International Research Journal of Agricultural Science and Soil Science (ISSN: 2251-0044) Vol. 3(1) pp. 24-29, January 2013
Available online http://www.interesjournals.org/IRJAS

Full Length Research Paper

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

***Ibatsam Khokhar, Muhammad Saleem Haider, Irum Mukhtar, Amna Ali, Sobia Mushtaq, Muhammad Ashfaq**

First Fungal Culture Bank of Pakistan, Institute of Agriculture Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan

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 gremination were *P. dendriticum*, *P. herquei*, *P. notatum*, and *P. oxalicum*. Results revealed that he 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. variabile* 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 and 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.

*Corresponding Author E-mail: ibatsamk@yahoo.com

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): $NaNO_3$ (2), K_2HPO_4 (1), KCl (0.5), $MgSO_4.7H_2O$ (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 ± 2ºC for 15 days. Fungal filtrate was collected by filtration through Whatman filter paper No. 4. Filtrate was stored at 4ºC 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℃) 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:

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

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

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

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

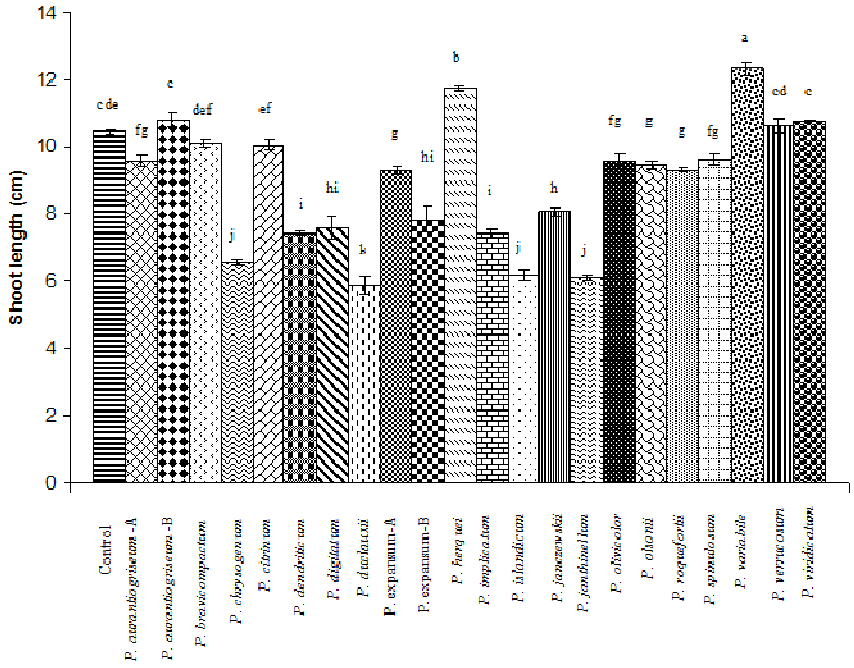

Figure 1. Effect of *Penicillium* spp. culture filtrates on shoot length of wheat seedlings. Bars topped by different letters are significantly different at $P < 0.05$

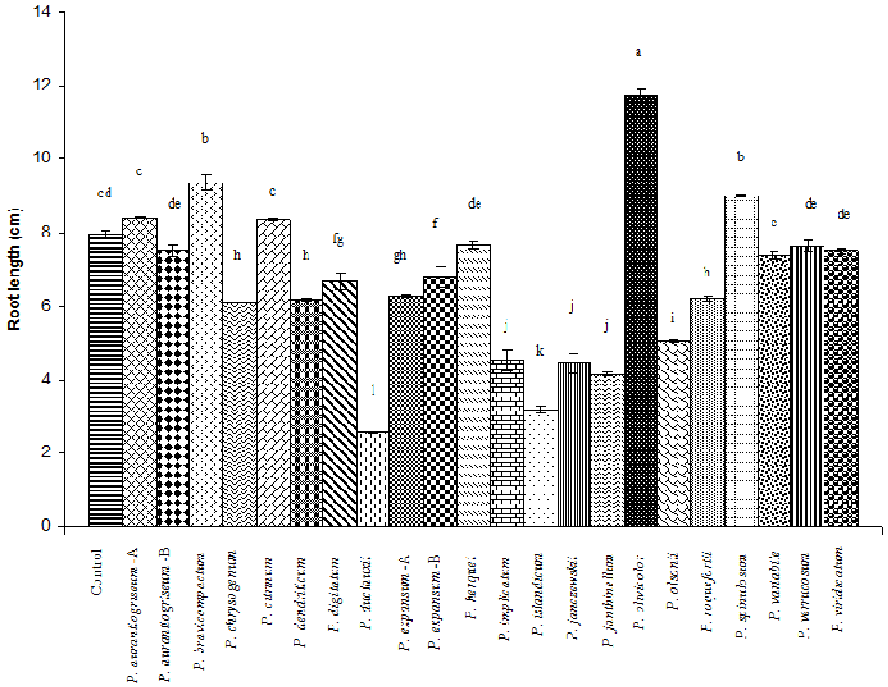

Figure 2. Effect of *Penicillium* spp. culture filtrates on root length of wheat seedlings. Bars topped by different letters are significantly different at $P < 0.05$

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. bravicompactum*, *P. islandicum* and *P. implicatum* had higher values for dry matter as compare to other *Penicillium* spp. (Figure 4).

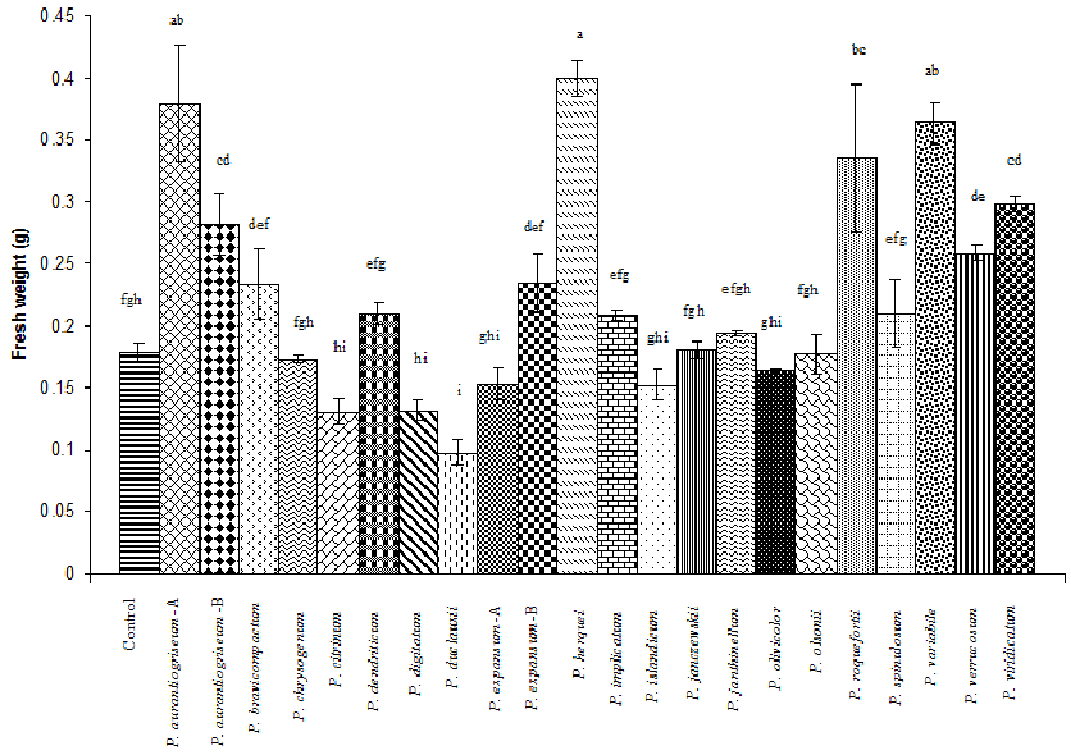

Figure 3. Effect of *Penicillium* spp. culture filtrates on fresh weight of wheat seedlings. Bars topped by different letters are significantly different at $P < 0.05$

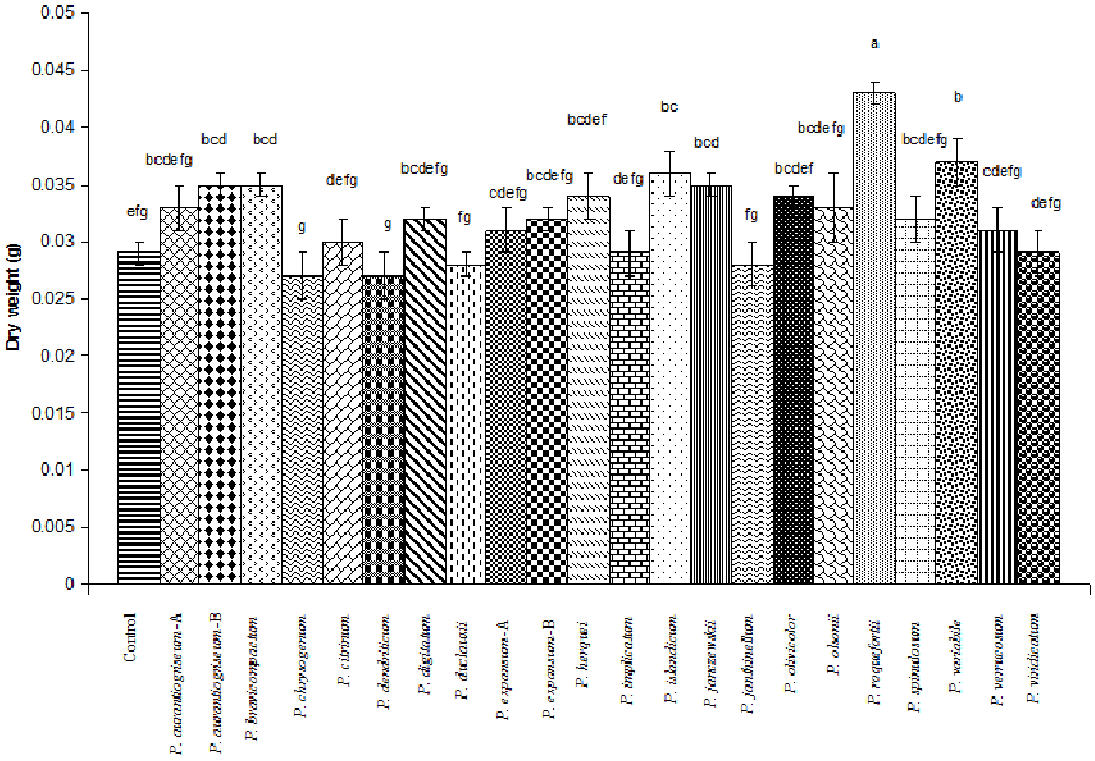

Figure 4. Effect of *Penicillium* spp. culture filtrates on dry weight of wheat seedlings. Bars topped by different letters are significantly different at $P < 0.05$

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.

REFERENCES

Altmore C, Norvell WA, Bjorkman T, Harman GE (1999). Solubilization of phosphates and micronutrients by the plant growth promoting and biocontrol fungus *trichoderma harzianum* Rifai 1295-22 // Applied and Environmental Microbiology.vol. 65, p. 2926-2933

Association of Official Seed Analysts (AOSA) (1983). Seed Vigor Testing Handbook. Contribution No. 32. Association of Official Seed Analysts. Lincoln, NE, USA. p. 88

Choi W Y, Rim S O, Lee J H, Lee J M, Lee I J, Cho K J, Rhee I K, Kwon J B, Kim J G (2005). Isolation of gibberellins-producing fungi from the root of several *Sesamum indicum* plants // Journal of Microbiology and Biotechnology. vol. 15, p. 22-28

Domsch KH, Gams W, Anderson TW (1993). Compendium of soil fungi. – Eching, Germany, vol. 1, p. 672

Dubey SC, Suresh M, Singh B (2007). Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt // Biological Control. vol. 40, p. 118-127

Dutta T, Sahoo R, Sengupta R (2008). Novel cellulases from an extremophilic filamentous fungi *Penicillium citrinum*: production and characterization // Journal of Industrial Microbiology and Biotechnology. vol. 35, iss. 4, p. 275-282

Hamayun M, Khan SA, Kim HY, Chaudhary MF, Hwang YH, Shin DH, Kim IK, Lee BH, Lee IJ (2009). Gibberellin production and plant growth enhancement by newly isolated strain of *Scolecobasidium tshawytschae* // Journal of Microbiology and Biotechnology. vol. 19, iss. 6, p. 560–565

Hasan HAH (2002). Gibberellin and auxin-indole production by plant root-fungi and their biosynthesis under salinity-calcium interaction // Rostlina vyroba. vol. 48, iss. 3, p. 101-106

Hyakumachi M (1994). Plant–growth–promoting fungi from turfgrass hizosphere with potential for disease suppression // Soil Microorganisms. vol. 44, p. 53-68

International Seed Testing Association [ISTA] (1999). International Rules for Seed Testing. Seed Science and Technology. vol. 27, p. 340

International Seed Testing Association [ISTA] (1993). International rules for seed testing. Seed Science and Technology. vol. 21, p. 160-186

Khan SA, Hamayun M, Yoon H, Kim H, Suh S, Hwang S, Kim J, Lee I, Choo Y, Yoon U, Kong W, Lee B, Kim J (2008). Plant growth promotion and *Penicillium citrinum* // BMC Microbiology. vol. 8, p. 231–240.

Krasil'nikov NA (1958). Soil microorganisms and higher plants // Academy of Sciences of the USSR Moscow.

Kucey RMN (1987). Increased phosphorus uptake by wheat and field beans inoculated with a phosphorus-solubilizing *Penicillium bilaji* strain and with vesicular-arbuscular mycorrhizal fungi // Applied Environmental Microbiology. vol. 53, iss. 12, p. 2699–2703

Kucey RMN (1988). Effect of *Penicillium bilaji* on the solubility and uptake of P and micronutrients from soil by wheat // Canadian Journal of Soil Science. vol. 68, p. 261–270

Narisawa R, Quimio TH (2004). Soil mycoflora of black pepper rhizosphere in the Philippines and their *in vitro* antagonism against *Phytophthora capsici* // Indonesian Journal of Agricultural Sciences. vol. 5, iss. 1, p. 1–10

Orchard T (1977). Estimating the parameters of plant seedling emergence // Seed Science and Technology. vol. 5, p. 61-69

Phuwiwat W, Soy tong K (2001). The effect of *Penicillium notatum* on plant growth. Fungal Diversity 8: 143-148

Wakelin SA, Warren RA, Ryder MH (2004a). Effect of soil properties on growth promotion of wheat by *Penicillium radicum* // Australian Journal of Soil Research. vol. 8, p. 897–904

Wakelin SA, Warren RS, Harvey PR, Ryder M (2004b). Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots // Biology and Fertility of Soils. vol. 40, p. 36–43

Wakiyama M, Tanaka H, Yoshihara K, Hayashi S, Ohta K (2008). Purification and properties of family-10 endo-1,4-β-xylanase from *Penicillium citrinum* and structural organization of encoding gene // Journal of Biosciences and Bioengineering. vol. 105, iss. 4, p. 367-374

Whitelaw MA, Harden TJ, Bender GL (1997). Plant growth promotion of wheat inoculated with *Penicillium radicum* sp. nov. // Australian Journal of Soil Research. vol. 35, p. 291–300

Whitelaw MA, Harden TJ, Helyar KR (1999). Phosphate solubilization in solution culture by the soil fungus *Penicillium radicum* // Soil Biology and Biochemistry. vol. 31, iss. 5, p. 655–665