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Enhancing Effect of *Shimizuomyces paradoxus* on Seed Germination and Seedling Growth of Canola, Plant Growth of Cucumber, and Harvest of Tomato

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***Shimizuomyces paradoxus* showed no inhibitory effect against plant pathogen fungi, such as *Fusarium oxysporum* f. sp. *lycopersici* and *Alternaria solani*. The *S. paradoxus* culture filtrate showed higher seed germination and seedling growth rates in canola than distilled water and potato-dextrose broth. A conidial suspension of 1.0×10^4/mL resulted in the highest growth stimulating effects on total plant length, and fresh and dry weight of shoots and roots in cucumber, when compared to the highest suspension concentration. Total plant length and shoot weight increased with the foliar spray treatment, and root length and root weight increased by simultaneous treatments of soil drenching and foliar spray in cucumber. Lower concentrations of the *S. paradoxus* conidial suspension increased the harvest of tomato fruit.**

KEYWORDS : Conidial suspension, Growth promotion, Harvest, Plant growth, Seed germination

Shimizuomyces paradoxus and *S. kibiana* were first reported in Japan from mature fruits of *Smilax* spp. [1, 2]. *Shimizuomyces* species are morphologically similar to *Cordyceps* s. l. Other than Japan, *S. paradoxus* has been reported only in Korea [3, 4]. Cultural characteristics of *S. paradoxus* have been recently reported by Sung *et al*. [5]. Morphologically and phylogenetically, *Shimizuomyces* is placed in the family Clavicipitaceae [1, 6, 7]. Microorganisms are known to have different effects on plant growth. Hence, it was of interest to observe the effect of an *S. paradoxus* conidial suspension on non-host plants. The results showed enhanced effect on seed germination and seedling growth of canola, plant growth of cucumber, and harvesting of tomato.

Materials and Methods

Fungal isolate. Multi-ascospore isolates were derived from *S. paradoxus* specimen EFCC C-5280 on potato dextrose agar (PDA; potato 200 g, dextrose 20 g and agar 20 g per 1,000 mL) plates and were used in this study. The specimen was collected from Mt. Chundeung of Chungcheong-do on July 23, 2000. The specimen and isolates were preserved in the entomopathogenic fungal culture collection of Kangwon National University, Korea.

Effect of *S. paradoxus* on seed germination and seedling growth of canola (*Brassica campestris*). Before observing the effect of *S. paradoxus* on plant growth, its inhibitory effect against two fungal pathogens was tested. Isolates of *Fusarium oxysporum* f. sp. *lycopersici* and *Alternaria solani*, obtained from the Plant Pathology Department of Rural Development Administration (RDA), Suwon, Korea were inoculated with an *S. paradoxus* isolate on opposite sides of PDA agar plates and incubated at 25°C. The plates were observed after 20 days of incubation with *F. oxysporum* f. sp. *lycopersici* and after 25 days of incubation with *A. solani*.

To prepare *S. paradoxus* liquid cultures five mycelial PDA agar plate discs (5 mm) were inoculated in PDA broth (PDA without agar) and incubated at 25°C for 30 days, then filtered through a syringe filter (pore size, 0.45 µm; Advantec MFS, Inc., Dublin, CA, USA). The filtrate was sterilized at 121°C under 1.2 psi pressure for 20 min. Twenty-five canola seeds were sown on filter paper at 25°C for 15 days in the presence of 5 mL distilled water, PD broth culture, and sterilized and non-sterilized culture filtrates separately. Two mL of distilled water was sprayed on the seeds every day to maintain humidity. Germination rate, total plant height, root length, leaf length, and leaf width were measured at the end of the incubation.

Effect of *S. paradoxus* on seedling growth of cucumber (*Cucumis sativus*). Different concentrations of conidial suspension, such as 1.0×10^5, 0.2×10^5, 1.0×10^4, and 0.7×10^4 per mL, were prepared to observe the effect of an *S. paradoxus* conidial suspension on cucumber plant

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growth. The original suspension of 1.0×10^7/mL was used as the control. Seeds of cucumber var. Eunseong Baekdadagi were sown in a biosoil bed in 72-cell trays. After 13 days, the seedlings were transferred to 20-cm diameter pots. After transferring to pots, 20 mL of conidial suspension of each concentration was drenched on the soil every 5 days. Total plant height, root length, fresh weight, and dry weight of the shoots and roots were measured after 1 mon. Dry weight was measured after drying the material at 60°C for 48 hr.

The 1.0×10^4/mL conidial suspension was used in different ways, such as drenching in soil, drenching in soil and spraying on leaves, and only spraying on leaves. Seeds of cucumber of var. Eunseong Baekdadagi were sown in 36-cell trays. After 13 days, each seedling was transferred to 20-cm diameter pot, and 20 mL of conidial suspension was drenched on the soil, and 10 mL was sprayed on the leaves every 5 days for 1 mon. Twenty mL of distilled water was added to the soil every 5 days as a control. Total plant height, root length, and fresh and dry weights were measured. Dry weight was measured as mentioned above.

Effect of *S. paradoxus* on plant growth of tomato (*Solanum lycopersicum*). Conidial suspensions of 1.0×10^5, 0.2×10^5, 1.0×10^4, and 0.7×10^4 per mL were used to observe the effect of *S. paradoxus* on tomato fruit The 1.0×10^7 conidia per mL concentration was used as the control. Seeds of tomato var. 630 were sown in biosoil bed in 36-cell trays and only distilled water was used in the soil. The seedlings were transferred to 20-cm diameter pots after 60 days of sowing. After 7 days, 100 mL of suspension of each concentration was added to the soil, and 50 mL was sprayed on the leaves every wk for 4 wk. Two-hundred mL was added to the soil, and 100 mL was sprayed every wk for 4 more wk. Subsequently, the harvest was started and continued for 2 mon. The number of fruits, fruit weight and diameter, and sugar content (degrees Brix, °Bx) were measured.

Results and Discussion

Effect of *S. paradoxus* on canola plant growth. *S. paradoxus* showed no inhibitory effect against the fungal pathogens *F. oxysporum* f. sp. *lycopersici* and *A. solani* (Fig. 1). These two pathogens were used in this study, because they are very common in the field. However, further tests are required to show non-inhibitory effects of *S. paradoxus* against other fungal and bacterial plant pathogens.

The culture filtrate resulted in a good seed germination rate and plant height, root length, leaf length, and leaf width of seedlings (Table 1, Figs. 2 and 3). Furthermore, the non-sterilized culture filtrate resulted in a 42.1% and 53.8% increase in plant height, compared to distilled water and PD broth, respectively (Table 1). The culture filtrate

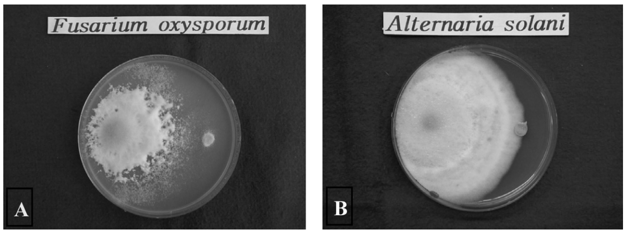

Fig. 1. Non-inhibitory effects of *Shimizuomyces paradoxus* isolate CRI C-5280 against *Fusarium oxysporum* f. sp. *lycopersici* (A) and *Alternaria solani* (B).

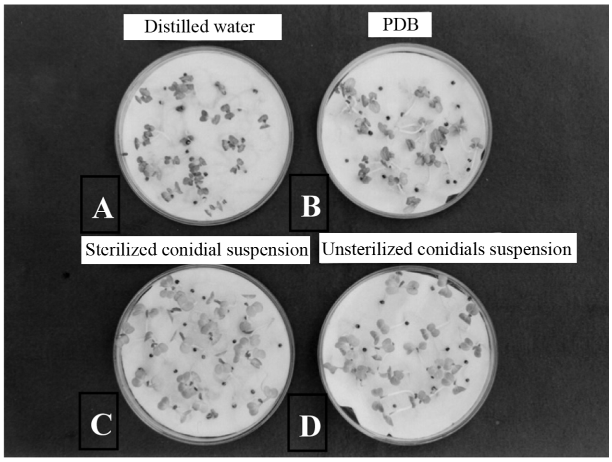

Fig. 2. Effect of a *Shimizuomyces paradoxus* CRI C-5280 conidial suspension on canola seed germination. Treatments with A, distilled water; B, potato-dextrose broth (PDB); C, sterilized conidial suspension; and D, unsterilized conidials suspension.

Table 1. Effect of a conidial suspension of *Shimizuomyces paradoxus* CRI C-5280 on canola seed germination and seedling growth

Treatment	Germination rate (%)	Total plant height (mm)	Root length (mm)	Leaf length (mm)	Leaf width (mm)
Distilled water	94.67	56.57	39.77	6.083	3.767
PD broth culture	94.67	52.27	33.27	6.967	4.717
Culture filtrate (sterilized)	94.67	69.87	49.77	8.683	6.083
Culture filtrate (non-sterilized)	97.33	80.37	58.00	8.150	5.750

PD, potato-dextrose.

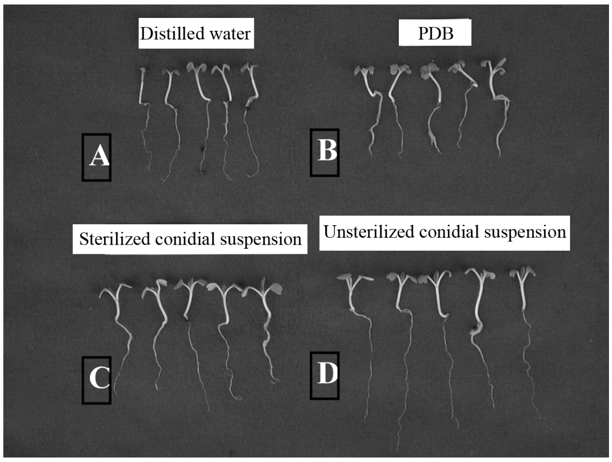

Fig. 3. Effect of *Shimizuomyces paradoxus* CRI C-5280 conidial suspension on canola seedling growth. Treatments with A, distilled water; B, potato-dextrose broth (PDB); C, sterilized conidial suspension; and D, unsterilized conidial suspension.

also showed similar plant growth stimulating activities even after autoclaving at 121°C for 20 min, indicating that the plant growth stimulating compound(s) in the filtrate was heat stable.

Effect of *S. paradoxus* on cucumber seedling growth. The *S. paradoxus* conidial suspension resulted in better growth of cucumber than the control (Table 2, Fig. 4). Plant growth was most positively affected with lower conidial suspension concentrations (Table 2, Fig. 4). However, reason for this relationship was unclear. Among the conidial suspensions, a concentration of 1.0×10^4/mL showed the best results. Concentrations of 0.2×10^5/mL produced the longest root length. All types of treatments produced better growth characteristics than the control (Table 3, Fig. 5). Root length and weight were best when treated with soil drenching and foliar spraying.

Fig. 4. Cucumber plants treated with *Shimizuomyces paradoxus* CRI C-5280 conidial suspensions. A, control; B, 1.0×10^5; C, 0.2×10^5; D, 1.0×10^4; and E, 0.7×10^4.

Effect of *S. paradoxus* on tomato fruit growth. Lower concentrations of *S. paradoxus* conidial suspension resulted in a better fruit harvest than the control (Table 4). How-

Table 2. Effect of *Shimizuomyces paradoxus* CRI C-5280 conidial suspension concentration on cucumber growth

Concentration (No. of conidia/mL)	Total plant height (mm)	Root length (mm)	Fresh weight (g)		Dry weight (g)	
			Shoot	Root	Shoot	Root
Control	45.83	16.333	4.540	0.4666	1.0433	0.1000
1.0×10^5	66.00	20.000	9.023	0.7033	1.5700	0.2600
0.2×10^5	75.67	23.000	10.740	0.8966	1.8700	0.3900
1.0×10^4	85.00	22.333	13.203	1.2066	2.1900	0.4766
0.7×10^4	65.33	19.833	10.640	1.0633	1.8533	0.4066

Table 3. Effect of *Shimizuomyces paradoxus* EFCC C-C5280 conidial suspension treatment type (1.0×10^4/mL) on cucumber growth

Treatment type	Total plant height (mm)	Root length (mm)	Fresh weight (g)		Dry weight (g)	
			Shoot	Root	Shoot	Root
Control	60.80	9.400	7.3240	0.1520	0.6640	0.0600
Soil drench	94.40	19.8	13.348	0.4740	1.5020	0.1260
Foliar spray	104.2	22.20	17.856	0.5980	1.9460	0.1220
Soil drench + foliar spray	96.60	23.20	13.174	0.9200	1.4220	0.1420

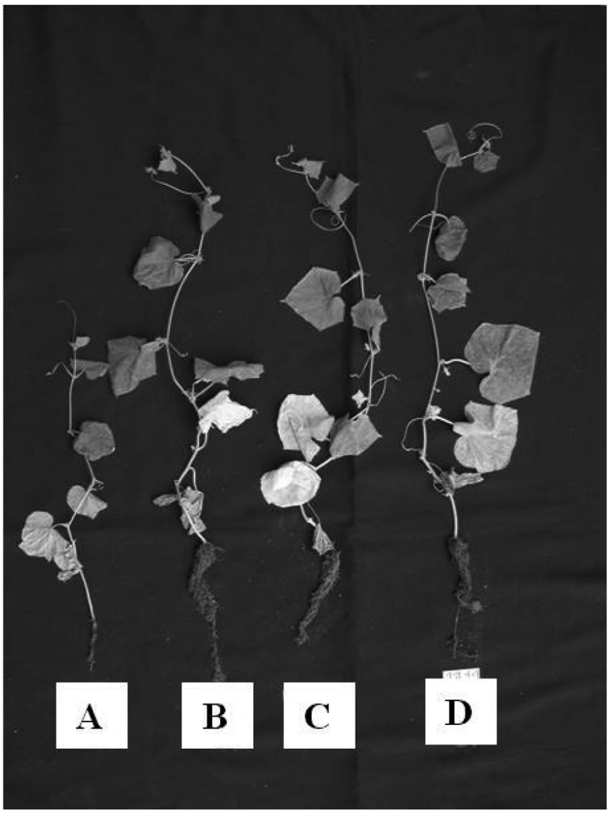

Fig. 5. Increased growth of cucumber treated with *Shimizuomyces paradoxus* EFCC C-C5280 conidial suspensions. A, control; B, soil drench; C, soil drench + foliar spray; and D, only foliar spray.

Table 4. Effect of various concentrations of *Shimizuomyces paradoxus* isolate CRI C-5280 conidial suspension soil drench and foliar spray on tomato fruit

Concentration (No. of conidia/mL)	No. of fruits	Fruit weight (g)	Fruit diameter (cm)	Degrees Brix (°Bx)
Control	28	73.56	5.193	3.875
1.0×10^5	32	84.75	5.306	4.241
0.2×10^5	29	88.65	5.469	4.252
1.0×10^4	31	89.42	5.487	4.274
0.7×10^4	33	85.05	5.403	4.112

ever, the harvest was similar among the lower concentrations of conidial suspensions (Table 4). Fruit weight was highest at a concentration of 0.7×10^4/mL.

The mechanism of the plant growth-promoting effects of *S. paradoxus* could not be understood from the results. Thus, it is necessary to study the anti-fungal effects of *S. paradoxus* against other plant pathogens. *S. paradoxus* is basically a pathogen of mature *Smilax sieboldi* fruits. During both the infection period of the host and further growth, *S. paradoxus* may produce some anti-microbial compounds that resist the effect of microorganisms present in the environment. Hence, it can be assumed that *S. paradoxus* enhances the growth of non-host plants due to its anti-microbial properties.

Many plant growth-promoting rhizobacteria and fungi show resistance to pathogens [8, 9]. Plant growth-promoting rhizobacteria such as *Pseudomonas* species enhance plant growth and crop yield [10-14]. Site competition, increased phosphate uptake, synthesis of plant growth promoting substances, and antagonism through the production of antibiotics and siderophores are some of the mechanisms for enhanced plant growth [10, 11, 15-17]. Powder formulations of rhizobacteria have also been prepared commercially promote rhizobacteria [12]. Frommel *et al*. [18] reported a significant increase in root number, root dry weight, haulm dry weight, stem length, leaf hair formation, secondary root branching, and total plant lignin content by applying nonfluorescent *Pseudomonas* sp.

Trichoderma spp. are the mostly studied fungal species as biocontrol agents [19]. They produce growth regulating factors that increase the rate of seed germination and shoot and stem dry weights [20]. *T. harzianum* has shown anti-fungal activity through enzymatic action [21]. Increased seedling emergence, root area, root length, and dry weight, as well as shoot length, leaf area and increased P, Fe, Zn and Mn concentrations have been reported with the use of *T. harzianum* [22]. Lo and Lin [23] reported root growth in cucurbitaceous plants, including seedling height, root exploration, leaf area, root dry weight, and chlorophyll concentrations in *Trichoderma* spp. These fungi utilize various nutrient sources and are resistant to chemicals and toxins; they can also degrade some toxins [24].

Microbial interactions have been discussed in detail by Whipps [25]. Non-pathogenic microorganisms improve nutrient acquisition, hormonal stimulation, disease suppression, and the induction of resistance [26, 27]. Siderophores, produced by most bacteria and fungi, control plant pathogens [28]. Further studies such as light and electron microscopic studies of leaf, stem, and root as well as biochemical (lignin content and chlorophyll content) and morphological (leaf area) studies are required for a more detailed assessment the effect of *S. paradoxus* on non-host plant growth.

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