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tongbao@im.ac.cn

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研究报告

Field Control Efficiency of a Microbial Seed Dressing Agent of *Bacillus* Strain on Soybean Fungal Root Rot

LI Jing[1,2*] ZHANG Shu-Mei[2] WANG Yu-Xia[2] ZHAO Xiao-Yu[2]

(1. *Department of Life Science and Engineering, Harbin Institute of Technology, Harbin, Heilongjiang* 150001, *China*)

(2. *Institute of Microbiology of Heilongjiang Academy of Sciences, Harbin, Heilongjiang* 150010, *China*)

Abstract: Soybean fungal root rot diseases are the most serious soybean disease problem in Heilongjiang province. The microbial seed dressing agent comprised of *Bacillus* strain B29 which showed strong antagonistic ability to the pathogens of *Fusarium oxysporum*, *Pythium* spp. and *Rhizoctonia solani* were applied to control soybean fungal root rot in field. The control efficiencies of the treatment T3, T4, T5, T6 on soybean fungal root rot after 35-day cultivation were 50.2%, 60.0%, 48.3%, 49.4% respectively, which were better than that of Carbendazim-Carbofuran-Thiram seed coating agent (T2). The second investigation after 50-day cultivation showed the control efficiency of T4 was the best (60.7%), while the control efficiency of T3 was not significantly different from T2. The control efficiencies of T5 and T6 were lower than that of T2. The microbial seed dressing agents were safe to soybean cultivation based on the investigation on seed germination rate, plant height, fresh weight and root nodules. The results based on 2-year demonstrating in field showed that soybean seeds germinating rate was more than 90% after seeds dressing by the microbial seed dressing agents, and the control efficiencies on soybean root rot diseases were 56.3% to 89.1%. It suggested that the microbial seed dressing agents were not only efficient to control soybean root rot diseases but also increased soybean productions.

Keywords: Microbial seed dressing agents, Control efficiency, Soybean fungal root rot

一种枯草芽孢杆菌 B29 菌株拌种剂对大豆根腐病的田间防效研究

李 晶[1,2*] 张淑梅[2] 王玉霞[2] 赵晓宇[2]

(1. 哈尔滨工业大学生命科学与工程系 黑龙江 哈尔滨 150001)

(2. 黑龙江省科学院微生物研究所 黑龙江 哈尔滨 150010)

摘 要: 根腐病是黑龙江省主栽作物大豆的主要病害之一。生防枯草芽孢杆菌 B29 菌株对引起大豆根腐病的镰刀菌、腐霉菌和立枯丝核菌具有强拮抗作用。以枯草芽孢杆菌为主要成分的微生物拌种剂对大豆根腐病的田间防效结果: 播种后 35 d 调查, 4 种不同处理的平均防效分别为 50.2%、60.0%、48.3%、49.4%, 均高于 30%多克福种衣剂的防效(44.9%)。第 2 次(50 d)调查, 处理 4 的防效(60.7%)明显好于化学种衣剂(48.6%), 处理 3 的防效与化学种衣剂的防效差异不显著。通过对生长期大豆出苗率、株高、鲜重和根瘤数等各项生理指标的调查, 结果表明枯草芽孢杆菌微生物拌种剂对大豆的生长和发育是安全的。经过黑龙江省 6 个不同县市的 2 年田间示范, 微生物拌种剂对大豆根腐病的田间防效达 56.3%~89.1%, 增产率为 9.4%~24.6%。

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***Corresponding author:** Tel/Fax: 86-451-84674876; ✉: lijing0706@hit.edu.cn

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关键词：微生物拌种剂，防效，大豆根腐病

Soybean fungal root rot diseases can and do occur each year in the world. Soybean plants with root rot are noticeable in fields because of uneven growth. Affected plants may appear yellow, stunted, and wilted. The plants may be scattered or found in large patches; they often occur in fields or areas with poor drainage[1,2]. Cool weather promotes these root rot problems because such a weather slows soybean root development and are ideal for some soilborne fungi. The early-season soybean diseases include those that cause seed decay, seedling blights, damping-off, and soybean root rots. Most of these early-season soybean diseases are caused by fungi singly or together that are found in the soil wherever soybean scultivited. *Fusarium*, *Pythium*, *Rhizoctonia* and *Phytophthora* are the most common of these early-season pathogens[3]. The causal fungus can survive well in the absence of host plants because it grows well in the soil, colonizes many types of plant debris and can also survive as resting mycelia or sclerotia in the soil. The extent of the damage due to soybean diseases in a given season depends on a number of factors, including the susceptibility of the soybean variety to the specific disease, the level of pathogen inoculum present and the environmental conditions during that season.

Fusarium root rot can occur at any time during the growing season, but is most common on seedlings and young plants. Fusarium root rot is caused by several *Fusarium* species, such as *Fusarium oxysporum* or *F. solani*. In fine-textured (heavy) soils or where root growth is restricted by compaction, root rot tends to be more severe and yields are reduced[4]. Fusarium root rot often is found in low spots in a field. Rhizoctonia root rot is caused by *Rhizoctonia solani* and readily identified by its typical elongate, sunken and reddish-brown lesions on basal stems. Small, brown or black sclerotia may form inside the stem and occasionally on the surface of older lesions. For Rhizoctonia root rot, damping-off may occur at the seedling stage; therefore, seedlings dying from root rot sometimes are evident in the field. Rhizoctonia root rot is frequently found in combination with other diseases such as soybean cyst nematode or Fusarium root rot. Damage from *Rhizoctonia* may be more severe when it occurs in combination with other diseases[3].

Pythium Root Rot is caused by various species of *Pythium*. *Pythium* spp. may cause disease at any time of the season, but seed rots and damping off diseases are most common. *Pythium* species typically infect young tissues and cause a soft decay that is initially water-soaked and become necrotic. Seeds, roots, and seedlings become soft and mushy and rapidly decay. Infected seedlings that emerge may wilt and die within the first few weeks of growth[4].

Phytophthora Root Rot is caused by the soilborne fungus *Phytophthora*. *Phytophthora* is widely distributed. It can cause seed decay or pre- and post-emergence damping-off and seedling blight of soybean. This disease is most common after soil temperature increases and high rainfall results in soils waterlogged for long periods. It is especially prevalent in low-lying areas of the field.

Soybean fungal root rot diseases are the most serious soybean disease problem in Heilongjiang province. Since its discovery, soybean fungal root rot has spread to the most areas in the north-east and middle-south of Heilongjiang province with large soybean acreages. Severe yield loss caused by these diseases is especially common in the succession cropping field. The most common soybean fungal root rots in Heilongjiang province are Fusarium root rot, Pythium root rot, Phytophthora rot, and Rhizoctonia root rot[5]. Each of these diseases may occur singly or in combination with the others. In general, a seed treatment with chemical fungicide is effective to control soybean fungal root rot diseases. But due to the long-term usage of the chemical fungicide, the side-effects such as resistance in pathogens populations[6], unsafety to the human and animals and contaminated to the environment have been more evident. Recently, the idea of sustainable development and environmental protection enhance the application of biological control agents against plant pathogens. *Trichoderma* spp. and *Bacillus* spp. have been reported to significantly suppressed soybean root-rot[7,8]. An antagonistic strain of *Bacillus* to inhibit *Fusarium oxysporum*, *Pythium* spp. and *Rhizoctonia solani* was isolated and showed strong antagonistic ability to the pathogens in greenhouse[9,10]. This study reports the field control efficiency of the *Bacillus* strain B29 on soybean root rot by seed dressing, which will not only supply guidance on application of the microbial seed dressing agent, but also constitute an addition to the scanty literature of biocontrol on soybean fungal root rot.

1 Materials and methods

1.1 Bacterial strain

Bacillus strain B29 was isolated from the soil samples of the cucumber rhizosphere, stored at −80°C in NYD (0.8% beef extract, 0.5% yeast extract, 1% glucose) amended with 15% glycerol.

1.2 Microbial seed dressing agent production

Bacillus strain B29 liquid cultures were incubated on a rotary shaker at 120 r/min at 30°C overnight, then fermented in the automatic fermentation jar at 150 r/min at 30°C for 48 h. The concentration of *Bacillus* strain B29 was 3.5×10^9 CFU/mL quantitated by the serial dilution agar plate procedure. The film-forming agent was added to produce soybean seed dressing agent comprised of 3.5×10^8 CFU/mL or 3.5×10^7 CFU/mL antagonistic strain.

1.3 Field experiment of the microbial seed dressing agents against soybean fungal root rot

The study was conducted at the experimental field of the Agriculture Technology Extension Center in LinDian county of Heilongjiang province, where soybean has been cultivated for 2 years and soybean fungal root rot diseases were severe last year. The area was divided into four blocks to represent the replication and the treatment plot size used was 100 m^2. The soil pattern of the test field was carbonate chernozem, its organic material content was 3%. The cultivated soybean variety was AN9-1577. Treatment applications were undertaken on April 28th, 2003 using the microbial seed dressing agents, the ratio of the seed dressing agents to soybean seeds was 1:80 (*V/W*). Then the treated seeds were planted on April 30th. All cultivated practices in soybean such as weeding, fertilization, and irrigation were routine. The investigation of control efficiency on soybean fungal root rot was undertaken on June 5th and June 20th respectively. The samples of each treatment were collected at five areas at random, and 30 plants were investigated at every sampling area. The disease index of soybean root rot was investigated, and then calculated the biocontrol efficiency.

The different treatments tested were as following:

T1- untreated control

T2-30% Carbendazim-Carbofuran-Thiram seed coating agent (chemical pesticide treated control)

T3-3.5×10^8 CFU/mL soybean seed dressing agent

T4-3.5×10^8 CFU/mL soybean seed dressing agent with 50 g/667 m^2 fertilizer

T5-3.5×10^7 CFU/mL soybean seed dressing agent

T6-3.5×10^7 CFU/mL soybean seed dressing agent with 50 g/667 m^2 fertilizer

1.4 Demonstration application of the microbial seed dressing agents

The microbial seed dressing agents were improved by adding up a kind of colorant and some kinds of microelement essential to the growth of soybean, then were applied to some counties of Heilongjiang province during 2004 and 2005. The demonstration areas included Yilan county, A'cheng town, Hulan district of Harbin city, Bayan county, Fangzheng county and Yanshou county, which showed severe soybean fungal root rot diseases last year. Before planting, soybean seeds were dressed with the microbial seed dressing agents by the ratio of 1:80 (*V/W*). All cultivated practices in soybean such as weeding, fertilization, and irrigation were followed as usual. During soybean growth period, the seeds germination rate and the control efficiencies on soybean root rot diseases were investigated. Soybean production increasing rates of all demonstration areas were investigated and calculated after plant harvesting.

1.5 Statistical analysis

All data were analyzed using SPSS 13.0 Statistical analysis software.

2 Results and discussion

2.1 The effect of the microbial seed dressing agents in field

The microbial seed dressing agents control efficiencies of the treatment T3, T4, T5, T6 on soybean fungal root rot after 35-day cultivation were 50.2%, 60.0%, 48.3% and 49.4% respectively, which were better than that of the Carbendazim-Carbofuran-Thiram seed coating agent (T2) (Table 1). The second investigation after 50-day cultivation showed the control efficiency of T4 was the best (60.7%), while the control efficiency of T3 was not significantly different from T2. The control efficiencies of T5 and T6 were lower than that of T2.

Despite of the control efficiencies of the microbial seed dressing agents on soybean fungal root rot, the influence of seed dressing agents on soybean growth was also investigated. From Table 2, the results showed that the germination rate of the different treatment was above 93%, and no symptoms of chemical damage on plants were observed in the tested soybean field. The plant height, fresh weight and root nodules in the treatment T3, T4, T5, T6 were no distinct difference, which were similar to or slightly better than that of control. In conclusion, the microbial seed dressing agents comprised of *Bacillus subtilis* were safe to soybean cultivation, which can be demonstrated in the soybean field and applied to soybean seeds.

2.2 Demonstration application of the microbial seed dressing agents

The microbial seed dressing agents of *Bacillus* strain were applied to six different counties of Heilongjiang province. The results of 2-year demonstrating in field showed that soybean seeds germination rates were more than 90% after seeds dressing; and the control efficiencies on soybean fungal root rot diseases were from 56.3% to 89.1% (Table 3). The microbial seed dressing agents were not only efficient to control soybean fungal root rot diseases but also increased soybean productions more than 9.4% up to 24.6%.

Table 1 The control efficiency of different treatments on soybean fungal root rot

Treatment \ Time	35-day cultivation		50-day cultivation	
	Average disease index (%)	Control efficiency (%)	Average disease index (%)	Control efficiency (%)
T1 (CK)	26.5		51.9	
T2	14.6	44.9c	24.2	53.4b
T3	13.2	50.2b	26.7	48.6b
T4	10.6	60.0a	20.4	60.7a
T5	13.7	48.3b	30.6	41.0c
T6	13.4	49.4b	28.7	44.7bc

Note: Data are averages of four replicates. CK indicates seeds untreated. Data followed by different letters in the same column are significant difference at 0.05 level.

Table 2 The influence of seed dressing agents on soybean growth

Treatment	Germination rate (%)	Plant height (cm)	Fresh weight(g)	Root nodules
T1 (CK)	95a	13.7a	1.70a	5.5b
T2	96a	14.3a	1.75a	4.1d
T3	94a	13.8a	1.71a	4.6c
T4	96a	14.1a	1.73a	6.3a
T5	93a	13.9a	1.70a	4.8c
T6	95a	14.2a	1.74a	5.4b

Note: Data are averages of four replicates. CK indicates seeds untreated. Data followed by different letters in the same column are significant difference at 0.05 level.

Table 3 The field demonstrating of the microbial seed dressing agents

Demonstrating areas	Germination rate (%)	Control efficiency (%)	Production increasing rate (%)
Yilan county	98.3a	89.1a	17.4b
A'cheng town	93b	56.3d	23.0a
Hulan district	96a	78.9b	24.6a
Bayan county	91b	65.7c	9.4c
Fangzheng county	93b	78.1b	16.2b
Yanshou county	98a	87.9a	13.28bc

Note: Data are averages of four replicates. Data followed by different letters in the same column are significant difference at 0.05 level.

The microbial seed dressing agents comprised of *Bacillus Subtilis* B29 can be applied to control soybean fungal root rot diseases after two years field demonstration, which should be extended to more and more soybean cultivated areas due to its efficient to control soybean fungal root rot diseases and safety to human, animals and the envoirnment.

3 Conclusion

Bacillus strain B29, a strong antagonistic bacterium against the pathogens of *Fusarium oxysporum*, *Pythium* spp. and *Rhizoctonia solani* showed better efficiency to control soybean fungal root rot in field and increased soybean productions. The treatment method of *Bacillus* strain B29 on soybean seeds by seed dressing was simple and safe to soybean cultivation. Although the demonstration application of the microbial seed dressing agents were extended to six different areas in Heilongjiang province, it also should be applied to other soybean cultivated areas of different provinces in China to demonstrate that *Bacillus* strain B29 would be a potential bacterium to control soybean fungal root rot diseases. The applications of the microbial seed dressing agents need to be extended to maize, rice and other crop seeds to control their fungal root rot diseases.

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REFERENCES

[1] Yang XB. Soybean root rot management, *Intergated Crop Management* 7/9/2001.

[2] Yang XB. Soybean root rot in 2003, *Intergated Crop Management* 7/14/2003.

[3] Missouri Pest Management Guide: Corn, Sorghum, Soybean, Winter Wheat. University of Missouri-Columbia 2006, pp.91–93.

[4] Howard RJ, Garland JA, Seaman WL. Diseases and pests of vegetable crops in Canada. Canada: The canadian phytopathological society and entomological society of Canada, 1994, pp.214–216.

[5] Li BY, Ma SM. Pathogens of soybean root rot and screening of resistant sources. *Acta Phytophylacica Sinica*, 2000, **27**(1): 91–92.

[6] Russell PE. Fungicide resistance: occurrence and management. *J Agric Sci*, 1995, **124**: 317–323.

[7] Wang GH, Zhou KQ, Jin J, *et al.* Antagonism on organism BRF-1 against soybean root rot. *Soybean Science*, 2004, **23**(3): 188–191.

[8] Guo RJ, Liu XZ, Yang HW, *et al*. Mechanism of rhizobacteria BH1 (*Bacillus* sp.) to suppress soybean root rot disease caused by *Fusarium* spp.. *Chinese Journal of Biological Control*, 2003, **19**(4): 180–184.

[9] Li J, Yang Q. Biological control of fusarium wilt with an antagonistic strain of *Bacillus subtilis.* In: Yang Q, Yu ZN. Study on plant pest and diseases biological control and bio-technology, Heilongjiang Science Technology Press, 2005, pp.229–236.

[10] Li J, Yang Q, Zhang SM, *et al*. Evaluation of biocontrol efficiency and security of a *Bacillus subtilis* strain B29 against cucumber Fusarium wilt in field. *China Vegetables*, 2009, **2**: 30–33.