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The effect of biological soil disinfestation on dry rot disease in industrial potatoes caused by *Fusarium oxysporum*

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Abstract

Objective

Biological soil disinfestation methods have been employed as an alternative to methyl bromide, as they are effective in eliminating soil-borne pathogens, environmentally safe, and cost-effective. The aim of this study was to evaluate the efficiency of the biological disinfestation method using three local organic materials in controlling the pathogenic fungus *Fusarium oxysporum* on industrial potato crops.

Materials and methods

Industrial potato tubers were collected from various potato-growing regions in Baghdad Province. Three tubers were used for each fungal isolate. The inoculated tubers were stored in an incubator at 15°C with 70–85% humidity for 30 days. This study identified 30 isolates of *Fusarium spp.* from infected potato tubers and roots, showing significant variation in growth rates, colony colors, and mycelia density. The severity of infection was calculated based on a scale related to root weight, and various growth parameters were measured, including the number of branches, branch length, number of tubers, tuber weight, and the fresh and dry weight of the root system. The experiment was conducted using a randomized complete block design (RCBD).

Results

Pathogenicity tests on potato buds revealed that isolates F1, F5, F8, F10, F15, F20, F21, F28, and F30 caused the highest infection severity, ranging from 75% to 100%. Similarly, the tests on potato tubers confirmed that all isolates could induce dry rot, with damaged tissue areas ranging

from 39.16 to 59.64 mm², significantly differing from the control treatment. In Biological disinfestation tests, wheat bran at concentrations of 100, 200, and 300 g/m² significantly reduced infection severity, ranging from 5% to 26%, compared to the 100% infection in the pathogenic fungus control. Sawdust also showed significant reductions, with infection severity between 46% and 60%, while corn husks reduced infection severity to a range between 60% and 80%. Wheat bran demonstrated superior performance in tuber weight, producing tuber weights of 282 g, 362.10 g, and 469.53 g per plant, significantly higher than the control (150 g per plant). In additional biological disinfestation tests, wheat bran reduced infection severity by 25% to 50%, sawdust by 50% to 75%, and corn husks by 66% to 91%, compared to the control with 100% infection.

Conclusions

Tuber weights for these treatments were also significantly higher than the control, with wheat bran showing the best results. Overall, biological soil disinfestation treatments, especially wheat bran, are promising methods for controlling *Fusarium* infections and improving potato tuber yield.

Keywords: Biological soil disinfestation, dry rot, *F. oxysporum*, industrial potatoes

Paper Type: Research Paper.

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Introduction

The potato (Solanum tuberosum) belongs to the nightshade family (Solanaceae) and is one of the most important staple crops in the world, ranking fourth globally after wheat, corn, and rice

(AL-Razaq et al. 2018). Potato crops are susceptible to various fungal diseases, with Fusarium species being among the most significant pathogens. They cause tuber rot in both field and storage conditions, leading to substantial damage, particularly to seed potatoes, which affects sprouting and plant growth, ultimately destroying the vegetative parts of the plant. Fusarium species are endemic in the soil and possess a high capacity to withstand unfavorable environmental conditions (Agrios 2005; Alsamir et al. 2020; Ahmed & Kameem 2020; Tiwari et al. 2022). Biological soil disinfestation methods have been employed as an alternative to methyl bromide, as they are effective in eliminating soil-borne pathogens, environmentally safe, and cost-effective (Guo et al. 2017). This method relies on adding easily degradable organic matter to the soil as a carbon source to promote rapid microbial growth. Various materials have been used, including rice bran and wheat bran. The soil is then watered to fill its pores and reduce oxygen levels, followed by covering the soil with polyethylene to prevent oxygen from penetrating the surface (Hewavitharana et al. 2019). The aim of this method is to create an anaerobic environment to encourage the growth of anaerobic microorganisms, including bacteria that utilize carbon as an energy source. Species such as Enterobacter spp. and Clostridium spp. produce various gases, including carbon dioxide (CO_2), hydrogen sulfide (H_2S), ammonia (NH_3), and methane (CH_4), as well as several organic acids, aldehydes, alcohols, ammonia, mineral ions, and organic compounds that can be toxic to many pests and pathogens (Hewavitharana et al. 2021).

Some studies have indicated the efficacy of biological soil disinfestation using wheat bran in plastic pots for 14 days in reducing the incidence of the pathogenic fungus Rhizoctonia solani, which causes black scurf and stem canker diseases in potatoes. This method also led to improvements in several growth parameters, such as the number of branches, branch length, tuber count, and tuber weight in potato crops in Iraq (Hashi & Kameem 2020). It also results in reducing the pathogenic fungus Rhizoctonia solani, responsible for root rot in radishes (Khadka & Miller 2021).

Additionally, the use of molasses against Fusarium oxysporum f.sp. lycopersici has also shown effectiveness in controlling vascular wilt in tomatoes (Shrestha et al. 2021). The incidence of the bacterium Ralstonia solanacearum decreased by 83% to 100% when using peanut bran (Mao et al. 2022). Furthermore, the application of wheat bran resulted in a 49% reduction in sclerotia of the Macrophomina phaseolina during the first season, and a 75% to 85% reduction in the second season on strawberries (Daugovish et al. 2023). The study aimed to evaluate the efficiency of the biological disinfestation method using three local organic materials in controlling the Fusarium oxysporum on industrial potato crops.

Materials and methods

Isolation and diagnosis-Isolation from infected tubers and roots of affected plants: Industrial potato tubers were collected from various potato-growing regions in Baghdad Province (Yusufiya, Abu Ghraib, and Jadiriyah). The tubers were washed under running tap water to remove soil residues. The infected portion of the tuber surface was cut into small pieces measuring 0.5 cm using a sterile, sharp knife. The infected tubers and roots were surface-sterilized with a 10% sodium hypochlorite solution (1% free chlorine) for two minutes, then rinsed with sterile distilled water for two minutes and dried on sterile filter paper. The plant pieces were transferred with sterile forceps to petri dishes, with four plant pieces per dish. The plates were incubated at 25°C ± 2 for three days.

Afterward, the fungal colonies growing around the plant pieces were examined. The pathogenic fungus was then subcultured in petri dishes containing PSA medium. The *Fusarium* spp. isolates were identified to the genus level based on macroscopic and microscopic characteristics, including colony color, spore-producing cells, small conidia (microconidia), large conidia (macroconidia), and chlamydospores, following the standard taxonomic principles and using the referenced identification keys (Hafizi et al. 2013).

Pathogenicity-pathogenicity test of *Fusarium spp.* isolates on potato tubers in the laboratory: Uniform-sized industrial potato tubers of the *Stet* cultivar were washed under running water for five minutes, then surface-sterilized with a 10% sodium hypochlorite solution (1% free chlorine) for five minutes. After rinsing with sterile distilled water, the tubers were allowed to dry.

A sterile cork borer was used to make holes in the center of each tuber (7 mm deep and 5 mm in diameter). Each hole was inoculated with a 5-day-old fungal colony disk from the *Fusarium spp.* isolates. For the control treatment, a disk of the growth medium without the pathogen was added to the holes (Chandana et al. 2022).

Three tubers were used for each fungal isolate. The inoculated tubers were stored in an incubator at 15°C with 70–85% humidity for 30 days (Al-Zubaie, 2000). The depth of fungal growth in the tuber tissue at each hole was recorded by cutting the tuber transversely through the hole. The standard disease index was used to assess the infection (Willocquet et al. 2023) with traditional formulas (Table 1).

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Table 1. Used formulas for assessing the infection

Parameter	Formulas	Components
Area of the damaged portion of the tuber	$r^2\pi + r \times h\pi$	r = radius of the infected area h = depth of the infected area
Area of the oval-shaped infected region	long radius×short radius×3.14	
Infection percentage	(Area occupied by the fungus/Total area of the tuber)×100	

These formulas are used to calculate the extent of fungal infection in potato tubers based on the size of the infected area compared to the total area of the tuber.

Testing the efficiency of biological disinfestation in protecting industrial potatoes from artificial contamination in the field: The field experiment was conducted in February 2022 at the experimental station of the Plant Protection Department, College of Agricultural Engineering Sciences - University of Baghdad. The land was prepared by plowing it twice in perpendicular directions, and the soil was finely leveled and divided into three blocks, each containing 11 ridges, 6 meters long and spaced 70 cm apart. A drip irrigation system (strip-type) was installed.

A trench of 10-15 cm deep was made along each ridge, and the fungal inoculum was added at a rate of 10 g per meter length for all treatments requiring contamination with the pathogen. The trench was then covered with soil from the same ridges, and the soil was moistened using the drip irrigation system for 10 minutes. Three days later, the amendments (wheat bran, sawdust, and maize cobs) were done.

The experiment included the following treatments:

Fusarium spp. pathogen treatment,

Control treatment without the pathogenic fungus,

Wheat bran amendment at 100 g/m² with the pathogenic fungus,

Wheat bran amendment at 200 g/m² with the pathogenic fungus,

Wheat bran amendment at 300 g/m² with the pathogenic fungus,

Sawdust amendment at 100 g/m² with the pathogenic fungus,

Sawdust amendment at 200 g/m² with the pathogenic fungus,

Sawdust amendment at 300 g/m² with the pathogenic fungus,

Maize cob amendment at 100 g/m² with the pathogenic fungus,

Maize cob amendment at 200 g/m² with the pathogenic fungus,

Maize cob amendment at 300 g/m² with the pathogenic fungus.

Each treatment was replicated three times, including a treatment with soil contaminated by the pathogenic fungus and a control treatment without the pathogen.

Uniform-sized industrial potato tubers of the Stet cultivar were planted at a depth of 10 cm and spaced 25 cm apart, with 24 tubers per ridge. For the control treatment, only untreated potato tubers were planted. Irrigation was carried out according to the plant's needs, and compound fertilizers were used as recommended, along with foliar fertilizers and acaricides to control pests and mites.

The severity of infection was calculated based on a scoring scale related to root weight, and various growth parameters were measured, including the number of branches, branch length, number of tubers, tuber weight, and the fresh and dry weight of the root system. The experiment was conducted using a randomized complete block design (RCBD).

Results and discussion

Diagnosis of isolates: The results of the laboratory isolation revealed 30 isolates of Fusarium spp. obtained from infected tubers and roots of industrial potato plants. These isolates exhibited noticeable variation in their growth rate on PSA medium and the density of their mycelium. Additionally, the color of Fusarium colonies on the plates varied between white, pink, and purple, with pigmentations likely due to genetic variations among the isolates.

The conidiophore length and conidia formation also varied. Large conidia (macroconidia) were spindle-shaped or crescent-like, with 3-5 septa. The apical cell was curved, and the basal cell was foot-shaped. Small conidia (microconidia) were spherical, non-septate, and formed in false heads on the conidiophores of the mono phialide type. Chlamydospores with rough walls were also observed. Based on microscopic examination of the reproductive structures produced by these fungal isolates, as well as the morphology of their hyphae (Figure 1), the isolates were identified as Fusarium oxysporum. These diagnostic characteristics are consistent with the findings of several researchers, including Gherbawy et al. (2019), who identified Fusarium oxysporum as responsible for dry rot in potatoes.

Pathogenicity-Pathogenicity test of Fusarium spp. isolates on industrial potato tubers in the laboratory: The results of the pathogenicity test on industrial potato tubers in the laboratory demonstrated that all studied isolates were capable of causing dry rot in industrial potato tubers (Table 2). The fungal isolates varied significantly in the extent of the damaged tissue on the potato tubers, with differences compared to the control treatment. The size of the tissue area damaged by Fusarium spp. isolates ranged between 39.16 mm^2 and 59.64 mm^2 for all tested isolates.

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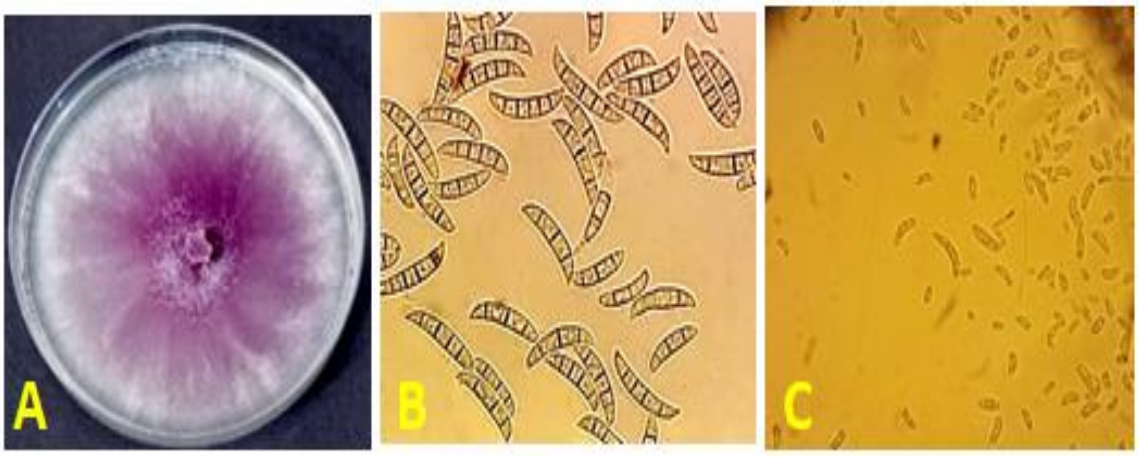

Figure 1. Reproductive structures of Fusarium spp. isolates. A - Fungal colony, B - Large spores (Macroconidia), C - Small spores (Microconidia) and large spores (Macroconidia)

This increase in the size of the area occupied by the fungus inside the tuber may be due to the fungus directly infecting the tuber, which provides a favorable nutrient source for fungal growth, leading to the rapid production of new spores. Additionally, the isolates may produce certain enzymes that degrade tuber tissues, negatively impacting the tuber. These results are consistent with those of Chen et al. (2020), and Azil et al. (2021), who demonstrated the pathogenicity of these isolates on potato tubers.

Testing the efficiency of biological disinfestation in protecting industrial potatoes in the field from artificial infection: The results of the biological disinfestation test using wheat bran at three concentrations (100, 200, 300 g/m²) demonstrated its ability to reduce infection severity (Table 3), ranging between 25% and 50%, with significant differences compared to the control treatment exposed to the pathogenic fungus, which recorded 100% infection severity. This was followed by the biological disinfestation treatment with sawdust at the same concentrations, which showed infection severity ranging between 50% and 75%, also significantly different from the pathogen control (100%). The corn cob treatment at the same concentrations also significantly reduced the infection severity caused by Fusarium oxysporum, lowering it to 66%-91%. Wheat bran treatments at the three concentrations (100, 200, 300 g/m²) achieved the highest control percentage, ranging from 37.5% to 68.75%. This was followed by the sawdust treatments, which achieved control percentages of 6.25%-37.5%.

Table 2. Pathogenicity test of Fusarium spp. isolates on industrial potato tubers in the laboratory

Isolation Number	Area Occupied by Fungus (mm^2)	Isolation Number	Area Occupied by Fungus (mm^2)
F1	53.11	F16	47.62
F2	40.13	F17	40.98
F3	40.18	F18	49.18
F4	46.13	F19	40.28
F5	56.76	F20	55.59
F6	47.21	F21	56.90
F7	49.15	F22	46.11
F8	59.64	F23	50.92
F9	39.22	F24	49.77
F10	53.66	F25	39.99
F11	39.65	F26	49.48
F12	50.19	F27	48.22
F13	39.16	F28	59.01
F14	45.39	F29	46.11
F15	58.43	F30	52.87
Control	0.00		
L. S. D	2.13		

The growth parameters of industrial potato plants in the biological disinfestation experiment showed significant differences in some growth metrics (Table 4). Wheat bran treatment at three concentrations (100, 200, 300 g/m^2) significantly outperformed other treatments in terms of the number of branches, branch length, fresh and dry weight, number of tubers, and tuber weight. (Table 3), These values were significantly different from the Fusarium oxysporum treatment.

For the number of branches, the wheat bran treatments recorded 5.75, 4.80, and 5.44 branches per plant, respectively, compared to 3.63 branches per plant in the Fusarium treatment. The branch lengths were 38.23, 31.69, and 33.15 cm/plant, respectively, compared to 21.35 cm/plant in the Fusarium treatment. Fresh weight was 14.04, 8.13, and 21.45 g/plant, respectively, compared to 5.29 g/plant for the Fusarium treatment. Dry weight was 4.68, 2.53, and 7.15 g/plant, compared to 1.72 g/plant in the Fusarium treatment. The number of tubers was 23.90, 21.30, and 32.63

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tubers/plant, respectively, compared to 13.60 tubers/plant in the Fusarium treatment. Tuber weight was 1.140, 1.130, and 1.340 g/plant, compared to 0.670 g/plant in the Fusarium treatment.

Table 3. Severity of infection in the biological disinfestation experiment based on artificial contamination in the field

Treatment No.	Treatment	Infection Severity (%)	Control Efficiency (%)
1	Wheat Bran 100 + Pathogenic Fungus	41	48.75
2	Wheat Bran 200 + Pathogenic Fungus	50	37.5
3	Wheat Bran 300 + Pathogenic Fungus	25	68.75
4	Sawdust 100 + Pathogenic Fungus	50	37.5
5	Sawdust 200 + Pathogenic Fungus	58	27.5
6	Sawdust 300 + Pathogenic Fungus	75	6.25
7	Corn Cobs 100 + Pathogenic Fungus	75	6.25
8	Corn Cobs 200 + Pathogenic Fungus	91	0
9	Corn Cobs 300 + Pathogenic Fungus	66	17.5
10	Control with Pathogen	80	0
11	Control without Pathogen	60	0
L.S.D. 0.05	L.S.D. 0.05	5.11	2.28

Sawdust came in second, showing significant improvement in growth parameters. The number of branches was 5.25, 4.99, and 6.55 branches per plant, respectively, compared to 3.63 branches in the Fusarium treatment. Branch lengths were 37.75, 33.40, and 30.37 cm, respectively, compared to 21.35 cm in the Fusarium treatment. Fresh weight was 7.64, 14.16, and 8.55 g/plant, respectively, compared to 5.29 g/plant in the Fusarium treatment. Dry weight was

2.54, 4.90, and 2.85 g/plant, compared to 1.72 g/plant in the Fusarium treatment. The number of tubers was 17.63, 19.21, and 15.80 tubers/plant, respectively, compared to 13.60 tubers/plant in the Fusarium treatment, and tuber weights were 1090, 1040, and 970 g/plant, respectively, compared to 670 g/plant in the Fusarium treatment.

Table 4. Effect of biological disinfestation method on some growth parameters of industrial potato plants based on artificial contamination in the field

ID	Treatment	Branch per Plant	Branch Length (cm)	Average Wet Root Weight g/plant	Average Dry Root Weight g/plant	Average Number of Tubers per Plant	Tuber Weight g/plant
1	Wheat Bran 100 + Pathogenic Fungus	5.75	38.23	14.04	4.68	23.9	1140
2	Wheat Bran 200 + Pathogenic Fungus	4.80	31.69	8.13	2.53	21.3	1130
3	Wheat Bran 300 + Pathogenic Fungus	5.44	33.15	21.45	7.15	32.63	1340
4	Sawdust 100 + Pathogenic Fungus	5.25	37.75	7.64	2.54	17.63	1090
5	Sawdust 200 + Pathogenic Fungus	4.99	33.4	14.16	4.9	21.16	1040
6	Sawdust 300 + Pathogenic Fungus	6.55	30.37	8.55	2.85	19.21	972
7	Corn Cobs 100 + Pathogenic Fungus	5.51	32.24	10.9	3.63	15.8	970
8	Corn Cobs 200 + Pathogenic Fungus	5	27.58	21.31	7.1	18.5	960
9	Corn Cobs 300 + Pathogenic Fungus	5.07	24.44	19.97	6.65	16.03	1000
10	Control with Pathogen	4.1	22.65	7.79	2.59	14.04	670
11	Control without Pathogen	3.63	21.35	5.29	1.72	13.6	830
	L.S.D. 0.05	0.8	4.24	0.9	0.39	2.25	320

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Conclusions: The study concludes that *Fusarium* spp. isolates from infected potato plants exhibit significant variation in pathogenicity, with several isolates (F1, F5, F8, F10, F15, F20, F21, F28, and F30) causing the highest infection severity in potato buds and tubers. All isolates were capable of causing dry rot in tubers, with varying degrees of tissue damage. Biological soil disinfestation treatments, particularly wheat bran, effectively reduced infection severity, demonstrating a significant decrease compared to untreated control plants infected with *Fusarium oxysporum*. Wheat bran was the most effective, reducing infection severity to as low as 5% while significantly increasing tuber weight. Sawdust and corn husks also showed positive effects, though less pronounced than wheat bran. Overall, biological soil disinfestation treatments, especially wheat bran, are promising methods for controlling *Fusarium* infections and improving potato tuber yield.

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Conflict of Interest: There is no conflict of interest.

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تاثیر ضد عفونی بیولوژیکی خاک بر بیماری پوسیدگی خشک در سیب زمینی صنعتی ناشی از قارچ *Fusarium oxysporum*

مآب هشی

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چکیده

هدف: روش‌های بیولوژیکی ضد عفونی خاک به عنوان جایگزینی برای متیل بروماید استفاده شده است، زیرا آن‌ها در از بین بردن پاتوژن های موجود در خاک موثر هستند و از نظر زیست محیطی ایمن و مقرون به صرفه هستند. هدف از این مطالعه بررسی کارایی روش ضد عفونی بیولوژیکی با استفاده از سه ماده آلی محلی در کنترل قارچ بیماریزا *Fusarium oxysporum* بر روی محصولات سیب زمینی صنعتی بود.

مواد و روش‌ها: غده‌های سیب زمینی صنعتی از مناطق مختلف پرورش سیب زمینی در استان بغداد جمع آوری شد. برای هر جدایه قارچی از سه غده استفاده شد. غده‌های تلقیح شده در انکوباتور در دمای ۱۵ درجه سانتی گراد با رطوبت ۷۰ تا ۸۵ درصد به مدت ۳۰ روز نگهداری شدند. این مطالعه ۳۰ جدایه گونه‌های فوزاریوم را شناسایی کرد. از غده‌ها و ریشه‌های آلوده سیب‌زمینی، تغییرات قابل‌توجهی در نرخ رشد، رنگ کلنی و تراکم میسلیوم نشان می‌دهد. شدت آلودگی بر اساس مقیاس مربوط به وزن ریشه محاسبه شد و پارامترهای مختلف رشد شامل تعداد شاخه، طول شاخه، تعداد غده، وزن غده و وزن تر و خشک سیستم ریشه اندازه‌گیری شد. آزمایش با استفاده از طرح بلوک‌های کامل تصادفی (RCBD) انجام شد.

نتایج: آزمایش‌های بیماری‌زایی روی جوانه‌های سیب‌زمینی نشان داد که جدایه‌های F1، F5، F8، F10، F15، F20، F21، F28 و F30 بیشترین شدت آلودگی را داشتند که از ۷۵٪ تا ۱۰۰٪ متغیر بود. به طور مشابه، آزمایش‌ها روی غده‌های سیب‌زمینی

تأیید کرد که همه جدایه‌ها می‌توانند باعث پوسیدگی خشک، با مناطق بافت آسیب‌دیده از ۳۹/۱۶ تا ۵۹/۶۴ میلی‌متر مربع، که به طور قابل‌توجهی با تیمار شاهد متفاوت است شوند. در آزمایش‌های ضد عفونی بیولوژیکی، سبوس گندم در غلظت‌های ۱۰۰، ۲۰۰ و ۳۰۰ گرم در متر مربع، شدت عفونت را به طور قابل‌توجهی، در محدوده ۵٪ تا ۲۶٪، در مقایسه با عفونت ۱۰۰٪ در کنترل قارچ بیماری‌زا کاهش داد. خاک اره نیز کاهش قابل توجهی را نشان داد، با شدت عفونت بین ۴۶ تا ۶۰ درصد، در حالی که پوسته ذرت شدت عفونت را بین ۶۰ تا ۸۰ درصد کاهش داد. سبوس گندم عملکرد بهتری، با تولید غده‌های ۲۸۲، ۳۶۲/۱۰ و ۴۶۹/۵۳ گرم در بوته در وزن غده نشان داد که به طور قابل توجهی بیشتر از شاهد (۱۵۰ گرم در بوته) بود. در آزمایش‌های ضد عفونی بیولوژیکی اضافی، سبوس گندم شدت آلودگی را بین ۲۵ تا ۵۰ درصد، خاک اره را ۵۰ تا ۷۵ درصد و پوسته ذرت را ۶۶ تا ۹۱ درصد در مقایسه با شاهد با ۱۰۰ درصد آلودگی کاهش داد.

نتیجه‌گیری: وزن غده برای این تیمارها نیز به طور قابل توجهی بالاتر از شاهد بود و سبوس گندم بهترین نتایج را نشان داد. به طورکلی، درمان‌های بیولوژیکی ضد عفونی خاک، به ویژه سبوس گندم، روش های امیدوارکننده‌ای برای کنترل عفونت‌های فوزاریوم و بهبود عملکرد غده سیب زمینی هستند.

واژه‌های کلیدی: پوسیدگی خشک، سیب‌زمینی صنعتی، ضد عفونی بیولوژیکی خاک، F. oxysporum

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