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-molded 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...
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, 2008; Gende et al., 2008; Rathod et al., 2010; Yassin et al., 2010).

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) (Soysu 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 (Agil 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) and 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).

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 acetone: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:

Reduction in linear growth (%) = \((R1 - R2)/R1\) × 100

Table 1

| No. | Penicillium Species  | Source | Aumc No.* |
|-----|----------------------|--------|----------|
| 1   | *P. asperosporum*    | Apple  | 7965     |
| 2   | *P. aurinnotreus*    | Peanut | 5860     |
| 3   | *P. brevicompactum*  | Walnut | 7934     |
| 4   | *P. chermesinum*     | Popcorn| 5847     |
| 5   | *P. chrysogenum*     | Peanut | 5846     |
| 6   | *P. citrinum*        | Apple  | 7732     |
| 7   | *P. duchesnii*       | Corn   | 5965     |
| 8   | *P. expansum*        | Grape  | 7576     |
| 9   | *P. funiculosum*     | Corn   | 5966     |
| 10  | *P. griseofulvum*    | Sorghum| 5905     |
| 11  | *P. glatrum*         | 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).

Table 2

| 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 linear growth of *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. asperosporum* 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. fumiculosum* except 5% for ginger.

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**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 | 959.49 | 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. fumiculosum*                    |     |     |         |     |
| 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   | 8461.35 | 952.96 | 0.000 |
| Interaction (P * C)                    | 12  | 282.57 | 31.75 | 0.000 |
| Error                                  | 57  | 8.90 |
| 11- *P. glabricum*                     |     |     |         |     |
| 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*                       |     |     |         |     |

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

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**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**                     | 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**                    | 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 | | |

**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 |
|-------------------|----------------|---------------|------|
|                   | Cloves         | Control      | 90   |
|                   |               | 5%           | 85.5 |
|                   |               | 10%          | 71   |
|                   |               | 15%          | 63.75|
|                   |               | 20%          | 73   |
|                   | Cinnamon       | Control      | 90   |
|                   |               | 5%           | 61.5 |
|                   |               | 10%          | 53   |
|                   |               | 15%          | 32.75|
|                   |               | 20%          | 73   |
|                   | Turmeric       | Control      | 90   |
|                   |               | 5%           | 80.25|
|                   |               | 10%          | 73   |
|                   |               | 15%          | 63.5 |
|                   |               | 20%          | 46.25|
|                   | Ginger         | Control      | 90   |
|                   |               | 5%           | 90   |
|                   |               | 10%          | 83.75|
|                   |               | 15%          | 65.5 |
|                   |               | 20%          | 59.5 |
|                   | Mean           |              | 77.75|

**Table 5**

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. aurintogriseum* | Plant Extracts | Concentration | Mean |
|---------------------|----------------|---------------|------|
| Cloves              | Control        | 85            | 70   |
|                    | 5%             | 70            | 51   |
|                    | 10%            | 51            | 33.5 |
|                    | 15%            | 33.5          | 11.5 |
|                    | 20%            | 11.5          | 50.20|
| Cinnamon            | Control        | 85            | 73.5 |
|                    | 5%             | 73.5          | 53.25|
|                    | 10%            | 53.25         | 20.25|
|                    | 15%            | 20.25         | 111  |
|                    | 20%            | 111           | 48.60|
| Turmeric            | Control        | 85            | 71.5 |
|                    | 5%             | 71.5          | 68   |
|                    | 10%            | 68            | 61.5 |
|                    | 15%            | 61.5          | 44.75|
|                    | 20%            | 44.75         | 66.15|
| Ginger              | Control        | 85            | 84.25|
|                    | 5%             | 84.25         | 81.5 |
|                    | 10%            | 81.5          | 79.75|
|                    | 15%            | 79.75         | 77   |
|                    | 20%            | 77            | 81.50|
| Mean                |                | 7481          | 78.44|
| LSD for interaction | 3.8            | LSD for extract | 1.7 |
| LSD for Concentration | 1.9            | LSD for Concentration | 1.9 |

| *P. brevicompactum* | Extracts of | Concentration | Mean |
|---------------------|-------------|---------------|------|
| Cloves              | Control     | 90            | 76   |
|                    | 5%          | 64.75         | 54   |
|                    | 10%         | 64.75         | 12.25|
|                    | 15%         | 12.25         | 59.4 |
| Cinnamon            | Control     | 90            | 72.25|
|                    | 5%          | 72.25         | 54.75|
|                    | 10%         | 54.75         | 44.25|
|                    | 15%         | 44.25         | 22   |
|                    | 20%         | 22            | 56.65|
| Turmeric            | Control     | 90            | 65.75|
|                    | 5%          | 65.75         | 57.75|
|                    | 10%         | 57.75         | 45   |
|                    | 15%         | 45            | 42   |
|                    | 20%         | 42            | 60.1 |
| Ginger              | Control     | 90            | 84.75|
|                    | 5%          | 84.75         | 75.25|
|                    | 10%         | 75.25         | 70.75|
|                    | 15%         | 70.75         | 61   |
|                    | 20%         | 61            | 76.35|
| Mean                | 76.35       | 90            | 74.68|
| LSD for interaction | 4.06         | LSD for plant extract | 1.82 |
| LSD for Concentration | 2.03         | LSD for Concentration | 2.03 |

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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, *b*-elemene, curcumin, germacrone, curcumol in the extract of Turmeric (*Curcuma longa*). Which were effective against *Penicillium pallidum* and other fungi.

| 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 | Mean |

| **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 | Mean |

| **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 | Mean |

| 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** | Plant Extracts | Concentration | Control | 5% | 10% | 15% | 20% | Mean |
| | Cloves | 81.75 | 36.5 | 34.75 | 19.5 | 13.25 | 37.15 |
| | Cinnamon | 81.75 | 62.45 | 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 | Mean |

| **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 | Mean |

| **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 | Mean |

![Table 5](image-url)
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*.

| Plant Extracts | Concentration |
|----------------|---------------|
|                | Control | 5% | 10% | 15% | 20% | Mean |
| Cloves         | 62.75   | 52.25 | 45.75 | 36 | 9 | 38.65 |
| Cinnamon       | 72.25 | 53 | 44.25 | 37.25 | 22.25 | 25.37 |
| Turmeric       | 67 | 64 | 54 | 44.25 | 43.5 | 44.05 |
| Ginger         | 67 | 57.43 | 45.5 | 36.43 | 26.75 | 34.05 |

LSD for interaction = 4.18 LSD for plant extract = 1.87 LSD for Concentration = 2.09

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*.

| Plant Extracts | Concentration |
|----------------|---------------|
|                | Control | 5% | 10% | 15% | 20% | Mean |
| Cloves         | 58 | 45.5 | 45.25 | 30.25 | 30.25 | 40.85 |
| Cinnamon       | 67 | 64 | 54 | 44.25 | 43.5 | 54.45 |
| Turmeric       | 67 | 64 | 54 | 44.25 | 43.5 | 44.75 |
| Ginger         | 67 | 57.43 | 45.5 | 36.43 | 26.75 | 34.75 |

LSD for interaction = 3.97 LSD for plant extract = 1.77 LSD for Concentration = 1.98

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*.

| Plant Extracts | Concentration |
|----------------|---------------|
|                | Control | 5% | 10% | 15% | 20% | Mean |
| Cloves         | 63.5 | 47.75 | 42.75 | 37.25 | 22.25 | 33.85 |
| Cinnamon       | 67 | 64 | 54 | 44.25 | 43.5 | 44.05 |
| Turmeric       | 67 | 64 | 54 | 44.25 | 43.5 | 44.05 |
| Ginger         | 67 | 57.43 | 45.5 | 36.43 | 26.75 | 34.95 |

LSD for interaction = 4.0 LSD for plant extract = 1.79 LSD for Concentration = 2.0
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  | 36.45    | 68.6     | 78.30  | 57.68|
| 9 P. fusciformis  | 90.00   | 47.40  | 36.45    | 60.40    | 70.45  | 57.5 |
| 10 P. griseofulvum | 90.00  | 38.65  | 43.9     | 64.25    | 61.15  | 50.17|
| 11 P. glabrum     | 72.25   | 38.95  | 36.35    | 51.50    | 54.45  | 46.62|
| 12 P. implicatum  | 67.00   | 34.65  | 45.9     | 52.05    | 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

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   |         |     |    |

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 mycoxin analysis. A M E, SA, and MMH 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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