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Control of Lentil Seedling Blight Caused by *Botrytis cinerea* Using Microbial Seed Treatments

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ABSTRACT

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Indoor and field experiments were conducted to assess the efficacy of 36 bacterial or fungal agents for suppression of seedling blight of lentil caused by *Botrytis cinerea*. When applied as seed treatment prior to planting, 26 of the microbial agents were effective in reducing incidence of seedling blight, thereby increasing seedling emergence compared to the untreated control. Two bacterial isolates, *Pantoea agglomerans* LRC 954, and *Pseudomonas fluorescens* LRC 1788, and two fungal isolates, *Penicillium aurantiogriseum* LRC 2450 and *Trichoderma harzianum* LRC 2428, were selected for further testing in the greenhouse and results showed that they were effective in increasing seedling emergence and plant height of lentil. These biocontrol agents did not interfere with normal development of root nodules of lentil by *Rhizobium leguminosarum*. The same four isolates were tested in three field experiments, one in 2000 and two in 2001, and two of the three experiments showed significant increases in seedling emergence of lentil. The study concludes that microbial seed treatment with certain biocontrol agents is an effective and environmentally sustainable method for control of botrytis seedling blight of lentil.

Key words : Lentil, *Lens culinaris*, seedling blight, damping-off, *Botrytis cinerea*, biological control, *Trichoderma harzianum, Penicillium aurantiogriseum*, *Pantoea agglomerans*, *Pseudomonas fluorescens*

INTRODUCTION

Botrytis cinerea Pers.:Fr. is a plant pathogenic fungus with world-wide distribution[5] and broad host range[13]. It can infect plants at various stages of development including pre-emergence, seedling, vegetative growth, flowering, fruiting, and post-harvest stages[11]. *B. cinerea* was found on lentil (*Lens culinaris* Medik.) in western Canada [6,7,8,14], where it caused seedling blight or stem and blossom blight, depending on the plant stage at which infection occurred. Outbreaks of botrytis blights during cool, wet weather can cause serious reductions in seed yield and quality of lentil. No botrytis-resistant cultivars of lentil are available. Although fungicidal seed treatment may be effective in preventing seedling blight [3], its use may not always be economically feasible, because lentil is primarily grown in low-input agricultural systems in Canada. In addition, environmental concerns regarding the non-sustainability of fungicides have made their use less desirable. Current practices for control of botrytis diseases of lentil include crop rotation and use of clean, healthy seed.

Studies such as those conducted by Bardin *et al*. [1] a Liang *et al*. [12] indicated that seed treatment with antagoni rhizobacteria was an effective method for preventing seedl diseases of pulse and oilseed crops. Another study conduc by Huang and Erickson [9] showed that foliar application bacterial or fungal biocontrol agents was effective suppressing botrytis stem and blossom blight of lentil. T purpose of this study was to determine the efficacy of s treatment with indigenous fungal or bacterial biocont agents, for control of botrytis seedling blight of lentil.

MATERIALS AND METHODS

Screening of microbial agents (indoor experiments

The 11 fungal and 25 bacterial agents used for this st were selected from those isolated during a previous study

biocontrol of botrytis stem and blossom blight of lentil, and were identified in that study as antagonistic to *B. cinerea* [9]. The bacterial agents were two *Bacillus cereus* Frankland & Frankland (isolates LRC 805 and LRC 1195), three *Bacillus circulans* Jordan (isolates LRC 1667, LRC 1669 and LRC 1675), three *Bacillus subtilis* (Ehrenberg) Cohn (isolates LRC 886, LRC 1105 and LRC 1786), one *Erwinia rhapontici* (Millard) Burkholder (isolate LRC 946), three *Paenibacillus polymyxa* (Prazmowski) Ash *et al*. (isolates LRC 806, LRC 959 and LRC 1784), three *Pantoea agglomerans* (Beijerinck) Gavini *et al*. (isolates LRC 954, LRC 1672 and LRC 1787), seven *Pseudomonas fluorescens* (Trevisan) Migula (isolates LRC 939, LRC 941, LRC 1192, LRC 1654, LRC 1673, LRC 1788 and LRC 8192), and three *Pseudomonas putida* (Trevisan) Migula (isolates LRC 945, LRC 1190 and LRC 1785). The fungal agents were *Coniothyrium minitans* Campbell isolate LRC 2137, *Epicoccum purpurascens* Ehrenb.:Schlect. isolate LRC 2250, *Gliocladium catenulatum* Gilman & Abbott isolate LRC 2087, *Gliocladium roseum* (Link.) Bainier isolate LRC 2090, *Penicilllium aurantiogriseum* Dierckx isolate LRC 2450, two *Penicillium griseofulvum* Dierckx (isolates LRC 2461 and LRC 2464), *Talaromyces flavus* (Klöcker) A. C. Stock & R. A. Sampson isolate LRC 2152, *Trichoderma harzianum* Rifai isolate LRC 2428, *Trichoderma virens* (Miller, Giddens & Foster) von Arx isolate LRC 2425 and *Trichothecium roseum* (Pers.:Fr.) Link isolate LRC 2424.

Botrytis cinerea isolate LRC 2421 was grown on potato dextrose agar (PDA) in Petri dishes at 20℃ under continuous fluorescent light for 21 days. Spores were harvested by flooding each dish with 3 ml of sterile distilled water, scraping the culture gently with a spatula, and straining the resulting suspension through four layers of cheesecloth. The concentration of the resulting suspension was adjusted to 2×10^7 spores/ml. Soil (sandy clay loam, pH 7.5) was artificially infested with *B. cinerea* by adding 40 ml of spore suspension per kg soil and mixing thoroughly. Rootrainers™ (Spencer-Lemaire Industries, Edmonton, Alberta, Canada) were configured to provide grids of 6×16 cells, and were filled with the *B. cinerea*-infested soil to the halfway mark.

Biocontrol agents were grown on PDA in Petri dishes at 20℃ under continuous fluorescent light for 3 days for bacterial agents or 21 days for fungal agents. To each culture, 3 ml of 1% methyl cellulose (Aldrich Chemical, Milwaukee, WI, USA) was added, and the bacterial cells or fungal spores were re-suspended by gently scraping the agar with a spatula. The resulting slurries had concentrations of 1-6×10^9 colony forming units (cfu)/ml for bacteria, and 2-7×10^7 spores/ml for fungi, except for *E. purpurascens*, which was 6×10^6 spores/ml. Lentil seeds cv. Laird were soaked in the slurry for 20 min and placed directly into the half-filled rootrainers™, one seed per cell. Seeds were covered with the remaining infested soil to a depth of 2 cm, the soil was packed tight, and the rootrainers™ were watered and placed in propagation chambers (The Stewart Company, Croydon, Surrey, UK) to maintain high moisture. The seedlings were maintained in a greenhouse at 20±4℃, and watered lightly as needed during the experiment. Biocontrol agents were tested in groups of six per test, with each treatment consisting of four replicates, 24 seeds per replicate, arranged in a completely randomized design. Seeds soaked in water were included in each test as an untreated control. Each experiment was performed twice.

Seedling emergence was recorded five days after planting, and then every two days for the next ten days. Unemerged lentil seeds and wilted seedlings were collected, surface sterilized in 70% ethanol for 90 sec, plated on PDA, and incubated at 20℃ for 10 days to verify infection by *B. cinerea* and development of the disease. Seedling emergence data were standardized across all experimental groups by expressing them as percentage of the untreated control, and analysis of variance was performed on the transformed (percentage) data. Treatment means were compared using Duncan's multiple range tests at P=0.05 level. Analysis was conducted separately on data from each run, and on the combined data from both runs. Seedling emergence data were reverse-transformed for presentation in the Tables. All statistical analyses were performed using SAS version 8.2 [15].

Effect of microbial seed treatments on plant growth and root nodulation of lentil (indoor experiments)

Based on the results of the greenhouse screening, two bacteria: *Pantoea agglomerans* LRC 954 and *Pseudomonas fluorescens* LRC 1788; and two fungi: *Penicillium aurantiogriseum* LRC 2450 and *Trichoderma harzianum* LRC 2428; were selected for further studies. Seeds were treated with biocontrol agents and planted in botrytis-infested soil. Seedling emergence was recorded as previously described. Fifteen days after planting, the height of each lentil plant was measured, and plants were transplanted to pots filled with a mixture of 50% Cornell peat-lite mix [2] and 50% field soil (sandy clay loam, pH 7.5).

Four weeks after transplanting, lentil plants were removed from pots, and their roots were washed in a sieve with running tap water in order to count the number of Rhizobium nodules on each plant. The experiment was performed twice. Seedling emergence, plant height and root nodulation data were analysed using analysis of variance, and comparison of treatment means was accomplished using Duncan's multiple range test at P=0.05 level. Analysis was conducted separately on data from each run, and on the combined data from both runs.

Biocontrol of botrytis seedling blight (field experiments)

The four biocontrol agents *Pantoea agglomerans* LRC 954, *Pseudomonas fluorescens* LRC 1788, *Penicillium aurantiogriseum* LRC 2450, and *Trichoderma harzianum*

LRC 2428 were evaluated for control of botrytis seedling blight in the field experiments conducted at the Fairfield Farm of the Agriculture and Agri-Food Canada Lethbridge Research Centre during 2000 and 2001. The experiments were performed once in 2000 and twice in 2001. For each location-year, the experiment was conducted in a fallowed area of the field. The field was fertilised with ammonium nitrate (34-0-0; N-P-K) prior to seeding, at a rate of 200 kg/ha.

Inoculum of *B. cinerea* LRC 2421 was prepared by mixing 150 g wheat bran (Ellison Milling, Lethbridge, Alberta, Canada) and 150 g corn meal (McCormick, London, Ontario, Canada) with 300 ml distilled water in an enclosed aluminum foil tray, autoclaving twice at 121℃ for 30 min with a 48-h interval, and placing 20 agar plugs (8 mm diameter) of a 14-d PDA culture of *B. cinerea* on the surface of the mixture. The trays were covered with aluminium foil and incubated at room temperature (20±2℃) for two weeks. The colonised bran/corn meal mixture was air-dried at room temperature for 5 d and ground to powder using a Thomas-Wiley model 4 laboratory mill (Thomas Scientific, Philadelphia, PA, USA) with a 1-mm mesh screen. The resulting mycelium/spore powder was made into beads by mixing 2.5 g with 50 ml of 2% alginate (Sigma, St. Louis, MO, USA), and dropping into an aqueous solution of 2% calcium chloride (Fisher, Fair Lawn, NJ, USA). The beads were collected in a strainer, rolled in the spore powder, and air-dried overnight. The experimental field was artificially infested with *B. cinerea* by spreading the alginate beads onto the area at a rate of 100 g/m^2 and mixing them into the soil to a depth of less than 5 cm using a Triple K cultivator (Kongskilde, Exeter, Ontario, Canada).

Lentil seeds cv. Laird were coated with biocontrol agents as previously described, and air-dried overnight at room temperature on a metallic mesh, which was placed over paper towel to absorb excess slurry. Seeds were planted on 7 June 2000 and 30 May 2001, in 5-m long×0.9-m wide plots composed of 4 rows of 100 seeds per row. Seeding was done using a four-row plot seeder set at a depth of 4 cm. Treatments were arranged in a randomized block design with four replications. Untreated controls and fungicide controls were included in each run of the experiment. The fungicide used was Crown^{TM} (92 g/L carbathiin + 58 g/L thiabendazole) (Gustafson, Calgary, Alberta, Canada), applied to the seed prior to planting, at the rate of 0.15 L per 25 kg seed.

The percentage of seedling emergence was recorded 3 weeks after planting. Unemerged lentil seeds and wilted seedlings were collected and plated on PDA as previously described, to verify the infection was caused by *B. cinerea*. The data of seedling emergence were analysed using analysis of variance, and treatment means were compared using Duncan's multiple range tests at P=0.05 level. Analysis was conducted separately on data from each location-year.

RESULTS

Control of seedling blight of lentil by microbial se treatment (indoor experiments)

Treatment of lentil seeds with bacterial agents prior planting resulted in a significant (P<0.05) increase in seedl emergence in 16 of the 25 bacterial isolates tested (Table Seedling emergence for the effective bacteria ranged from to 90%, compared to 38 to 65% for the ineffective bacte and 44% for the untreated control. The effective bacter agents included *Bacillus circulans* (2 isolates), *Bacil subtilis* (2 isolates), *Paenibacillus polymyxa* (3 isolate *Pantoea agglomerans* (2 isolates), *Pseudomonas fluoresc* (6 isolates), and *Pseudomonas putida* (1 isolate). The m

Table 1. Control of seedling blight of lentil caused by *Botr cinerea* by seed treatment with bacterial agents (greenho experiments).

Bacterial isolate[1]	Seedling Emergence (%)
Pantoea agglomerans LRC 1672	90 a[2]
Pseudomonas fluorescens LRC 1788	90 a
Bacillus subtilis LRC 1786	84 a
Pseudomonas fluorescens LRC 1654	82 a
Pseudomonas fluorescens LRC 939	81 ab
Pseudomonas fluorescens LRC 1673	81 ab
Pseudomonas putida LRC 1785	80 ab
Bacillus subtilis LRC 1105	78 ab
Pseudomonas fluorescens LRC 1192	78 ab
Bacillus circulans LRC 1669	76 b
Paenibacillus polymyxa LRC 1784	76 b
Pseudomonas fluorescens LRC 8192	75 bc
Pantoea agglomerans LRC 1787	74 bc
Bacillus circulans LRC 1675	69 c
Paenibacillus polymyxa LRC 959	67 c
Paenibacillus polymyxa LRC 806	66 c
Pantoea agglomerans LRC 954	65 cd
Bacillus cereus LRC 1195	63 cd
Pseudomonas putida LRC 1190	63 cd
Bacillus circulans LRC 1667	62 d
Bacillus cereus LRC 805	55 d
Bacillus subtilis LRC 886	51 de
Pseudomonas fluorescens LRC 941	47 de
Untreated control	44 de
Erwinia rhapontici LRC 946	42 e
Pseudomonas putida LRC 945	38 e
Standard Error	3.0

[1] Lentil cv. Laird seeds were soaked for 20 min in a bacte slurry of 10^9 cfu/ml suspended in 1% aqueous methyl cellul and planted in soil artificially infested with *B. cinerea* at the r of 8×10^8 spores/kg soil.

[2] Means within a column followed by the same letter are significantly different at P = 0.05 level (Duncan's multiple ra test). Data presented are the combined results of two runs of experiment.

effective bacterial isolate was *Pantoea agglomerans* LRC 1672, which showed an emergence rate of 90%.

For the fungal biocontrol agents, seed treatment prior to planting resulted in a significant (P<0.05) increase in seedling emergence in 10 of the 11 isolates tested (Table 2). Seedling emergence for the effective fungi ranged from 60 to 79%, compared to 32% for the untreated control. The effective fungal agents included *Coniothyrium minitans* (1 isolate), *Epicoccum purpurascens* (1 isolate), *Gliocladium catenulatum* (1 isolate), *Gliocladium roseum* (1 isolate), *Penicillium aurantiogriseum* (1 isolate), *Penicillium griseofulvum* (2 isolates), *Talaromyces flavus* (1 isolate), *Trichoderma harzianum* (1 isolate), and *Trichoderma virens* (1 isolate). The most effective fungal isolate was *Penicillium griseofulvum* LRC 2464. Compared to 32% of seedling emergence in the untreated control, seed treatment with *Trichothecium roseum* LRC 2424 significantly reduced seedling emergence of lentil, resulting in emergence of only 14%.

Table 2. Control of seedling blight of lentil caused by *Botrytis cinerea* by seed treatment with fungal agents (greenhouse experiments).

Fungal isolate[1]	Seedling Emergence (%)
Penicillium griseofulvum LRC 2464	79 a[2]
Gliocladium roseum LRC 2090	76 a
Trichoderma harzianum LRC 2428	75 a
Penicillium griseofulvum LRC 2461	69 ab
Coniothyrium minitans LRC 2137	68 ab
Talaromyces flavus LRC 2152	68 ab
Trichoderma virens LRC 2425	68 ab
Gliocladium catenulatum LRC 2087	63 bc
Epicoccum purpurascens LRC 2250	61 bc
Penicillium aurantiogriseum LRC 2450	60 bc
Untreated control	32 d
Trichothecium roseum LRC 2424	14 e
Standard Error	2.7

[1] Lentil cv. Laird seeds were soaked for 20 min in a slurry of 1% aqueous methyl cellulose and 10^6 spores/ml for *E. purpurascens*, or 10^7 spores/ml for the other fungi, and were planted in soil artificially infested with *B. cinerea* at the rate of 8×10^8 spores/kg soil.

[2] Means within a column followed by the same letter are not significantly different at P = 0.05 level (Duncan's multiple range test). Data presented are the combined results of two runs of the experiment.

Effect of microbial seed treatments on plant growth and root nodulation of lentil (indoor experiments)

Lentil plants arising from seeds treated with the biocontrol agents *P. agglomerans* LRC 954, *P. aurantiogriseum* LRC 2450, *P. fluorescens* LRC 1788, or *T. harzianum* LRC 2428, grew significantly taller (P<0.05) than plants arising from untreated seeds (Table 3). Average height of the 15 day-old plants ranged from 11.0 to 12.5 cm for the treatments with biocontrol agents, compared to 9.7 cm for the untreated control. Lentil plants arising from seeds treated with the biocontrol agents also had a significantly higher number of nodules per plant, compared to the untreated control. The number of nodules per plant for the biocontrol treatments ranged from 20 to 22 nodules/plant, compared to 9 nodules/plant for the untreated control.

Biocontrol of botrytis seedling blight of lentil (field experiments)

Seed treatment with the biocontrol agents *P. agglomerans* LRC 954, *P. aurantiogriseum* LRC 2450, *P. fluorescens* LRC 1788, or *T. harzianum* LRC 2428, or with the fungicide Crown™, significantly (P<0.05) improved seedling emergence compared to the untreated control in two out of three field experiments (Table 4). In the experiment in 2000, seedling emergence for the untreated control was 40%, compared to 55 to 69% for the biocontrol treatments, and 69% for the fungicide treatment. The microbial seed treatments of *T. harzianum* or *P. agglomerans* resulted in higher seedling emergence than the treatments of *P. aurantogriseum* or *P. fluorescens*, and were not significantly different than the fungicide treatment (Table 4).

Table 3. Control of seedling blight of lentil caused by *Botrytis cinerea* by seed treatment with bacterial or fungal agents (greenhouse experiments).

Treatment[1]	Seedling emergence (%)	Seedling height (cm)	No. nodules per plant
Pseudomonas fluorescens 1788	94 a[2]	12.5 a	21 a
Pantoea agglomerans 954	97 a	11.3 b	22 a
Penicillium aurantiogriseum 2450	95 a	11.0 b	21 a
Trichoderma harzianum 2428	95 a	11.8 ab	20 a
Untreated control	33 b	9.7 c	9 b
Standard Error	3.1	0.5	2.1

[1] Lentil cv. Laird seeds were soaked for 20 min in a slurry of 1% aqueous methyl cellulose and 10^7 spores/ml for fungi, or 10^9 cfu/ml for bacteria, and were planted in a mixture of 50% soil and 50% Cornell mix, artificially infested with *B. cinerea* at the rate of 8×10^8 spores/kg mixture.

[2] Means within a column followed by the same letter are not significantly different at P = 0.05 level (Duncan's multiple range test). Data presented are the combined results of two runs of the experiment.

Table 4. Effect of seed treatment with biocontrol agents or fungicide on seedling emergence of lentil in a field artificially infested with *Botrytis cinerea* (field experiments, 2000-2001).

Treatment[1]	Seedling emergence (%)		
	2000	2001 run 1	2001 run 2
Fungicide (Crown™)	69 a[2]	83 a	98 a
Trichoderma harzianum 2428	69 a	56 a	77 b
Pantoea agglomerans 954	68 a	65 a	72 b
Penicillium aurantiogriseum 2450	59 b	57 a	75 b
Pseudomonas fluorescens 1788	55 b	68 a	77 b
Control	40 c	62 a	51 c
Standard Error	1.0	6.2	1.8

[1] Lentil cv. Laird seeds were soaked for 20 min in a slurry of 1% aqueous methyl cellulose and 10^7 spores/ml for fungi, or 10^9 cfu/ml for bacteria, and were planted in a field artificially infested with alginate beads of *B. cinerea* at the rate of 100g/m^2.

[2] Means within a column followed by the same letter are not significantly different at $P = 0.05$ level (Duncan's multiple range test). Data presented are the combined results of two runs of the experiment.

For the experiment in 2001 (run 2), seedling emergence for the untreated control was 51%, compared to 72 to 77% for the biocontrol treatments, and 98% for the fungicide treatment (Table 4). All of the biocontrol seed treatments were equally effective in suppressing botrytis seedling blight, but none were as effective as the fungicide treatment. For run 1 of the 2001 experiment, lower disease pressure and higher variability in seedling emergence resulted in no significant differences (P>0.05) between treatments.

DISCUSSION

The indoor and field experiments carried out during this study demonstrate that the bacteria *P. agglomerans* LRC 954 and *P. fluorescens* LRC 1788, and the fungi *P. aurantiogriseum* LRC 2450 and *T. harzianum* LRC 2428, have potential as microbial seed treatments for control of seedling blight of lentil caused by *B. cinerea*. While lentils are vulnerable to infection by *B. cinerea* during seed germination and seedling growth, the increases in seedling emergence in the microbial-treated seeds observed in this study may be due to occupation of the rhizosphere niche by biocontrol agents and competitive exclusion of the pathogen from that niche. In addition, the production of antibiotic substances has been suggested as another possible mechanism for suppression of *B. cinerea* by antagonistic bacteria or fungi [(9)]. Further studies are required to understand the mechanisms involved in the control of botrytis blight of lentil by each of the biocontrol agents, and to apply the knowledge to improve efficiency of the seed treatment techniques.

This study shows that the ability to suppress botrytis seedling blight could be found among a wide range of bacterial and fungal species. It also demonstrates that this t is isolate-specific and not species-specific. For examp within the tested isolates of each of the bacterial spec *Bacillus circulans*, *Bacillus subtilis*, *Pantoea agglomera Pseudomonas fluorescens*, and *Pseuodomonas putida*, so isolates were effective in suppressing botrytis seedling bli of lentil but other isolates of the same species were ineffect (Table 1). This suggests that screening and selection superior isolates is critical in ensuring the success biocontrol of botrytis seedling blight of lentil.

Although *Trichothecium roseum* LRC 2424 v suppressive to *Botrytis cinerea in vitro* (Huang and Ericks unpublished), this study showed that it decreased ler seedling emergence when applied as a seed treatment prio planting. *Trichothecium roseum* is a mycoparasite *Sclerotinia sclerotiorum* (Lib.) de Bary [(10)] and a we pathogen of a wide range of higher plants [(4)] including legu crops such as bean [(17)] and lentil [(16)]. These findings sugg that *T. roseum* has no potential for use as a biocontrol ag for botrytis blight of lentil because of its ineffectiveness a its pathogenicity on higher plants.

The finding that suppression of *B. cinerea* is a relativ common trait among soilborne bacteria and fungi sugge that a high level of microbial biodiversity in soil may desirable for suppression of plant pathogens. Plant dise control in the past century has generally been focused on use of chemical pesticides, which practice has been recer criticized due to negative impacts on human health a environmental sustainability. This study suggests t biological control has potential as an environmenta friendlier alternative to use of chemicals. Further researcl this area and other areas of soil microbial ecology worthwhile, in order to improve the sustainability agricultural practices with respect to plant disease control.

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摘 要

黃鴻章[1,2]、Erickson, R. S.[1] 2005. 種子處理拮抗微生物防治扁豆幼苗立枯病 (*Botrytis cinerea*). 植病會刊 14:35-40. ([1] 加拿大農業及農業食品部 Lethbridge 研究中心；[2] 聯絡作者，電子郵件：huangh@agr.gc.ca；傳真：+1-403-382-3156)

本研究於溫室及田間進行試驗，評估種子處理拮抗真菌或細菌對於扁豆幼苗立枯病 (由 *Botrytis cinerea* 引起) 的防治效果。溫室初步試驗結果顯示，供試的 36 個拮抗真菌與細菌菌株中，有 26 個菌株可以有效降低發病率與增加出苗率。進一步選取其中兩個細菌菌株 *Pantoea agglomerans* LRC954 和 *Pseudomonas fluorescens* LRC1788、兩個真菌菌株 *Penicillium aurantiogriseum* LRC2450 和 *Trichoderma harzianum* LRC2428 於溫室進行栽培試驗，結果發現四個受測菌株不但可以增加株高與出苗率，而且不會影響扁豆根系的根瘤數。於西元 2000 和 2001 年間在田間進行三次病害防治試驗，顯示這些菌株在其中兩次試驗具有顯著降低病害與增加出苗率的效果。由此可見，利用拮抗微生物處理扁豆種子是防治扁豆幼苗立枯病的一種有效且對環境無害的良方。

關鍵詞：扁豆、幼苗立枯病、生物防治、木黴菌 (*Trichoderma harzianum*)、青黴菌 (*Penicillium aurantiogriseum*)、團泛菌 (*Pantoea agglomerans*)、螢光假單胞菌 (*Pseudomonas fluorescens*)