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Application of *Penicillium* spp. Produced in Waste Materials to Control Neck Root Rot Diseases Caused by *Sclerotium rolfsii* Sacc. on Chili

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The research was conducted to know the ability of *Penicillium* spp. grown in various substrats to suppress neck root rot disease caused by *Sclerotium rolfsii* on Chili. The research was arranged in Randomized Completely Design with 11 treatments and 3 replications as consisted of control, *Penicillium* spp. isolates P8 and P10 produced in combination of substrates of tapioca dregs+bran+bunch of plam oil; coconut dregs+bran+bunch of palm oil; tapioca dregs+bran+sawdust; coconut dregs+bran+sawdust; and Yeast extract+sukrose+aquadest. The result showed that application of *Penicillium* spp. effectively reduced the neck root rot disease caused by *S.rolfsii* on chili. Seedlings treated with *Penicillium* spp. grown by various substrates significantly (0,05%) reduced disease severity ranged from 61,01-94,91%. Based on the results, *Penicillium* spp has potential as biocontrol agent against *Sclerotium rolfsii* on Chili.

Key words: Biocontrol, *Penicillium* sp.; *Sclerotium rolfsii*; Chili

INTRODUCTION

Neck root rot diseases caused by *Sclerotium rolfsii* Sacc is one of the most destructive and economically damaging diseases of chili. Species of *S. rolfsii* as soil-borne pathogen cause a variety of diseases on many different type of plant such as paddy (Purwanti *et al*., 1997), green bean, alfalfa, peanut, and bean (Caresini, 1999); papaya and corn (Uchida, 2007). Prayoga (2007), reported that, the persentage of diseases incidence caused by *S. Roflsii* on Chili in Pemulutan Sub-District, District of Ogan Ilir was 2.6%. Other survey conducted by Akbar (2007) reported that the persentage of diseases incidence caused by *S. Roflsii* on Chili in Pangkalan Benai Sub-District, District of Banyuasin ranged from 28% - 80% .

Soil microorganisms are ideal for use as biocontrol agents against soil-borne diseases. Previous research demonstrated that *Penicillium oxalicum* spp as soil inhabitants effectively reduced Fusarium will of tomato caused by *Fusarium oxysporum* .f.sp. *lycopersici* through induced resistance (De Cal A, et al., 1995; De Cal, A., et al., 1997; De Cal, A., et al., 2000). Koike et al. (1997) reported that *Penicillium* spp beside could reduced antracnose disease caused by *Colleltotrichum orbiculare* and bacterial leaf blight caused by *Pseudominas syringae* pv. *Lachrymans* on cucumber, it also could increase plant growth.

The objective of this research was to evaluate *Penicillium* spp produced in waste materials for control of neck root rot disease caused by *Sclerotium rolfsii* on chilli.

MATERIALS AND METHODS

Fungi

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Biocontrol agent used in this study was *Penicillium* spp.(isolates P8 and P10) as the Plant Growth Promoting Fungi (PGPF) isolated from rhizosphere of chili plant cultivated in low land area. *Sclerotium rolfsii* Sacc was obtained from an infected chili plant was used as the pathogen.

Plant

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

Inoculum Preparation

For inoculums of *Penicillium spp* isolates: For solid inoculum, each isolates of *Penicillium* spp (isolate P8 and P10) was cultured on potato dextrose agar (PDA) for 3 days at 25°C in the dark. 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 substrats (1:1, dry various substrats/distilled water, w/v) contained in a 500 ml Erlenmeyer flask e.i : 1). tapioca dregs+bran+bunch of palm oil (TBP); 2). coconut dregs+bran+bunch of palm oil (CBP); 3). tapioca dregs+bran+sawdust (TBS): 4). coconut dregs+bran+sawdust (CBS); 5).Yeast extract+sukrose+aquadest (YSA). The cultures were incubated in the dark for 10 days at 25°C and shaken regularly to aid even colonization. The infested media substrates were air-dried for 7 days and stored at 4°C until used. While for liquid media, each isolates of *Penicillium* spp (isolate P8 and P10) was cultured on potato dextrose agar (PDA) for 3 days at 25°C in the dark. Two-Three mycelial disks (5 mm) of the isolates cut from the edges of three-day old cultures were added to liquid media contain 15 g *yeast extract* and 20 g sukrosa per liter distilled water. The cultures were incubated in the dark for 5 days at 25°C at statis condition. The conidia were harvested by filter the culture and then used for this study.

For inoculums of pathogen, *Sclerotium rolfsii*. The procedures was prepared similar to solid inoculums of *Penicillium* spp. described above, except the media substrates used for pathogen was bran+corn+rice-straw with comparison 4 : 3 : 1 for each material, respectively.

Assay of *Penicillium* spp produced by various substrates for control

The inoculums of *Penicillium* spp. 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+kompos). The liquid inoculums were applied to potting medium in the concentration 10^7 conidia/g potting medium. Small polybags were filled with approximately 20 g with potting medium amended with inoculums *Penicillium* spp. One surface-sterilized chili seed was sown in each small polybag. The seedlings were allowed to grow for 21 days. The treated seedlings with *Penicillium* spp. were transferred to polibag (20x15 cm) which filled with potting soil. The inoculums of pathogen was then inoculated in soil surround the seedlings (1 g pathogen inoculums per seedling). The seedlings were kept in greenhouse to allow their grow for the next 14 days. The seedlings not treated with *Penicillium* spp. and challenged with *S. rolfsii* were set up as control. Treatments were replicated 3 times and each replicate consists of 5 plants.

Disease severity based on the foliar symptom was assessed using a scale of 0 to 4; 0 = healthy; 1 = 0-25% yellowing; 2 = 25-50% yellowing; 3 = 50-75% yellowing; 4 = >75% yellowing or dead plant. The percentage of disease severity in each replication within the treatment were calculated using the formula :

$$K = \frac{\Sigma(nxv)}{ZxN} x100\%$$

Where::

K = Disease Severity (%)

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n = number of seedlings infected by pathogen in each scale
v = Diseases scale (0-4)
Z = The highest of disease scale
N = total seedlings

Data analysis

The experiments were carried out in randomized completely design. Treatments means obtained for percentage disease severity were compared using honest significant difference (HSD) at $P = 0.05$ and $P = 0.01$.

RESULTS AND DISCUSSION

On the whole, all application of *Penicillium* spp produced in waste materials significantly reduced disease severity compare to control. Reduction of disease severity by *Penicillium* spp isolates, however, differed depending on *Penicillium* isolates and kinds of waste materials used for inoculums production. However, Statistically, there are no significantly different among treatment with *Penicillium* spp (Table 1). Seedlings treated with *Penicillium* spp. provided the reduction of disease severity was ranged from 61%-94,91%. The highest reduction against disease severity was provided in the treatment TBP P10 (94.91%), followed by TBS P8 and CBP P8 (88,13%), while the lowest was performed by YSA P10 (61,01%) and YSA P8 (69,49%).

Table 1. The effect of treatment with *Pnicillium* spp. produced by various substrates against disease severity of neck root rot of chili caused by *Sclerotium rolfsii* Sacc [a)]

Treatments	Disease severity (%)	HSD (0.05)	Reduction (%)
Control	100	a [b)]	
YSA P10	38.34	b	61.01
YSA P8	30.00	b	69.49
CBP P10	28.34	b	71.18
TBS P10	20.00	b	79.66
CBS P10	18.34	b	81.35
CBS P8	16.67	b	83.04
TBP P8	13.34	b	86.43
TBS P8	11.67	b	88.13
CBP P8	11.67	b	88.13
TBP P10	5.00	b	94.91

a). Data were taken 8 days after inoculation of pathogen
b). Mean of 3 replication with 5 plants per replication. Values followed by the same letter in each column do not differ significantly (P = 0.05) according to Honest significant different test. Data were analyzed after transformation to arc sin √x

In these study, all treatments using *Penicillium* produced in waste materials were effective in reducing disease of neck root rot disease on chili caused by *S. rolfsii* Sacc. under greenhouse condition (Table 1). This study support previous result conducted by some researchers who demonstrated that that *Penicillium* spp effectively reduced Fusarium will of tomato caused by *Fusarium oxysporum* .f.sp. *lycopersici* (De Cal, A. et al., 1995); bacterial angular leaf spot caused *Pseudomonas syringae* pv. *lachrymans* and Fusarium wilt caused by *Fusarium*

oxysporum f.sp. *cucumerinum* on cucumber through induced systemic resistance by the increasing lignin accumulation, soproxide generation and chemiluminescence activit (Koike et al. (2001). The biocontrol ability of *Penicillium* spp against neck root rot of chili obtained in this study holds a great possibility for their use as protective agents against Sclerotium diseases.

Its application as a waste materials (tapioca dregs; coconut dregs; bran; bunch of palm oil; sawdust) medium preparation that serves as a food base probably contributed to their successful establishment. It suggested that when the antagonists were introduced into small pot for preparing chili seedlings, it became establish in the rhizosphere and root area before transplanting in pathogen-infested soil. This ability might trigger host defense reaction, which was then transferred to the whole root or might be stem and leaf against neck root rot of chili De Cal et al. (1997) reported that, tomato plants treated with *Penicillum oxalicum* reduced disease severity of fusarium wilt of tomato when the antagonist and pathogen were inoculated in different points of tomato roots. Biles and Martyn (1989) observed that, prior inoculation of watermelon root wi avirulent *Fusarium oxysporum* f.sp. *niveum* induced resistance in both local and systemic, in that induced watermelon plants were protected from both fusarium wilt and anthracnose. Muslim et al. (2003a,b,c) reported that prior treatment of seedlings with *Hypovirulent Binucleate Rhizoctonia* (HBNR) in paper pot during seedling stage before transplanting into bigger pot contained pathogen-infested soil, effectively reduced Fusarium diseases of tomato and spinach. The mechanisms of biological control of the Fusarium diseases using HBNR might be related to competition for colonization site or nutrient and induced resistance

The effectiveness of *Penicillium* spp produced in waste materials against neck root rot of chili were also might be related to the contain of the waste materials used as medium were plenty of nutrient which increase its growth. Pareira (2008) reported that tapioca dregs and bran contain protein and carbohydrate,. Furthermore, Wahyono (2007) reported that bunch of palm oil contain nutrient such as nitrogen (0,4 %), $P_2 0_5$ (0,029 – 0,05), and $K_2 0$ (0,15 – 0,2 %). Widiastoety (2008) reported that coconut dregs contain essensial nutrients such as K, P, Ca, Mg and N. It also contain organic material, ash. Pectin, hemyselulosa, selulosa, pentosa and legnin..

CONCLUSION

The conclusion of this study is seedlings treated with *Penicillium* spp produced in waste materials based on tapioca dregs; coconut dregs; bran; bunch of palm oil; and sawdust, effectively reduced disease severity of neck root rot of chili caused by *Sclerotium rolfsii* Sacc. ranged from 61%-94,91%.

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