

Nature of Protection of Chilli Seedling from Rhizoctonia Damping-off
by Plant Growth Promotion Fungi¹⁾

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ABSTRACT

Protection of chili seedlings from Rhizoctonia damping-off by Plant Growth Promotion Fungi (PGPF) i.e.: *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi was investigated in laboratory and greenhouse studies. The PGPF failed to show antagonistic interaction when grown in dual culture on agar media with *Rhizoctonia solani* causing damping-off disease. All PGPF isolates did not inhibit the growth of *Rhizoctonia solani*. Treatment of chili seedlings with PGPF for 10 days and then the treated chili roots were surface sterilized with 70% ethanol for 30 sec. When the treated seedlings were replanted and subsequently inoculated with pathogen, however, the protective capability against *R. solani* was still maintained. . Seedlings treated with *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi reduced disease severity ranged from 30.0-95.9%; 32.6-71.4%; 16.3-65.3%; 69.4-95.9%, respectively. These results suggest that the mechanism of protection in this system might be induced resistance.

Key words: Biocontrol, Plant Growth Promotion Fungi, Rhizoctonia damping-off; Chili

INTRODUCTION

Damping-off seedling caused by *Rhizoctonia solani* Kuhn (teleomorph= *Thanatephorus*) is economically important due to substantial losses it caused to farmers. Rhizoctonia diseases occur throughout the world. They cause losses on almost all vegetables and flowers. Very young seedling may be killed before or soon after they emerge from the soil (Agrios, 2000). Moulani (2005) reported that percentage of *pre-emergence damping-off*, *pre-emergence damping-off* and disease severity was ranged from 1-35%; 12.6-51.0%; 10.9-47.8%, respectively.

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Conventional methods of control for *R. Solani* include cultural practices and fungicidal treatment. Frisina and Benson (1988). However, with changing farming methods (e.g. reduced till, direct drill and reduced crop rotation), increased concern regarding the effect of synthetic pesticides in the environment and the development of fungicide resistance in pathogenic strains prompted to farmers to choose alternative disease control management strategies. One such method involves biological control by the introduction of selected microorganisms to the soil (Campbell, 1989).

A Variety of soil microorganisms have demonstrated their potential as biocontrol agents against various diseases. Hyakumachi (1994) and Shivana et al (1996) reported that sterile fungi, *Phoma*, *Trichoderma*, *Fusarium* and *Penicillium* effectively reduced damping-off diseases and Fusarium wilt of cucumber and also take-all disease of wheat.

The objectives of this research were: 1) to know the ability of PGPF *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi against the growth *Rhizoctonia solani* and their interaction on agar. 2) to evaluate the effectiveness of seedling treated with PGPF *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi in suppressing *Rhizoctonia* damping-off after sterilizing the treated roots with 70% ethanol for 30 sec.

MATERIALS AND METHODS

Fungi

Biocontrol agent used in this study was *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi indicated as the Plant Growth Promoting Fungi (PGPF). These isolates were isolated from rhizosphere of chilli cultivated in low land area. *Rhizoctonia solani* Kuhn was obtained from an infected chili plant was used as the pathogen.

Plant

All chilli seeds were surface-sterilized with 1% hydrochloric acid for 15 min and rinsed three times in sterile distilled water before sowing.

Inoculums Preparation

For application in greenhouse, inoculums of PGPF and pathogen isolates were prepared in solid inoculums, each isolates of PGPF and pathogen was

cultured on potato dextrose agar (PDA) for 7 days at room temperature. Five mycelial disks (5 mm) of the isolates cut from the edges of three-day old cultures were added to 100 g moist autoclaved combinations of various substrates (1:1, dry various substrates/distilled water, w/v) contained in a 500 ml Erlenmeyer flask. The combination substrates used in this study was bran+corn+rice-straw with comparison 4 : 3 : 1 for each material, respectively. The cultures were incubated in room temperature for 10 days and shaken regularly to aid even colonization. The infested media substrates were air-dried for 7 days and stored at 4°C until used.

Assay of colony interaction between PGPF and *Rhizoctonia solani*

The influence of PGPF *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi against *R. solani* in culture was examined by inoculating paired combination between PGPF and *R. solani*, 3 cm apart on potato dextrose agar (PDA) in three replicate 9 cm petri dishes. Interactions were assessed using a key based on the observations of Skidmore and Dickinson (1976). They recognized five separate modes of interaction colony growth: A) Mutually intermingling growth where both fungi grew into one another without any macroscopic signs of interaction (score 1); Bi) Intermingling growth where the fungus being observed is growing into the opposed fungus either above or below or above and below its colony, and its corollary (score 2); Bii) intermingling growth where the fungus under observation has ceased growth and is being overgrown by another colony (score 3); C) Slight inhibition where the fungi approached each other until almost in contact and a narrow demarcation line, c. 1-2 mm, between the two colonies was clearly visible (score 4); D) Mutual inhibition at a distance of > 2 mm (score 5). The percentage of inhibition against *R. solani* by PGPF also was recorded by formula: $R1-R2/R1 \times 100\%$, R1= the mycelial growth of *R. solani* in the position to the edge of petri dish and R2= the mycelia growth of *R. solani* in the opposed of PGPF isolates.

Assay of PGPF treatment in suppressing disease severity of *Rhizoctonia damping-off*

The inoculums of PGPF were pulverized in a blender for about 30 sec. (1 to 2 mm particle size) and mixed (1.5%, w/w) with sterilized potting medium (soil+compost). The procedure used in this study was followed the method of Cardosa and Echandi (1987) with modification. The seedling were treated with each

PGPF *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi tested in the concentration 2% in plastic tray for 14 days. The treated seedling was then gently take away from soil. The treated root with PGPF was sterilized with 70% ethanol for 30 sec. The sterilized root of treated seedling were transferred to small plastic pot which filled with infested potting soil with pathogen *R. solani* in the concentration 1%. The seedlings not treated with PGPF and challenged with *R. solani* were set up as control. Treatments were replicated 4 times and each replicate consists of 5 plants.

Disease severity based on the damping-off or root lesion was assessed using a scale of 0 to 5; 0 = healthy; 1 = one or two light brown lesion (1mm) on the crown root; 2 = light brown-dark brown lesion 2-10 mm on root; 3 = dark brown lesion 10-25 mm ; 4 = dark lesion \geq 26 mm water soaked of the hypocotyls; 5= collapsed hypocotyls with wilted leaves or dead seedlings. The percentage of disease severity in each replication within the treatment were calculated using the formula :

$$K = \frac{\sum(n \times v)}{Z \times N} \times 100\%$$

Where::

K = Disease Severity (%)

n = number of seedlings infected by pathogen in each scale

v = Diseases scale (0-5)

Z = The highest of disease scale

N = total seedlings

RESULTS AND DISCUSSION

All of the interaction observed between PGPF *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi against *R. solani* in the score 1 or 2, which mean that Mutually intermingling growth where both fungi grew into one another without any macroscopic signs of interaction for score 1 and for score 2 is Intermingling growth where the fungus being observed is growing into the opposed fungus either above or below or above and below its colony, and its corollary (Skidmore and Dickinson, 1976). We did not observed any inhibition effect against *R. solani* by PGPF *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi. This results indicated that there are no antagonistic mechanism such as antibiosis or mycoparasite occur this study.

In the assay of PGPF treatment in suppressing disease severity of *Rhizoctonia* damping-off, after the treated chili roots were surface sterilized with 70% ethanol for 30 sec. The results showed that all treatments with PGPF *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi still have various ability to suppress disease

severity caused by *Rhizoctonia* damping-off of chilli. Seedlings treated with *Penicillium* spp. provided the reduction of disease severity was ranged from 30-95,9%. Isolate *Penicillium* P11 showed the highest of the reduction effect on disease severity (95,9%) (Figure 1). Treatment with *Fusarium* sp. provided the percentage of reduction on disease severity ranged from 32.6-71.4%. The highest reduction was recorded on F15 and F11 (Figure 2). Treatment with *Phoma* isolates reduced moderately disease severity. They provided the percentage of reduction on diseases severity 16,3% for PH1 and 65,3% for PH4 . Seedlings treated with sterile fungi effectively reduced disease severity. The percentage of reduction on diseases severity ranged from 68,4-95,9% (Figure 3). This results showed that even though the presence of PGPF was eliminated from root surface by soaking the root in ethanol 70% for 30 sec, the reduction effect against *Rhizoctonia* damping-off still occurred. This results indicated that treatment with PGPF *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi could induce resistance in chilli against *Rhizoctonia* damping-off. This results supported previous research conducted by Koike et al (2001), where the application of 5 PGPF isolates, *Trichoderma*, *Fusarium*, *Penicillium*, *Phoma* and sterile fungi on root prepared in barley grain, mycelia, and culture filtrate inoculums effectively induced systemic resistance in cucumber against angular leaf spot caused by *Pseudomonas syringae* pv. *lachrymans*, Fusarium wilt caused by *Fusarium oxysporum* f.sp. *cucumerinum* through the increasing of lignifications, superoxide generation and chemiluminescence activity. Merra et al (1994) and Merra (1994) also reported that, PGPF isolates, *Phoma* and sterile fungi effectively induced systemic resistance in cucumber against antracnose disease caused by *Colletotrichum orbiculare* through the enhancing the activity of chitinase, β -1,3-glucanase, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase. Another experiment conducted by De Cal, A., et al. (2000) reported that *Penicillium oxalicum* spp as soil inhabitants effectively reduced Fusarium will of tomato caused by *Fusarium oxysporum* .f.sp. *lycopersici* through induced resistance

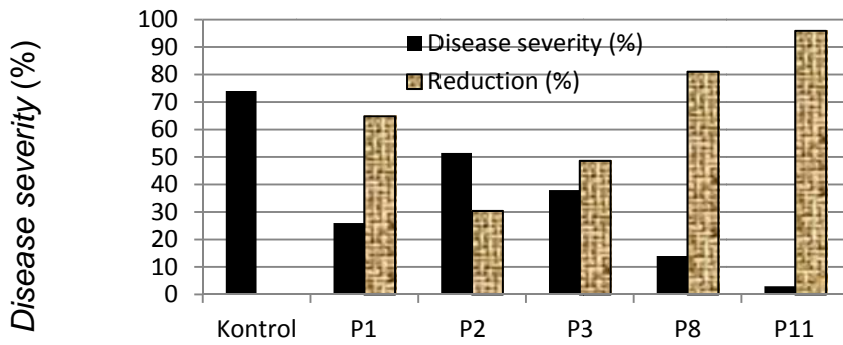


Figure 1. The effect of treatment with *Penicillium* spp. in supressing disease severity of Rhizoctonia damping-off of chili caused by *Rhizoctonia solani* Kuhn



Figure 2. The effect of treatment with *Fusarium* spp. in supressing disease severity of Rhizoctonia damping-off of chili caused by *Rhizoctonia solani* Kuhn

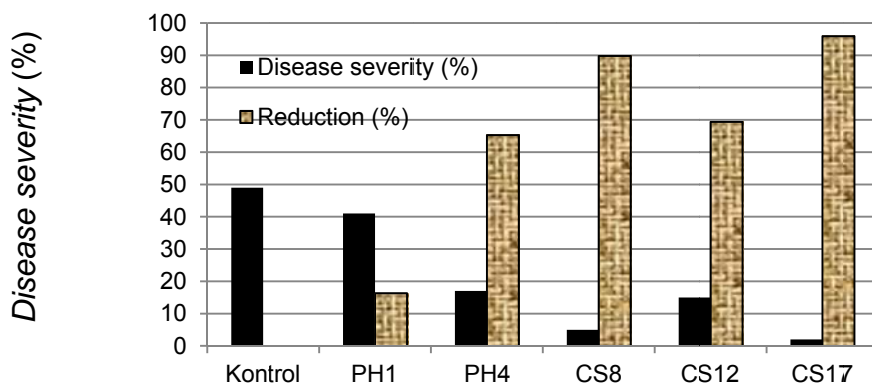


Figure 3. The effect of treatment with *Phoma* spp and sterile fungi. in supressing disease severity of Rhizoctonia damping-off of chili caused by *Rhizoctonia solani* Kuhn

CONCLUSION

The conclusion of this study is the interaction between PGPF *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi against *R. solani* in the score 1 or 2, which mean that Mutually intermingling growth where both fungi grew into one another without any macroscopic signs of interaction (score 1) and Intermingling growth where the fungus being observed is growing into the opposed fungus either above or below or above and below its colony, and its corollary (score 2). We also observed that there are no antagonistic mechanism such as antibiosis or mycoparasite occur in this study. Even though the presence of PGPF was eliminated from root surface by soaking the root in ethanol 70% for 30 sec, the reduction effect against Rhizoctonia damping-off still occurred. This is indicated that treatment with PGPF *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi could induce resistance in chilli against Rhizoctonia damping-off.

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