Fungicidal Activity of Some Metallic Ions, Fungicides and Essential Oils for Preventing Biodeterioration of Old Manuscripts

F. Sahab, Ahmed1*, M. Sidkey, Nagwa2, N. Abed, Nermine2 and Mounir, Ayah3

1Department of Plant Pathology, National Research Center, Giza, Egypt.
2Department of Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt.
3Conservation and Microfilm Centre, General Book Organization of Egypt, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author FSA designed the study and fungal identification and wrote the protocol, Author MSN wrote the manuscript and contributed to critical reading of the manuscript, Author NAN managed the literature searches. Author MA performed the laboratory work and the statistical analysis. All authors read and approved the final manuscript.

ABSTRACT

Aims: The main goal of this work to investigate the fungicidal activity of some metallic ions and essential oils which were to be applied as alternative protective of synthetic fungicides for old manuscripts and documents.

Place and Duration of Study: Sample: Studies were conducted at three floors (as indoor sampling sites) of the National Library and Archives of Dar El-Kottob, Egypt during one year, between November 2012 to October 2013.

Methodology: By using the Food Poisoned Technique 11 metallic ion, 5 fungicides and 5 essential oils were used to investigate their effective against the two selected fungal isolates Fusarium oxysporum and Trichoderma viride to evaluate these protective agents by measuring their effect on fungal mycelial (linear) growth and cellulolytic activity.

Results: The results revealed that of 11 metallic ions tested only metallic ions of CoCl2, FeSO4,
NiCl₂, CuSO₄ and ZnSO₄ at 100 mM were completely inhibited the linear growth of *F. oxysporum*, while the same concentration of CoCl₂, FeSO₄, FeCl₃ and NiCl₂ also completely inhibit the growth of *T. viride*. For cellulases activity, all metallic ions at different concentrations inhibit the activity, except CoCl₂, which increased the avicelase enzyme activity of *F. oxysporum*. Results for *T. viride* were little different than *F. oxysporum*. In vitro effect of fungicides, formaldehyde was found to be toxic to *F. oxysporum* and *T. viride* causing complete inhibition at all concentrations with percentage inhibition of 100%. Present study also indicated that, all tested essential oils were found to highly effective and gave 100% reduction in the growth of the two tested fungi at the higher concentration of 0.4%. The Anise essential oil was most effective against *F. oxysporum* and *T. viride* responsible for 91.80 and 100 mean % inhibition respectively followed by Rocket essential oil responsible for 82.90 and 89.97 mean % inhibition respectively.

**Conclusion:** Metallic ions (CH₃COOH)₂ Pb completely inhibited the linear growth of *F. oxysporum* and *T. viride* at low concentration (10 mM), as well as formaldehyde which gave percentage inhibition of 100% for both organisms. Anise essential followed by Rocket gave 100% reduction in the growth of the two tested fungi at the higher concentration of 0.4%.

**Keywords:** Metallic ions; fungicides; essential oils; linear growth; cellulolytic activity.

1. **INTRODUCTION**

Although several heavy metal ions are trace elements necessary for the growth of fungi, however at high concentrations they are toxic. The toxic effect of metals upon the growth and activity of microorganisms may result from the fact that metals can bind to various biomolecules by covalent and coordinate bonds. Metals may also un-specifically affect many cell structures and influence metabolic processes through a blockage of enzyme activity [1,2]. The ions of Fe, Mn at concentrations of 10 and 20 ppm did not cause decreased in the growth of *Trichoderma viride* [3]. Several researches reported that, 100% growth of *T. viride* up to the concentration of 3g/L of Cu (II) only 55% and 14% growth were observed at 4 and 5g/L of Cu (II) respectively. Also, the responses of *Trichoderma* isolates to zinc ions were connected with concentrations of this metal [4]. Zinc ions at 1000 and 3000 ppm inhibited the mycelial growth and spore germination of *Trichoderma*, while Zn at 16 ppm did not decrease the growth [3]. In addition, zinc apparently inhibited mycelial growth, whereas manganese ions stimulated spore germination of *T. viride* [5].

Additional work declared that, calcium chloride at concentrations of 1% and 2% gradually reduced mycelial growth of *F. solani* and *F. oxysporum* by 22.2% and 33.3%, respectively, whereas no further reductions were observed at a higher concentration (4%) [6]. On the other hand, some researchers found that Fe”, Ca” and Cu”; Mg” metallic ions had slight activation effect on cellulolytic activity of *F. vasinfectum* at 0.1 mM, while Mg” at higher concentrations (1.0 and 10.0 mM)) and Mn”, Zn” at all concentrations tried (0.1, 1.0 and 10.0 mM) had no effect on activation [7].

Endoglucanase activity of *Trichoderma viride* was stimulated slightly by Mg”, Co”, Fe”, Ca” and Zn”, and strongly by Co+2 and reached the highest level when activated by 75 mM of Mg+2, [8,9]. Cellulase activity of *T. harzianum* was greatly inhibited by Cu”, Ag+ and Hg+2 [10], while Fpase (Filter paperase) and CMCase (Carboxy Methyl Cellulase) enzymes were completely inhibited in the presence of 10 μM Hg+2. The inhibitory effect of Hg+2 on purified cellulases of *T. viride* were shown whereas; the enzymes were activated by Co” and Mn” at a concentration of 1 mM [11].

A fungicide is an agent or a chemical that kills the fungi. Therefore, it was necessary to use a less toxic biocide or scheme but with an equally efficient effect on microbiota [12]. Specialists in the laboratories of museums, libraries, and archives most often applied biocides used in other fields: medical disinfectants and chemical protection of plants [13].

Fumigation by formaldehyde at 1.5% has been used in the treatment of 8.1 million books in Russia, but it was restricted due to its toxicity and irritation effect [14]. He also reported that, thymol vapor was used extensively by many conservators using a chamber named “thymol cabinets.” This compound is no longer used because of its health hazard and deleterious effects on the objects. Rizolex and Topsin have been used successfully against *F. oxysporum* [15] and *Trichoderma* spp. [2,16]; they gave
complete inhibition in-vitro at 100 ppm for blocking mycelium growth and spore germination. Mancozeb (Dithane M-45) was the most effective chemical against \( F. \) oxysporum and \( F. \) solani, only at higher concentrations of 50 and 100 ppm [17]. On the other hand, mancozeb could not inhibit the growth \( T. \) viride [18]. Copper oxychloride showed weak inhibition against \( T. \) harzianum [19,20].

Great importance is given to essential oils by the industry and scientific research for their antifungal activity and safety which make them useful as natural preservatives [21,22,23,24]. The antifungal activity of essential oils of thyme, rosemary, lemongrass, armoise, clove, boldo, eucalyptus, ravensare, lavender, tea tree, thuya, wormseed and their main components were tested in the vapour phase [25,26,27].

Thus, the present study explore the in-vitro efficacy of some metallic ions, fungicides and essential oils for their antifungal and cellulases activities against \( F. \) oxysporum and \( T. \) viride isolated from deteriorated old documents.

2. METHODOLOGY

2.1 Fungal Culture

Through studies on fungal isolation involved on biodeterioration of ancient manuscripts, two isolates of fungi namely \( F. \) oxysporum and \( T. \) viride were isolated and identified [28]. The two fungal isolates were maintained on Czapek’s agar medium supplemented with 5% avicel and were further confirmed by the Department of plant pathology, NRC of Cairo, Egypt.

2.2 Antifungal Activity

Antifungal activity of microelements, fungicides, and essential oils were studied on the selected fungal isolates by mixing them with the culture using a contact assay method.

2.2.1 Effect of microelements on fungal mycelial (linear) growth

Sterilized PDA solid medium amended with serial concentrations of some metallic ions, as \( \text{MgSO}_4 \), \( \text{MnSO}_4 \), \( \text{CuSO}_4 \), \( \text{NiCl}_2 \), \( \text{ZnSO}_4 \), \( \text{CoCl}_2 \), \( \text{CaCl}_2 \), \( \text{FeSO}_4 \), \( \text{FeCl}_3 \), \( \text{NaCl} \) and \( (\text{CH}_3\text{COOH})_2\text{Pb} \) at concentrations of 10, 50, and 100 mM was tested. Microelements were added to the medium before autoclaving and the experiment was performed in triplicates. The linear growth was estimated until any plate was completely covered by fungal growth. The efficacy of the microelements was expressed as % of inhibition of mycelial growth over control, using formula by [29].

\[
\text{IP} (%) = \left[ \frac{(C - T)}{C} \right] \times 100
\]

Where:

\( \text{IP} \) = Inhibitory percentage (%).
\( C \) = Average colony diameter in Check (control).
\( T \) = Average colony diameter in treatment.

2.2.2 Effect of fungicides on fungal mycelial (linear) growth

The efficacy of each of five fungicides (Rizolex-T50, Topsin-M70, mancozeb-64%, Cuprosan 35% WP, and Formaldehyde) against selected fungal isolates was conducted with the method recommended for laboratory tests with fungicides [30]. Chemical formulas of these tested fungicides are given in Table 1.

Potato dextrose agar (PDA) molten medium was mixed with different fungicides at concentrations 0, 200, 400, 600, 800 and 1000 ppm of each, and a set of three replicates were used for each concentration. The radius of the mycelia growth in each concentration and the inhibition percentage was calculated as mentioned before.

2.2.3 Effect of essential oils on fungal mycelial (linear) growth

The efficacy of each of five essential oils (Anise, Fennel, Rosemary, Rocket and Tea tree oil) against the selected fungal isolates were evaluated using formula of [29]. Potato dextrose agar (PDA) molten medium was mixed with different essential oils at concentrations of 0, 0.05, 0.1, 0.2 and 0.4% (v/v) of each, and a set of three replicates were used for each concentration. The radius of the mycelia growth and the percentage of inhibition were calculated as mentioned before.

2.2.3.1 Preparation of essential oil

Seeds (250 g) of Anise (\textit{Pimpinella anisum}), Fennel (\textit{Foeniculum vulgare}) and Rocket (\textit{Eruca sativa}), and the leaves of Rosemary...
Table 1. Fungicides applied, its trade name, common name, and active ingredients

| Fungicide   | Trade Name | Common Name         | Active Ingredients                                      |
|-------------|------------|---------------------|---------------------------------------------------------|
| Rizolex-T50 | 20% Rizolex-T: tolclofos-(ethylyuro-o-dimethyl)-o-(2,6 dichloro-4-methylphenyl) o,odimethylphosphorothioate. 30% Thiram (TMDD): bis(dimethylthiocarbamoyl) disulphide. |
| Topsin-M70  | Dimethyl [(1, 2 phenylene) bis (iminocarbonothiole)] bis[carbonate]; dimethyl 4,4'-o-phenelenebis [3-thioaliphonate]. |
| Mancozeb 64%| Mancozeb [1, 2 Ethaznediybis (carbamodithio) (-2)] manganese zinc salt. Mancozeb is an ethylene bisdithiocarbamate protective fungicide which can inhibit pyruvic acid being oxidated so as to kill the fungi. |
| Cuprosan 35%wp | Copper oxychloride | Formaldehyde |
| Formalin 37% |                                      |

(Rosmarinus officinalis), and Tea tree (Melaleuca alternifolia) obtained from Department of Medicinal and Aromatic Plants Research, National Research Center, were subjected to hydrodistillation for 3 h using a Clevenger type apparatus to obtain essential oil according to [31]. Two layers were formed, upper organic layer of oil and lower aqueous layer of water. Lower aqueous layer was discarded and upper layer of oil was collected. The resulted essential oil of each treatment was separately dehydrated with anhydrous sodium sulfate [32] and preserved in a sealed vial at 4°C until further analysis of alkanes.

2.3 Statistical Analysis

The collected data were statistically computed using the software Mstate-c for Windows. Results were expressed with the standard error of the treatment means for 95% confidence limits.

3. RESULTS AND DISCUSSION

The two isolates of Fusarium oxysporum and Trichoderma viride isolated from deteriorative old documents in the storage area of National Library and Archives of Dar El-Kottob, Egypt were used for the experiment. The choice of Czapek's medium, pH and temperature were made from our earlier work as the most potent growth medium and environmental factors for the test in terms of radial growth rate.

3.1 Influence of Metallic Ions on F. oxysporum and T. viride

3.1.1 Influence of metallic ions on growth

The effects of thirteen aforementioned metal ions at different concentrations on the growth and cellulolytic activity of F. oxysporum and T. viride were evaluated. As seen there was a general trend to reduce the linear growth of the two fungi as the dose of metallic ions applied to medium increased, except MgSO₄, NaCl and CaCl₂ which showed the same linear growth of T. viride as control Table 2. Metallic ion of (CH₃COOH)₂Pb at low concentration (10 mM) proved in this concentration to be the most effective metallic ion as it completely stopped the linear growth of both fungi. Data also showed that, the growth rate of F. oxysporum significantly decreased at the higher level (100 mM) of CoCl₂, FeSO₄, NiCl₂, CuSO₄, FeCl₃ and ZnSO₄ metallic ions, while the growth of T. viride at the same concentration of CoCl₂, FeSO₄, FeCl₃ and NiCl₂ was also completely inhibited. The other metallic ions were less effective on the growth of both fungi at different concentrations. The results were in agreement with those recorded by many authors [33,34]. Similar results were also observed by [17,35] who emphasized that, Zn, Mn, and Cu as sulphate reduced the linear growth and sporulation of F. oxysporum and T. viride. Also, present results are greatly supported by [36,37] who stated that, although micronutrients are essential for microbes, however elevation of concentrations above certain threshold rendered them toxic to microorganisms.

3.1.2 Influence of metallic ions on cellulolytic activity

It was shown from Table 2 that avicelase activity of F. oxysporum was increased by adding NaCl at (10 mM), CoCl₂ at (100 mM), and MgSO₄ at (100 mM) more than the control. On the other hand, the addition of other used metal ions to the medium affected adversely the activity of avicelase produced by F. oxysporum. Concerning T. viride the same result was obtained for the same metal ions of CoCl₂ at (50 & 100 mM), CaCl₂ at (10 and 50 mM), NiCl₂ at (10 mM), and MnSO₄ at (10, 50 and 100 mM). It is clear also that all metallic ions added to the culture media of F. oxysporum and T. viride
affected drastically the CMCase activity in the culture filtrate, except, metallic ion of CoCl$_2$ at (100 mM) which increased the CMCase activity of T. viride more than the control. While, the effect of these metallic ions on Fpase was little different, as the Fpase activity of F. oxysporum was slow increased by adding CoCl$_2$ at all concentrations (10, 50 and 100 mM), MgSO$_4$ at (10 and 50 mM), CaCl$_2$ at (10 mM). On the other hand, data showed that, the addition of other used metal ions to the medium affected adversely the activity of Fpase. Concerning T. viride results showed that, there was an increase in the activity of Fpase in the filtrate more than the control by adding CaCl$_2$ and NaCl at (10 mM), MgSO$_4$ and MnSO$_4$ at (50 and 100 mM), while the other tested metals affected badly the activity of Fpase.

These results are in agreement with other scientific researchers, who stated that; the salts had been noticed directly related to metabolism, stimulation or inhibiting enzyme production in microorganism [38]. Previous studies detected an increase in the activity of cellulases of T. viride by adding cobalt up to 10 ppm and then declined as cobalt was further increased to 100 ppm [3].

The production of endoglucanase (CMCase) by F. oxysporum was enhanced in the presence of MgCl$_2$, CoCl$_2$, MnCl$_2$ and CaCl$_2$ metal cations at 10 mM, while inhibition was occur to some extent by FeCl$_2$ and to great extent by HgCl$_2$ [39].

The effect of balance between different metal ion concentrations could be more important than their individual effects [40].

### 3.2 Effect of Fungicides on the mycelial Growth

The effect of systemic and non-systemic fungicides (Topsin M70, Rizolex, Mancozeb and Cuprosan) and formaldehyde were evaluated for their efficacy on mycelial growth of F. oxysporum and T. viride by food poisoning technique. Based on the experiment it was found that the effect of fungicides upon growth depends on both the concentration and the fungal isolate (Tables 3 and 4).

#### 3.2.1 Effect on F. oxysporum mycelial (linear) Growth

The analysis of the obtained results revealed that formaldehyde caused complete inhibition to F. oxysporum at all concentrations with percentage inhibition of 100%, so it was considered the most effective fungicides (Table 3). The present results agreed with Mishchenko et al. [41] who stated that, formaldehyde was the most effective in suppressing growth of several fungi.

Generally, growth of tested fungi was reduced gradually as the fungicide concentration increased. Mancozeb and Topsin gave complete inhibition to F. oxysporum at concentrations of 200 ppm and 1000 ppm, respectively with percentage inhibition of 100%. While, Rizolex and Cuprosan applied to the medium significantly decreased the linear growth from 90 mm in control to 12.5 and 16.3 mm at 1000 ppm with percentage inhibition of 86.1% and 81.1%, respectively. The results of this investigation partially confirm those of previous authors [42,43,44].

The inhibitory effect of Cuprosan on F. oxysporum was also observed by many investigators [45,46]. As reported by Dłużniewska [2] fungicides containing copper ions block the enzyme activity in the energy processes.

#### 3.2.2 Effect on T. viride mycelial (linear) growth

The analysis of obtained results revealed that Topsin M 70% was considered the best fungicide causing complete reduction of mycelial growth at low concentration and Mancozeb at 800 ppm with percentage inhibition of 100% (Table 4). Rizolex and Cuprosan at higher concentration of 1000 ppm caused percentage inhibition of 72.2%, and 65% respectively. Almost, similar results were obtained by several investigators [2,47,19,48]. The differential response of T. viride to various fungicides in the present study might be due to their inherent resistance to the fungicides, and their ability to degrade these chemicals [48].

### 3.3 Antifungal Activity of Essential Oils

Essential oils were also evaluated in the laboratory for their inhibition of mold growth of the two tested fungi. The analysis of the obtained results revealed that there was a wide variation in the linear growth of F. oxysporum which ranged from 7.33 to 58 mm (Table 4 and Plate1) and between 0.0 to 75mm in T. viride (Table 4 and Plate 2) according to the essential oil and its concentration.
Table 2. Effect of different metallic ions on linear growth and cellulolytic activity of *F. oxysporum* and *T. viride*

| Metallic ions | Conc. (mM) | Linear growth (Ømm) | Cellulolytic activity (Ømm) | Filter paperase |
|---------------|-----------|----------------------|----------------------------|-----------------|
|               |           | *F. oxysporum* | *T. viride* | *F. oxysporum* | *T. viride* | *F. oxysporum* | *T. viride* | *F. oxysporum* | *T. viride* |
| Control       | 0.0       | 90.00 a           | 90.00a          | 17.00 fgghi     | 17.20 fghi   | 18.00 b         | 13.30 ghi     | 13.90 gh      |
| NaCl          | 10        | 64.00 I           | 90.00a          | 17.50 efgf      | 18.30 cd ef g| 15.50 d efghi   | 15.30 efg hi  | 10.00 n       | 14.00 g       |
| CaCl₂         | 50        | 68.00 ghi         | 90.00a          | 16.50 Hijk      | 15.80 j k l m| 13.00 m n o p   | 13.50 k l m n o| 10.00 n       | 14.00 g       |
|               | 100       | 81.75 b           | 90.00a          | 14.00 pqr s     | 15.20 k l m o| 12.00 q r s     | 13.20 m n o p q| 10.00 n       | 12.00 k l      |
| CaCl₂         | 10        | 58.00 j           | 90.00a          | 12.00 wxy       | 17.50 efg h  | 13.80 j k l m n| 15.80 c d e f g| 14.00 g       | 15.80 e f      |
|               | 50        | 75.00 e f         | 90.00a          | 12.50 u w x y   | 17.30 g h i k| 14.50 g h i k j| 13.70 j k l m n| 10.00 n       | 12.50 i j k    |
|               | 100       | 90.00 a           | 90.00a          | 13.00 t u v w x | 16.50 h i j k| 15.30 e f g h   | 12.80 n o p q r| 10.00 n       | 12.30 i j k    |
| CaCl₂         | 10        | 50.50 l m         | 76.00 de        | 15.00 m n o p   | 20.30 b      | 13.00 m n o p q r| 16.50 c d e    | 10.00 n       | 10.00 n       |
| MnSO₄         | 50        | 50.50 l m         | 22.00 stu       | 14.10 o p q r s t| 19.00 b c d  | 13.90 j k l m n| 16.00 c d e f | 10.00 n       | 17.00 c        |
|               | 100       | 43.25 no          | 21.00 tu        | 13.40 r s t u w v| 17.50 e f g h| 14.40 h i j k   | 15.50 d e f g h| 10.00 n       | 15.00 f        |
| MgSO₄         | 10        | 80.33 bc          | 90.00 a         | 18.30 c d e f g | 16.00 i k l m| 16.50 c d e    | 13.80 j k l m n| 16.00 d e      | 13.00 h i j    |
|               | 50        | 79.50 b c d       | 90.00 a         | 15.00 m n o p   | 16.50 h i j k| 15.00 f g h i j| 14.80 f g h i j| 14.00 g       | 16.00 d e      |
|               | 100       | 76.50 c d e       | 90.00 a         | 14.80 m n o p q r| 18.50 c d e f| 13.80 j k l m n| 16.00 c d e f  | 13.70 g h      | 10.00 n       |
| FeCl₃         | 10        | 32.0 q            | 56.5jk          | 11.80 x y       | 16.50 h i j k| 16.83 b c d    | 13.80 j k l m n| 0.00 o         | 0.00 o         |
|               | 50        | 26 rs             | 0.0 W           | 13.00 t u v w x | 14.30 p q r s t| 10.00 t       | 13.50 k l m n o| 0.00 o         | 0.00 o         |
| FeCl₃         | 100       | 0.0 W             | 0.0 W           | 14.0 p q r s t   | 13.50 q r s t u v| 10.00 t       | 12.50 o p q r s| 0.00 o         | 0.00 o         |
| FeCl₃         | 10        | 37.75 P           | 90.0 a          | 14.00 p q r s t | 16.00 i k l m| 14.00 i k l m n| 13.00 m n o p q r| 10.00 n       | 11.75 k l      |
| FeCl₃         | 50        | 0.0 w             | 23.4 r s t      | 13.50 q r s t u v| 15.50 k l m n o| 14.00 i k l m n| 12.73 n o p q r s| 10.00 n       | 10.80 m n      |
| CuSO₄         | 10        | 39.00 op          | 90 a            | 13.20 s t u v w | 13.5 q r s t u v| 13.00 m n o p q r| 12.80 n o p q r s| 10.00 n       | 10.00 n        |
| CuSO₄         | 50        | 27.00 r           | 90 a            | 12.90 t u v w x x| 13.50 q r s t u v| 12.50 o p q r s| 13.80 j k l m n o| 10.00 n       | 10.00 n        |
| NiCl₂         | 10        | 58.00 j           | 52.6 k l        | 13.00 t u v w x x| 17.70 d e f g h| 13.00 m n o p q r| 15.50 d e f g h| 10.00 n       | 11.80 k l      |
| Metallic ions | Conc. (mM) | Linear growth (Ømm) | Cellulolytic activity (Ømm) | Filter paperase (Ømm) |
|--------------|-----------|---------------------|-----------------------------|------------------------|
|              |           | F. oxysporum | T. viride | Avicelase | CMCase | F. oxysporum | T. viride | F. oxysporum | T. viride |
| ZnSO₄        | 50        | 0.0 w        | 18.0 U     | 13.50qrstuv | 14.50 opqrs | 15.00 fghij | 12.30 pqrs | 10.00 n     | 11.50 lm |
|              | 100       | 0.0 w        | 0.0 w      | 15.50klmno  | 12.30 wxwy  | 17.00 bc    | 11.50 s     | 10.00 n     | 10.80 mn |
|              | 10        | 58.00 J      | 52.6 kl    | 13.00 tuvwx | 17.70 defgh | 13.00 mnonpqr | 15.50 defgh | 10.00 n     | 11.80 kl |
|              | 50        | 12.50 V      | 38.5 P     | 14.50nopqrs | 13.00 tuvwx | 13.00 mnonpq | 12.50 opqrs | 0.00 w      | 10.00 N  |
|              | 100       | 0.0 W        | 17.8U      | 12.90tuvwxyz | 12.50 uwwxy | 13.00 mnonpq | 11.80 rs    | 0.00 w      | 10.00 N  |
| (CH₃COOH)₂Pb | 10        | 0.0 w        | 0.0 w      | 0.00 w      | 12.00 wxwy  | 0.00 w      | 12.50 opqrs | 0.00 w      | 0.00 w   |
|              | 50        | 0.0 w        | 0.0 w      | 0.00 w      | 11.50 y     | 0.00 w      | 12.50 opqrs | 0.00 w      | 0.00 w   |
|              | 100       | 0.0 w        | 0.0 w      | 0.00 w      | 11.50 y     | 0.00 w      | 12.50 opqrs | 0.00 w      | 0.00 w   |

- Each figure represents average of three replicates, incubated at 28±2°C for 9 days (solid) PDA medium.
- In each column, values followed by the same letters don't differ significantly (P ≥ 0.05) according to Duncan's multiple range test.

Table 3. Effect of different fungicides on the linear growth (mm) of *Fusarium oxysporum* and their percent inhibition on PDA medium

| Fungicides | Concentration (ppm) |
|------------|---------------------|
|            | 0.0 (Control) | 200 | 400 | 600 | 800 | 1000 | 400 | 600 | 800 | 1000 | 400 | 600 | 800 | 1000 |
|            | Ø mm | Inhibition % | Ø (mm) | Inhibition % | Ø (mm) | Inhibition % | Ø (mm) | Inhibition % | Ø (mm) | Inhibition % | Ø (mm) | Inhibition % | Ø (mm) | Inhibition % | Ø (mm) |
| Formaldehyde | 90A | 0 | 0M | 100 | 0M | 100 | 0M | 100 | 0M | 100 | 0M | 100 | 0M | 100 | 0M | 100 |
| Rizolex | 90A | 0 | 13.5KL | 85 | 13.2L | 85.3 | 13L | 85.5 | 13L | 85.5 | 12.5L | 86.1 |
| Topsin | 90A | 0 | 19.6GH1 | 78 | 17.8HIJ | 80.2 | 17IJ | 81.1 | 16.6J | 81.5 | 0M | 100 |
| Cuprosan | 90A | 0 | 53.8B | 40.2 | 22.4FG | 75.1 | 20.3GH | 77.4 | 18.3HIJ | 79.6 | 16.3JK | 81.8 |
| Mancozeb | 90A | 0 | 0M | 100 | 0M | 100 | 0M | 100 | 0M | 100 | 0M | 100 | 0M | 100 | 0M | 100 |

- Each figure represents average of three replicates, incubated at 28±2°C for 9 days (solid) PDA medium.
- In each column, values followed by the same letters don’t differ significantly (P ≥ 0.05) according to Duncan’s multiple range test.
Table 4. Effect of different fungicides on the linear growth (mm) of *Trichoderma viride* and their percent of inhibition on PDA medium

| Fungicides   | Concentration (ppm) | 0.0 (Control) Ø (mm) | Inhibition % Ø (mm) | 200 | Inhibition % Ø (mm) | 400 | Inhibition % Ø (mm) | 600 | Inhibition % Ø (mm) | 800 | Inhibition % Ø (mm) | 1000 | Inhibition % Ø (mm) |
|--------------|---------------------|----------------------|---------------------|-----|---------------------|-----|---------------------|-----|---------------------|-----|---------------------|------|---------------------|
| Formaldehyde | 90A                 | 0                    | 0M                  | 100 | 100                 | 0M  | 100                 | 0M  | 100                 | 0M  | 100                 | 0M   | 100                 |
| Rizolex      | 90A                 | 0                    | 35.4C               | 60.6| 34.2CD              | 62  | 28.5E               | 68.3| 28.4E               | 68.4| 25F                | 72.2 |
| Topsisn      | 90A                 | 0                    | 0M                  | 100 | 0M                  | 100 | 0M                  | 100 | 0M                  | 100 | 0M                  | 100  |
| Cuprosan     | 90A                 | 0                    | 90A                 | 0   | 90A                 | 0   | 90A                 | 0   | 35.4C               | 60.6| 31.5D               | 65   |
| Mancozeb     | 90A                 | 0                    | 90A                 | 0   | 20GHI               | 77.8| 13L                 | 85.6| 0M                  | 100 | 0M                  | 100  |

- Each figure represents average of three replicates, incubated at 28±2°C for 9 days (solid) PDA medium.

- In each column, values followed by the same letters don't differ significantly (P ≥ 0.05) according to Duncan’s multiple range test.

Table 5. Effect of different essential oils on the linear growth (mm) of *Fusarium oxysporum* and *Trichoderma viride* on PDA medium

| Essential oil   | *Fusarium oxysporum* Concentration (%) | *Trichoderma viride* Concentration (%) |
|-----------------|----------------------------------------|----------------------------------------|
|                 | 0.0 (Control) 0.05 0.1 0.2             | 0.0 (Control) 0.05 0.1 0.2             |
| Rosemary        | 90A 58DE 46FG 38HI                     | 90A 74.00BC 66.00CD 24JKL              |
| Fennel          | 90A 40GHI 35.33I 26J                    | 90A 63.00D 45.33FGH 20JKLMNO           |
| Anise           | 90A 34.33I 16.00LMNOP 7.33QR            | 90A 75.00B 48.00FG 0.00R               |
| Rocket          | 90A 23.33JKLM 22JKLM 15MNOQ             | 90A 16.33LMNOP 15.00MOPQ 9.00PQ        |
| Tea tree oil    | 90A 25JK 22.33JKLM 17.33KLNO           | 90A 52.33EF 38.00HI 13.33OPQ          |

- Each figure represents average of three replicates, incubated at 28±2°C for 9 days (solid) PDA medium.

- In each column, values followed by the same letters don’t differ significantly (P ≥ 0.05) according to Duncan’s multiple range test.
Anise and Rocket showed the highest effect on the linear growth of *F. oxysporum*, as the linear growth were 34.33, 16.00 and 7.33 mm at 0.05, 0.1 and 0.2% respectively for Anise and were 23.33, 22.0 and 15.0 mm respectively for Rocket. The same trend was also observed on the effect of Anise and Rocket on *T. viride*, although the higher concentration (0.2%) of the two essential oils caused complete inhibition to linear growth of *T. viride*, these results are conformity with the previous studies [28,49,50,51]. Anethole was found in anise as the main compound, and this compound has more fungicidal effect, the same results were also reported before [52-60]. Besides, Shukla and Tripathi [61] reported that *trans*-anethole from anise essential oil was found to be responsible for its antifungal activity. Also, Sabry [51] and Khoobchandani et al. [62] reported that, erucin followed by Carvacrol and Thymol were the main components of rocket essential oil as erucin accounted for approximately 78.69% of the rocket extracts.
which play an important role as an antifungal agent. Previous studies have highlighted rocket as a rich source of glucosinolate compounds which have a great role in the antimicrobial activity [63].

In contrast to Saleem et al. [64] who reported that, essential oil of anise show moderate and weak inhibition effect against T. viride.

It is well known that, essential oils have antimicrobial properties [25,65]. Antimicrobial activity of these oils can be attributed to the presence of an aromatic nucleus and a phenolic OH group that are known to be reactive and can form hydrogen bonds with –SH groups in the active sites of target enzymes, resulting in deactivation of enzymes in fungi [66,67]. They thought it may be the result of phenolic compounds of essential oils that cause an altering of microbial cell permeability by interaction with membrane proteins. This would cause a deformation in cell membrane and functionality, and permit the loss of macromolecules from their interior [68,69].

The Tea tree essential oil comes at the third level against the growth of the two fungi as the linear growth of F. oxysporum and T. viride were 17.33 and 13.33mm at the higher concentration (0.2%) respectively. The antifungal activity of tea tree oil had been reported by many authors [28,70]. Contrary with Yang et al. [25] who reported that, Tea tree vapors did not inhibit the growth of mold fungi (Aspergillus niger, Trichoderma viride, and Penicillium chrysogenum).

Present work showed that Rosemary and Fennel have moderate effect on the growth of F. oxysporum and T. viride, as the linear growth of F. oxysporum were 38.0 and 26.0 mm at concentration of 0.2% respectively and were 24.0 and 20 mm for T. viride at the same concentration respectively. Similarly, the antifungal effects of Rosmarinus officinalis oil can be attributed to the monoterpenes combination and in particular α-pinene whose antifungal effects of this combination has been proved by Okamura et al. [71,72]. Rosemary vapor was found by other workers to be inhibiting in-vitro against T. viride and P. chrysogenum for 12 weeks. These findings suggest that ketone volatilization may play a role in preventing spore germination for rosemary oil [25].

4. CONCLUSION

The main goal of this work to investigate the fungicidal activity of different microelements (metallic ions), commercial fungicides and essential oils. Present study revealed (CH3COOH)\textsubscript{2}Pb, completely inhibited the linear growth of F. oxysporum and T. viride at low concentration (10 mM). The present study also detected that Formaldehyde followed by Mancozeb were the most effective fungicides inhibiting the growth of the two fungi at low concentration (200 ppm). Regarding to essential oils the study noted that, Anise and Rocket oils showed the highest effect on the linear growth of F. oxysporum and T. viride.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Badura L, Piotrowska-Seget Z. Heavy metals in the environment and their impact on soil microorganisms. Chem Inż Ekol. 2000;7(11):1135-1142.
2. Dłużniewska J. Reaction of fungi of Trichoderma genus to selected abiotic factors. Electronic Journal of Polish Agricultural Universities (EJPAU). 2003; 6(2):1-15.
3. Mandels M, Reese ET. Induction of cellulase in Trichoderma viride as influenced by carbon source and metals. Journal of Bacteriology. 1957;73(2):269-278.
4. Anand P, Isar J, Savan S, Saxena PK. Bioaccumulation of Copper by Trichoderma viride. Bioresour. Technol. 2006;97:1018-1025.
5. Sierota Z. Wpływ niektórych soli mineralnych na rozwój Trichoderma viride Pers. ex Fr. in vitro [Effect of some mineral salts on Trichoderma viride Pers. ex Fr. development in vitro]. Prace Inst Bad Leśnictwa. 1982;611:67-78. [Polish].
6. Abdel-Kader MM, El-Mougy NS, Embaby EI, Lashin SM. Occurrence of sclerotinia foliage blight disease of cucumber and pepper plants under protected cultivation system in Egypt I. Chemical and Biological Control Measures in vitro. Advances in Life Sciences. 2012;2(1):20-27.
7. Sampathnarayanan A, Shanmugasundaram RB. Studies on cellulase of the cotton wilt pathogen Fusarium vasinfectum atk. Mycopathologia et Mycologia applicata. 1970;41(3-4):223-232.
8. Huang X, Ge J, Fan J, Chen X, Xu X, Li J, et al. Characterization and optimization of xylanase and endoglucanase production by *Trichoderma viride* using response surface methodology (RSM). African Journal of Microbiology Research. 2013; 7(36):4521-4532.

9. Kandari V, Vajpayee I, Kumar D, Gupta S. Cellulase and β-Glucosidase production by *Trichoderma viride* and *Aspergillus wentii* in Sub-Merged Fermentation Utilizing. International J of Science and Research. 2013;3(11):2122-2128.

10. Sidhu MS, Kalra MK, Sandhu DK. Purification and characterization of cellulolytic enzymes from *Trichoderma harzianum*. Folia Microbiol. 1986;31:293-302.

11. Halliwell G, Griffin M. The nature and mode of action of the cellulolytic component of *Trichoderma koningii* on native cellulose. Biochem J. 1973;135:587.

12. Dobroussina SA, Velikova TD. Mass disinfection of documents affected by microorganisms: One practical experience. 65th IFLA Council and General Conference, Bangkok; 1999.

13. Velikova T, Trepova E, Rozen T. The use of biocides for the protection of library documents: Before and now. Science against Microbial Pathogens: Communicating Current Research and Technological Advances. 2011;152-159.

14. Nittérus M. Fungi in archives and libraries, a literary survey. Restaurator. 2000;21:25-40.

15. Rashid MT. Studies on damping-off and root-rot diseases of green bean. MSc. Thesis In Agric. Sci Dept of Plant Pathology. Fac of Agr, Cairo Univ, Egypt; 2012.

16. Muhammad AA. Variability in *Fusarium oxysporum* f. sp. *ciceris* for Chickpea Wilt resistance in Pakistan. PhD. Thesis in Microbiology, Dept. of Microbiol, Fac of Biol Sci, Quaid-i-Azam Univ, Islamabad, Pakistan; 2010.

17. Hyakumachi M, Hassan N, Elsharkawy MM, Shimizu M. Control of root rot and wilt diseases of roselle under field conditions. Mycobiology. 2014;42(4):376-384.

18. Sobha RI, Dorcas M. Seed mycoflora associated with ragi (*Eleusine coracana* L.) earthin. (J of Innovations in Pharmaceuticals and Biological Sci.) JIPBS. 2016;3(2):1-6.

19. Tapwal A, Kumar S, Gautam N, Pandey S. Compatibility of *Trichoderma viride* for selected fungicides and botanicals. International Journal of Plant Pathology. 2012;3:89-94.

20. Veena GA, Eswara RNP, Bhasakara RBV, Prasanthi L. Potential of *Trichoderma* spp. as biocontrol agents against *Rhizoctonia bataticola* causing dry root rot of chickpea. Int J of Plant, Animal and Environmental Sci. 2014;4(1):78-81.

21. Dung NT, Kim JM, Kang SC. Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr and Perry buds. Food Chem Toxicol. 2008;(46):3632–3639.

22. Gachkar L, Yadegari D, Rezaei MB, Taghizadeh M, Astanee SA, Rasooli I. Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chem. 2007;102(3):898-904.

23. Matan N, Matan N. Antifungal activities of anise oil, lime oil, and tangerine oil against molds on rubber wood (*Hevea brasiliensis*). Int Biodeterio Biodegrad. 2008;62:75-78.

24. Siripornvisal S, Rungprom W, Sawatdikarn S. Antifungal activity of essential oils derived from some medicinal plants against grey mould (*Botrytis cinerea*). As J Food Ag-Ind. 2009;229-233.

25. Yang VW, Clausen CA. Inhibitory effect of essential oils on decay fungi and mold growth on wood. Proceedings for American Wood Protection Society, Birmingham, AL. 2008;103:62-70.

26. Rakotonirainy MS, Lavedrine B. Screening for antifungal activity of essential oils and related compounds to control the biocontamination in libraries and archives storage areas. International Biodeterioration and Biodegradation. 2005; 55:141–147.

27. Rakotonirainy M, Heude E, Lavedrine B. Isolation and attempts of biomolecular characterization of fungal strains associated to foxing on a 19th century book. Journal of Cultural Heritage. 2007; 8(2):126-133.

28. Sahab AF, Sidkey NM, Abed NN, Mounir A. Studies on indoor air quality in the repositories of the national library and archives of Egypt. International Journal of
Science and Research (IJSR). 2014; 3(11): 2122-2128.

29. Farzaneh M, Hadian J, Peighami S, Sharifi TA, Ghorbanoop M. Evaluation of antifungal activity of some plant essential oils against the grey mold of apple caused by Botrytis cinerea. Agric Res: Water, Soil, Plant Agric. 2007;7(3):1-10.

30. Wójdyla AT. Chemical control of Fusarium avenaceum 9 Cda ex Fr. Sacc on carnations. 1- Effectiveness of fungicides in-vitro and their use for stem protection against Fusarium avenaceum. Roczniki Nauk Rolniczych E. 1993;23:35-40.

31. General Organization for Governmental Printing Office, Ministry of Health, Cairo, Egypt, Egyptian Pharmacopoeia. 1984;31-33.

32. Ozcan MM, Chalchat JC. Effect of collection time on chemical composition of the essential oil of Foeniculum vulgare sub sp. Piperitum growing wild in Turkey. Eur Food Res Technol. 2006;224:279-281.

33. Barajas-Aceves M, Grace C, Ansorena J, Dendooven L, Brookes PC. Soil microbial biomass and organic C in a gradient of Zinc concentrations in soils around a mine spoil tip. Soil Biology and Biochemistry. 1999;31(6):867–876.

34. Pečiuliytė D, Dirginčiutė-Volokdišienė V. Effect of zinc and copper on cultivable populations of soil fungi with special reference to entomopathogenic fungi. EKOLOGIJA. 2012;58(2):65–85.

35. Vaidilutė DV, Dalė P. Increased soil heavy metal concentrations affect the structure of soil fungus community. Agriculturae Conspectus Scientificus. 2011;76(1):27-33.

36. Aelion CM, Davis HT, McDermott S, Lawson AB. Soil metal concentrations and toxicity: Associations with distances to industrial facilities and implications for human health. Science of the Total Environment. 2009;407(22):2216-2223.

37. Hartikainen ES, Lankinen P, Rajasärkkä J, Koponen H, Virta M, Hatakka A, et al. Impact of copper and zinc on the growth of saprotrophic fungi and the production of extracellular enzymes. Boreal Environ Res. 2012;17:210-218.

38. Viniegra G, Favela E, Aguilar C, Romero S, Díaz G, Augur C. Advantages of fungal enzyme production in solid state over liquid fermentation systems. Biochem Eng J. 2003;13(2-3):157-167.

39. Dar RA, Saba I, Shahnawaz M, Sangale MK, Ade AB, Rather SA, et al. Isolation, purification and characterization of carboxymethyl cellulase (CMCase) from endophytic Fusarium oxysporum producing podophyllotoxin. Advances in Enzyme Research. 2013;1(4):91-96.

40. Mandels M, Reese ET. Fungal cellulases and the microbial decomposition of cellulosic fabric. J Ind Microbiol Biotechnol. 1999:22:225-240.

41. Mishchenko VN, Osipov VV, Prokopenko SL, Serenko EG. Physicochemical methods of preservation and treatment of archive documents/ Libraries of the National Academies of Sciences: Functioning problems, progress tendencies). Kiev. 2005;(3)3-20. (Russian).

42. Gehad MM, Saida MA. Application of salicylic acid and some fungicides as seed treatment for controlling damping-off and root-rot diseases of squash and cantaloupe plants under field conditions. J Plant Prot and Path. Mansoura Univ. 2014; 5(12):1025-1043.

43. Hefnawy MA, Omima AE, Nora ME. Impact of the fungicide rizolix T50% on the antagonistic activity of Trichoderma harzianum and Trichoderma koningii. International Journal of Science and Research (IJSR). 2014;3(9):1767-1770.

44. Hagagg HEK, Elshahawy IE, Abd-El-Khair H. Antagonistic activity of Bacillus and Pseudomonas isolates alone or in combination with fungicides against some soil borne plant pathogens under laboratory and greenhouse conditions. Middle-East Journal of Scientific Research. 2015;23(10):2354-2365.

45. Arunodhayam K, Eswara RNP, Bhasakara RBV. Isolation and identification of fungicidal compatible antagonist against Fusarium oxysporum f. sp. ciceris inciting wilt of chickpea. Current Biotica. 2015; 9(1):36-44.

46. Kumhar KC, Babu A, Bordoloi M, Banerjee P, Dey T. Biological and chemical control of Fusarium solani, causing dieback disease of tea Camellia sinensis (L): An in-vitro study. Int J Curr Microbiol App Sci. 2015;4(8):955-963.

47. Gupta A, Kerni PN, Gupta A. In-vitro evaluation of different chemicals against T. viride isolated from button mushroom. Res. Dev. Rep. 1995;12:44-47.

48. Vasundara P, Rangaswamy V, Johnson M. Compatibility studies with fungicides, insecticides and their combinations on Trichoderma viride in-vitro conditions.
International Journal of Scientific & Engineering Research. 2015;6(2).

49. Ross SA, El-Keltawi NE, Megalla SE. Antimicrobial activity of some Egyptian aromatic plants. Fitoterapia. 1980;51:201-205.

50. Singh G, Maurya S, de Lampasona MP, Catalán C. Chemical constituents, antimicrobial investigations and antioxidative potential of volatile oil and acetone extract of star anise fruits. J Sci Food Agric. 2006;86(1):111-121.

51. Sabry BA. Evaluation of some plant extracts as antioxidants and protectors against the harmful effects of aflatoxins on albino rat. PhD. Thesis. Fac of Sci, Benha Univ, Egypt; 2011.

52. Bas'er KHC, Özek T. Essential oil of pimpinella aromatic Bieb. from Turkey. J Essent Oil Res. 1996;8:463-464.

53. Askari F, Sefidkon F, Mirza M. Quantitative and qualitative study of components in essential oil of Pimpinella anisum L. Journal of Pajouhes –Va- Sazandegi. 1998;38:70-77.

54. Hansel R, Sticher O. Steinegger E. Pharmacokognosie-Phytopharmazie. 6th ed. Springer-Verlag, Berlin.1999;692-695.

55. Omidbaigi R, Hadjiakhoondi A, Saharkhiz M. Changes in content and chemical composition of Pimpinella anisum oil at various harvest time. J Essent Oil Bearing Plants. 2003;6(1):46-50.

56. Waumans D, Bruneel N, Tytgat J. Anise Oil as a precursor for 2-alkoxy-5-methoxybenzaldehydes. Microgram Journal. 2004;2(1-4):4-10.

57. Kosalec I, Pepeljnjak S, Kustrak D. Antifungal activity of fluid extract and essential oil from anise fruits (Pimpinella anisum L., apiaceae). Acta Pharmaceut. 2005;55(4):377-385.

58. Tuncturk M, Yildirim B. Effect of seed rates on yield and yield components of anise (Pimpinella anisum). Indian J Agric Sci. 2006;76(11):679-681.

59. Takayuki S, Mami S, Azizi M, Yoshiharu F. Antifungal effects of volatile compounds from black zira (Bunium persicum) and other spices and herbs. Journal Chemistry Ecology. 2007;33:2123-2132.

60. Orav A, Raal A, Arak E. Essential oil composition of Pimpinella anisum L. fruits from various European countries. Natural product Res. 2008;22(3):227-232.

61. Shukla HS, Tripathi SC. Antifungal substance in the essential oil of anise (Pimpinella anisum L.). Agric Biol Chem. 1987;51:1991-1993.

62. Khooobchandani M, Ojeswi K, Ganesh N, Srivastava M, Gabbanini S, Matera R, et al. Antimicrobial properties and analytical profile of traditional Eruca sativa seed oil: Comparison with various aerial and root plant extracts. Food Chem. 2010;120:217–224.

63. Kim SJ, Jin S, Ishii G. Isolation and structural elucidation of 4-(beta-d-glucopyranosylsulfanyl) butyl glucosinolate from leaves of rocket salad (Eruca sativa L.) and its antioxidative activity, Biosci Biotechnol Biochem. 2004;68:2444-2450.

64. Saleem A, El-Said AHM, Moharram AM, Hamed A. Cellulose decomposing fungi and cellulase activity as affected by amistar and moncut fungicides. Afr J Microbiol Res. 2012;6(21):4457-4470.

65. Ziani M, Mortabit D, El Abed S, Remmal A, Koraichi SI. Antifungal activity of five plant essential oils against wood decay fungi isolated from an old house at the medina of Fez. Int Res J. of Microbiology. 2011;2(3):104-108.

66. Velluti A, Sanchis V, Ramos AJ, Egdio J, Marín S. Inhibitory effect of cinnamon, clove, lemon grass, oregano and palmarose essential oils on growth and fusonisin B1 production by Fusarium proliferatum in maize grain. J Food Microbiol. 2003;89:145-154.

67. Alam MS, Kaur G, Jabbar Z, Javed K, Athar M. Eruca sativa seeds possess antioxidant activity and exert a protective effect on mercuric chloride induced renal toxicity. Food Chem Toxicol. 2007;45(6):910–920.

68. Rattanapitigorn P, Arakawa M, Tsuro M. Vanillin enhances the antifungal effect of plant essential oils against Botrytis cinerea. The Int J of Aromatherapy. 2006;16:193-198.

69. Pramila DM, Xavier R, Marimuthu K, Kathiresan S, Khoo ML, Senthilkumar M, et al. Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (Mentha piperita). J of Medicinal Plants Res. 2012;6:331–335.

70. Martins JAS, Sagata E, Santos VA, Juliatti FC. Evaluation of the effect of Melaleuca alternifolia oil on mycelial growth of
71. Okamura N, Haraguchi H, Hashimoto K, Yagi A. Flavonoids in *Rosmarinus officinalis* leaves. Phytochem. 1994; 37(5):1463-1466.

72. Moghtader M, Salari H, Farahmand A. Evaluation of the antifungal effects of rosemary oil and comparison with synthetic borneol and fungicide on the growth of *Aspergillus flavus*. J Ecol Natural Environ. 2011;3(6):210-214.