treatment	*Rhizopus nigricans*	Non-sporulating species	Bacteria	Healthy seeds
C1	0.0 c	0.0 b	2.0 e	7.5 f
C2	0.5 c	0.0 b	0.0 e	95.5 a
F	0.5 c	0.0 b	1.0 e	94.5 a
B	7.5 ab	0.0 b	0.0 e	0.0 i
P	0.5 c	0.0 b	1.0 e	0.0 i
OP1	0.5 c	0.0 b	0.0 e	3.0 gh
OP2	0.0 c	0.0 b	0.0 e	13.0 e
FOP1	0.0 c	0.0 b	3.0 e	21.5 d
FOP2	0.0 c	0.5 b	6.5 d	85.0 b
FOP3	9.0 ab	0.0 b	0.0 e	1.0 i
BOP1	15.0 a	0.0 b	0.0 e	0.0 i
BOP2	9.0 ab	0.0 b	0.0 e	0.0 i
BOP3	8.5 ab	0.0 b	0.0 e	0.0 i
POP1	8.0 ab	0.0 b	0.0 e	0.0 i
POP2	6.0 b	0.0 b	0.0 e	0.0 i
POP3	10.0 ab	0.0 b	0.0 e	0.0 i
HP1	7.0 ab	0.0 b	64.0 a	0.0 i
HP2	1.0 c	0.5 b	45.0 b	22.5 d
FHP1	1.0 c	0.0 b	11.0 d	84.5 b
FHP2	0.0 c	0.0 b	28.0 c	52.0 c
BHP1	10.0 ab	3.0 a	22.5 c	3.5 fg
BHP2	0.5 c	0.0 b	24.5 c	1.5 hi
PHP1	6.5 ab	0.0 b	6.5 d	0.0 i
PHP2	9.0 ab	0.0 b	58.0 a	0.0 i

After osmopriming (OP1), the incidence of *Penicillium* spp. increased to 97.0%. A significant increase occurred in seed infestation with *Penicillium* spp. (77.0%) after sterilisation of osmoprimed seeds (OP2) compared with C2 (1.5%). *Botrytis cinerea* Pers ex Fr. occurred at this treatment at low level. The percentage of healthy seeds in OP1 significantly decreased compared with C1. A considerable reduction in the percentage of healthy seeds was observed also after sterilisation of osmoprimed seeds (OP2) compared with C2.

After hydropriming, the lower incidence of *Penicillium* spp. (39.0%) was observed than that in control 1. However, more fungi were detected: *A. alternata*, *B. cinerea*, *Cladosporium* sp. and *R. nigricans*. After sterilisation of hydroprimed seeds (HP2), the incidence of *Penicillium* spp. was only 7.0%. The percentage of seeds infested with *A. alternata*, *B. cinerea* and *R. nigricans* did not differ significantly from C2. On the other hand, the incidence of bacteria in HP1 and HP2 significantly increased (64.0% and 45.0% respectively) compared with C1, C2, OP1 and OP2 (Tab. 2).

Treating seeds with fungicides during osmopriming (FOP2) resulted in the significant decrease in seed infestation with *Penicillium* spp. compared with control 1 (C1) and osmopriming alone (OP1). At the same time, the percentage of healthy seeds considerably raised and was 85.0%. FOP3 treatment was the worst one from among FOP1, FOP2 and FOP3 treatments.

In treatments in which Biozym was combined with osmopriming (BOP1, BOP2, BOP3), the incidence of *Penicillium* spp. was very high (100%), higher than after osmopriming (97.0%). The healthy seeds in these three treatments were not observed. Promot combined with PEG treatment showed the same results as Biozym combined with

PEG treatments. In all these treatments higher percentage of seeds infested with *R. nigricans* than that in OP1 was observed.

Among all treatments in which fungicides and biological preparations were combined with hydropriming, the fungicide treatments showed the best results. The incidences of *Penicillium* spp. in FHP1 and FHP2 were 0 and 17.0% respectively. The percentages of healthy seeds were 84.5% and 52.0% respectively. The differences in the incidence of *Penicillium* spp. between BHP1 and BHP2 treatments were not statistically significant. However, the number of healthy seeds in BHP1 was higher than those in BHP2 and HP1. More other fungi were detected in BHP2 treatment than in control 1 (C1) and Biozym (B) treatment: *Cladosporium* sp. (29.0%), *Epicoccum purpurascens* (1.5%), *A. alternata*, *B. cinerea* and *R. nigricans*. The incidence of the three last fungi was 0.5%. The healthy seeds were not observed in treatments in which Promot was combined with hydropriming. The difference in seed infestation with *Penicillium* spp. between PHP1 (88.5%) and PHP2 (35.0%) treatments was statistically significant. The number of the bacteria in PHP2 was the highest (58.0%) from among all combined treatments that included hydropriming.

3 Discussions

This study showed that the percentage of onion seeds colonised by *Penicillium* spp. was significantly lower after hydropriming than that of untreated seeds at 10 ℃. However, the percentage of seeds infested with bacteria was increased. Li[7] found that hydropriming of onion seeds at very high R. H. (about 100%) significantly increased their infestation with *Penicillium* spp., but this treatment reduced the incidence of *Botrytis allii*.

The incidence of *Penicillium* spp. increased after priming seeds in PEG alone at 10 ℃. This finding was supported by the results of previous reports. Zhang[8] found that osmopriming of onion seeds significantly increased their infestation with *Penicillium spp*. Tylkowska and Biniek[9] studied the effect of carrot and parsley seeds conditioning in PEG 6000 alone and combined with Iprodione and/or Thiram on seed-borne fungi and seed germination. After seed osmopriming, a considerable increase in seed infestation with fungi was observed. It seems possible that fungi, which were already present in the seed samples, could multiply and were distributed among the seeds during the procedure. Leaching of seed exudates can stimulate microbial activity. Therefore, during seed soaking in the osmotic solution, seed leakage could contribute to the increase of fungal proliferation[10] or some fungi could also occur in the osmotic system[11]. Tylkowska and Van den Bulk[12] reported that in the carrot seed lots with intermediate or high incidence levels of *Alternaria* spp., priming resulted in significantly higher levels of contamination. Moreover, fungi invaded inner parts of the seeds, especially after PEG priming. These findings indicate that the level of fungal contamination of seeds should be evaluated prior to the selection of seed lots for priming. Habdas et al.[13] pointed out that matriconditioning stimulated development of fungi in cucumber, China aster and onion seeds. In non-endospermic seeds (cucumber, China aster) strong hydrolysis of storage proteins and lysis of tissues of embryo by developing fungus were observed. In endospermic seeds (onion) the strongest changes such as hydrolysis of storage substances and disintegration of tissue occurred in seed coat and in endosperm.

Sterilisation treatment resulted in a considerable reduction of *Penicillium* spp. and higher level of healthy seeds at 10 ℃. It can be concluded that the majority of the fungi——*Penicillium* spp. were surface contaminants.

Two microbial preparations were used in the experiment: Biozym and Promot. These preparations did not control *Penicillium* spp. when they were applied alone or in combination with osmopriming. Biological control of plant diseases by seed treatment with microorganisms has been directed mainly against soil-borne organisms. Both fungi and bacteria have been tested for this purpose. Biological control is more variable and less effective than control obtained with manufactured pesticides often due to the poor ecological competence of the biocontrol microorganisms, i.e. the ability of the organisms to survive and compete in nature. The ability of such organisms to colonize root surfaces is known as rhizosphere competence and their intrinsic ability to grow on and colonize seed surfaces is called spermosphere competence[14]. Seed treatment applications of biologically active organisms have been shown to be effective against the fungus for considerable periods (12-16 weeks) in naturally infested soil in some situations, but in other seed treatments a less effective method than the application of biocontrol organisms as soil amendments or in pellet form[15]. The treatment of seeds with microorganisms which are beneficial to plant growth or which achieve disease control has been the subject of considerable investigation for many years, often with mixed results[16].

The effects of Promot and Biozym treatments combined with osmopriming and hydropriming on onion seed health have never been investigated. Hence, further research is needed.

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Research on chaotic characteristics of the disaster rate of crops and its GA-BPNN forecasting model

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Abstract: The chaos theory is used to test chaotic characteristics of the disaster rate of wheat rust certain part of Hubei province. Then the forecasting model is established to forecast the disaster rate by combining BP-NN with GA. With reconstruction of phase space, determining the input numbers and values and the optimized BP algorithms, the disaster rate has been successfully forecasted.

Key words: wheat disaster rate forecast; chaos character; reconstruction of phase space; GA-BPNN model

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引发剂、杀真菌剂和生物制剂对洋葱种子的抑菌作用

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[摘 要] 以洋葱种子"Wolska"为材料, 研究了在 10 ℃时杀真菌剂、生物制剂、PEG 8000 和水引发单独处理及复合处理对洋葱种子的抑菌效果。结果表明, 未处理的对照种子主要受青霉菌感染; 杀真菌剂(单独处理)在很大程度上可减少青霉菌的感染率; 而生物制剂(Biozym 生物合剂, Promot 助长剂分别单独处理)未能很好地控制青霉菌的感染; 水引发剂处理后, 种子青霉菌的感染率极显著地低于未处理的对照种子; 在杀真菌剂与 PEG 8000 同时处理种子和杀真菌剂先于水引发剂处理种子的 2 种复合处理中, 可检测到高比例的未染菌种子。

[关键词] 洋葱种子; 引发剂; 杀真菌剂; 生物制剂
[中图分类号] S436.33