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Influence of different species of *Penicillium* and their culture filtrates on seed germination and seedling growth of sorghum

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Abstract

The present investigations influence of *Penicillium* species and their culture filtrates on the seed germination and seedling growth of sorghum was studied. Culture filtrate of *P. nordicum*, *P. verrucosum*, *P. chrysogenum, P. commune*, *P. citrinum*, *P.camemberti* and *P. digitatum* were comparatively more toxic and significantly inhibited the seed germination and seedling growth varied with the species. However, pathogenicity of different inoculum of *Penicillium* species also exhibited varying seedling growth inhibition with *P. citrinum*, *P. chrysogenum, P. commune, P. italicum, P. verrucosum* and *P. expansum* assayed by water agar method. A significant phytotoxicity of these species inhibited coleoptile 32-70%, radicle 10-87% and leaf growth 20-86% with correlation coefficient 0.65, 0.67 and 0.79%, and were observed respectively. In-vitro mycotoxin production was assayed by culture filtrates of major mycotoxigenic strains revealed production of ochratoxin A (OTA), cyclopiazonic acid (CPA), rubratoxin B (RTB), griseofulvin (GRI), citrinin (CIT), patulin (PAT), penitrem A (PENA) and mycophenolic acid (MPA) screened by TLC/HPLC. Toxicity of species of *Penicillium* on seed germination, coleoptile, radicle, and leaf elongation inhibition may be attributed to the toxin-chemotypes produced by the species of *Penicillium*. However, even non-toxigenic strains of *Penicillium* also caused mild inhibition which may be attributed to the presence of other toxin-chemotypes.

Keywords: *Penicillium*, sorghum, seed germination, coleoptile length, radicle length, leaf length, mycotoxin-chemotypes, TLC, HPLC.

Introduction

Sorghum (*Sorghum bicolar* (L.) Moench) commonly known as "Jowar" is extensively used for feed and fodder production, and staple food for humans in many states of India particularly in Telangana. Crop productivity is adversely affected by environmental stress of both biotic and abiotic factors. Among the biotic factors contributing to the yield loss of the crop may be attributed to diseases. Different species of *Penicillium* such as *P. expansum, P. digitatum, P. allii*, *P. ulaiense* are reported to be associated with many economically important crop diseases (Balgrie 2003; Valdez et al. 2006; Youssef et al. 2010).

A wide variety of microorganisms are reported to be associated with the seeds of sorghum (Erpelding and Prom 2006, Girish et al. 2004). Proliferation of fungi is favored by several factors such as field, storage conditions, relative humidity, temperatures and insect damage and contributes to the production of mycotoxins. Seed-borne fungi are reported to cause quantitative and qualitative changes in chemical composition of the seeds and may contribute for health problem of man (Makun et al. 2012). The major effect on seeds include reduced germination, discoloration, visible mould growth, musty or sour odour, dry matter loss, caking and mycotoxins elaboration. Contaminated agricultural products particularly cereals are the main source of mycotoxins in the animal and human food chain (Bryden 2007).

The genera of *Penicillium, Aspergillus* and *Fusarium* are commonly associated with crop plants are known to produce diverse toxic substances causing different diseases in plants, animals including humans (Singh et al. 2007). A large number of instances can be cited in regard to the seed transmission of plant diseases both within the country and outside the country (Hartman 1999). A large number of fungi associated with the spike of sorghum on living plant and cause even failure of seed germination (Bhat et al. 2000). The inhibitory effect of the seed-borne fungi on seed germination, radicle and coleoptile growth has been attributed to the production of certain enzymes and toxins produced by fungi in different crops (Ramalingam et al. 2003; Rajput et al. 2010).

In spite of several studies on the effect of *Penicillium* species, much more studies are required (Bennett and Klich 2003; Jonar et al. 2011) the problems of mycotoxins was realized in the last few years and probably they are associated with

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several mysterious diseases (Richard 2007). Mycotoxins are secondary metabolites of various moulds comprise diverse group of compounds different in their chemical properties and toxicity. The substrates for these mycotoxigenic fungi include plants grown and stored for human or animal consumption as well as processed food. Unfortunately no originated efforts been made to address the problems relevant to the association of *Penicillium* species with sorghum seeds. The present study aimed to understand the nature of association and role of these fungi in sorghum seed and possible health problem. Effect of culture filtrates of some common species of *Penicillium* associated with sorghum seed on seed germination and seedling growth was studied. Pathogenicity of these species to sorghum was also assessed by inoculating to its germinating seed.

Materials and methods

Chemicals

All the chemicals used in the present investigations were purchased from Merck manufactures (Mumbai, India) and acetic acid, acetonitrile and water were HPLC grade Sigma Aldrich (Mumbai, India).

Fungal cultures

P. aethiopicum (GSMBKU-P1), *P. alli* (GSMBKU-P2), *P.aurantiogriseum* (GSMBKU-P3), *P. brevicompactum* (GSMBKU-P4), *P. camemberti* (GSMBKU-P5), *P. caseifulvum* (GSMBKU-P6), *P. chrysogenum* (GSMBKU-P7), *P. citrinum* (GSMBKU-P8), *P. commune* (GSMBKU-P9), *P. crustosum* (GSMBKU-P10), *P. digitaum* (GSMBKU-P11), *P. dipodomyis* (GSMBKU-P12), *P. discolor* (GSMBKU-P13), *P. expansum* (GSMBKU-P14), *P. flavigenum* (GSMBKU-P15), *P. griseofulvum* (GSMBKU-P16), *P. italicum* (GSMBKU-P17), *P. nalgiovense* (GSMBKU-P18), *P. nordicum* (GSMBKU-P19), *P. olsonii* (GSMBKU-P20), *P. roqueforti* (GSMBKU-P21), *P. rubrum* (GSMBKU-P22), *P. tricolor* (GSMBKU-P23) and *P. verrucosum* (GSMBKU-P24) were isolated from poultry feed samples of India (Koteswara Rao et al. 2011) employed for the present study were maintained on malt extract agar (MEA) slants. These *Penicillium* species were deposited in Department of Microbiology herbarium, Kakatiya University.

Influence of culture filtrates on seed germination and seedling growth

The toxic effect of *Penicillium* species was determined on sorghum seeds. The surface sterilized seeds (0.1% mercuric chloride and were rinsed three times in sterile distilled water) by soaking in the culture filtrates of different species of *Penicillium*. Different *Penicillium* species (listed above) were grown in 250ml of Erlenmayer conical flask containg 100ml CYA broth for 12 days on rotary shaker (LM-450D) at 27±2°C. At the end of incubation period, culture filtrates were filtered through Whatman filter paper no. 1 and centrifuged at 12,000g to get cell-free filtrates. Hundred healthy surface sterilized seeds were suspended in 50ml of culture filtrates and incubated at 27±2°C for 24 hours and transferred to sterile-petri plates containing three layered wet blotter paper and incubated for 5 days under illumination. Seeds soaked in un-inoculated broth were served as control. At the end of incubation period, seed germination, coleoptile and radicle length were measured over control and their inhibition percentage was calculated with the formulae. Each experiment was run in triplicate (*n=3*) and the results are statistically anaysed and expressed mean and standard deviation (Std.Dev).

Percentage of seed germination inhibition=

$$100 - \frac{\text{Germination in treated seed}}{\text{Germination in control seed}} \times 100$$

Percentage of radicle elongation inhibition=

$$100 - \frac{\text{Radicle elongation inhibition in treated}}{\text{Radicle elongation inhibition in control}} \times 100$$

Percentage of coleoptile elongation inhibition=

$$100 - \frac{\text{Coleoptile elongation inhibition in treated}}{\text{Coleoptile elongation inhibition in control}} \times 100$$

Percentage of leaf elongation inhibition=

$$100 - \frac{\text{Leaf elongation inhibition in treated}}{\text{Leaf elongation inhibition in control}} \times 100$$

Influence of species of Penicillium on growth of sorghum seedlings

Water-agar method as described by Girisham et al. (1985) was employed for testing the pathogenic potential of different *Penicillium* species. Surface sterilized sorghum seeds were placed on two percent (2%) sterilized water-agar (WA) in culture tubes along with seven days old culture of *Penicillium* species aseptically inoculated and incubated at 27±2°C for two weeks under light. Surface disinfected seeds without fungal inoculum were served as control. The coleoptile, radicle and leaf length were measured and their percentage of inhibition was calculated described earlier. Each experiment was run in triplicate (*n=3*) and the results are statistically anaysed and expressed mean and standard deviation (Std.Dev).

Profiling of toxin chemotypes of species of Penicillium
TLC analysis of toxin chemotypes

Single spore cultures of different species of *Penicillium* were grown in CYA broth for 12 days at 27±2^{0}C on rotary shaker (Yihder LM-450 D) at 120 rpm. At the end of incubation period, culture filtrates were filtered through Whatman No. 1 filter paper and centrifuged at 12,000g to get cell-free filtrates. The filtered samples (25ml) were acidified with 0.1M *o*-Phosphoric acid and extracted twice with chloroform (1:1, v/v). The chlorogenic fraction was concentrated by rotary evaporator and eluted in 500µl of chloroform. The TLC plates Merck (Mumbai, India) were activated by immersing in oxalic acid solution (10% oxalic acid in methanol) for 5-10min and heated at 110°C for 2min and allowed to cool, later developed in a mobile phase of toluene: ethyl acetate: formic acid (6:3:1). After separation of the compounds on chromatographic plates,

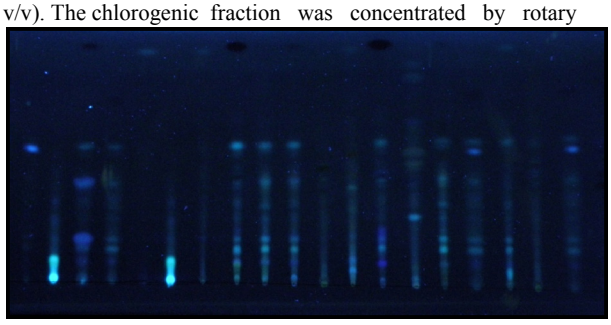

Figure 1. TLC plate showing bluish fluorescence of OTA under UV light

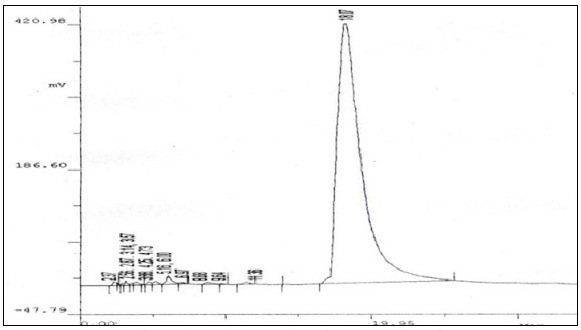

Figure 2. HPLC detection of OTA showing retention time at 18.07 min

fluorescence observations were made under long wavelength ultraviolet light (365nm) as well as compared with the *Rf* values of standard OTA (Fig 1). Different mycotoxins produced by *Penicillium* species were further confirmed by chemical tests our previous report (Koteswara Rao et al. 2011).

High performance liquid chromatography (HPLC)
Extraction and quantification of OTA

Twenty five ml (25ml) of culture filtrates of ochratoxin producing species of *Penicillium* were acidified with 0.1 M o-phosphoric acid and equal volume of chloroform was added and shaken thoroughly and extracted twice. The chlorogenic fraction of OTA was concentrated on rotary evaporator.

The resultant extract eluted in 500 µl of acetonitrile and OTA was estimated by RP-HPLC (Koteswara Rao et al. 2013). Quantification of OTA by using liquid chromatography (*JASCO-975*-HPLC, Japan), C18 isocratic reverse phase, Luna column (250X4.6mm internal diameter, 5µM particle size), mobile phase was acetonitrile: water: acetic acid (99:99:2, v/v/v) and flow rate 1ml/min. by injecting 20µl of extract under ultraviolet (UV) detector at 333 nm was showed at retention time (rt) at18.07 min (Fig 2).

Statistical analysis

All treated seeds were performed in triplicate and the obtained data was analysed statistically to test the significant difference (P<0.005) between different treatments by applying't' test and correlation analysis using GraphPad InStat Version 50.3 (GraphPad Software, Inc.,) was performed to compare seed germination, coleoptile, radicle and leaf length inhibition by specific *Penicillium* species.

Results

Effect of culture filtrates on seed germination

The culture filtrate of *P.nordicum* was highly toxic and caused significant percentage of seed germination inhibition followed by *P. verrucosum*, *P. chrysogenum*, *P. camemberti*, *P. citrinum*, *P. commune*, *P. roqueforti*, *P. griseofulvum*, *P. expansum* and *P. brevicompactum* were also toxic and inhibited seed germination in a decreasing order. Least percentage of seed germination inhibition was recorded with culture filtrates of *P. alli, P. discolor, P. caseifulvum, P. dipodomyis, P. digitatum* and *P. olsonii.* The toxicity of *P. crustosum, P. nalgiovense*, *P. italicum* and *P. aethiopicum* culture filtrates were intermediate in their toxicity (Table 1). Seed germination inhibition of sorghum seeds were statistically analysed and their mean, minimum, maximum and standard deviations (Std.Dev) were tabulated (Table 2).

The mean inhibition of germination 37.9% and ranged between 17% and 84% recoded with culture filtrates of species of *Penicillium* under study. The correlation 0.32, (P=0.06) was observed between the seed germination and radicle growth inhibition. The highest radicle growth inhibition was recorded with culture filtrates of *P. commune* followed by *P. camemberti, P. digitatum*, *P. nordicum* and *P. caseifulvum* in a descending order. *P.alli, P.discolor, P.aethiopicum, P.crustosum* and *P.olsonii* exhibited least percentage of radicle elongation inhibition. Rest of *Penicillium* species was intermediate in the degree of inhibition towards the radicle growth. The mean inhibition of radicle growth 69% and ranged between 21% and 78% was recorded with the culture filtrates of species of *Penicillium*. The coefficient variation 16.41% with correlation 0.42 (P<0.05) was observed between the coleoptile and radicle growth inhibition.

The coleoptile elongation inhibition recorded with the culture filtrates of *P. nordicum. P. nalgiovense*, *P. digitatum*, *P. commune*, *P. dipodomyis* and *P. citrinum* were the next toxic, while, *P.discolor, P.alli, P.tricolor, P.griseofulvum* and *P.caseifulvum* were responsible for least inhibition of coleoptile elongation. However, *P. aurantiogriseum*, *P. verrucosum*, *P. flavigenum*, *P. italicum* and *P. expansum* were intermediate in their toxicity as they were responsible for moderate coleoptile growth inhibition. All the species tested against their efficacy towards coleoptile growth were statistically analysed and significant inhibition was observed. The mean inhibition of coleoptile growth 63.1% and ranged

Table 1. Effect of culture filtrates on seed germination and seedling growth elongation inhibition of sorghum

Penicillium sp.	Seed germination inhibition (%)	Radicle elongation inhibition (%)	Coleoptile elongation inhibition (%)
P.aethiopicum	28.12±0.59	62.31±0.37	52.79±0.30
P.alli	17.64±0.49	21.80±0.30	43.16±0.70
P.aurantiogriseum	25.20±0.59	76.26±1.68	74.07±0.52
P.brevicompactum	37.22±0.17	72.38±1.99	53.68±0.37
P.camemberti	65.09±0.77	78.66±0.83	62.44±0.70
P.caseifulvum	20.01±0.14	76.41±0.43	48.52±0.50
P.chrysogenum	74.25±0.66	75.37±0.30	59.46±0.76
P.citrinum	61.20±1.17	71.43±1.16	76.48±0.43
P.commune	42.39±0.65	78.77±1.26	81.56±1.22
P.crustosum	34.51±0.54	63.32±0.18	59.78±1.40
P. digitatum	22.88±0.25	76.90±0.52	83.43±0.92
P.dipodomyis	19.95±0.59	69.86±0.54	79.18±0.13
P. discolor	17.69±0.55	60.18±0.55	37.14±0.60
P.expansum	37.47±0.56	71.96±0.77	63.74±2.22
P.flavigenum	25.21±0.56	74.64±0.48	69.05±0.44
P.griseofulvum	41.96±0.64	69.58±0.50	44.06±0.36
P. italicum	28.77±0.29	67.98±0.30	68.52±0.50
P.nalgiovense	28.85±0.42	65.59±0.66	83.61±0.56
P.nordicum	84.97±0.65	76.74±0.63	85.59±0.76
P.olsonii	23.16±0.42	65.37±0.28	60.94±0.62
P.roqueforti	42.05±0.81	71.54±0.78	58.44±0.19
P.rubrum	25.77±0.16	70.44±0.74	55.41±0.29
P.tricolor	25.56±0.20	71.85±0.73	43.84±0.34
P.verrucosum	80.85±0.35	66.69±0.58	71.70±0.81

Results are mean and standard deviation (Std.Dev) of three replicate experiments and significant differences at (P<0.005).

between 37% and 85% recoded with the culture filtrates treatments of all the species *Penicillium* assayed. The coefficient variation 22.62% with correlation 0.32 (P<0.018) was observed between the seed germination and coleoptile growth inhibition.

Effect of Penicillium species (inoculum) on radicle growth

The present attempts to screen the pathogenicity different species of *Penicillium* on radicle, coleoptile and leaf elongation inhibition were precised in table 3. The highest radicle growth inhibition was recorded with the inoculum of *P. citrinum* followed by *P. chrysogenum*, *P. commune P. italicum P. expansum, P. griseofulvum*, *P. rubrum* and *P. nordicum* in a descending order. The degree of inhibition by *P. camemberti, P. brevicompactum, P. alli, P. aurantiogriseum* and *P. tricolor* was least percentage of inhibition. On the other hand, *P.caseifulvum*, *P. discolor*, *P. verrucosum, P.crustosum* and *P.roqueforti* were intermediate in their pathogenicity.

Seven day old inoculum of individual species tested against their efficacy towards radicle growth were statistically analysed are precised in table 2. The mean inhibition of radicle growth 36.63% and ranged from 10.8% to 87.9% recoded with the pure culture of *Penicillium* tested with a significant correlation 0.67 (P<0.0002) was observed between the radicle and coleoptile growth inhibition.

The highest coleoptile elongation inhibition was recorded in seeds inoculated with *P. commune* followed by *P. citrinum*, *P. italicum*, *P. crustosum*, *P. caseifulvum*, *P. verrucosum*, *P. nalgiovense*, *P. dipodomyis*, *P. nordicum* and *P. griseofulvum* in a descending order. On the other hand, *P.alli, P.camemberti, P. brevicompactum, P.digitatum* and *P. olsonii* caused limited coleoptile growth inhibition. Rest of the *Penicillium* species were intermediate in their toxicity on coleoptile growth. The mean inhibition of coleoptile growth 50.5% and ranged between 27.6% and 70.9% was recorded with a significant and positive correlation 0.65 (P<0.0002) was observed between the coleoptile and leaf growth inhibition.

Maximum leaf elongation inhibition was recorded in seeds inoculated with *P. citrinum*, *P. commune, P. expansum*, *P. verrucosum, P. nordicum*, *P. chrysogenum* and *P. rubrum* in a descending order. On the other hand, *P. aethiopicum*, *P. aurantiogriseum*, *P. camemberti* and *P. flavigenum* failed to inhibit or exhibit toxicity to the leaf growth. *P. italicum*, *P. crustosum*, *P.caseifulvum*, *P.roqueforti*, *P.dipodomyis* and *P. griseofulvum* were intermediate in their degree of toxicity. The mean inhibition of coleoptile growth

43.5% and ranged between 20.5% and 86.1% was recorded. The coefficient variation 42% with a significant correlation 0.65 (P<0.0000) was observed between the radicle and leaf growth inhibition. A positive correlation was observed among the radicle, coleoptile and leaf elongation inhibition and degree of toxicity of species of *Penicillium*.

Table 2. Statistical analysis of seed germination and seedling growth of Sorghum

	Water agar method						Culture filtrate method				
	Mean	Coefficient	Std.error	t-value	P- value		Mean	Coefficient	Std.error	t -value	P-value
Root	36.63	-19.55	13.40	1.4586	0.0794	SG**	37.94	-1.80	25.35	0.07	0.47
Shoot	50.4	1.11	0.26	4.2854	0.0002	Root	69.00	0.57	0.362	1.58	0.06
	SEE*	8.40					SEE*	19.69			
	R^{**}	0.67					R^{**}	0.32			
	R^{2***}	0.43					R^{2***}	0.10			
Shoot	50.41	3.29	4.50	7.3946	0.0000	Root	69.00	8.34	18.53	0.45	0.32
Leaf	43.59	0.39	0.09	4.1146	0.0002	Shoot	63.19	0.46	0.28	1.63	0.05
	SEE*	8.45					SEE*	19.63			
	R^{**}	0.65					R^{**}	0.42			
	R^{2***}	0.43					R^{2***}	0.10			
Root	36.63	0.79	5.84	0.3446	0.3668	SG**	37.94	47.64	9.88	4.82	0.0000
Leaf	43.59	10.96	0.12	6.4162	0.0000	Shoot	63.19	0.33	0.15	2.21	0.0188
	SEE*	0.80					SEE*	10.47			
	R^{**}	0.65					R^{**}	0.32			
	R^{2***}	0.79					R^{2***}	0.42			

SEE* Standard error estimate; R^{**} Correlation; R^{2***}Regression, SG**Seed germination

Production of toxin-chemotypes

Analysis of culture filtrates of different species of *Penicillium* revealed production of ochratoxin A (*P. verrucosum* and *P. nordicum*), citrinin (*P. verrucosum*, *P. citrinum* and *P. expansum*), rubratoxin B (*P. rubrum),* penitrem A (*P. flavigenum*), mycophenolic acid (*P. brevicompactum*), cyclopiazonic acid (*P. camemberti, P. commune* and *P. digitatum*) and penicillic acid (*P. aurantiogriseum*). Some of the species like *P.verrucosum* (OTA, CIT)*, P.griseofulvum* (GRI, PAT and CIT) *P.expansum* (PAT and CIT) and *P. roqueforti* (roquefortine C and P.R. toxin) were able to produce more than one mycotoxin-chemotypes. Quantification of OTA (In-vitro toxin production) was assayed by 25ml of same culture filtrate (50ml used for seed germination and seedling growth), and amounted (31.0±1.2µ/ml and 27.2±0.9µ/ml) with *P. verrucosum* and *P. nordicum* respectively.

Discussion

The present attempts to screen the different species of *Penicillium* in their toxic effect on sorghum seed germination and seedling, significantly retard the seed germination, radicle, coleoptiles and leaf elongation. Toxic fungal metabolites also induce adverse effects on plants such as inhibition of seed germination, malformation and reducing seedlings. Many fungal toxins are known to be phytotoxic and their role in plant

pathogenesis (Desjardins and Hohn 1997). Very little information is available on phytotoxic effects of *Penicillium* species. In the present investigations, we recorded 74-85% toxic effects caused by culture filtrates of *P. nordicum* and *P. verrucosum* on seed germination of sorghum seeds. However, chemical analysis of these species produced OTA and CIT which are directly or indirectly involved in the seed germination and seedling growth. Recently Rashmi (2011) have also made similar kind of observation on sorghum seed germination inhibition by its seed-borne fungi. The present findings are in agreement with those of Rajput et al. (2010) who also reported the inhibition of seed germination of cereals studied by them. Ibatsam et al. (2013) reported the toxic effect of *P. bravicompactum* extract on wheat seedlings.

The present investigations culture filtrates of *P. commune* retard the highest radicle elongation inhibition, while culture filtrates of *P. nordicum* retard the coleoptile length are in agreement with the reports of Akbar and Javaid (2012) who also reported that phytotoxicity of culture filtrates of *D. hawaiiensis, D. holmii, D. biseptata, D. australiensis* towards two cumbersome weeds (*C. album* and *A. fatua*) of wheat. The significant percentage of coleoptile inhibition was recorded with *P. nordicum*, *P. nalgiovense*, *P. digitatum*, *P. commune* and *P. citrinum*. Similarly Madhosing (1995) have reported inhibition of seed germination and coleoptile elongation inhibition studied by them.

Table 3. Effect of *Penicillium* species on seedling growth elongation inhibition of sorghum

Penicillium sp.	Radicle elongation inhibition (%)	Coleoptile elongation inhibition (%)	Leaf elongation inhibition (%)
P. aethiopicum	35.79±0.65	40.19±0.88	20.57±0.50
P. alli	15.01±0.42	27.65± 0.64	36.44±0.28
P. aurantiogriseum	17.77±1.64	52.18±0.25	22.26±0.33
P. brevicompactum	11.84±0.62	36.70±0.37	23.36±0.34
P. camemberti	10.84±0.43	32.45±0.34	22.28±0.35
P. caseifulvum	44.49±1.06	59.56±0.97	45.09±0.87
P. chrysogenum	64.98±0.47	47.87±0.49	52.60±0.19
P. citrinum	87.91±0.55	70.39±0.39	86.12±0.51
P. commune	54.82±0.75	70.97±0.49	77.30±0.07
P. crustosum	39.00±0.40	63.03±0.55	47.99±0.40
P. digitatum	24.27±0.92	39.69±0.39	31.82±0.50
P. dipodomyis	25.36±0.51	53.77±0.54	44.46±0.34
P. discolor	42.05±0.71	46.10±0.70	38.03±0.50
P. expansum	48.65±0.42	47.81±0.74	76.29±0.05
P. flavigenum	20.54±1.04	48.58±0.38	22.54±0.47
P. griseofulvum	47.76±0.77	52.57±0.20	38.14±0.53
P. italicum	48.66±1.05	64.00±0.39	48.46±0.42
P. nalgiovense	28.03±0.52	55.84±0.48	32.81±0.76
P. nordicum	44.61±0.09	53.11±0.71	61.86±0.56
P. olsonii	23.90±0.98	40.01±0.61	27.35±0.22
P. roqueforti	36.07±0.29	46.36±0.26	44.66±0.10
P. rubrum	46.98±0.77	51.96±0.45	49.23±0.76
P. tricolor	20.51±1.12	50.66±0.38	34.69±0.37
P. verrucosum	39.29±0.52	58.46±0.18	61.91±0.49

Results are mean and standard deviation (Std.Dev) of three replicate experiments and significant differences at ($P<0.005$).

The present findings are significant as they are common in agricultural products like cereals, pulses, oil seeds, feeds (Erpelding and Prom 2006; Fakhrunnisa and Ghaffar 2006). Pathogenicity studies of different species of *Penicillium* revealed *P. citrinum*, *P. chrysogenum*, *P. commune, P. rubrum* and *P. caseifulvum* were responsible for significant seedling growth elongation inhibition. Our findings are positively correlated (Vijayan and Rehill 1990; Gachomo et al. 2004; Narasimha Rao et al. 2006) reported that 70% of seed samples were infected with different pathogens by their culture filtrates and reduced the germination. Marley (2004) could find a positive correlation between the incidence fungi and reduction of seedling growth. In-vitro quantification of OTA production by *P.verrucosum* and *P. nordicum* were assessed by HPLC method reveled that, 25 and 30µg/ml of OTA respectively by both the species of *Penicillium* was recorded. This amount of OTA may inhibit seed germination about 80-85%, coleoptile 66-76% and radicle 71-85% growth of sorghum by culture filtrate method. Comparatively, water agar method reduced the radicle 39-44%, coleoptile 53-58% and leaf 61% growth with both ochratoxigenic strains of *Penicillium*. Tekle et al. (2013) reported that more than 2ppm DON-amended water agar retard the seedling growth and alter the morphology of oat seed. The culture filtrate method was positively correlated with water agar method. However, in water agar method fungal mycelium may enter into internal part of seeds. Gwary et al. (2003) with the help of microtome section located the internal fungal mycelium in apparently healthy grains of sorghum. On the other hand, culture filtrate may directly and/or indirectly involved in germination and suppress/alter the gene cluster of sorghum. Similar findings reported on *F. graminearum* mycelium and spores have been detected on endosperm as well as under the husk of barley (Schwarz et al. 2001).

The present observation pathogenicity of CPA inhibited 42.6%, GRI 42%, CIT 60%, PAT 35% and MPA 37% were significantly recorded on seed germination and seedling growth of sorghum was varied with species and toxin-chemotype. However, other types of toxins like rubratoxin B penicillin, PR toxin, roquefortine C and penitrem A producing species were also inhibiting the seed germination and seedling growth of sorghum. Mycotoxins affected seedling growth, coleoptile, and radicle elongation due to different toxin-chemotypes and varying their toxicity on diffident cereals has been reported (Bruins et al. 1993, McLean 1996, Masuda et al. 2007). In addition to untapped biodiversity and genomes that have many gene clusters are silent possibility to produce many more compounds and several factors can stimulate or alter metabolic pathway genes (Klejnstrup et al. 2012).

Conclusions

From the present investigations it can be concluded that species of *Penicillium* varied in their toxicity in congrnance of mycotoxins produced. Many species of *Penicillium* were significantly toxic and inhibited the seed germination and seedling growth of sorghum. In present observations, non toxigenic strains of *Penicillium* strains also showed significant effects on radicle and coleoptile growth of sorghum seeds. Further studies are also needed to reveal the quantitative mechanisms of other toxin induced germination and pathogenicity of sorghum in comparison with non toxigenic strains.

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