

## Research Article



## *In vitro* and *In vivo* Antifungal Activity of Selected Fungal Species against the Fusarium Wilt of *Solanum lycopersicum*

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**Abstract** | Fusarium wilt of tomato is a devastating fungal disease with several infectious strategies leading to the complete death of plant. None of the strategies in controlling the wilt have shown fruitful results. Dual culture assay was employed in this study to identify the fungal species with their potent antifungal activity against the Fusarium wilt of tomato. Six distinct fungal species including *Penicillium* sp., *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma harzianum*, *Alternaria solani* and *Pythium aphanidermatum* showed growth inhibition (%) against the pathogen. *Penicillium* sp. showed maximum growth inhibition ( $63.85 \pm 3.26$  %) and its extract showed  $9.20 \pm 0.14$  mm zone of inhibition at  $1000 \text{ mg}\cdot\text{L}^{-1}$  against the Fusarium wilt. Based on its higher *in vitro* antifungal activity, *in vivo* screen house experiments were conducted. Plants given the treatment as *Penicillium* + *Fusarium* showed slight yellowing of the leaves after 60<sup>th</sup> days of cultivation. Present results may help in exploiting the *Penicillium* sp. extract for isolation and structural characterization of its antifungal compounds.

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### Introduction

Several pathogenic species of *Fusarium* cause diseases in plants with great diversity. These fungal species can be air borne, soil borne or reside inside the plant and can be recovered from any part of the plant (Summerell et al., 2003). *Fusarium oxysporum* f.sp. *lycopersici* (Fol) is a pathogenic fungus that can cause Fusarium wilt in tomato and lead to economic loss (Agrious, 2005). Control of wilt diseases depends mainly on biological control or synthetic fungicides (Singha et al., 2011). Fungal bio-control agents have a narrow host range and they act slowly (Copping and Menn, 2000) whereas use of synthetic fungicides against phytopathogenic fungi has been questioned due to their environmental concern and several micro-

organisms have developed resistance to these chemical fungicides (Gaigole et al., 2011). Therefore, control of these pathogenic fungi has become important (Bernal et al., 2002) and there is a need to work on the development of natural product based fungicides.

Natural products, generally known as secondary metabolites show no harmful effects on the producing organisms while they have deterrent effects on predators and competitors (Schafer and Wink, 2009). The possible use of the natural products as pharmaceuticals has been extensively investigated, and it is estimated that 50% of the clinically approved drugs in today's global market are derived from natural products (Butler, 2008). Fungal novel bioactive compounds have contributed advances in agriculture and

human health with impacts on societal and economic growth (Goler, 2007). Therefore, present study was designed to isolate distinct fungal species and to test their antifungal efficacy against the *Fusarium* wilt of tomato. Moreover, *in vivo* screening of the bioactive extract was also conducted in pot experiments.

## Materials and Methods

### Isolation of fungal species from soil samples

Fungal species were isolated using soil serial dilution method (Warcup, 1950). The mixed fungal colonies were further purified by sub culturing the inoculum from each colony to new petri dish (Freitas et al., 2011).

### Isolation of endophytes

Healthy plant segments (Stem and leaves) were first washed with a running tap water to remove dust and debris. Small pieces of the segments (0.5 to 1 cm in length) were surface sterilized with 75 % methanol and by dipping them in mercuric chloride (HgCl<sub>2</sub>) solution inside biosafety cabinet. The segments were then rinsed with sterile distilled water twice and were dried on blot paper. Finally, 5 to 6 segments were placed on agar plate and the dishes were incubated at 27 °C for 7-10 days. The plates with mixed fungal colonies were further purified by sub culturing the inoculum from each colony to new petri dish (Arnold et al., 2000).

### Identification of fungal Species

Fungal species were identified on the basis of their morphological characteristics and microscopic examination (Diba et al., 2007). Length and width of the colony, presence and absence of mycelium, color and any pigment production were observed as morphological characteristics whereas spore structures were observed under the light microscope. The fungal species were identified with the help of Dr. Shaukat Husain (Mycologist) at the Department of Plant Pathology, The University of Agriculture, Peshawar, Pakistan and relevant literature (Ilhan et al., 2006).

### Isolation of *Fusarium oxysporum* f.sp. *lycopersici* (Fol)

Infected tomato plants were selected to isolate *Fol* as described (Singha et al., 2011). Fungal growth micro conidia were observed under the microscope (Agrios, 2005). The pure culture was stored at 4 °C. The fungus was re-cultured every second month.

### Dual culture assays

Dual culture technique was used to test the antifun-

gal efficacy of the isolated fungal species against *Fol* (Mishra, 2010). The petri dishes with PDA media were first inoculated with the mycelial plugs (5 mm in diameter) of the isolated fungal species and after 2 days of incubation at 28 °C, the same plates were inoculated with the mycelial plug (5 mm in diameter) of the *Fol* placed 5 cm away from the antagonists. The antagonist and the pathogen were incubated for additional 9 days at 28 °C. The growth of the pathogen in both the test and control experiments was recorded and % inhibition of the radial growth was calculated as:

$$\% \text{ Inhibition} = \frac{G1-G2}{G1} \times 100$$

Where; G1 = growth of the pathogen in control and G2 = growth of the pathogen in dual culture.

### Well diffusion method

Well diffusion method was used for *in vitro* antifungal assay of the crude extracts as described (Sonawane et al., 2015). Crude extracts (20 µL) dissolved in methanol (10 mg·mL<sup>-1</sup>) were poured in wells. Mancozeb and methanol were used as positive and negative control respectively. The plates were incubated at 27 °C for 7 days and zone of inhibition (mm) was measured.

### In vivo screen house experiments of tomato

*In vivo* antifungal activity of the extract was carried out in screen house as described (Lee et al., 2001). The pots were filled with sterilized soil (1 kg) in each pot. After 10 days of cultivation, 5 mL of the treatment suspension was applied using hypodermic syringe to the plants as Fungal Extract (FE), *Fusarium* (Fus), Fungal Extract (FE) + *Fusarium* (Fus), Mancozeb + *Fusarium* (Man+Fus), Fungal Extract (Sprayed) + *Fusarium* (FE<sub>sp</sub> + Fus) and Mancozeb (Sprayed) + *Fusarium* (Man<sub>sp</sub> + Fus).

### Statistical analysis

Means and standard deviations were calculated using Statistix 8.1.

## Results and Discussion

### Isolation of fungal species from soil and plants

Total nine fungal species including *Penicillium* sp., *Aspergillus flavus*, *Aspergillus niger*, *Alternaria solani*, *Trichoderma harzianum*, *Rhizopus stolonifer* and *Paecilomyces lilacinus* from soil samples were isolated whereas *Pythium aphanidermatum* was isolated from

the plant samples. *Fusarium oxysporum* f.sp. *lycopersici* was isolated from the diseased tomato plant showing the symptoms of wilt. Geographical location and sampling methods are important factors in the isolation of fungal species (Gaddeyya et al., 2012). It has been reported that occurrence (%) of *Aspergillus* and *Fusarium* was more than other fungal species at Wazirabad and Mandi Bahudin, Punjab, Pakistan (Raza et al., 2013). The environmental factors of soil such as moisture, nutrients, temperature and pH affect the diversity of fungal population (Yu et al., 2007).

Soil microflora have an important role in various biochemical transformations and mineralization activities of the soil and plants. Fungi, a diversified kingdom plays an important role in soil ecosystem especially in agricultural soils for organic matter decomposition and release of minerals (Ainsworth and Bisby, 1995).

### Dual culture assays

Isolated fungal species were tested for their antagonistic activities against *Fusarium oxysporum* f.sp. *lycopersici*. It was observed that out of the nine fungal species, six of them including *A. solani*, *A. niger*, *Penicillium* sp., *P. aphanidermatum*, *T. harzianum* and *A. flavus* showed antagonistic activity in dual culture assay. It is clear from Table 1 that the maximum % inhibition ( $63.85 \pm 3.26$ ) was shown by *Penicillium* sp. followed by *A. niger* ( $51.54 \pm 1.09$ ) and *A. solani* ( $46.15 \pm 2.18$ ). *A. flavus* showed minimum % inhibition ( $20.00 \pm 2.18$ ) against the pathogen while *P. aphanidermatum* and *T. harzianum* showed % inhibition  $36.16 \pm 1.09$  and  $33.85 \pm 4.35$  respectively.

**Table 1:** Inhibition (%) of six different fungal species against *Fusarium oxysporum* f.sp. *lycopersici* in dual culture assay.

Fungal Species	Pathogen	Inhibition % $\pm$ SD
<i>Aspergillus niger</i>	<i>Fusarium oxysporum</i> f.sp. <i>lycopersici</i>	$51.54 \pm 1.09$
<i>Alternaria solani</i>		$46.15 \pm 2.18$
<i>Penicillium</i> sp.		$63.85 \pm 3.26$
<i>Trichoderma harzianum</i>		$33.85 \pm 4.35$
<i>Pythium aphanidermatum</i>		$36.15 \pm 1.09$
<i>Aspergillus flavus</i>		$20.00 \pm 2.18$

A zone of inhibition was observed during the dual culture assay of *A. niger*, *Penicillium* sp., *T. harzianum*, *P. aphanidermatum* and *A. flavus* against *Fusarium oxysporum* (Fol). This zone of inhibition is often pro-

duced by the antagonists without hyphae contact by releasing the inhibitory metabolites such as the secretion of diffusible non-volatile inhibitory substances known as extracellular exochitinases (Brunner et al., 2003; Kullning et al., 2000). Hajieghrari et al. (2008) carried out dual culture assay of *T. harzianum* against *Fusarium oxysporum* (Fol) and zone of inhibition between them was suggested as the secretion of exochitinases. Cell fragments of the hosts are dissolved by diffusion of these enzymes and which in turn stimulate further enzymes for rapid growth of *Trichoderma* sp. (Zeilinger et al., 1999). Alwathnani et al. (2012) reported in his study that *P. citrinum* inhibited *F. oxysporum* f. sp. *phaseoli* with 53.5 % inhibition. This may be due to the production of inhibitory substances such as viridian, terricin and terric acid etc. (Mondal et al., 2000; Xiao Yan et al., 2006).

### In vitro antifungal activity

Based on highest antifungal activity of *Penicillium* sp. in dual culture assay, *in vitro* antifungal activity of the crude extract of *Penicillium* sp. was carried out at three different concentrations i.e. 1000, 500 and 250 mg·L<sup>-1</sup> (Table 2). The extract showed maximum zone of inhibition ( $9.20 \pm 0.14$  mm) against the wilt pathogen at 1000 mg·L<sup>-1</sup>. Mancozeb, a commercial fungicide showed  $11.70 \pm 0.57$  mm zone of inhibition. Mancozeb is a well-known agricultural non-systemic dithiocarbamate fungicide, which is a mixture of Maneb and Zineb (Gullino et al., 2010). Its higher antifungal activity at 250 mg·L<sup>-1</sup> shows that antifungal compounds in the crude extract of *Penicillium* sp. might be in lower concentration and therefore showed less activity than Mancozeb even at 1000 mg·L<sup>-1</sup>. Saadabi and El Amin (2007) investigated the effects of cultural filtrates of different fungi *in vitro* against *F. oxysporum*.

**Table 2:** Mean zone of inhibition (mm)  $\pm$  SD of *Penicillium* sp.

Fungal Species	Media	Concentration (mg·L <sup>-1</sup> )	Zone of Inhibition (mm) $\pm$ SD
<i>Penicillium</i> sp.	GPYB	1000	$9.20 \pm 0.14$
		500	$4.45 \pm 0.21$
		250	$2.40 \pm 0.28$
Positive Control	Mancozeb	250	$11.70 \pm 0.57$

\*Whereas GPYB stands for Glucose Peptone Yeast Extract Broth.

### In vivo screen house pot experiment

Due to the higher *in vitro* antifungal activity of the crude extract of *Penicillium* sp. against the pathogen, *in vivo* screen house pot experiments were carried



out. The experiment was conducted twice.

**First growing season:** *Penicillium* sp. was selected for pot experiments based on its higher *in vitro* antifungal activity against *Fol*. The efficacy of its crude extract was tested in pot experiment. The Roma variety of tomato was selected for pot experiment. The plant started showing symptoms of wilting after 20 days of cultivation. The disease severity was calculated as (Grattidge and O'Brien, 1982): 0 = no symptom, 1 = slight yellowing or wilting, 2 = moderate yellowing or wilting, 3 = complete wilting and 4 for 100% leaves yellowed and wilted (Table 3). The plants were given scale 0 (no symptom) to all the treatments after 20 days. After 30 days of cultivation scale 0 was given to control and *Penicillium* (Pen) crude whereas *Penicillium* + *Fusarium* (Pen+Fus), Mancozeb + *Fusarium* (Man+Fus) while *Fusarium* (Fus), *Penicillium* crude (Sprayed) + *Fusarium*, Mancozeb (Sprayed) + *Fusarium*, *Penicillium* (Filtrate) + *Fusarium* showed scale 1 (slight yellowing). After 60 days of cultivation, all of the treatments showed 100 % wilting of the leaves (Figure 1). Pot experiments showed mixed results in replicates and no clear idea was drawn from the trail. The same experiment was repeated in 2nd growing season of tomato for clear outcomes.

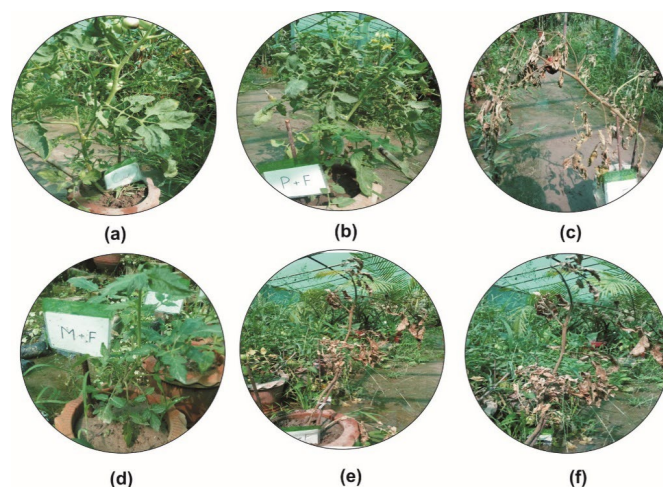
**Table 3:** Average disease severity scale given to different treatments in pot experiments during the first growing season.

Treatments	Given Scale				
	20 <sup>th</sup> Day	30 <sup>th</sup> Day	40 <sup>th</sup> Day	50 <sup>th</sup> Day	60 <sup>th</sup> Day
Control	0	0	0	0	0
<i>Penicillium</i> (Crude)	0	0	0	0	0
<i>Penicillium</i> + <i>Fusarium</i>	0	1	2	3	4
<i>Fusarium</i>	0	1	2	3	4
Mancozeb + <i>Fusarium</i>	0	1	2	3	4
<i>Penicillium</i> (Sprayed) + <i>Fusarium</i>	0	1	2	3	4
Mancozeb (Sprayed) + <i>Fusarium</i>	0	1	2	3	4
<i>Penicillium</i> (Filtrate) + <i>Fusarium</i>	0	1	2	3	4

0: no symptom; 1: slight yellowing or wilting; 2: moderate yellowing or wilting; 3: complete wilting; 4: 100% leaves yellowed and wilted.

**Second growing season:** The experiment was repeated in second season due to its inconsistent results in first season to get reliable and reproducible results. The pot experiments of tomato plants were carried out during the month of March-April. The plant started to show symptoms of *Fusarium* after 20

days of cultivation. The disease severity was calculated according to the scale described by Grattidge and O'Brien (1982): 0 = no symptom, 1 = slight yellowing or wilting, 2 = moderate yellowing or wilting, 3 = complete wilting and 4 for 100% leaves yellowed and wilted.



**Figure 1:** The non-wilted and the wilted plants (a) Control; (b) Pen+Fus; (c) Fus; (d) Man+Fus; (e) Sfil Pen; (f) 60 days of plantation.

It was observed that control plants and the plants given the treatment as only *Penicillium* (Extract) did not show symptoms even after 60<sup>th</sup> day of cultivation (Table 4). On the 20<sup>th</sup> day of cultivation all the other treated plants did not show wilting symptoms. On the 30<sup>th</sup>, 40<sup>th</sup>, 50<sup>th</sup> and 60<sup>th</sup> day of cultivation, the plant infested with *Fusarium* showed slight yellowing or wilting, moderate yellowing, complete yellowing and 100% leaves yellowed and wilted respectively. The plants given the treatments as *Penicillium* + *Fusarium* (Pen+Fus) did not show wilting symptoms till 40<sup>th</sup> day however it showed slight yellowing/wilting on the 50<sup>th</sup> and 60<sup>th</sup> day. The plants given the treatments as *Penicillium* (Sprayed) + *Fusarium* and Mancozeb (Sprayed) + *Fusarium* showed complete wilting of the plants after 60<sup>th</sup> of cultivation while Mancozeb + *Fusarium* showed moderate yellowing/wilting.

The *in vivo* pathogenicity test showed yellowing of the older leaves as an early symptoms of the tomato wilt disease. Most of the foliage are affected by turning yellow and ultimately leads to wilting. The wilting continues until the plant dies. It has been commonly reported that tomato plants are attacked by *Fusarium* wilt within a growth period of 60 days during flower initiation. The pathogen proliferates in vascular tissues through the roots of the plant and breakdown the water and food supply of the infected plants (Agrios, 2005).

**Table 4:** Average disease severity scale given to different treatments in pot experiments during the second growing season.

Treatments	Given Scale				
	20 <sup>th</sup> Day	30 <sup>th</sup> Day	40 <sup>th</sup> Day	50 <sup>th</sup> Day	60 <sup>th</sup> Day
Control	0	0	0	0	0
<i>Penicillium</i> (Extract)	0	0	0	0	0
<i>Penicillium</i> + <i>Fusarium</i>	0	0	0	1	1
<i>Fusarium</i>	0	1	2	3	4
Mancozeb + <i>Fusarium</i>	0	0	0	1	2
<i>Penicillium</i> (Sprayed) + <i>Fusarium</i>	0	0	1	2	3
Mancozeb (Sprayed) + <i>Fusarium</i>	0	0	1	2	3

0: no symptom; 1: slight yellowing or wilting; 2: moderate yellowing or wilting; 3: complete wilting; 4: 100% leaves yellowed and wilted.

## Conclusions and Recommendations

Nine fungal species were isolated from soil and plant samples. Dual culture assays revealed six antifungal species against *Fusarium oxysporum* f.sp. *lycopersici*. *Penicillium* sp. showed maximum % inhibition against the wilt pathogen. The crude extract of the *Penicillium* sp. showed  $9.20 \pm 0.14$  mm zone of inhibition at 1000 mg·L<sup>-1</sup>. *In vivo* pot experiments showed mixed results during the first growing season. During the second season even after 60 days of cultivation the tomato plant showed slight yellowing or wilting with the application of *Penicillium* extract. These *in vitro* and *in vivo* experiments may help in exploiting the crude extract for the identification and isolation of antifungal compounds in future.

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## Author's Contribution

The present research is a part of doctoral study of Zulqarnain who performed experiments and wrote the article under the supervision of Zafar Iqbal.

## References

- Agrios, G. 2005. Plant pathology. 5<sup>th</sup> ed. Academic Press, New York, USA.
- Ainsworth, G. and G. Bisby. 1995. Dictionary of the Fungi. 8<sup>th</sup> ed. Common Wealth Mycological Institute Kew, Surrey.
- Alwathnani, H.A., K. Perveen, R. Tahmaz and S. Alhaqbani. 2012. Evaluation of biological control potential of locally isolated antagonist fungi against *Fusarium oxysporum* under in vitro and pot conditions. Afr. J. Microbiol. Res. 6: 312-319. <https://doi.org/10.5897/AJMR11.1367>
- Arnold, A.E., Z. Maynard, G.S. Gilbert, P.D. Coley and T.A. Kursar. 2000. Are tropical fungal endophytes hyperdiverse? Ecol. Lett. 3: 267-274. <https://doi.org/10.1046/j.1461-0248.2000.00159.x>
- Bernal, G., A. Illanes and L. Ciampi. 2002. Isolation and partial purification of a metabolite from a mutant strain of *Bacillus* sp. with antibiotic activity against plant pathogenic agents. Electro. J. Biotechnol. 5: 7-8. <https://doi.org/10.2225/vol5-issue1-fulltext-4>
- Brunner, K., C.K. Peterbauer, R.L. Mach, M. Lorito, S. Zeilinger and C.P. Kubicek. 2003. The Nag1 N-acetylglucosaminidase of *Trichoderma atroviride* is essential for chitinase induction by chitin and of major relevance to biocontrol. Curr. Genetics 43: 289-295. <https://doi.org/10.1007/s00294-003-0399-y>
- Butler, M.S. 2008. Natural products to drugs: natural product-derived compounds in clinical trials. Nat. Prod. Rep. 25: 475-516. <https://doi.org/10.1039/b514294f>
- Copping, L.G. and J.J. Menn. 2000. Biopesticides: a review of their action, applications and efficacy. Pest Manage. Sci. 56: 651-676. [https://doi.org/10.1002/1526-4998\(200008\)56:8<651::AID-PS201>3.0.CO;2-U](https://doi.org/10.1002/1526-4998(200008)56:8<651::AID-PS201>3.0.CO;2-U)
- Diba, K., P. Kordbacheh, S. Mirhendi, S. Rezaie and M. Mahmoudi. 2007. Identification of *Aspergillus* species using morphological characteristics. Pak. J. Med. Sci. 23: 867.
- Freitas, R.S., K.C. Dantas, C.N. Pereira, J.E. Levi and J.E.C. Martins. 2011. Preservation methods of fungi in 35 years old stock culture storages: A comparative study. Afr. J. Microbiol. Res. 5: 555-561.
- Grattidge, R. and R. O'Brien. 1982. Occurrence

- of a third race of *Fusarium wilt* of tomatoes in Queensland. Plant Dis. 66: 165-166.
- Gaddeyya, G., P.S. Niharika, P. Bharathi and P.R. Kumar. 2012. Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. Adv. Appl. Sci. Res. 3: 2020-2026.
- Gaigole, A., G. Wagh and A. Khadse. 2011. Anti-fungal activity of Trichoderma species against soil borne pathogen. Asiat. J. Biotech. Resour. 2: 461-465.
- Gloer, J. 2007. 15 Applications of Fungal Ecology in the Search for New Bioactive Natural Products. Environ. Microb. Relat. 4: 257. [https://doi.org/10.1007/978-3-540-71840-6\\_15](https://doi.org/10.1007/978-3-540-71840-6_15)
- Gullino, M.L., F. Tinivella, A. Garibaldi, G.M. Kemmitt, L. Bacci and B. Sheppard. 2010. Mancozeb: past, present, and future. Plant Dis. 94: 1076-1087. <https://doi.org/10.1094/PDIS-94-9-1076>
- Hajieghrari, B., M. Torabi-Giglou, M.R. Mohammadi and M. Davari. 2008. Biological potential of some Iranian Trichoderma isolates in the control of soil borne plant pathogenic fungi. Afr. J. Biotechnol. 7: 967-972.
- İlhan, S., R. Demirel, A. Asan, C. Bayçu and E. KINACI. 2006. Colonial and Morphological Characteristics of Some Microfungal Species Isolated from Agricultural Soils in Eskişehir Province (Turkey). Turk. J. Bot. 30: 95-104.
- Kullnig, C., R.L. Mach, M. Lorito and C.P. Kubicek. 2000. Enzyme diffusion from Trichoderma atroviride (= T. harzianum P1) to Rhizoctonia solani is a prerequisite for triggering of Trichoderma ech42 gene expression before mycoparasitic contact. Appl. Environ. Microbiol. 66: 2232-2234. <https://doi.org/10.1128/AEM.66.5.2232-2234.2000>
- Lee, S.E., B.S. Park, M.K. Kim, W.S. Choi, H.T. Kim, K.Y. Cho, S.G. Lee and H.S. Lee. 2001. Fungicidal activity of piperonaline, a piperidine alkaloid derived from long pepper, Piper longum L., against phytopathogenic fungi. Crop Prot. 20: 523-528. [https://doi.org/10.1016/S0261-2194\(00\)00172-1](https://doi.org/10.1016/S0261-2194(00)00172-1)
- Mishra, V. 2010. In vitro antagonism of Trichoderma species against Pythium aphanidermatum. J. Phytol. 2: 28-35.
- Mondal, G., P. Dureja and B. Sen. 2000. Fungal metabolites from Aspergillus niger AN27 related to plant growth promotion. Ind. J. Exp. Biol. 38: 84-87.
- Raza, M., M.U. Ghazanfar, M. Hussain, Z. Iqbal and Y. Iftikhar. 2013. Pathogenic and non-pathogenic fungi isolated from soil and roots of tobacco. Pak. J. Phytopathol. 25: 95-100.
- Saadabi, A.M. and Z.N. El-Amin. 2007. In vitro Studies on the Toxicity of Culture Filtrates of Different Fungi on the Growth of Fusarium oxysporum f. sp. vasinfectum. J. Biol. Sci. 7: 429-432. <https://doi.org/10.3923/jbs.2007.429.432>
- Schafer, H. and M. Wink. 2009. Medicinally important secondary metabolites in recombinant microorganisms or plants: progress in alkaloid biosynthesis. Biotechnol. J. 4: 1684-1703. <https://doi.org/10.1002/biot.200900229>
- Singha, I.M., Y. Kakoty, B.G. Unni, M.C. Kalita, J. Das, A. Naglot, S.B. Wann and L. Singh. 2011. Control of Fusarium wilt of tomato caused by Fusarium oxysporum f. sp. lycopersici using leaf extract of Piper betle L.: a preliminary study. World J. Microbiol. Biotechnol. 27: 2583. <https://doi.org/10.1007/s11274-011-0730-6>
- Sonawane, A., M. Mahajan and S. Renake. 2015. Antifungal activity of a fungal isolates against Pomegranate wilt pathogen Fusarium. Int. J. Curr. Microbiol. Appl. Sci. 2: 48-57.
- Summerell, B.A., B. Salleh and J.F. Leslie. 2003. A utilitarian approach to Fusarium identification. Plant Dis 87: 117-128. <https://doi.org/10.1094/PDIS.2003.87.2.117>
- Warcup, J. 1950. The soil-plate method for isolation of fungi from soil. Nat. 166: 117-118. <https://doi.org/10.1038/166117b0>
- Xiao-Yan, S., S. Qing-Tao, X. Shu-Tao, C. Xiu-Lan, S. Cai-Yun and Z. Yu-Zhong. 2006. Broad-spectrum antimicrobial activity and high stability of Trichokonins from Trichoderma koningii SMF2 against plant pathogens. FEMS Microbiol. Lett. 260: 119-125. <https://doi.org/10.1111/j.1574-6968.2006.00316.x>
- Yu, C., D. Lü, S. Qin, G. Du and G. Liu. 2007. Microbial flora in Cerasus sachalinensis rhizosphere. J. Appl. Ecol. 18: 2277-2281.
- Zeilinger, S., C. Galhaup, K. Payer, S.L. Woo, R.L. Mach, C. Fekete, M. Lorito and C.P. Kubicek. 1999. Chitinase Gene Expression during Mycoparasitic Interaction of Trichoderma harzianum with Its Host. Fungal Genet. Biol. 26: 131-140. <https://doi.org/10.1006/fgbi.1998.1111>