# Effect of Some Bioagents, Fungicides and Fertilizers on Bean Root Rot Caused by *Sclerotium rolfsii*

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## ABSTRACT:

*Bean is subjected to infection with damping-off and root rot diseases causing great loss in seedling emergence and seed yield. Under laboratory conditions, the most effective bioagent on decreasing linear growth and number of selerotica of Sclerotium rolfsii was T. viride followed by Azotobacter chrococcum (genetically modified), Pseudomonas fluorecense and Bacillus megaterum, respectively. While, A. chrococcum and Candida spp. were the least effective ones. Under greenhouse conditions, the bioagent T. viride was the most effective on reducing incidence and severity of the infection with S. rolfsii and gave the highest crop yield followed by A. chrococcum; P. fluorecense and B. megaterum, respectively. While, A. chrococcum and Candida spp. were the least effective ones.*

*The most effective fungicide on reducing the linear growth of S. rolfsii in vitro was Ronilan followed by Vitavax/Thiram. While, Euparein and Switch gave the lowest effect. Under greenhouse conditions, the same results were obtained using these fungicides against damping-off disease.*

*Applying different levels of fertilizers (nitrogen, phosphorus and potassium) decreased incidence of post emergence damping-off at different degrees. Using the previous fertilizers at levels of 100:200:100 kg/feddan and 150:300:150 kg / feddan significantly reduced the disease than the other levels.*

**Key words:** Bean, *Phaseolus vulgaris*, root rot, *Sclerotium rolfsii*, control.

## INTRODUCTION

Bean (*Phaseolus vulgaris L.*) is one of the most important vegetable crops in Egypt. This legume crop is widely cultivated for local consumption and exportation as green vegetable pods and dry seeds. Bean crop recorded the second level in the vegetable exportation during the year of 2003 with 7 million Egyptian pounds as exportation value. Therefore, special attention has been given to increase the cultivated area of this crop in Egypt. The cultivated area was clearly increased from 39721 feddan in 2002 to 61217 feddan in 2003 (Economical Statistic Report, Agricultural Statistics, Ministry of Agriculture and Land Reclamation, Economic Affairs Sector, Volume2, March 2002).

Bean is subjected to attack by numerous pathogenic microorganisms at all stages of growth and wherever the crop is grown, which seriously affect the quality and quantity of the yield. Soil-borne fungal pathogens are particularly considered as serious disease agents which affect seed germination, seedling emergence and root rot disease causing great losses in the yield (Furuya, 1982; Diaz-Franco. 1984 and Rusuku, 1992). In Egypt, root rot is considered the most destructive disease on bean. However, little attempts were carried out to investigate bean root rot disease and its control, particularly caused by *S. rolfsii*.

*Trichoderma harzianum* was considered the major biocontrol agent used by many investigators to control damping off and root rot disease of beans. Early reports mentioned that root rot disease caused by *S. rolfsii* could be controlled by antagonistic micro-organisms, *i.e. Trichoderma lignorum* Fawcett (1935). *T. harzianum* effectively controlled damping-off and root rot of bean, groundnut, eggplant, tomato and cotton when the soil infested with *S. rolfsii* or *R. solani* (Chet and Elad, 1982). The number of the fungal propagules of *T. harzianum* in nature was not sufficient as biocontrol purpose as well as *T. harzianum* was marked affected with soil acidity and temperature Ahmed and Baker (1987). *Bacillus. subtilis* str. F-29-3 make *S. rolfsii* to form bulging hyphae on potato dextrose agar and to accumulate chitin-like substances in the hyphae, as well as the culture filtrate of *B. subtilis* stimulates sclerotial formation of *R. solani* (Tschen, 1987). Four strains of *T. harzianum* reduced root rot of bean caused by *S. rolfsii* by 30-50 % (Papavizas and Lewis 1989). In greenhouse experiment at Italy, *T. viride* was more effective than other antagonistic fungi tested as seed treatment against *S. rolfsii* on bean plants (Roberti *et al.* 1993). Of 120 diverse bacterial strains tested for their ability to protect bean seedlings from pre-emergence damping-off, 9 bacterial strains on *P. ultimun.*, 5 on *R. s.* ...*ii* and 9 on *F. solani* f.sp. *phaseoli* provided significant suppression of disease severity compared with the non-bacterized control (Reddy *et al.* 1994). They

added that no relationship between the ability of bacterial strains to inhibit fungal vegetative growth on solid culture media and their ability to suppress pathogen activity in the greenhouse but for a few strains, disease reduction was linked to reduced growth of the pathogens in liquid media.

Carboxin was the most effective, inhibiting mycelial growth and sclerotial germination of *S. rolfsii*, isolated from bean roots. However, Thiram, Captan, PCNB (quintozene) and Lesan + quintozene were moderately efficient *in vitro* (Menten, 1980). Mixed treatment containing benomyl and placement in soil gave no significant efficiency on bean root rots caused by *S. rolfsii, R. solani, Pythium* spp., *Aphanomyces* sp. and *F. solani* f.sp. *phaseoli* (Brien *et al.* 1991). Under greenhouse conditions, the best control of *S. rolfsii* on French bean obtained by seed treatment with benomyl, benalaxyl + copper oxychloride and thiabendazole (Thakur *et al.*, 1991). Treatment of bean seeds with DCT (diazinon + captan + thiophanate methyl) improved germination and reduced seed and hypocotyls infection with *Pythium ultimum* and *S. rolfsii* (Tu and Zheng1993). Using fungicides and bioagents led to best results against root rot on French bean (Mukherjee *et al,* 2001).

The purpose of this investigation was to study the efficiency of different bioagents, fungicides and fertilizers controlling root rot of bean caused by *S. rolfsii.*

## MATERIALS AND METHODS:

*Sclerotium rolfsii* was isolated from the infected roots on Potato Dextrose Agar (PDA) medium.

Inoculum of *S. rolfsii* was multiplied on sand wheat bran medium (1: 3, v/v) in 250 ml glass bottles and watered with 50 ml water. The bottles were autoclaved at 121°C for 30 minutes. The bottles were inoculated with the fungus. All bottles were incubated at 28°C for 15 days. The bottles were daily shaken to spread the fungal inoculum on the medium.

**A- Effect of some bioagents:**

This investigation was carried out for studying the efficiency of the following bioagents as follows:

1. *Candida* spp.
2. *Trichoderma viride.*
3. *Azotobacter chrococcum*
4. *A. chrococcum* (genetically modified and kindly provided by Dr. A. A. Ali, Microbial Genetic Department, N RC, Egypt.)
5. *Bacillus* sp.
6. *Bacillus megaterum.*
7. *Bacillus subtilis.*
8. *Pseudomonas peutida*
9. *Pseudomonas fluorescens*

The aforementioned bioagents were isolated from the rhizosphere of bean plants and the fungi isolated on PDA medium. While, bacteria were isolated on nutrient glucose agar medium. The aforementioned fungal bioagents were grown in liquid potato dextrose, while bacterial bioagents were grown in nutrient glucose broth for one week.

***In vitro* experiment:**

The antagonistic ability of different bioagent was tested against *S. rolfsii* on PDA medium. One ml of culture filtrate of bioagent was placed 20 mm apart from the edge of PDA plates (90 mm). A disk of the pathogen was placed 50 mm away from the biocontrol agent. Cultures were incubated in the dark at 28°C until the growth of the pathogen completely covered the check plates. The inhibition of the pathogen growth was taken as an index of antagonistic ability, which was calculated by comparing radial growth of the pathogen colony directly to the bioagent colony with maximum radial growth.

***In vivo* experiment:**

Autoclaved sterilized soil was inoculated at sowing time with the pathogen (3% v/v). Bioagents were inoculated at sowing time (3% v/v of each). Five seeds were sown in each pot. Each treatment consists of five replicates. Check treatment inoculated only with the pathogen only.

**B- Effect of some fungicides:**

Five fungicides, i.e. Euparein (Diclofluanid at 250g/100 L), Ronilan (Vinclozaline at150g/100 L), Switch (Fludioxanil + Cyprodinil at 75g/100 L), Telidor (Fenhexamid at 600cc/fed.) and Vitavax/Thiram (Carboxin at 250 g/100 L) were used. These fungicide treatments were tested in soil, previously infested with S., rolfsii to investigate their efficiency on controlling pre- and post-emergence damping-off disease of Giza 3 beans cultivar, under greenhouse conditions. Ten seeds were sown in each pot. Each treatment was replicated five times. Pots containing soil without fungicides were served as control treatment. The aforementioned fungicides were also tested in the laboratory to study their efficiency on inhibiting the linear growth of *S. rolfsii* at1-25 ppm using PDA medium.

**C- Effect of some fertilizers:**

Three fertilizers namely ammonium nitrate (N), calcium phosphate (P) and potassium sulfate (K) were added to soil previously infested with the tested

pathogen at different rates in different combinations as mentioned in Table (5). Ten seeds of Giza 3 bean cultivar were sown per pot (25 cm in diameter). Each treatment was replicated five times. The percentage of dead plants was calculated 30 days after sowing. Survived plants were also calculated 60 days after sowing. Averages of plant height, number and weight of pods per plant were also estimated.

**Disease assessment and Statistical analysis:**

Pre-emergence damping-off was calculated 14 days after sowing and post-emergence damping-off was calculated after 21days. Root rot severity was determined after 60 days according to Horsfall and Barrtt (1945).

All obtained data were subjected to the proper statistical analysis using the MSTAT statistical software. Comparisons were made following Fishers LSD (0.05).

## RESULTS AND DISCUSSION:

It is clear from Table (1) that the bioagent T. viride was the most antagonistic one against *S. rolfsii* on beans under greenhouse conditions and resulted in the highest pod yield during both seasons (2002 and 2003), followed by modified A. chrococum P. flourocens, Bacillus megaterum and Bacillus spp., respectively. On the other hand, the bioagents Candida spp. and A. chrococum were the least effective ones and resulted in the lowest pod yield.

The aforementioned data are confirmed with those obtained by Chet and Elad (1982) who found that T. harzianum effectively controlled damping-off and root rot of some crops when applied in the form of bran culture to soil infested with *S. rolfsii* or R. solani. Papavizas and Lewis (1989) reported that T. harzianum reduced root rot of bean seedling caused by *S. rolfsii*. Roberti et al. (1993) and Montcalegre and Leranas (1995) found that T. viride were more effective than other antagonistic fungi tested as seed treatments against *S. rolfsii* of bean.

Data shown in Table (2) indicate that the most effective bioagent on decreasing the mean linear growth, number of selerotica of *S. rolfsii* was T. viride, followed by P. fluorecense, modified A. chrococcum, B. megatarem and Bacillus spp., consequently. The least effective one was A. chrococcum, and Candida spp. Tschen (1987) stated that the culture filtrate of B. subtilis stimulated sclerotial formation of *S. rolfsii*. Moreover, Reddy et al. (1994) tested 120 bacterial strains for their ability to protect been seedlings from pre- and post-emergence damping-off. They found that all strains provided significant suppression of disease severity.

Data presented in Table (3) indicate that the most effective fungicide on inhibiting the linear growth of *S. rolfsii* was Ronilan followed by Vitavax/Thiram. While Euparin and Switch were of the lowest effect ones.

Data presented in Table (4), show that the most effective fungicides against *S. rolfsii* under greenhouse conditions were Ronilan and Vitavax/Thiram. Meanwhile, the least effective ones were Telidor and Switch.

Table (1): Effect of some bioagents on the infection with *S. rolfsii* of bean under greenhouse conditions, 60 days after sowing.

| Treatments | 2002 | | 2003 | |
|---|---|---|---|---|
| | Root rot severity % | ** Pod yield (gm) | Root rot severity% | ** Pod yield (g) |
| ***Candida* spp.** | 32.0 | 9.4 | 34.0 | 8.5 |
| ***Trichoderma viride*** | 12.0 | 18.5 | 13.3 | 17.55 |
| ***Azotobacter chrococcum*** | 36.0 | 9.3 | 46.0 | 8.0 |
| ***A.. Chrococcum* (Modefied)** | 16.0 | 18.25 | 20.0 | 16.65 |
| ***Bacillus* sp.** | 24.0 | 10.5 | 26.0 | 9.75 |
| ***Bacillus megaterum*** | 22.0 | 12.3 | 24.0 | 11.25 |
| ***Bacillus subtilis*** | 28.0 | 10.18 | 33.0 | 9.5 |
| ***Pseudomonas peutida*** | 30.0 | 9.75 | 35.0 | 9.25 |
| ***Pseudomonas fluorecense*** | 20.0 | 13.75 | 22.0 | 13.5 |
| **Control** | 42.0 | 9.0 | 66.0 | 7.5 |
| **L.S.D. at 5%** | 1.78 | 0.72 | 1.28 | 1.2 |

* Root rot caused by *S. rolfsii* on beans.

** Yield per plant in gram.

Table (2): Effect of some cultural filtrates of some bioagents on *S. rolfsii* linear growth on beans *in vitro* at 28 °C for 5 days.

| Treatments | Linear growth (mm) | % inhibition | No. of sclerotia from Edge of PDA | No. of sclerotia from 1 cm apart from the pathogen |
|---|---|---|---|---|
| ***Candida* spp.** | 48.7 | 45.89 | 34.75 | 23.75 |
| ***Trichoderma viride*** | 25.0 | 72.2 | 0.0 | 1.75 |
| ***Azotobacter chrococcum*** | 70.7 | 21.4 | 43.75 | 22.5 |
| ***A.. Chrococcum* (Modefied)** | 31.7 | 64.78 | 12.5 | 6.26 |
| ***Bacillus* sp.** | 38.0 | 57.78 | 19.75 | 8.5 |
| ***Bacillus megaterum*** | 36.7 | 59.2 | 12.25 | 13.25 |
| ***Bacillus subtilis*** | 42.7 | 52.56 | 19.5 | 20.75 |
| ***Pseudomonas peutida*** | 44.7 | 50.3 | 24.5 | 16.75 |
| ***Pseudomonas fluorecense*** | 33.0 | 63.3 | 15.0 | 9.5 |
| **Control** | 90.0 | 0.0 | 48.75 | 46.0 |
| **L.S.D. at 5%** | 0.58 | -- | 13.0 | 10.8 |

Table (3): Effect of some fungicides on the linear growth of *S. rolfsii in vitro* at different concentrations 5 days after incubation at 28±1°C.

| Fungicides | Linear growth (cm) at (ppm) | | | | | % Inhibition at (ppm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1.0 | 3.0 | 6.0 | 10 | 25 | 1.0 | 3.0 | 6.0 | 10 | 25 |
| **Euparin** | 9.0 | 9.0 | 6.1 | 3.6 | 0.0 | 0.0 | 0.0 | 32.2 | 60.0 | 100.0 |
| **Ronilan** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| **Switch** | 9.0 | 6.4 | 3.1 | 1.5 | 0.0 | 0.0 | 43.2 | 65.6 | 83.3 | 100.0 |
| **Telidor** | 9.0 | 7.2 | 4.5 | 2.4 | 0.0 | 0.0 | 20.0 | 50.0 | 73.3 | 100.0 |
| **Vitavax/Thiram** | 2.0 | 1.0 | 0.0 | 0.0 | 0.0 | 77.8 | 88.9 | 100.0 | 100.0 | 100.0 |
| **Control** | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **L.S.D. at 5%** | 2.6 | | | | | 7.3 | | | | |

Table (4): Chemical control of bean damping-off caused by *S. rolfsii* under greenhouse conditions.

| Fungicides | Time of adding the fungicides to the soil | | | |
|---|---|---|---|---|
| | 7 days before planting | | 7 days after planting | |
| | Pre-emergence* | Post-emergence** | Pre-emergence | Post-emergence |
| **Euparin** | 14 | 12.0 | 10.0 | 10.0 |
| **Ronilan** | 10.0 | 20.0 | 8.0 | 8.0 |
| **Switch** | 24.0 | 18.0 | 12.0 | 16.0 |
| **Telidor** | 26.0 | 18.0 | 14.0 | 14.0 |
| **Vitavax/Thiram** | 12.0 | 20.0 | 10.0 | 10.0 |
| **Control** | 70.0 | 70.0 | 70.0 | 70.0 |
| **Untreated check** | 84.0 | 80.0 | 88.0 | 80.0 |
| **L.S.D. at 5%** | 3.1 | 2.7 | 1.8 | 2.3 |

* Assessed 14 days after sowing.
* Assessed 21 days after sowing.

Table (5): Different levels of fertilizers (g.) added to the pots.

| Treatments | Rate of application | | |
|---|---|---|---|
| | Ammonium nitrate (N) | Calcium superphosphate (P) | Potassium sulphate (K) |
| | g/pot | g/pot | g/pot |
| $N_0 P_0 K_0$ | 0 | 0 | 0 |
| $N_1 P_0 K_0$ | 0.42 | 0 | 0 |
| $N_1 P_1 K_0$ | 0.42 | 0.66 | 0 |
| $N_1 P_1 K_1$ | 0.42 | 0.66 | 0.42 |
| $N_2 P_1 K_1$ | 0.84 | 0.66 | 0.42 |
| $N_2 P_2 K_1$ | 0.84 | 1.32 | 0.42 |
| $N_2 P_2 K_2$ | 0.84 | 1.32 | 0.84 |
| $N_3 P_2 K_2$ | 1.26 | 1.32 | 0.84 |
| $N_3 P_3 K_2$ | 1.26 | 1.98 | 0.84 |
| $N_3 P_3 K_3$ | 1.26 | 1.98 | 1.26 |

Table (6): Effect of different levels of fertilizers on bean plants inoculated with *S. rolfsii* under greenhouse conditions.

| Treatments | %Post-emergence | Survived plants | Av. plant height (cm) | No. of pods/ plant | Weight of pods (g)/ plant |
|---|---|---|---|---|---|
| $N_0 P_0 K_0$ | 21.3 | 10.5 | 51.0 | 4.0 | 20.0 |
| $N_1 P_0 K_0$ | 12.8 | 22.0 | 76.25 | 11.0 | 96.0 |
| $N_1 P_1 K_0$ | 18.0 | 22.0 | 63.75 | 8.0 | 57.0 |
| $N_1 P_1 K_1$ | 14.4 | 22.5 | 60.0 | 14.0 | 108.0 |
| $N_2 P_1 K_1$ | 18.0 | 22.0 | 55.0 | 6.0 | 44.5 |
| $N_2 P_2 K_1$ | 15.8 | 24.0 | 63.75 | 8.0 | 51.5 |
| $N_2 P_2 K_2$ | 11.3 | 17.3 | 55.0 | 7.0 | 70.0 |
| $N_3 P_2 K_2$ | 13.7 | 18.5 | 42.5 | 3.0 | 11.0 |
| $N_3 P_3 K_2$ | 11.6 | 17.0 | 58.25 | 11.0 | 124.0 |
| $N_3 P_3 K_3$ | 13.6 | 17.5 | 63.5 | 9.0 | 44.0 |
| L.S.D. at 5% | 3.9 | 6.4 | 2.8 | 0.81 | 2.79 |

It is obvious from the obtained data that adding the tested fungicides 7 days after planting caused the best results in controlling *S. rolfsii* on bean. Data obtained in this experiment are agreement with those reported by Brien et al. (1991), Thakur et al. (1991), Tu and Zheng (1993). Also, Mukherjee et al, (2001) found that using fungicides and bioagents led to best results against root rot on French bean, and Bhoraniy et al. (2003) reported that treatments including bioagent T. harzianum and fungicides were effective in controlling stem rot on bean.

Data presented in Table (6) reveal that applying the tested fertilizers at the rate of (N2P2K1) resulted in the highest number of survived plants followed by (N1P1K1), N2P1K1, N2P1K1, N1P1K0 and N1P0K0, respectively. According to plant height, it was obvious that the most effective one was N1P0K0 followed by N1P1K1, N2P2K1 and N3P3K3. Rates of N1P1K1, N3P3K2 and N1P0K0 gave significant differences in the number of pods / plant. It is also clear that adding rates of N3P3K2, N1P1K1 resulted in the highest pod weight/ plant. While, applying rates of N3P2K2 and N3P0K0 resulted in pod weight/ plant. Data obtained are in parallel with those of Hoynes et al. (1999) who found that treatment with soil fertilizers together with conidia of some isolates of T. viride significantly reduced the sclerotial viability of S. rolfesii.

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## الملخص العربى

# تأثير بعض الكائنات المضادة والمبيدات والأسمدة على عفن جذور الفاصوليا المتسبب عن الفطر إسكليروشيم رولفزياى

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**معهد بحوث أمراض النباتات – مركز البحوث الزراعية – جيزة – مصر**

تم دراسة تأثير استخدام بعض الكائنات المضادة على مقاومة مرض عفن الجذور على نباتات الفاصوليا المتسبب عن الفطر سكليروشيم رولفزياى وقد اتضح أن أقوى هذه الكائنات تأثيراً على المرض هو الفطر ترايكودرما فيردى والكائن الحيوى أزوتوباكتر كروكوكم(المعدل وراثيا) بينما كان الفطر كانديدا أقلها تأثيراً على المرض. كذلك كان تأثير الفطر ترايكودرما فيردى إيجابياً على زيادة المحصول الناتج كذلك كانت النتائج متشابهة تحت ظروف المعمل والصوبة.

وقد تم دراسة تأثير بعض المبيدات الفطرية على المرض وتبين أن المبيد رونيلان بتركيز ١٥٠ جم/١٠٠لتر ماء يليه فيتافاكس/ثيرام بتركيز ٢٥٠جم/كيلو جرام بذرة أقواها تأثيراً بينما كان أقلها تأثيرا مبيد الايوبارين بمعدل ٢٥٠جم/١٠٠لتر ماء، المبيد سويتش بتركيز ٧٥جم/١٠٠لتر ماء.

وقد أدى استخدام الأسمدة الأرضية المركبة من العناصر الأساسية (نتروجين، بوتاسيوم، فوسفور) إلى وخفض النسبة المئوية للمرض وزيادة معنوية بالنسبة للمحصول الناتج. وقد أدى استخدام السماد المركب بنسبة ١٥٠كج/ فدان نتروجين، ٣٠٠كج/ فدان فوسفور، ١٥٠كج / فدان بوتاسيوم إلى تقليل المرض وزيادة كمية المحصول الناتج.