

Effect of *Penicillium* species culture filtrate on seedling growth of wheat

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Abstract

Penicillium spp. produce a variety of beneficial metabolites for plant growth and survival, as well as defend their hosts from attack of certain pathogens. In present study 20 *Penicillium* spp. culture filtrates were screened for growth activity in wheat seedlings. Germination percentage of wheat seeds was enhanced by *Penicillium* spp. culture extracts. Although, the most active inhibitors of seed germination were *P. dendriticum*, *P. herquei*, *P. notatum*, and *P. oxalicum*. Results revealed that the highest seedling root length was attained by *P. olivicolor* followed by *P. bravicompactum* and *P. spinulosum*. While, the lowest root growth was attained from *P. duclauxii* and *P. islandicum* among other *Penicillium* culture filtrate treatments. The highest seedling shoot length was observed with *P. variable* and *P. herquei* followed by *P. aurantiogriseum* - A and *P. viridicatum* while other *Penicillium* cultural filtrates significantly suppressed the shoot length as compared to control value ($P < 0.05$). Fresh and dry weight of wheat seedling were also significantly higher in *Penicillium* treatments as compared to control ($P < 0.05$). Although, in *P. dendriticum* treatment, fresh and dry weight of seedling was considerably poor. Whereas, *P. roquefortii*, *P. bravicompactum*, *P. islandicum* and *P. implicatum* had higher values for seedling dry matter. Results revealed that *Penicillium* spp. are effective for growth parameters and can be used to further investigations and raise the yield of wheat and other cereal crops.

Keywords: *Penicillium*, Metabolites, Inhibitors, Wheat, Filtrates, Seedling Growth.

INTRODUCTION

Fungi may play an important role in plant survival by enhancing nutrient uptake and producing growth-promoting metabolites such as gibberellins and auxins. *Penicillium* and *Trichoderma* species are known to produce a variety of beneficial compounds to suppress the pathogens (Hyakumachi et al., 1994; Narisawa et al., 2004; Dubey et al., 2007) and stimulate plant growth by the production of phytohormones (Hasan, 2002) and/or degradation of complex substrates (Altmore et al., 1999). On the other hand, *Penicillium* and *Aspergillus* species have been reported to produce gibberellin which is a growth regulating hormone in higher plants (Hasan, 2002; Hamayun et al., 2009). *Penicillium* species

generally occur at greater soil depths than species of other genera, and have low concentrations in rhizosphere soils (Domsch et al., 1993). However all strains of *Penicillium* so far has been tested to solubilise metaphosphates and utilize them as phosphorus sources (Phuwiwat and Soy Tong, 2001). However, very little is known about plant growth stimulants produced by *Penicillium* spp. in wheat. Therefore, the objective of this study is to investigate the potential of *Penicillium* species culture filtrate on seedling growth of wheat.

MATERIALS AND METHODS

Seed selection. Wheat seeds of local cultivars were used in the experiment. Healthy seeds were obtained from Institute of Agricultural Sciences, University of the Punjab, Lahore.

Fungal isolate. *Penicillium* species (Table 1) were obtained from the First Fungal Culture Bank of the Pakistan (FCBP), Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. These isolates were maintained on Czapek's agar (g/l): NaNO₃ (2), K₂HPO₄ (1), KCl (0.5), MgSO₄·7H₂O (0.5), agar (17) and 1000ml of distilled water. These cultures were kept at 4°C for further studies.

Fungal filtrates. For crude fungal filtrates preparation, a disc of 1 cm from a 7-day culture of each *Penicillium* sp. on Czapek's agar was inoculated into Erlenmeyer flasks (250 cc) containing 100 ml of Malt extract (ME) broth. Culture broth flasks were incubated at 20 ± 2°C for 15 days. Fungal filtrate was collected by filtration through Whatman filter paper No. 4. Filtrate was stored at 4°C for further use.

In vitro, germination and early seedling growth. Wheat seeds were soaked in the each fungal filtrate for 30 minutes for the seed treatment. For control, seeds were soaked in distilled water. Ten seeds from each treatment were placed in 90-mm-diameter Petri dishes on whatman No.1 filter paper moistened with 3 ml of distilled sterile water. Treated seed was kept at room temperature (25°C) under normal light. Germination progress was recorded daily up to 7 days. The germination percent (GP) of germinated seeds was recorded after 7 days of planting (ISTA, 1993; 1999). At harvesting, the seedling height, root length, fresh and dry weights of the root and shoots, germination percentage (GP), germination index (GI), seedling vigour index, were measured.

Germination percentage (GP) was calculated using the following formulas:

$$\text{Germination percentage (GP)} = \frac{\text{number of germinated seeds}}{\text{Number of total seeds}} \times 100$$

The germination index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) by following formula:

$$GI = \frac{\text{No. of germinated seed}}{\text{Days of first count}} + \frac{\text{No. of germinated seed}}{\text{Days of final count}}$$

The seedling vigor index was calculated according to equation (Orchard 1977).

Seedling vigor index (SVI) = [seedling length (cm) × germination percentage].

The average mean of growth parameters from three lab experiments (each experiment was carried out in three replicates) were subjected to analysis of variance and treatment means were computed by Duncan's multiple range test (DMRT) at $P = 0.05$.

RESULTS AND DISCUSSION

According to the results, all studied seed growth parameters were affected by the treatments and there

was completely significant difference between control and treated seeds (Table 2).

Germination percentage (GP) was enhanced by *Penicillium* spp. culture extracts. *P. dendriticum* and *P. bravicompactum* extract reduce germination up to 40% in wheat. *Penicillium* has shown the poisoning effect on the seedlings and of cereals. The most active inhibiting effect was observed with *P. notatum*, and *P. oxalicum*. The formation of toxins by fungi has also been reported, which act on cereals growth in soil (Krasil'nikov, 1958).

The lowest germination index of wheat seed was attained with *P. dendriticum* (4.00) and *P. herquei* (5.33), respectively. There was significant difference between control and *Penicillium* extract treatments so as germination index from treated seed was more than control (Table 2). The probable reason for early emergence of the treated seed may be due to the effect of *Penicillium* filtrate extract on pre-germinating metabolic activities of seed that make radicle protrusion and the extract treated seed germinated soon after planting compared with untreated seed. These positive effects are probably due to the stimulatory effects of *Penicillium* extract on the early stages of germination process by mediation of cell division in germinating seeds. Whitelaw et al. (1997) also reported that *Penicillium* spp. can stimulate plant growth in cereal crops. The improvement in germination and vigour of normal/low-vigour seed might be due to reserve mobilization of food material, activation and re-synthesis of some enzymes, DNA and RNA synthesis start due to effect of *Penicillium* metabolites. A similar action mechanism has been reported for *P. bilaii* and *P. radicum*, two fungal species noted for their ability to solubilize phosphorus and promote vegetable growth (Kucey, 1987, 1988; Whitelaw et al., 1997; 1999; Wakelin et al., 2004 a; 2004 b).

Variance analysis results showed that there was significant difference between *Penicillium* extract treated seeds and control treatment from the aspect of root and shoot growth of wheat seedlings. Seedling growth promotion by tested *Penicillium* was significantly higher than the control ($P < 0.05$). The highest seedling shoot length was observed with *P. variable* and *P. herquei* followed by *P. aurantiogriseum* and *P. viridicatum* (Figure 1) while other *Penicillium* cultural filtrates significantly suppressed the shoot length as compare to control value ($P < 0.05$).

Results revealed that the highest seedling root length was attained by *P. olivicolor* followed by *P. bravicompactum* and *P. spinulosum* while, the lowest root growth was attained from *P. duclauxii* and *P. islandicum* among other *Penicillium* filtrate treatments (Figure 2). Current findings confirmed the previous reports of shoot length promotion by fungal culture filtrate application (Choi et al., 2005; Khan et al., 2008). However, *Penicillium* spp. has been being explored for different secondary metabolites that can produce plant

Table 1. *Penicillium* spp. used in this study

Sr. No.	<i>Penicillium</i> spp.	Sr. No.	<i>Penicillium</i> spp.
1	<i>P. aurantiogriseum</i> - A	12	<i>P. implicatum</i>
2	<i>P. aurantiogriseum</i> - B	13	<i>P. islandicum</i>
3	<i>P. bravicompactum</i>	14	<i>P. janczewskii</i>
4	<i>P. chrysogenum</i>	15	<i>P. janthinellum</i>
5	<i>P. citrinum</i>	16	<i>P. olivicolor</i>
6	<i>P. dendriticum</i>	17	<i>P. olsonii</i>
7	<i>P. digitatum</i>	18	<i>P. roquefortii</i>
8	<i>P. duclauxii</i>	19	<i>P. spinulosum</i>
9	<i>P. expansum</i> - A	20	<i>P. variable</i>
10	<i>P. expansum</i> - B	21	<i>P. verrucosum</i>
11	<i>P. herquei</i>	22	<i>P. viridicatum</i>

Table 2. Germination %, index and vigor of wheat seeds

<i>Penicillium</i> species	Germination age (%)	Germination index	Seed vigor
Control	90	9.00	1657.8
<i>P. aurantiogriseum</i> - A	100	10.0	1799.0
<i>P. aurantiogriseum</i> - B	100	8.00	1832.0
<i>P. bravicompactum</i>	70	7.00	1364.3
<i>P. chrysogenum</i>	90	9.00	1141.2
<i>P. citrinum</i>	100	9.50	1841.0
<i>P. dendriticum</i>	60	4.00	816.0
<i>P. digitatum</i>	90	9.00	1283.4
<i>P. duclauxii</i>	80	7.00	675.2
<i>P. expansum</i> - A	100	10.00	1557.0
<i>P. expansum</i> - B	100	10.00	1463.0
<i>P. herquei</i>	70	5.33	1359.4
<i>P. implicatum</i>	100	10.00	1193.0
<i>P. islandicum</i>	100	10.00	936.0
<i>P. janczewskii</i>	100	10.00	1252.0
<i>P. janthinellum</i>	90	9.00	918.9
<i>P. olivicolor</i>	100	10.00	2133.0
<i>P. olsonii</i>	70	7.00	1017.1
<i>P. roquefortii</i>	100	8.50	1552.0
<i>P. spinulosum</i>	100	10.00	1863.0
<i>P. variable</i>	90	8.50	1773.9
<i>P. verrucosum</i>	100	10.00	1825.0
<i>P. viridicatum</i>	90	9.00	1643.4

growth associated benefits (Dutta et al., 2008; Wakiyama et al., 2008).

Fresh weight, dry weight and wheat seedling were also significantly higher in *Penicillium* culture filtrate treatments as compared to control ($P < 0.05$). Although,

in *P. dendriticum* treatment, fresh and dry weight of seedling was considerably poor as compared to control (Figures 3 and 4). Maximum fresh weight of seedling was recorded with *P. herquei*, *P. aurantiogriseum* – A and *P. viridicatum* treatment that was followed by *P.*

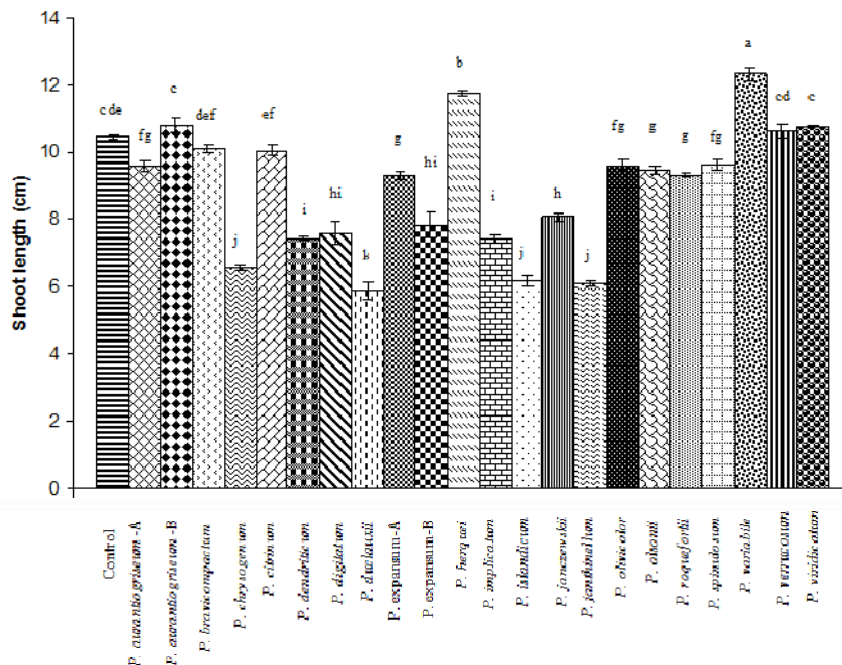


Figure 1. Effect of *Penicillium* spp. culture filtrates on shoot length of wheat seedlings. Bars topped by different letters are significantly different at $P < 0.05$

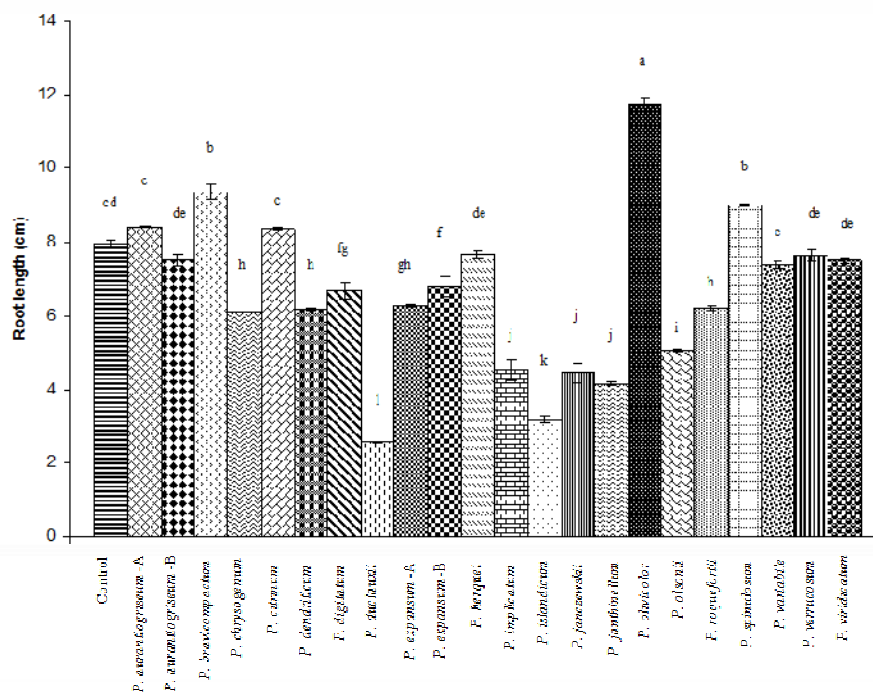


Figure 2. Effect of *Penicillium* spp. culture filtrates on root length of wheat seedlings. Bars topped by different letters are significantly different at $P < 0.05$

roquefortii which was significantly higher than the control ($P < 0.05$). However, *P. duclauxii*, *P. digitatum* and *P. citrinum* were least effective to stimulate fresh weight than the control (Figure 3). However, results showed that

seedling inoculated with *P. roquefortii*, *P. brevicompactum*, *P. islandicum* and *P. implicatum* had higher values for dry matter as compare to other *Penicillium* spp. (Figure 4).

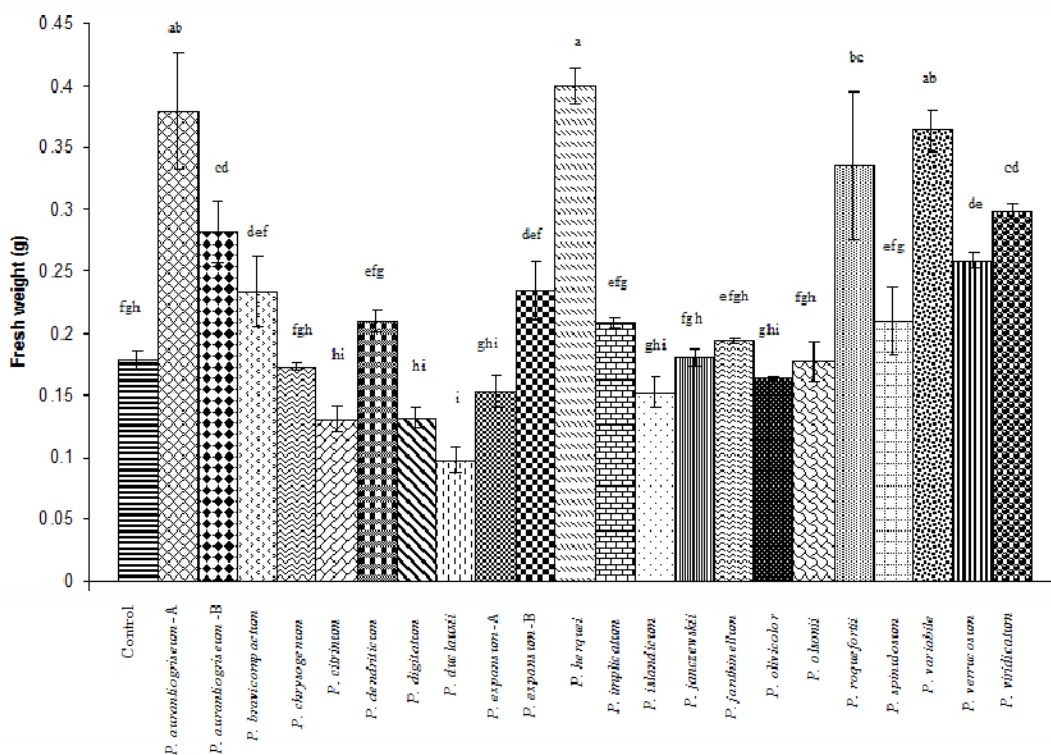


Figure 3. Effect of *Penicillium* spp. culture filtrates on fresh weight of wheat seedlings. Bars topped by different letters are significantly different at $P < 0.05$

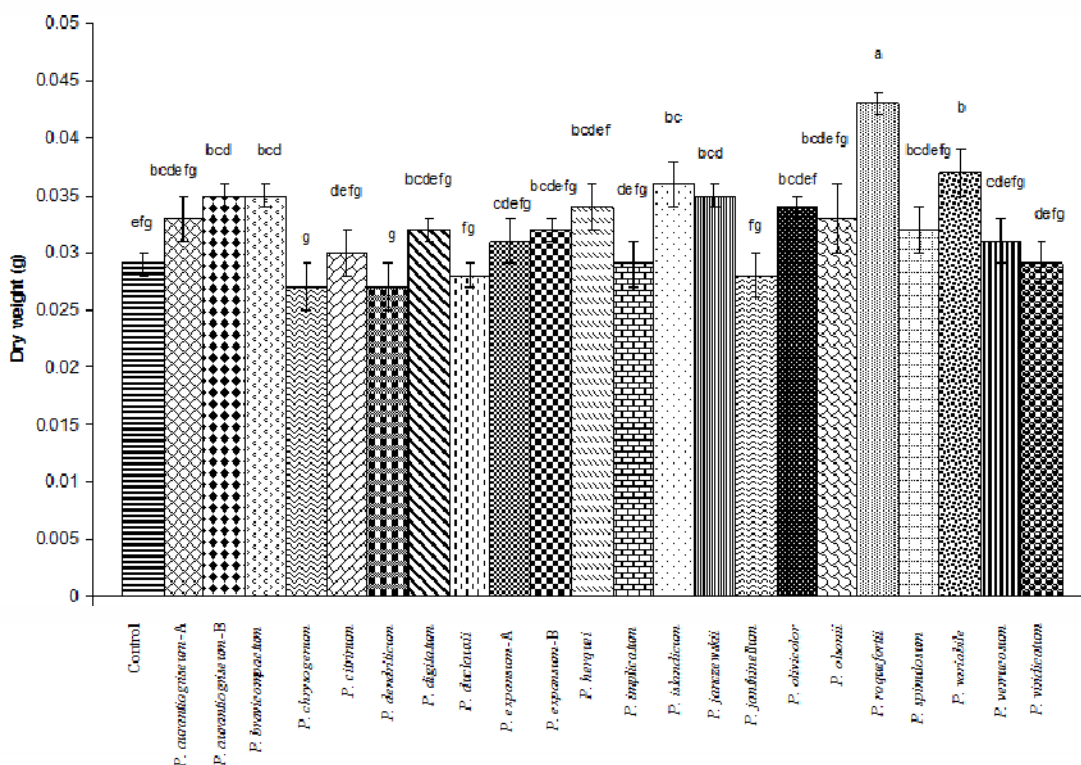


Figure 4. Effect of *Penicillium* spp. culture filtrates on dry weight of wheat seedlings. Bars topped by different letters are significantly different at $P < 0.05$

CONCLUSION

On the basis of results, it is evident that *Penicillium* spp. are effective for growth promotion in wheat. Further investigation can be carried out to determine the potential of *Penicillium* spp. on the growth promotion of other crops. Furthermore, the soil microbial communities are suitable for plant growth and could also be studied.

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