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# SUPPRESSION OF ROOT ROTTING FUNGI OF MUNGBEAN BY THE ENDOPHYTIC *PENICILLIUM* SPECIES UNDER SOIL AMENDMENT

**Faizah Urooj[1], Hafiza Farhat[1], Farzana Korejo[2], Hafiza Asma Shafique[1] Sharfun-Nahar[1] and Gulnaz Parveen[3]**

[1]*Department of Botany, University of Karachi, Karachi-75270, Pakistan*
[2]*Institurte of Plant Sciences, University of Sindh, Jamshoro, Sindh, Pakistan*
[3]*Department of Botany, Women University Sawabi, Pakistan*
[*]*Corresponding author's email: snahar@uok.edu.pk*

**ABSTRACT**

Endophytic fungi or bacteria, may enhance plant growth, help in uptake of nutrients and increase the ability to bear environmental stresses like salinity, drought, and also reduce biotic stresses. In current research antagonistic potential of endophytic *Penicillium* species was assessed in screen house alone or under soil amendment with neem cake and cotton cake against root rot pathogens on mungbean. *Macrophomina phaseolina, Rhizoctonia solani, F. solani,* and *Fusarium oxysporum* were significantly suppressed by many endophytic *Penicillium* isolates alone or under soil amendment. They produce healthy plants and improved plant length and weight. Endophytic *Penicillium* spp. associated with healthy plants may be a good biocontrol agent against root rot pathogens.

**KEYWORDS:** Root infecting fungi, Endophytic fungi, *Penicillium* spp., Plant growth, Cotton cake, Neem cake, Soil amendment.

## INTRODUCTION

To discover and create the best approach for sustainable agriculture and crop production system has now become very important to prevent or minimize the huge losses caused by plant disease and pests (Rai *et al.*, 2014). Endophytes are microorganisms that grow within the plant tissues for at least a part of their life cycle without causing any apparent symptoms to the host (Urooj *et al.*, 2018). Among beneficial endophytes, fungi are gaining importance because they may serve as biocontrol agent against plant disease, besides being source of novel biologically active compounds (Staniek *et al*., 2008; Farhat *et al.*, 2019). *Penicillium* sp., generally considered as contaminant of food and postharvest pathogen of fresh fruits, has also been reported as endophyte (Waller *et al.*, 2005; Urooj *et al.*, 2018). *Penicillium* species are known to have antifungal, algicidal and antibiotic activities and also induce resistance in their host plant against biotic and abiotic stresses (Meng *et al.*, 2011; Korejo *et al*., 2014).

Beneficial impact of organic matter on plant health have been demonstrated by several authors (Van Elsas and Postma, 2007; Noble and Coventry, 2005; Shafique *et al.*, 2017). Cover crop, crop rotation, soil tillage, organic amendments and use of biocontrol agents may have dramatic effects on overall soil fertility and particularly soil properties like soil nutrient availability, water holding capacity and soil erosion stability (Mader *et al*., 2002). Similarly, direct addition of organic matter improves soil health including soil structure, aeration, drainage, moisture and microbial population (Bailey and Lazarovits, 2003). Among various organic materials, neem cake has been reported to suppress root rot pathogens and parasitic nematodes affecting crop plants (Abbasi *et al*., 2005; Urooj *et al.*, 2018; Shafique *et al.*, 2016). Mungbean, an important pulse crop has been reported to be attacked by root rotting fungi and their control with conventional method is difficult (Farhat *et al*., 2017). We are here investigating the potential of endophytic *Penicillium* species alone or in soil amended with neem cake or cotton cake in suppressing the root rotting fungi of mungbean in screen house experiments.

## MATERIALS AND METHODS

Fourteen isolates of endophytic *Penicillium* species (Table 1) that have shown significant biocontrol potential against root rotting fungi on sunflower in our previous study (Urooj *et al.*, 2018) were further evaluated on mungbean. Sandy loam soil (naturally infested with root infecting fungi) used in this study were transferred in clay pots (12 cm diam.) at 1 Kg per pot. Aqueous suspension of test *Penicillium* (25mL/ pot) cfu $10^7$ /mL were applied in each pot. Whereas, in another set, inoculum of *Penicillium* were applied in pots containing amended soil with neem cake or cotton cake at 1% (soil was amended one week before and watered daily). Six seeds of mungbean (*Vigna radiata*) per pot were sown and four seedlings were kept in each pots after germination. Carbendazim (200 ppm) 25mL per pot served as positive control. Each treatment had 4 replicates and pots were randomized in block design observation were recorded after 45 days. Data on plant growth and biocontrol potential of endophytic *Penicillium* was determined as described by Habiba *et al*. (2016). Data were analyzed and significant level at $p<0.05$ was calculated using software COSTAT.

## RESULTS

**Root diseases and growth of mungbean plant treated with *Penicillium* spp., in soil amended with neem cake:** No infection of *F. oxysporum* was found in *P. lilacinum* (EPSML2), *P. purpurogenum* (EPSML3) and *P. duclauxi* (EPSML9) treatments when used in natural soil. Infection of *F. oxysporum* was also not found where *P. lilacinum*, *P. nigricans* and *P. duclauxi* used in neem cake amended soil. Significant reduction in infection of *F. solani* was observed in natural soil by all isolates whereas in neem cake amended soil all isolates also showed significant reduction except *P. citrinum*. No infection of *M. phaseolina* was found in *P. citrinum* treatment in both type of soil, whereas *P. restrictum* treated plants also showed no infection of *M. phaseolina* in natural soil. Less infection of *R. solani* was found in most of the treatments (Table 1)

Application of endophytic *P. lividum* with neem cake caused a significant increase in plant height while *P. nigricans*, *P. lilacinum*, *P. purpurogenum* (EPEHS7), *P. asperum*, *P. thomii*, *P. javanicum* and *P. purpurogenum* (EPAER14) also showed significant positive results in natural soil. *Penicillium purpurogenum* (EPEHS7) and *P. purpurogenum* (EPAER14) showed significant positive effect on shoot weight in natural soil. In natural soil greater root length was produced by *P. lilacinum* whereas in amended soil *P. restrictum*, *P. asperum*, *P. thomii* and *P. javanicum* produced larger root length (Table 2).

**Table 1. Effect of endophytic *Penicillium* spp. with neem cake on the infection of *Fusarium solani, F. oxysporum, Rhizoctonia solani* and *Macrophomina phaseolina* on mungbean roots in screen house experiments.**

| Treatments | Code # | Infection% | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ***F. oxysporum*** | | ***F. solani*** | | ***M. phaseolina*** | | ***R. solani*** | |
| | | **NS** | **AS** | **NS** | **AS** | **NS** | **AS** | **NS** | **AS** |
| Control | -- | 50 | 31.2 | 100 | 75 | 100 | 50 | 0 | 56.2 |
| Carbendazim | -- | 12.5 | 6.2 | 50 | 31.2 | 18.7 | 25 | 0 | 25 |
| *Penicillium decumbens* | EPAIR6 | 12.5 | 25 | 37.5 | 43.7 | 18.7 | 43.7 | 0 | 12.5 |
| *P. nigricans* | EPSLR4 | 6.2 | 0 | 50 | 18.7 | 12.5 | 18.7 | 0 | 0 |
| *P. regulosum* | EPAAR5 | 12.5 | 18.7 | 43.7 | 50 | 31.2 | 50 | 6.2 | 56.2 |
| *P. citrinum* | EPSMR1 | 6.2 | 6.2 | 43.7 | 75 | 0 | 0 | 6.2 | 6.2 |
| *P. lilacinum* | EPSMS2 | 0 | 0 | 50 | 12.5 | 31.2 | 6.2 | 18.7 | 6.2 |
| *P. purpurogenum* | EPSML3 | 0 | 25 | 37.5 | 50 | 25 | 25 | 43.7 | 18.7 |
| *P. duclauxi* | EPASS9 | 0 | 0 | 43.7 | 37.5 | 25 | 37.5 | 6.2 | 25 |
| *P. lividum* | EPMCL12 | 6.2 | 25 | 25 | 68.7 | 12.5 | 37.5 | 6.2 | 50 |
| *P. purpurogenum* | EPEHS7 | 6.2 | 12.5 | 37.5 | 31.2 | 18.7 | 18.7 | 6.2 | 25 |
| *P. restrictum* | EPCTS8 | 12. | 25 | 43.7 | 37.5 | 0 | 31.2 | 6.2 | 18.7 |
| *P. thomii* | EPAER11 | 6.2 | 6.2 | 43.7 | 25 | 12.5 | 31.2 | 0 | 0 |
| *P. purpurogenum* | EPAER14 | 6.2 | 12.5 | 37.5 | 31.2 | 18.7 | 18.7 | 6.2 | 25 |
| *P. javanicum* | EPSLR13 | 6.2 | 0 | 50 | 18.7 | 12.5 | 18.7 | 0 | 0 |
| *P. asperum* | EPHAL10 | 43.5 | 12.5 | 25 | 25 | 25 | 18.7 | 0 | 0 |
| $LSD_{0.05}$ | | Treatment=5.61[1] | | | Pathogen=2.80[2] | | | Soil Type=1.98[3] | |

[1]Difference greater than LSD values among means in column are significant at p<0.05
[2]Difference greater than LSD values among means in row are significant at p<0.05
[3]Mean values in the NS and AS column showing difference greater than LSD value are significantly different at p<0.05
NS= Natural soil; AS = Amended soil

**Table 2. Effect of endophytic *Penicillium* spp. and neem cake on the growth of mungbean in screen house experiments.**

| Treatments | Code # | Shoot length (cm) | | Shoot weight (g) | | Root length (cm) | | Root weight (g) | |
|---|---|---|---|---|---|---|---|---|---|
| | | **NS** | **AS** | **NS** | **AS** | **NS** | **AS** | **NS** | **AS** |
| Control | -- | 13.75 | 17.14 | 0.78 | 0.8 | 15.31 | 4.652 | 0.51 | 0.14 |
| Carbendazim | -- | 13.9 | 18.65 | 0.73 | 1.322 | 15.56 | 4.73 | 0.56 | 0.15 |
| *Penicillium decumbens* | EPAIR6 | 13.59 | 16.1 | 0.89 | 1.055 | 12.33 | 5.002 | 0.55 | 0.23 |
| *P. nigricans* | EPSLR4 | 14.63 | 14.52 | 0.77 | 0.31 | 11.25 | 6.375 | 0.31 | 0.11 |
| *P. regulosum* | EPAAR5 | 13.58 | 17.75 | 0.73 | 0.732 | 19.43 | 4.905 | 0.32 | 0.17 |
| *P. citrinum* | EPSMR1 | 12.99 | 16.06 | 0.59 | 0.617 | 16.5 | 4.77 | 0.39 | 0.16 |
| *P. lilacinum* | EPSMS2 | 14.8 | 16.85 | 0.83 | 0.662 | 25.1 | 4.175 | 0.46 | 0.22 |
| *P. purpurogenum* | EPSML3 | 12.99 | 16.06 | 0.59 | 0.617 | 16.5 | 4.77 | 0.39 | 0.16 |
| *P. duclauxi* | EPASS9 | 11.87 | 19.16 | 0.69 | 0.855 | 11.08 | 4.562 | 0.17 | 0.16 |
| *P. lividum* | EPMCL12 | 13.2 | 21.47 | 0.61 | 1.358 | 22.52 | 4.785 | 0.26 | 0.22 |
| *P. purpurogenum* | EPEHS7 | 14.48 | 19.17 | 0.92 | 1.115 | 15.43 | 4.45 | 0.59 | 0.16 |
| *P. restrictum* | EPCTS8 | 12.68 | 18.74 | 0.68 | 1.102 | 10.87 | 7.02 | 0.31 | 0.2 |
| *P. thomii* | EPAER11 | 14.63 | 17.9 | 0.77 | 1.203 | 11.25 | 7.025 | 0.31 | 0.24 |
| *P. purpurogenum* | EPAER14 | 14.48 | 19.17 | 0.92 | 1.115 | 15.43 | 4.45 | 0.59 | 0.16 |
| *P. javanicum* | EPSLR13 | 14.63 | 17.9 | 0.77 | 1.203 | 11.25 | 7.025 | 0.31 | 0.24 |
| *P. asperum* | EPHAL10 | 14.63 | 18.74 | 0.77 | 1.102 | 11.25 | 7.02 | 0.31 | 0.2 |
| $LSD_{0.05}$ | | 1.61[1] | 4.01[1] | 0.19[1] | 2.14[1] | 8.42[1] | 1.15[1] | 0.17[1] | 0.07[1] |

[1]Difference greater than LSD values among means in column are significant at p<0.05
[2]Difference greater than LSD values among means in row are significant at p<0.05
NS = Natural soil; AS = Amended soil

**Table 3. Effect of endophytic *Penicillium* spp., and cotton cake on the infection of *Fusarium solani, F. oxysporum Rhizoctonia solani* and *Macrophomina phaseolina* on mungbean roots in screen house experiment.**

| Treatments | Code# | Infection% | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ***F. oxysporum*** | | ***F. solani*** | | ***M. phaseolina*** | | ***R. solani*** | |
| | | **NS** | **AS** | **NS** | **AS** | **NS** | **AS** | **NS** | **AS** |
| Control | … | 50 | 50 | 100 | 75 | 100 | 75 | 0 | 18.7 |
| Carbendazim | … | 12.5 | 50 | 50 | 75 | 18.7 | 75 | 0 | 18.7 |
| *Penicillium decumbens* | EPAIR6 | 12.5 | 0 | 37.5 | 31.2 | 18.7 | 37.5 | 0 | 0 |
| *P. nigricans* | EPSLR4 | 6.2 | 18.7 | 50 | 43.7 | 12.5 | 37.5 | 0 | 0 |
| *P. rugulosum* | EPAAR5 | 12.5 | 6.2 | 43.7 | 12.5 | 31.2 | 18.7 | 6.2 | 0 |
| *P. citrinum* | EPSMR1 | 6.2 | 25 | 43.7 | 43.7 | 0 | 43.7 | 6.2 | 18.7 |
| *P. lilacinum* | EPSMS2 | 0 | 37.5 | 50 | 68.7 | 31.2 | 25 | 18.7 | 6.2 |
| *P. purpurogenum* | EPSML3 | 0 | 43.7 | 37.5 | 50 | 25 | 68.7 | 43.7 | 18.5 |
| *P. duclauxi* | EPASS9 | 0 | 31.2 | 43.7 | 56.2 | 25 | 56.2 | 6.2 | 6.5 |
| *P. lividum* | EPMCL12 | 6.2 | 12.5 | 25 | 25 | 12.5 | 25 | 6.2 | 0 |
| *P. purpurogenum* | EPEHS7 | 6.2 | 0 | 37.5 | 31.2 | 18.7 | 12.5 | 6.2 | 0 |
| *P. restrictum* | EPCTS8 | 12.5 | 31.2 | 43.7 | 31.2 | 0 | 31.2 | 6.2 | 6.5 |
| *P. thomii* | EPAER11 | 6.2 | 18.7 | 43.7 | 43.7 | 12.5 | 37.5 | 0 | 0 |
| *P. purpurogenum* | EPAER14 | 6.2 | 0 | 37.5 | 31.2 | 18.7 | 12.5 | 6.2 | 0 |
| *P. javanicum* | EPSLR13 | 6.2 | 18.7 | 50 | 43.7 | 12.5 | 37.5 | 0 | 0 |
| *P. asperum* | EPHAL10 | 43.7 | 37.5 | 25 | 31.2 | 25 | 56.2 | 0 | 12.5 |
| $LSD_{0.05}$ | | Treatment=5.89[1] | | | Pathogen=2.94[2] | | | Soil Type=2.08[3] | |

[1]Difference greater than LSD values among means in column are significant at $p<0.05$
[2]Difference greater than LSD values among means in row are significant at $p<0.05$
[3]Mean values in the NS and AS column showing difference greater than LSD value are significantly different at $p<0.05$
NS = Natural soil; AS = Amended soil

**Root diseases and growth of mungbean plant treated with *Penicillium* spp., in soil amended with cotton cake:** Endophytic *Penicillium* isolates alone or with cotton cake significantly reduced *M. phaseolina*, whereas, plants grown in soil treated with *P. nigricans*, *P. rugulosum*, *P. decumbens*, *P. purpurogenum* (EPEHS7), *P. thomii*, *P. lividum*, *P. javanicum* and *P. purpurogenum* (EPAER14) in cotton cake amended soil showed no infection of *R. solani* (Table 3). Most of the *Penicillium* species significantly suppressed *F. solani* and *F. oxysporum* both in natural and cotton cake amended soil (Table 3).

Cotton cake and *P. nigricans*, *P. thomii*, *P. javanicum* significantly increased root length and fresh root weight as compared to untreated control plants. Whereas mixed application of cotton cake and *P. decumbens* significantly increased fresh shoot weight (Table 4).

## DISCUSSION

Endophytic fungi may provide advantages to the host and may have with vast applications in agriculture and medicine (Clay *et al.*, 2005; Alvarez-Loayza, 2011). Endophytic fungi have beneficial effects on growth of plants as biocontrol agents because they suppress diseases by inhabiting internal tissues (Yuan *et al.,* 2017); similar site as plant pathogen (Berg *et al.*, 2005; Kado, 1992) and improve plant growth (Waqas *et al.,* 2015; Veja *et al*., 2008; Mendoza and Sikora, 2009 and Bahar *et al*., 2011).

**Table 4. Effect of endophytic *Penicillium* spp. and cotton cake on the growth of mungbean in screen house experiment.**

| Treatments | Code# | Shoot length (cm) | | Shoot weight (g) | | Root length (cm) | | Root weight (g) | |
|---|---|---|---|---|---|---|---|---|---|
| | | NS | AS | NS | AS | NS | AS | NS | AS |
| Control | … | 13.75 | 13.64 | 0.78 | 0.89 | 15.31 | 6.13 | 0.51 | 0.31 |
| Carbendazim | … | 13.9 | 13.98 | 0.73 | 1.06 | 15.56 | 6.99 | 0.56 | 0.38 |
| *Penicillium decumbens* | EPAIR6 | 13.59 | 14.7 | 0.89 | 1.42 | 12.33 | 7.9 | 0.55 | 0.39 |
| *P. nigricans* | EPSLR4 | 14.63 | 14.35 | 0.77 | 1.19 | 11.25 | 11.85 | 0.31 | 0.71 |
| *P. rugulosum* | EPAAR5 | 13.58 | 13.22 | 0.73 | 1.01 | 19.43 | 7.46 | 0.32 | 0.36 |
| *P. citrinum* | EPSMR1 | 12.99 | 13.18 | 0.59 | 1.93 | 16.5 | 9.61 | 0.39 | 0.37 |
| *P. lilacinum* | EPSMS2 | 14.8 | 14.38 | 0.83 | 1.16 | 25.1 | 10.96 | 0.46 | 0.45 |
| *P. purpurogenum* | EPSML3 | 12.99 | 13.18 | 0.59 | 1.93 | 16.5 | 9.61 | 0.39 | 0.37 |
| *P. duclauxi* | EPASS9 | 11.87 | 14.38 | 0.69 | 1.3 | 11.08 | 11.78 | 0.17 | 0.48 |
| *P. lividum* | EPMCL12 | 13.2 | 13.23 | 0.61 | 1.07 | 22.52 | 10.24 | 0.26 | 0.48 |
| *P. purpurogenum* | EPEHS7 | 14.48 | 12.875 | 0.92 | 1.07 | 15.43 | 9.33 | 0.59 | 0.41 |
| *P. restrictum* | EPCTS8 | 12.68 | 14.53 | 0.68 | 1.28 | 10.87 | 9.72 | 0.31 | 0.46 |
| *P. thomii* | EPAER11 | 14.63 | 14.35 | 0.77 | 1.19 | 11.25 | 11.85 | 0.31 | 0.71 |
| *P. purpurogenum* | EPAER14 | 14.48 | 12.875 | 0.92 | 1.07 | 15.43 | 9.33 | 0.59 | 0.41 |
| *P. javanicum* | EPSLR13 | 14.63 | 14.35 | 0.77 | 1.19 | 11.25 | 11.85 | 0.31 | 0.71 |
| *P. asperum* | EPHAL10 | 14.63 | 14.53 | 0.77 | 1.28 | 11.25 | 9.72 | 0.31 | 0.46 |
| $LSD_{0.05}$ | | 1.61[1] | 2.66[1] | 0.19[1] | 0.9[1] | 8.42[1] | 2.7[1] | 0.17[1] | 0.29[1] |

[1]Difference greater than LSD values among means in column are significant at $p<0.05$
NS = Natural soil; AS = Amended soil

In this study, application of endophytic *Penicillium* spp., significantly reduced infection by root rotting fungi and improved growth of mungbean alone or in soil amended with neem cake or cotton cake. Waqas (2015) reported that the *Penicillium citrinum* LWL4 significantly improved the growth of sunflower plant. It may be assumed that biocontrol fungi may restrict the disease progression and attenuate the diseases resulting in the improvement of plant growth (Mei and Flinn, 2010) and biomass via improved nutrient uptake (Muthukumarasamy *et al*., 2002). In our study, better growth of mungbean plant was achieved which may be due induction of systemic resistance as reported earlier (Urooj *et al.,* 2018; Shafique *et al.,* 2016). Our findings agree with the findings of Serfling *et al*. (2007) and Hamayun *et al*. (2010), who also reported plant growth promotion by endophytes.

For suppressing soil-borne diseases, improving crops and increasing agricultural productivity application of organic amendments is well known (Lazarovits, 2001; Stone *et al.,* 2003; Ikram and Dawar, 2015; Shafique *et al.,* 2016 and Sultana *et al.,* 2011; 2018). Organic soil amendments not only improve soil quality but also increase soil suppressiveness against soil-borne pathogens, thus bring positive effects on agriculture production and crop health (Bailey and Lazarovits, 2003). It has been reported by some studies that organic soil amendments can be very active against damages produced by fungal pathogens like *Fusarium* spp., *R. solani* and *M. phaseolina* (Urooj *et al.,* 2018; Parveen *et al.*, 2019), *Pythium* spp. and (McKellar and Nelson, 2003 and Veeken *et al.,* 2005). In the present study, use of organic fertilizers including cotton cake and neem alone or with *Penicillium* spp., showed suppressive effect on root rotting fungi comparable to chemical fungicide (carbendazim).

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