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Original article

Potency of plant extracts against Penicillium species isolated from different seeds and fruits in Saudi Arabia

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ABSTRACT

Antifungal activity of extracts of cinnamon (Cinnamomum zeylanicum), Cloves (Syzygium aromaticum), ginger (Zingiber officinale) and turmeric (Curcuma longa) were evaluated *in vitro* against 17 *Penicillium spp*. Seed disease and rotten fruit caused by these species cause considerable loss of quality for different agricultural products. Isolates of *Penicillium* spp. were screened for production of patulin an important serious mycotoxin. About 70.59% of *Penicillium* spp. produced this toxin in concentrations ranging from 4 to 31 ppb. The response of *Penicillium* spp.

to plant extracts differed according to the plant extract and concentration. Cinnamon extract showed the greatest effect on *P. asperosporum, P. aurintogriseum and P. brevicompactum*, and cloves extract produced the greatest effect on *P. chermesinum and P. duclauxii*. Turmeric extract had less effect on *P. duclauxii*. Cloves extract was the most effective in reducing the growth of *Penicillium spp*. On the other hand, ginger extract with all concentrations used had less effect against most *Penicillium* spp in the laboratory. Plant extracts are promising as natural sources of environmentally friendly compounds in laboratory studies.

1. Introduction

Corn seeds and fruits are subject to post-harvest diseases caused by fungi during storage. These diseases cause cuts, wounds and other physical damage during harvest, packing, transport, and storage. *Penicillium italicum* rot disease is a devastating post-harvest disease (Abramson et al., 2009; Agrios, 2005). This disease is found in produce during cooling, storage and marketing and the disease is exacerbated by wet conditions. Fungi on fruits exhibit dark blue round areas with mature fruiting bodies surrounded by white mycelia (Al-Rahmah et al., 2013; Al-Samarrai et al., 2013). Fruiting fungi are responsible for new infection in healthy produce. Blue mold disease losses are estimated to be 10–40% (Aqil et al., 2010).

Many fruits are exposed to post-harvest diseases in the field and during storage. Post-harvest disease injury are directly related to physical damage, such as cuts and wounds, during harvest, packing, transport, and storage. Corn seeds and fruits are infected by fungi during storage. Green-mold fungi on fruit exhibit dark blue round areas with mature fruiting bodies surrounded by the growth of white fungi from *P. italicum* (Ayoola et al., 2008). Blue fruits infected with fungi are responsible for the new infection in healthy fruits.

Several disease management options, including chemical control sprayed on fruits to reduce pathogenic fungal infection and increase storage periods, are available (Marzoug et al., 2011). Many fruits are exposed to many post-harvest diseases caused by field and storage fungi. The injuries associated with post-harvest diseases are directly related to mechanical damage, cuts, and wounds during harvest, packing, transport, and storage. The blue rot disease is the most devastating post-harvest disease caused by the fungus *Penicillium italicum* (Benkeblia, 2004; Boulenouar et al., 2012; Bowers and Locke, 2000). This disease manifests in gardens during cooling, storage, and marketing and becomes more serious in wet conditions. The green-molded fungi on the fruits exhibit

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dark blue round areas with mature germs surrounded by the growth of white fungi from *P. italicum* (Bragulat et al., 2008; Chen et al., 2018). The blue fruits infected with fungi are responsible for the new infection in healthy fruits. Humidity favors the development of the disease. Losses due to *Penicillium spp*. rotting disease are estimated to be approximately 15%–45% (Christian, xxxx; Dwivedi and Dwivedi, 2012).

Chemicals are, however, responsible for increasing risks to human health and environment, and their use leads to resistance to pesticides. Development of alternatives to fungicides is needed to help control post-harvest diseases. Biological control and adoption of natural products, including seed powders, water and alcohol extracts for many plants are possible options. Botanical extract products are environmentally friendly, inexpensive and may reduce losses by discouraging pathogen growth. Plant extracts contain active compounds that inhibit the growth of plant pathogens.

Penicillium spp. are the main cause of deterioration and decomposition of a wide range of plant products after harvest, especially fruits, such as grapes (Fki et al., 2005; Gende et al., 2008). These fungi are widespread, attacking various fruits, including grapes and especially during storage and often producing a variety of mycotoxins (Magnoli et al., 2003); (Moslem et al., 2011) . Harmful mycotoxins and carcinogenic compounds, such as citrinine, patulin, penicillic acid and other secondary metabolites are produced by *Penicillium* spp. (Abramson et al., 2009); (Santos et al., 2002) ; (Bragulat et al., 2008) . Effective control of fruit diseases can also be achieved through many non-chemical control strategies (Kanan and Al-Najar, 2008; (Sanzani et al., 2010). One popular non-chemical option for controlling plant diseases (Wang et al., 2004); (Soylu et al., 2005) is use of extracts and essential oils of herbaceous plants. Availability, low toxicity, and environmental friendliness make plant extracts attracted targets for investigation (Harris et al., 2001); (Fawzi et al., 2009) and (Aqil et al., 2010).

Several plant extracts possess antifungal properties and can be used to suppress decomposing fungi (Ismaiel, 2008). garlic is among the most promising natural plant materials with antifungal properties (Gende et al., 2008); Rathod et al. (2010); (Yassin et al., 2013) and Znini et al., 2011). Antifungal activity of plant extracts is noted against *Penicillium* spp. and other fungi, as well as reduced production of mycotoxins (Rezzi et al., 2001; Ismaiel, 2008); Taskeen et al., 2011 and (Minz et al., 2012).

The present study evaluated the efficacy of four plant extracts under laboratory conditions for antifungal activity against 17 *Penicillium* spp., isolated from fruits and seeds collected from Al-Riyadh markets; Saudi Arabia, and identified by the Assiut University Mycological Center, Egypt (AUMC).

2. Methods

2.1. Isolation of Penicillium spp.

Fruit and seed samples were collected from several locations (markets) in Al-Riyadh; capital of Saudi Arabia. Samples and the obtained samples were cut into small pieces, sterilized with 5% sodium hypochlorite solution for 5 min followed by washing in three changes of sterile distilled water. Samples were then dried between two filter papers for one minute. Samples were placed randomly onto potato dextrose agar (PDA) in three, 9 cm diameter, Petri dishes. Dishes were incubated at 28 °C and examined daily for seven days, after which colonies were counted. Isolates were purified either by single spore or hyphal tip methods and transferred to PDA slants. Identification of fungal isolates at the Mycological Center, Assiut University, Egypt. According to Pitt (1988), used morphological and microscopic characteristics (Table 1).

Table 1
Isolates of *Penicillium* spp. analyzed in this study.

No.	*Penicillium* Species	Source	Aumc No.*
1	*P. asperosporum*	Apple	7965
2	*P. aurintogriseum*	Peanut	5860
3	*P. brevicompactum*	Walnut	7934
4	*P. chermesinum*	Popcorn	5847
5	*P. chrysogenum*	Peanut	5846
6	*P. citrinum*	Apple	7732
7	*P. duclauxii*	Corn	5965
8	*P. expansum*	Grape	7576
9	*P. funiculosum*	Corn	5966
10	*P. griseofulvum*	Sorghum	5905
11	*P. glabrum*	Apple	7654
12	*P. implicatum*	Peanut	5866
13	*P. olsonii*	Peanut	5854
14	*P. oxalicum*	Corn	5950
15	*P. puberulum*	Grape	7934
16	*P. variabile*	Coffee bean	5560
17	*P. verrucosum*	Apple	8026

Aumc. No (Assiut University Mycological Center, Egypt).

2.2. Mycotoxins assays

Tested isolates of *Penicillium* spp. were grown on sterilized malt extract prepared in 100 ml flasks for 7–10 days at 27 ± 2 °C with three replicates per isolate (Yassin et al., 2010). Cultures were blended for 2 min using a high-speed homogenizer and filtered using glass filter paper. Patulin was extracted from the homogenized filtrate using acetonitrile:water (5:95 v:v) (liquid mobile phase) solution. The solvent was evaporated at 35 °C under vacuum. Dried residues containing patulin were dissolved in 1 ml of the same liquid mobile phase. This extract was passed through a 0.45 µm microfilter, and analyzed on an HPLC model PerkinElmer® Brownlee™ with a validated C18, 250 mm column. The HPLC was equipped with UV detector and compounds were detected with a UV detector at a wavelength at 280 nm. Total run time for the separation was approximately 25 min at a flow rate of 1 ml/min.

2.3. In vitro antifungal activity against 17- Penicillium spp.

Antifungal activity of four plant extracts of cinnamon (*Cinnamomum zeylanicum*), Cloves (*Syzygium aromaticum*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) (Table 2) were evaluated *in vitro* against 17 species of *Penicillium*. One hundred grams of plant materials were homogenized in 100 ml of distilled water (1:1W/V) for 5 min using a blender (Ismaiel, 2008). Obtained extracts were filtered through a sheath layer, and used immediately, or stored at 4 °C until use.

Different volumes of crude extracts were incorporated into PDA medium just before pouring into sterilized Petri dishes to obtain extract concentrations of 5%, 10%, 15%, and 20%. Petri dishes were centrally inoculated with 2 mm fungal plugs and incubated at 28 ± 2 °C for 7–10 days. Linear growth of fungi was measured at the time when pathogenic fungi completely covered medium surface in control treatments. Percentage inhibition was calculated as:

$$\text{Reduction in linear growth}\,(\%) = (R1 - R2/R1) * 100$$

Table 2
Showing the medicinal plants and their common and scientific names.

No.	Common Name	Scientific Name	Used parts
1	Cinnamon	*Cinnamomum zeylanicum*	Powder of Cinnamon
2	Cloves	*Syzygium aromaticum*	Aromatic Flower buds
3	Ginger	*Zingiber officinale*	Turmeric Rhizomes
4	Turmeric	*Curcuma longa*	Turmeric Rhizomes

R1 = The radius of control growth
R2 = The radius of fungal inhibited growth
% of inhibition of *Penicillium* spp.

2.4. Statistical analysis

Analysis of variance (ANOVA) performed with the MSTAT-C statistical package (Michigan State Univ., USA) was used to calculate least significant difference (LSD) to compare means.

3. Results

3.1. Mycotoxigenicity

Isolates of *Penicillium* spp. were screened for patulin production; 70.59% of *Penicillium* spp. produced the mycotoxin in concentrations that ranged from 4 to 31 ppb (Fig. 1). *P. chrysogenum* displayed the highest production of patulin and *P. brevicompactum* the least. *P. citrinum* and *P. oxalicum* produced similar amounts.

3.2. Antifungal activity of four plant extracts against 17 Penicillium spp.

Analysis of variance of the effects of plant extracts on the growth of *Penicillium* spp. showed that plant extract (P), concentrations (C) and their Interaction P × C were all highly significant sources of variation for all *Penicillium* spp. (Table 3). The significant interaction, P * C, indicated that the response in each species of *Penicillium* varied depending on plant source and concentrations.

Effects of tested plant extracts, concentrations, and their interactions on the linear growth of *P. asperosporum*, *P. aurintogriseum*, and *P. brevicompactum* were recorded (Table 4). *P. asperosporums* shows similar responses to effects of cloves and turmeric at concentrations 10 and 15%. *P. aurintogriseum* shows significantly different effects of all concentrations of plant extracts except for 5%. No significant difference in effects of turmeric at concentrations 15 and 20% were observed in *P. brevicompactum* (see Table 5.).

Concentrations of 20% for both turmeric and ginger showed a significant and similar effect in reducing linear growth of *P. chermesinum* and *P. chrysogenum*. Further, similar inhibitory effects were found at 5% and 15% concentrations of cloves and ginger extract against *P. citrinum*.

No significant differences were found between the activity of cloves and ginger at concentrations 5 and 10% against *P. duclauxii* (Table 6). All investigated concentrations for all extracts were effective in reducing the linear growth of *P. funiculosum* except 5% for ginger.

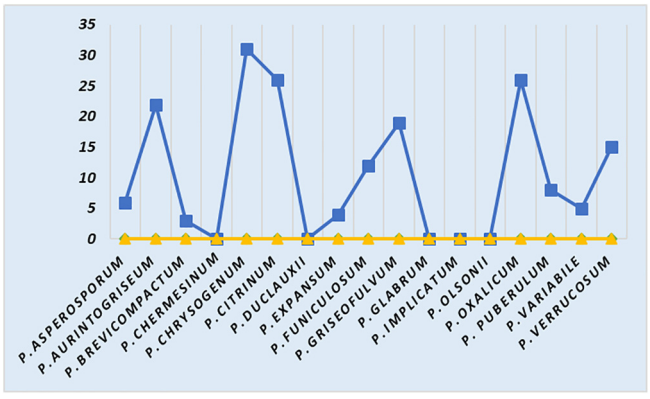

Fig. 1. Diagram showing the production of mycotoxin patulin by (ppb.) from 17 tested *Penicillium* spp. by Assiut University Mycological Center.

Table 3
ANOVA of the effects of plant extract(P). concentrations (C) and their interactions (P * C) on the linear growth of *Penicillium* spp.

Penicillium spp. **and source of variation**	**D.F**	**M.S**	**F. value**	**P. F**
1- *P. asperosporum*				
Replication	3	10.63	1.44	0.239
plant extract(P)	3	2455.43	333.55	0.000
Concentration(C)	4	7328.21	995.49	0.000
Interaction (P * C)	12	425.79	57.84	0.000
Error	57	7.36		
2- *P. aurintogriseum*				
Replication	3	30.54	1.5	0.224
plant extract(P)	3	4771.14	234.42	0.000
Concentration(C)	4	6171.16	303.20	0.000
Interaction (P * C)	12	767.76	37.72	0.000
Error	57	20.35		
3- *P. brevicompactum*				
Replication	3	0.350	0.042	0.989
plant extract(P)	3	1619.68	192.15	0.000
Concentration(C)	4	7231.08	857.88	0.000
Interaction (P * C)	12	393.09	46.63	0.000
Error	57	8.42		
4- *P. chermesinum*				
Replication	3	2.81	0.315	0.814
plant extract(P)	3	1616.61	181.28	0.000
Concentration(C)	4	8579.18	962.03	0.000
Interaction (P * C)	12	343.15	38.48	0.000
Error	57	8.91		
5- *P. chrysogenum*				
Replication	3	16.43	1.74	0.16
plant extract(P)	3	3218.10	342.09	0.000
Concentration(C)	4	6089.76	647.36	0.000
Interaction (P * C)	12	461.66	49.07	0.000
Error	57	9.40		
6- *P. citrinum*				
Replication	3	21.54	2.30	0.086
plant extract(P)	3	912.57	97.66	0.000
Concentration(C)	4	6003.53	642.49	0.000
Interaction (P * C)	12	292.88	31.34	0.000
Error	57	9.34		
7- *P. duclauxii*				
Replication	3	5.43	0.55	0.64
plant extract(P)	3	3664.24	374.63	0.000
Concentration(C)	4	4871.03	498.01	0.000
Interaction (P * C)	12	305.46	31.23	0.000
Error	57	9.78		
8- *P. expansum*				
Replication	3	19.61	1.96	0.12
plant extract(P)	3	2902.54	291.32	0.000
Concentration(C)	4	4378.81	439.49	0.000
Interaction (P * C)	12	433.33	43.49	0.000
Error	57	9.96		
9- *P. funiculosum*				
Replication	3	6.41	0.91	0.43
plant extract(P)	3	7338.81	1048.36	0.000
Concentration(C)	4	7980.56	1140.04	0.000
Interaction (P * C)	12	580.18	82.88	0.000
Error	57	7.00		
10- *P. griseofulvum*				
Replication	3	3.23	0.36	0.78
plant extract(P)	3	3610.83	405.71	0.000
Concentration(C)	4	8481.35	952.96	0.000
Interaction (P * C)	12	282.57	31.75	0.000
Error	57	8.90		
11- *P. glabrum*				
Replication	3	41.15	5.12	0.003
plant extract(P)	3	4237.91	527.36	0.000
Concentration(C)	4	4497.48	559.66	0.000
Interaction(P * C)	12	434.86	54.11	0.000
Error	57	8.03		
12- *P. implicatum*				
Replication	3	35.68	4.63	0.006
plant extract(P)	3	1526.15	198.17	0.000
Concentration(C)	4	4128.46	536.10	0.000
Interaction (P * C)	12	128.36	16.66	0.000
Error	57	7.70		
13- *P. olsonii*				

Table 3 (*continued*)

Penicillium spp. **and source of variation**	**D.F**	**M.S**	**F. value**	**P. F**
Replication	3	44.18	4.11	0.010
plant extract(P)	3	1476.41	137.52	0.000
Concentration(C)	4	3854.73	359.04	0.000
Interaction (P * C)	12	407.07	37.91	0.000
Error	57	10.73		
14- *P. oxalicum*				
Replication	3	11.87	1.84	0.150
plant extract(P)	3	2070.91	321.10	0.000
Concentration(C)	4	4302.48	667.11	0.000
Interaction (P * C)	12	328.42	50.92	0.000
Error	57	6.44		
15- *P. puberulum*				
Replication	3	10.74	1.31	0.27
plant extract(P)	3	1273.07	156.05	0.000
Concentration(C)	4	3113.32	381.62	0.000
Interaction (P * C)	12	140.26	17.19	0.000
Error	57	8.15		
16- *P. variabile*				
Replication	3	14.58	2.42	0.075
plant extract(P)	3	4454.68	739.74	0.000
Concentration(C)	4	6957.79	1155.40	0.000
Interaction(P * C)	12	524.94	87.17	0.000
Error	57	6.02		
17- *P. verrucosum*				
Replication	3	4.55	0.74	0.50
plant extract(P)	3	4470.95	770.27	0.000
Concentration(C)	4	6709.95	1156.01	0.000
Interaction (P * C)	12	453.06	78.06	0.000
Error	57	5.80		

P. griseofulvum shows the same responses to effect cinnamon and turmeric at concentrations 5 and 15%, and *P. implicatum* shows the same response to similar concentrations of turmeric and ginger extracts (Table 7). *P. glabrum* shows significant responses to all extracts and all concentrations except 5 %ginger.

Cloves and cinnamon extracts showed a significant effect in reducing the linear growth of *P. olsonii, P. oxalicum* and *P. uberulum* at all concentrations (Table 8). Equal effects of turmeric extracts were similar at concentrations of 10% with effects of ginger extracts at concentrations 15% against P. *olsonii* and *P. oxalicum*.

Cinnamon at concentrations 10% and turmeric at concentrations 15% showed similar impacts against *P. variabile* (Table 9). Turmeric at concentrations of 20% and ginger at concentrations of 15% also show similar impacts on *P. verrucosum*.

ANOVA (Table 10) for linear growth (mm) of *Penicillium* spp. demonstrated highly significant impacts of plant extracts (p = 0.000). LSD was calculated to compare *Penicillium* ssp. mean growth for each plant extract.

Responses of *P. brevicompactum, P. chermesinum, and P. griseofulvum* to turmeric extract are almost equal but responses of other species it responded to the other extracts were significantly different. *P. implicatum* and *P. olsonii* showed significant response to all extracts except ginger extract. On the other hand, *P. funiculosum* and *P. variabile* show significant response to all extracts except turmeric extract. (Table 11)

A phenogram based on average linkage cluster analysis of the response of *Penicillium* spp. to different plant extracts shows three distinct groups of isolated *Penicillium* spp. (Fig. 2) Each is divided into two subgroups; strongly and positively associated *Penicillium* spp. were grouped in the same cluster. The grouping pattern of the *Penicillium* spp. in the cluster analysis did depend on the source of the *Penicillium* isolate.

4. Discussion

Different strategies are employed for controlling a serious plant pathogenic fungi worldwide; one important approach is employing plant extracts. Such extracts are considered safe and effective alternatives (Al-Rahmah et al., 2013) and (Al-Samarrai et al., 2013); (Aqil et al., 2010); (El-Samawaty et al., 2013) and (Abramson et al., 2009). Four plant extracts showed significant variation for inhibition of mycelial growth for all the investigated *Penicillium* spp. *in vitro*. Production of mycotoxins also fluctuated among *Penicillium* spp.

Isolates of *Penicillium* spp. were screened for production of the mycotoxin, patulin; 70.59% of species produced patulin in varying amounts depending on species. These results are consistent with (Yassin et al., 2010) and (Moslem et al., 2011) who investigated fungal ochratoxin production on different plant materials and sug-

Table 4
Effects of plant extract(P) and concentrations (C) and their interactions (P * C) on the linear growth (mm) of *P. asperosporum, P. aurintogriseum and P. brevicompactum*.

P. asperosporum	**Plant Extracts**	**Concentration**					**Mean**
		Control	**5%**	**10%**	**15%**	**20%**	
	Cloves	90	85.5	71	63.75	13	64.65
	Cinnamon	90	61.5	53	32.75	20.75	51.6
	Turmeric	90	80.25	73	63.5	46.25	70.6
	Ginger	90	90	83.75	65.5	59.5	77.75
	Mean	90	79.31	70.19	56.37	34.87	
LSD for interaction = 3.8 LSD for plant extract = 1.7 LSD for Concentration = 1.9							
P. aurintogriseum	Plant Extracts	Control	5%	10%	15%	20%	Mean
	Cloves	85	70	51	33.5	11.5	50.20
	Cinnamon	85	73.50	53.25	20.25	11	48.60
	Turmeric	85	71.5	68	61.5	44.75	66.15
	Ginger	85	84.25	81.5	79.75	77	81.50
	Mean	85	74.81	63.44	48.75	36.06	
LSD for interaction = 6.32 LSD for plant extract = 2.82 LSD for Concentration = 3.16							
P. brevicompactum	**Extracts of**	Control	5%	10%	15%	20%	Mean
	Cloves	90	76	64.75	54	12.25	59.4
	Cinnamon	90	72.25	54.75	44.25	22	56.65
	Turmeric	90	65.75	57.75	45	42	60.1
	Ginger	90	84.75	75.25	70.75	61	76.35
	Mean	90	74.68	63.12	53.5	34.31	
LSD for interaction = 4.06 LSD for plant extract = 1.82 LSD for Concentration = 2.03							

Table 5
Effects of plant extract (P), concentration (C), and their interactions (P * C) on the linear growth (mm) of *P. chermesinum, P. chrysogenum, and P. citrinum*

P. chermesinum	**Plant Extracts**	**Concentration**					
		Control	**5%**	**10%**	**15%**	**20%**	**Mean**
	Cloves	90	50.5	42.5	40	9	46.40
	Cinnamon	90	70	50	22.75	17.25	50
	Turmeric	90	64.75	55.5	48.5	45	60.75
	Ginger	90	70.25	65	58.5	44.25	65.6
	Mean	90	63.87	53.25	42.43	28.87	
LSD for interaction = 4.18 LSD for plant extract = 1.87 LSD for Concentration = 2.09							
P. chrysogenum	Extracts of	Control	5%	10%	15%	20%	Mean
	Cloves	74.25	50.5	44.75	27.5	10.25	41.45
	Cinnamon	74.25	64.5	29.5	20.75	9	39.6
	Turmeric	74.25	71	54	50.25	47	59.3
	Ginger	74.25	70.75	61.5	53.25	44.5	60.85
	Mean	74.25	64.18	44.93	37.93	27.68	
LSD for interaction = 4.29 LSD for plant extract = 1.92 LSD for Concentration = 2.15							
P. citrinum	Extracts of	Control	5%	10%	15%	20%	Mean
	Cloves	82.75	80.5	71.25	65	16	63.1
	Cinnamon	82.75	69.5	56	42.25	21.5	54.4
	Turmeric	82.75	66.25	62.25	54.75	41	61.4
	Ginger	82.75	79	77.75	63.75	51	70
	Mean	82.75	73.81	66.81	56.43	32.37	
LSD for interaction = 4.28 LSD for plant extract = 1.91 LSD for Concentration = 2.14							

Table 6
Effects of plant extract(P). concentrations (C) and their interactions (P * C) on the linear growth (mm) of *P. duclauxii, P. expansum, and P. funiculosum*

P. duclauxii		**Concentration**					
	Plant Extracts	**Control**	**5%**	**10%**	**15%**	**20%**	**Mean**
	Cloves	81.75	36.5	34.75	19.5	13.25	37.15
	Cinnamon	81.75	64.25	52.5	47.25	26.75	54.5
	Turmeric	81.75	75	62.5	56.75	54.25	66.05
	Ginger	81.75	68.75	67.5	64.25	45.5	65.55
	Mean	81.75	61.12	54.31	46.93	34.93	
LSD for interaction = 4.38 LSD for plant extract = 1.96 LSD for Concentration = 2.19							
P. expansum	Extracts of	Control	5%	10%	15%	20%	Mean
	Cloves	67	52.5	25.75	17	9.75	34.25
	Cinnamon	67	65.75	41	34	18.5	45.25
	Turmeric	67	65.75	65	44.5	36.75	55.8
	Ginger	67	63.5	62.5	59.5	51.75	60.85
	Mean	67	61.87	48.56	38.75	29.18	
LSD for interaction = 4.42 LSD for plant extract = 1.98 LSD for Concentration = 2.21							
P. funiculosum	Extracts of	Control	5%	10%	15%	20%	Mean
	Cloves	90	63.5	39	35.5	9	47.4
	Cinnamon	90	41.75	30.5	21	9	38.95
	Turmeric	90	78.25	68.75	56	50	68.6
	Ginger	90	86.5	81.5	73.5	60	78.3
	Mean	90	67.5	54.93	46	32	
LSD for interaction = 3.7 LSD for plant extract = 1.66 LSD for Concentration = 1.85							

gested this toxin as an important factors for reducing self-life in Saudi Arabia.

Antifungal activity of four plant extracts against 17 *Penicillium* spp. showed that plant extract, concentrations and their interaction were all highly significant sources of variation in the inhibition of examined species. The significant interaction of extract and concentrations indicated that both factors contributed to variation in *Penicillium* spp. test. Earlier workers investigated effects of different plant extracts on controlling pathogenic fungi and observed that concentrations of extracts is a critical factor for reduction in mycelia growth (Wang et al., 2004; Soylu et al., 2005; Ismaiel, 2008 and Taskeen-Un-Nisa and Mir, 2010).

The activity of cinnamon (*C. zeylanicum*) extract against *penicillium spp*. could be attributed to the presence of Cinnamaldehyde, eugenol and cinamic acid in addition to flavonoids, alkaloiks, tannins and saponins suggested by some investigators as antifungal agents. Mahmoud (2012). Clove (*S. aromaticum*) extract also found to be very active against the tested *penicillium spp*. This activity could be attributed to the presence of phenolic compounds such as eugenol are highly active against microorganisms. Laila Muñoz Castellanos et al. (2020). Phenolic compounds such as gingerol, cedrene, zingiberene in ginger (Z. officinale) extract were determined as the most effective antifungal components; which play the vital role in growth inhibition of phytopathogenic fungi (Mostafa et al., 2011); (Al-Rahmah et al., 2013). Chen et al., 2018. They found the following compounds curdione, isocurcumenol, curcumenol, curzerene, β-elemene, curcumin, germacrone, curcumol in the extract of Turmeric *(Curcuma longa)*. Which were effective against *Penicillium pallidum* and other fungi.

Table 7
Effect of plant extract(P). concentrations (C) and their interactions (P * C) on the linear growth (mm) of *P. griseofulvum*, *P. glabrum*, and *P.* implicatum

P. griseofulvum		Concentration					
	Plant Extracts	**Control**	**5%**	**10%**	**15%**	**20%**	**Mean**
	Cloves	90	41.25	34.75	18.25	9	38.65
	Cinnamon	90	77.5	62.5	40.5	32	60.5
	Turmeric	90	73.5	54.25	44.5	40.75	60.5
	Ginger	90	86	68.25	61.25	46.75	70.45
	Mean	90	69.56	54.93	41.12	32.12	
LSD for interaction = 4.18 LSD for plant extract = 1.87 LSD for Concentration = 2.09							
p. glabrum	Extracts of	Control	5%	10%	15%	20%	Mean
	Cloves	72.25	53	33.25	27.25	9	38.95
	Cinnamon	72.25	53.25	31.75	14.75	9.75	36.35
	Turmeric	72.25	66.75	65.5	60	56.75	64.25
	Ginger	72.25	69.75	64.75	53.5	45.5	61.15
	Mean	72.25	60.68	48.81	38.87	30.25	
LSD for interaction = 3.97 LSD for plant extract = 1.77 LSD for Concentration = 1.98							
p. implicatum	Extracts of	Control	5%	10%	15%	20%	Mean
	Cloves	67	43.5	31	22.25	9.6	34.65
	Cinnamon	67	58.75	44.25	37.25	22.25	45.9
	Turmeric	67	64	52.75	42	31.75	51.5
	Ginger	67	63.5	54	44.25	43.5	54.45
	Mean	67	57.43	45.5	36.43	26.75	
LSD for interaction = 3.89 LSD for plant extract = 1.74 LSD for Concentration = 1.94							

Table 8
Effects of plant extract(P). concentrations (C) and their interactions (P * C) on the linear growth (mm) of *P. olsonii*, *P.oxalicum*, and *P. uberulum*.

P. olsonii		Concentration					
	Plant Extracts	**Control**	**5%**	**10%**	**15%**	**20%**	**Mean**
	Cloves	62.75	52.25	45.75	36	9.5	41.25
	Cinnamon	62.75	58.0	50.75	11	9	38.3
	Turmeric	62.75	60.0	53.5	45.25	32.75	50.85
	Ginger	62.75	60.0	58.0	53.5	50.25	56.9
	Mean	62.75	57.56	52.0	36.43	25.37	
LSD for interaction = 4.59 LSD for plant extract = 2.05 LSD for Concentration = 2.29							
P. oxalicum	Extracts of	Control	5%	10%	15%	20%	Mean
	Cloves	58.75	46.25	11.75	9	9	26.95
	Cinnamon	58.75	52.75	33	11	9	32.9
	Turmeric	58.75	51.75	45	40.25	29.5	45.05
	Ginger	58.75	55	51.75	45	32.75	48.65
	Mean	58.75	51.43	35.37	26.31	20.06	
LSD for interaction = 3.56 LSD for plant extract = 1.59 LSD for Concentration = 1.78							
P. uberulum	Extracts of	Control	5%	10%	15%	20%	Mean
	Cloves	63.5	46.5	27.75	22	9.75	33.9
	Cinnamon	63.5	47.75	37.75	32.75	30.5	42.45
	Turmeric	63.5	43.75	35.75	34	27.5	40.90
	Ginger	63.5	57.75	51.75	49.25	43.75	53.2
	Mean	63.5	48.93	38.25	34.5	27.87	
LSD for interaction = 4.0 LSD for plant extract = 1.79 LSD for Concentration = 2.0							

Table 9
Effects of plant extract(P). concentrations (C) and their interactions (P * C) on the linear growth (mm) of *P. variabile and P. verrucosum.*

P. variabile		**Concentration**					
	Plant Extracts	**Control**	**5%**	**10%**	**15%**	**20%**	**Mean**
	Cloves	90	60.75	57.75	44.5	9.5	52.5
	Cinnamon	90	71	61.75	52.75	9	56.9
	Turmeric	90	75.25	68	61.75	41.25	67.25
	Ginger	90	90	88.75	88	73.5	86.05
	Mean	90	74.25	69.06	61.75	33.31	
LSD for interaction = 3.44 LSD for plant extract = 1.54 LSD for Concentration = 1.72							
P. verrucosum	Extracts of	Control	5%	10%	15%	20%	Mean
	Cloves	90	56.25	45.5	31.5	9.75	46.6
	Cinnamon	90	77.5	60.75	40.75	19.25	57.65
	Turmeric	90	90	86.5	76.25	63.25	81.20
	Ginger	90	75.25	66.5	63.25	53.25	69.65
	Mean	90	74.75	64.81	52.93	36.37	
LSD for interaction = 3.37 LSD for plant extract = 1.51 LSD for Concentration = 1.69							

Table 10
ANOVA of the effects of plant extract (P), *Penicillium* spp. (P.S) and their interactions (P.S * P) on the linear growth (mm) of *Penicillium* spp.

Source of variation	D.F	M.S	F.value	P F
Replication	3	23964.11	257.21	0.000
plant extract(P)	3	9760.17	104.76	0.000
Penicillium spp. **(P.S)**	4	1445.22	15.51	0.000
Interaction (P.S * P)	12	196.33	2.10	0.000
Error	57	93.16		

Table 11
Effects of plant extract(P). *Penicillium* spp. (P.S) and their interactions (P.S * P) on the linear growth (mm) of *Penicillium* spp.

	Penicillium spp.	**Plant Extracts**					**Mean**
		Control	**Cloves**	**Cinnamon**	**Turmeric**	**Ginger**	
1	*P. asperosporum*	90.00	64.65	51.6	70.60	77.75	66.15
2	*P. aurintogriseum*	85.00	50.20	48.6	66.15	81.50	61.61
3	*P. brevicompactum*	90.00	59.00	56.65	60.10	76.35	63.02
4	*P. chermesinum*	90.00	46.40	50.00	60.75	65.60	55.68
5	*P. chrysogenum*	74.25	41.45	35.60	59.30	60.85	49.30
6	*P. citrinum*	82.75	63.10	54.4	61.40	70.85	62.43
7	*P. duclauxii*	81.75	37.15	54.5	66.05	65.55	55.81
8	*P. expansum*	67.00	34.25	43.70	55.80	60.85	48.65
9	*P. funiculosum*	90.00	47.40	36.45	68.6	78.30	57.68
10	*P. griseofulvum*	90.00	38.65	60.50	60.40	70.45	57.5
11	*P. glabrum*	72.25	38.95	36.35	64.25	61.15	50.17
12	*P. implicatum*	67.00	34.65	45.9	51.50	54.45	46.62
13	*P. olsonii*	62.75	41.25	38.3	50.85	56.90	46.82
14	*P. oxalicum*	58.75	26.95	32.9	45.05	48.65	38.38
15	*P. puberulum*	63.5	33.75	42.45	40.90	53.20	42.57
16	*P. variabile*	90.00	52.50	56.90	67.25	86.05	65.67
17	*P. verrucosum*	90.00	46.60	57.65	81.20	69.65	63.77
	Mean	79.11	44.52	47.20	60.59	66.94	

LSD for interaction = 2.98 LSD for plant extract = 1.45 LSD for Concentration = 1.66

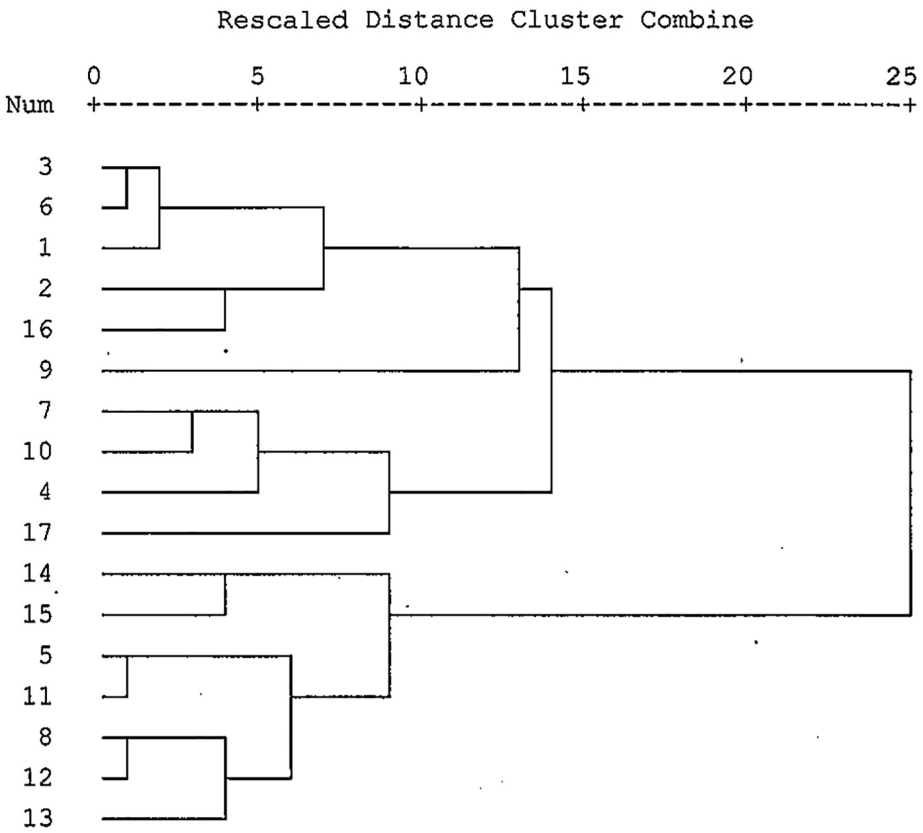

Fig. 2. Phenogram based on average linkage cluster analysis of the response of *Penicillium* spp. to plant extracts.

Analysis of variance for linear growth (mm) of *Penicillium* spp. showed highly significant impacts from exposure to plant extracts. These findings are consistent with results of Bowers et al., 2000; Obagwu and Korsten, 2003; Dwivedi, et al., 2012; (El-Samawaty et al., 2013) and (Al-Rahmah et al., 2013). Further, a phenogram based on average linkage cluster shows three distinct groups of isolated *Penicillium* spp. with strongly and positively associated *Penicillium* spp. grouped into the same cluster. The grouping pattern is similar to observed by earlier workers (Omar et al., 2007) and (Peng et al., 2012)who indicated that geographical origin didn't correlate with the source of isolated fungi and variations in results of grouping may due to genetic variation among isolates.

5. Conclusion

The present study shows the natural and ecological diversity of plants with anti-microbial activity. Comprehensive explorations are needed to identify more plants with these properties. Active compounds can then be identified, formulated and made available to farmers for use as pesticides to reduce the harmful effects of using fungicides.

Author contributions

A M E and D A E carried out isolation and mycotoxin analysis. A M E, SA, and MHM designed the study, performed the statistical analysis, and participated in the manuscript drafting.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abramson, D., Lombaert, G., Clear, R.M., Sholberg, P., Trelka, R., Rosin, E., 2009. Production of patulin and citrinin by Penicillium expansum from British Columbia (Canada) apples. Mycotoxin Res. 25 (2), 85–88.

Agrios, G.N., 2005. Plant pathology. Elsevier Academic Press, Burlington, Ma. USA, pp. 79–103.

Al-Rahmah, A.N., Mostafa, A.A., Abdel-Megeed, A., Yakout, S.M., Hussein, S.A., 2013. Fungicidal activities of certain methanolic plant extracts against tomato phytopathogenic fungi. African J. Microbiol. Res. 7 (6), 517–524.

Al-Samarrai, G.F., Singh, H., Syarhabil, M., 2013. Extracts some plants on controlling green mold of orange and on postharvest quality parameters. World Appl. Sci. J., Deira. 22 (4), 564–570.

Aqil, F., Zahin, M., Ahmad, I., Owais, M., Khan, M.S., Bansal, S.S., Farooq, S., 2010. Antifungal activity of medicinal plant extracts and phytocompounds: A review. InCombating Fungal Infections 2010, pp. 449–484. Springer, Berlin, Heidelberg.

Ayoola, G.A., Lawore, F.M., Adelowotan, T., Aibinu, I.E., Adenipekun, E., Coker, H.A., Odugbemi, T.O., 2008. Chemical analysis and antimicrobial activity of the essential oil of Syzigiu aromaticum (clove). African J. Microbiol. Res. 2 (7), 162–166.

Marzoug, H.N., Romdhane, M., Lebrihi, A., Mathieu, F., Couderc, F., Abderraba, M., Khouja, M.L., Bouajila, J., 2011. Eucalyptus oleosa essential oils: chemical composition and antimicrobial and antioxidant activities of the oils from different plant parts (stems, leaves, flowers and fruits). Molecules 16 (2), 1695–1709.

Benkeblia, N., 2004. Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). LWT-food Sci. Technol. 37 (2), 263–268.

Boulenouar, N., Marouf, A., Cheriti, A., Belboukhari, N., 2012. Medicinal Plants Extracts as Source of Antifungal Agents against Fusarium oxysporum f. sp. albedinis. J. Agr. Sci. Tech. 14, 659–669.

Bowers, J.H., Locke, J.C., 2000. Effect of botanical extracts on the population density of Fusarium oxysporum in soil and control of Fusarium wilt in the greenhouse. Plant Dis. 84 (3), 300–305.

Bragulat, M.R., Abarca, M.L., Cabanes, F.J., 2008. Low occurrence of patulin-and citrinin-producing species isolated from grapes. Lett. Appl. Microbiol. 47 (4), 286–289.

Chen, C., Long, L., Zhang, F., Chen, Q., Chen, C., Yu, X., et al., 2018. Antifungal activity, main active components and mechanism of Curcuma longa extract against Fusarium graminearum. PLoS ONE 13, (3). https://doi.org/10.1371/journal.pone.0194284 e0194284.

Christian, G. HPLC tips and tricks. Great Britain at the Iden Press.

Dwivedi, S.K., Dwivedi, N., 2012. Antifungal activity of some plant extracts against guava wilt pathogen. Int. J. Environ. Sci. 3 (1), 412–420.

El-Samawaty, A.E., Yassin, M.A., Moslem, M.A., Omar, M.R., 2013. Effectiveness of Some Plant Extracts against Fusarium spp Causing Cotton Seedlings Damping-off. Life Sci. J. 10 (4), 510–515.

Fawzi, E.M., Khalil, A.A., Afifi, A.F., 2009. Antifungal effect of some plant extracts on Alternaria alternata and Fusarium oxysporum. Afr. J. Biotechnol. 8 (11).

Fki, I., Allouche, N., Sayadi, S., 2005. The use of polyphenolic extract, purified hydroxytyrosol and 3, 4-dihydroxyphenyl acetic acid from olive mill wastewater for the stabilization of refined oils: a potential alternative to synthetic antioxidants. Food Chem. 93 (2), 197–204.

Gende, L.B., Floris, I., Fritz, R., Eguaras, M.J., 2008. Antimicrobial activity of cinnamon (Cinnamomum zeylanicum) essential oil and its main components against Paenibacillus larvae from Argentine. Bull. Insectol. 61 (1), 1.

Harris, J.C., Cottrell, S.L., Plummer, S., Lloyd, D., 2001. Antimicrobial properties of Allium sativum (garlic). Appl. Microbiol. Biotechnol. 57 (3), 282–286.

Ismaiel, A.A., 2008. Inhibitory effect of garlic extract of penicillic acid production and growth of *Penicillium hirsutum*. In: Third Environmental Conference Faculty of Science. Zagazig University, pp. 45–58.

Kanan, G.J., Al-Najar, R.A., 2008. In vitro antifungal activities of various plant crude extracts and fractions against citrus post-harvest disease agent Penicillium digitatum. Jordan Journal of Biological Sciences. 1 (3), 89–99.

Laila Muñoz Castellanos, Nubia Amaya Olivas , Juan Ayala-Soto, Carmen Miriam De La O Contreras, Miriam Zermeño Ortega, Fabiola Sandoval Salas, and Leon Hernández-Ochoa, 2020. In Vitro and In Vivo Antifungal Activity of Clove (Eugenia caryophyllata) and Pepper (Piper nigrum L.) Essential Oils and Functional Extracts Against Fusarium oxysporum and Aspergillus niger in Tomato (Solanum lycopersicum L.) International Journal of Microbiology, Article ID 1702037, 8 pages.

Magnoli, C., Violante, M., Combina, M., Palacio, G., Dalcero, A., 2003. Mycoflora and ochratoxin-producing strains of Aspergillus section Nigri in wine grapes in Argentina. Lett. Appl. Microbiol. 37 (2), 179–184.

Minz, S., Samuel, C.O., Tripathi, S.C., 2012. The effect of plant extracts on the growth of wilt causing fungi Fusarium oxysporum. J. Pharm. Biol. Sci. 4 (1), 13–16.

Moslem, M.A., Yassin, M.A., El-Samawaty, A.M., Sayed, S.R., 2011. New toxigenic Penicillium species associated with apple blue mold in Saudi Arabia. Fresenius Env. Bull. 20, 3194–3198.

Mostafa, A.A., Al-Rahmah, A.N., Adel-Megeed, A., 2011. Evaluation of some plant extracts for their antifungal and antiaflatoxigenic activities. J. Med. Plant. Res. 517, 4231–4238.

Obagwu, J., Korsten, L., 2003. Integrated control of citrus green and blue molds using Bacillus subtilis in combination with sodium bicarbonate or hot water. Postharvest Biol. Technol. 28 (1), 187–194.

Omar, M.R., El-Samawaty, A.M., El-Wakil, D.A., 2007. Suppression of Pythium ultimum involved in cotton seedling damping-off by Trichoderma spp. Egypt. J. Phytopath. 35 (2), 111–124.

Peng, L., Yang, S., Cheng, Y.J., Chen, F., Pan, S., Fan, G., 2012. Antifungal activity and action mode of pinocembrin from propolis against Penicillium italicum. Food Sci. Biotechnol. 21 (6), 1533–1539.

Pitt JI, Hocking AD. A laboratory guide to common Penicillium species. North Ryde, NSW Australia: CSIRO Food Research Laboratory; 1988.

Rathod, L.R., Jadhav, M.D., Awate, M.K., Surywanshi, A.M., Deshmukh, P.S., 2010. Utilization of medicinal plants to control seed borne pathogens of selected legumes seeds. Inter. J. Advan. Biotechnol. and Res. 1 (2), 57–59.

Rezzi, S., Cavaleiro, C., Bighelli, A., Salgueiro, L., da Cunha, A.P., Casanova, J., 2001 Feb 1. Intraspecific chemical variability of the leaf essential oil of Juniperus phoenicea subsp. turbinata from Corsica. Biochem. Syst. Ecol. 29 (2), 179–188.

Mahmoud, Sameer N., 2012. Antifungal Activity of Cinnamomum zeylanicum and Eucalyptus microtheca Crude Extracts Against Food Spoilage Fungi. Euphrates. J. Agric. Sci. 4 (3), 26–39.
Santos, I.M., Abrunhosa, L., Venâncio, A., Lima, N., 2002. The effect of culture preservation techniques on patulin and citrinin production by Penicillium expansum Link. Lett. Appl. Microbiol. 35 (4), 272–275.
Sanzani, S.M., Schena, L., De Girolamo, A., Ippolito, A., González-Candelas, L., 2010. Characterization of genes associated with induced resistance against Penicillium expansum in apple fruit treated with quercetin. Postharvest Biol. Technol. 56 (1), 1.
Soylu, E.M., Tok, F.M., Soylu, S., Kaya, A.D., Evrendilek, G.A., 2005. Antifungal activities of the essential oils on post-harvest disease agent Penicillium digitatum. Pak. J. Biol. Sci. 8 (1), 25–29.
Taskeen-Un-Nisa, W.A., Mir, R.A., 2010. Antimycotic activity of plant extracts on the spore germination of some pathogenic fungi. Mycopathology. 8, 65–69.
Wang, W., Ben-Daniel, B.H., Cohen, Y., 2004. Control of plant diseases by extracts of Inula viscosa. Phytopathology. 94 (10), 1042–1047.
Yassin, M.A., El-Samawaty, A.R., Bahkali, A., Moslem, M., Abd-Elsalam, K.A., Hyde, K. D., 2010. Mycotoxin-producing fungi occurring in sorghum grains from Saudi Arabia. Fungal Diversity. 44 (1), 45–52.
Yassin, M.A., Moslem, M.A., El-Samawaty, A.E., El-Shikh, M.S., 2013. Effectiveness of Allium sativum in controlling sorghum grain molding fungi. J. Pure Appl. Microbiol. 7 (1), 101–107.
Znini, M., Cristofari, G., Majidi, L., Mazouz, H.A., Tomi, P., Paolini, J.U., Costa, J., 2011. Antifungal activity of essential oil from Asteriscus graveolens against postharvest phytopathogenic fungi in apples. Nat. Prod. Commun. 6 (11), 1763–1768.