Journal of Advances in Microbiology

3(2): 1-14, 2017; Article no.JAMB.33853
ISSN: 2456-7116

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

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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.

Article Information

DOI: 10.9734/JAMB/2017/33853
Editor(s):
(1) Ana Claudia Correia Coelho, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Mugdha Ambatkar, Ramniranjan Jhunjhunwala College, Mumbai, India.
(2) Daniela Hanganu, "Iuliu Hatieganu"University of Medicine and Pharmacy, Romania.
Complete Peer review History: http://www.sciencedomain.org/review-history/19274

Original Research Article

Received 1st May 2017
Accepted 19th May 2017
Published 31st May 2017

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 $CoCl_2$, $FeSO_4$,

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$NiCl_2$, $CuSo_4$ and $ZnSO_4$ at 100 mM were completely inhibited the linear growth of *F. oxysporum*, while the same concentration of $CoCl_2$, $FeSO_4$, $FeCl_3$ and $NiCl_2$ also completely inhibit the growth of *T. viride*. For cellulases activity, all metallic ions at different concentrations inhibit the activity, except $CoCl_2$, 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^{+2}, Co^{+2}, Fe^{+3}, Ca^{+2} and Zn^{+2}, 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^{+2}, 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^{+2} and Mn^{+2} 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 *Trichoderma* spp, as *T. viride* and *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 *Fusarium oxysporum* and *Trichoderma 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 $MgSo_4$, $MnSO_4$, $CuSO_4$, $NiCl_2$, $ZnSO_4$, $CoCl_2$, $CaCl_2$, $FeSO_4$, $FeCl_3$, NaCl and $(CH3COOH)_2Pb$ 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].

$$IP\ (\%) = [(C - T) / C] \times 100$$

Where:

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 (*Pimpinella anisum*), Fennel (*Foeniculum vulgare*) and Rocket (*Eruca sativa*), and the leaves of Rosemary

Table 1. Fungicides applied, its trade name, common name, and active ingredients

Rizolex-T50	20%Rizolex-T: tolclofos-(ethyluro-o-dimethyl)-o-(2,6dichloro-4-methylphenyl) o,odimethylphosphorothioate. 30%Thiram (TMTD):bis(dimethylthiocarbamoyl)disulphide.
Topsin-M70	Dimethyl [(1, 2 phenylene) bis (iminocarbonothiole)] bis[carbonate]; dimethyl 4,4'-o-phynelenebis [3-thioallophanate].
Mancozeb 64%	Mancozeb [1, 2Ethaznediybis (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
Formalin 37%	Formaldehyde

(*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 $MgSO_4$, NaCl and $CaCl_2$ which showed the same linear growth of *T. viride* as control Table 2. Metallic ion of $(CH_3COOH)_2Pb$ 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 $CoCl_2$, $FeSO_4$, $NiCl_2$, $CuSO_4$, $FeCl_3$ and $ZnSO_4$ metallic ions, while the growth of *T. viride* at the same concentration of $CoCl_2$, $FeSO_4$, $FeCl_3$ and $NiCl_2$ 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), $CoCl_2$ at (100 mM), and $MgSO_4$ 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 $CoCl_2$ at (50 & 100 mM), $CaCl_2$ at (10 and 50 mM), $NiCl_2$ at (10 mM), and $MnSO_4$ 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 86.1and 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)					
				Avicel$_{ase}$		CMC$_{ase}$		Filter paper$_{ase}$	
		F. oxysporum	*T. viride*	*F. oxysporum*	*T. viride*	*F. oxysporum*	*T. viride*	*F. oxysporum*	*T. viride*
Control	0.0	90.00 a	90.00a	17.00fghij	17.20 fghi	18.00b	18.00 b	13.30ghi	13.90gh
NaCl	10	64.00l	90.00a	17.50efgh	18.30 cdefg	15.50defgh	15.30efghi	10.00 n	14.00 g
	50	68.00 ghi	90.00a	16.500Hijk	15.80jklmn	13.00mnop	13.50klmnop	10.00n	14.00g
	100	81.75b	90.00a	14.00pqrs	15.20klmno	12.00 qrs	13.20lmnopq	10.00 n	12.00kl
$CaCl_2$	10	58.00j	90.00a	12.00wxy	17.50efgh	13.80jklmn	15.80cdefg	14.00g	15.80ef
	50	75.00ef	90.00a	12.50 uvwxy	17.30fghi	14.50ghijkl	13.70jklmno	10.00n	12.50ijk
	100	90.00a	90.00a	13.00 tuvwx	16.50hijk	15.30efghi	12.80nopqrs	10.00n	12.30ijk
$CoCl_2$	10	90.00a	51.00lm	13.50 qrstuv	16.30hijkl	11.50 s	13.30lmnopq	14.00g	10.00n
	50	26.37r	47.50mn	13.50qrstuv	19.50bc	12.00 qrs	16.50cde	13.70gh	10.00n
	100	0.00W	0.00W	18.80cde	24.00a	18.00b	20.50a	13.50gh	10.00n
$MnSO_4$	10	71.50 fg	76.00 de	15.00lmnop	20.30b	13.00 mnopqr	16.50 cde	10.00 n	10.00 n
	50	50.50 lm	22.00 stu	14.10opqrst	19.00bcd	13.90jklmn	16.00cdef	10.00 n	17.00 c
	100	43.25 no	21.00 tu	13.40rstuvw	17.50 efgh	14.40 hijkl	15.50 defgh	10.00 n	15.00 f
$MgSO_4$	10	80.33bc	90.00 a	18.30 cdefg	16.00 ijklm	16.50 cde	13.80 jklmno	16.00 de	13.00 hij
	50	79.50 bcd	90.00a	15.00lmnop	16.50 hijk	15.00 fghij	14.80 fghijk	14.00 g	16.00 de
	100	76.50 cde	90.00 a	14.80mnopqr	18.50 cdef	13.80 jklmno	16.00 cdef	12.00 kl	17.30c
$FeCl_3$	10	32.0 q	56.5jk	11.80 xy	16.50 hijk	16.83 bcd	13.80 jklmno	0.00 o	0.00 o
	50	26 rs	0.0 W	13.00 tuvwx	14.30pqrst	10.00 t	13.5 klmnop	0.00 o	0.00 o
	100	0.0 W	0.0 W	14.0pqrst	13.50qrstuv	10.00 t	12.50 opqrs	0.00 o	0.00 o
$FeSO_4$	10	37.75 P	90.0a	14.00 pqrst	16.00 ijklm	14.00 ijklmn	13.00 mnopqr	10.00 n	11.75 kl
	50	0.0 w	23.4rst	13.50qrstuv	15.50 klmno	14.00 ijklmn	12.73 nopqrs	10.00 n	10.80mn
	100	0.0 w	0.0 w	13.50qrstuv	13.30 stuvw	13.60 klmnop	12.00 qrs	10.00 n	10.30n
$CuSO_4$	10	39.00 op	90 a	13.20stuvw	13.5 qrstuv	13.00mnopqr	12.80 nopqrs	10.00 n	10.00 n
	50	27.00 r	90 a	12.90tuvwxy	13.50qrstuv	12.50 opqrs	13.80 jklmno	10.00 n	10.00 n
	100	0.0 w	23.0 rst	12.30vwxy	13.50qrstuv	12.50 opqrs	14.0 ijklmno	10.00 n	10.00 n
$NiCl_2$	10	58.00 j	52.6 kl	13.00tuvwx	17.70 defgh	13.00 mnopqr	15.50 defgh	10.00 n	11.80 kl

Metallic ions	Conc. (mM)	Linear growth (Ømm)		Cellulolytic activity (Ømm)					
		F. oxysporum	*T. viride*	Avicel$_{ase}$		CMC$_{ase}$		Filter paper$_{ase}$	
				F. oxysporum	*T. viride*	*F. oxysporum*	*T. viride*	*F. oxysporum*	*T. viride*
	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 vwxy	17.00 bc	11.50 s	10.00 n	10.80 mn
$ZnSO_4$	10	58.00 J	52.6 kl	13.00 tuvwx	17.70 defgh	13.00 mnopqr	15.50 defgh	10.00 n	11.80 kl
	50	12.50 V	38.5 P	14.50nopqrs	13.00 tuvwx	13.00 mnopq	12.50 opqrs	0.00 w	10.00 N
	100	0.0 W	17.8U	12.90tuvwxy	12.50 uvwxy	13.00 mnopq	11.80 rs	0.00 w	10.00 N
$(CH_3COOH)_2$ Pb	10	0.0 w	0.0 w	0.00 w	12.00 wxy	0.00 w	12.50 opqr	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 9days (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	
	Ø mm)	Inhibition %	Ø (mm)	Inhibition %	Ø (mm)	Inhibition %	Ø (mm)	Inhibition %	Ø (mm)	Inhibition %	Ø (mm)	Inhibition %
Formaldehyde	90A	0	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	19.6GHI	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

-Each figure represents average of three replicates, incubated at 28±2°C for 9days (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)		200		400		600		800		1000	
	Ø (mm)	Inhibition %	Ø (mm)	Inhibition %	Ø (mm)	Inhibition %	Ø (mm)	Inhibition %	Ø (mm)	Inhibition %	Ø (mm)	Inhibition %
Formaldehyde	90A	0	0M	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
Topsin	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 9days (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*				*Trichoderma viride*			
	Concentration (%)				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	15MNOPQ	90A	16.33LMNOP	15.00NOPQ	9.00PQ
Tea tree oil	90A	25JK	22.33JKLMN	17.33KLMNO	90A	52.33EF	38.00HI	13.33OPQ

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

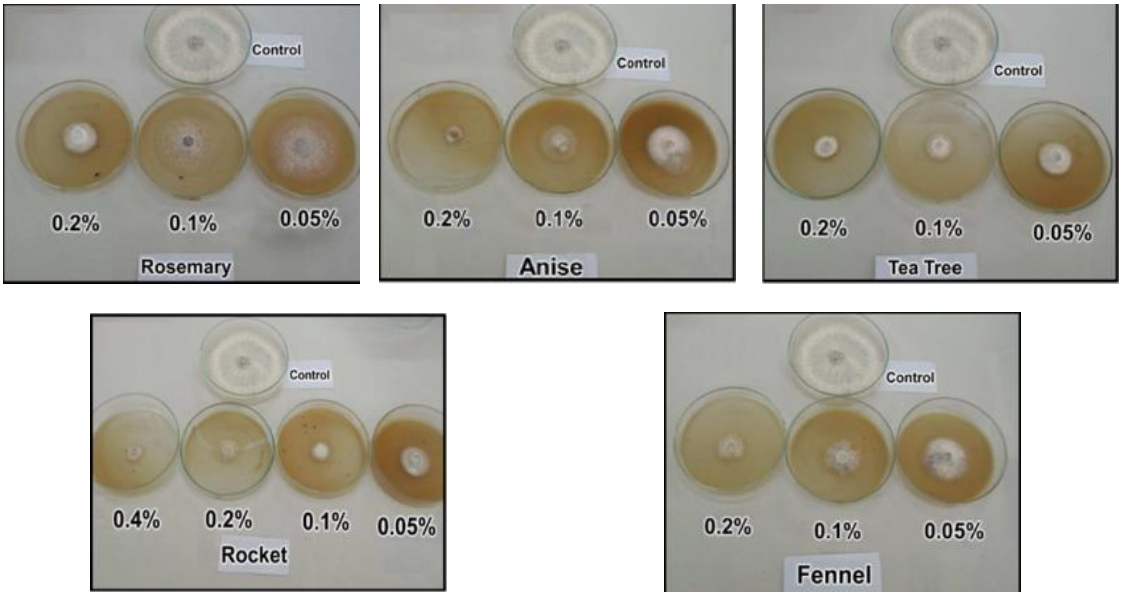

Plate 1. Effect of essential oils on the linear growth (mm) of *F. oxysporum*

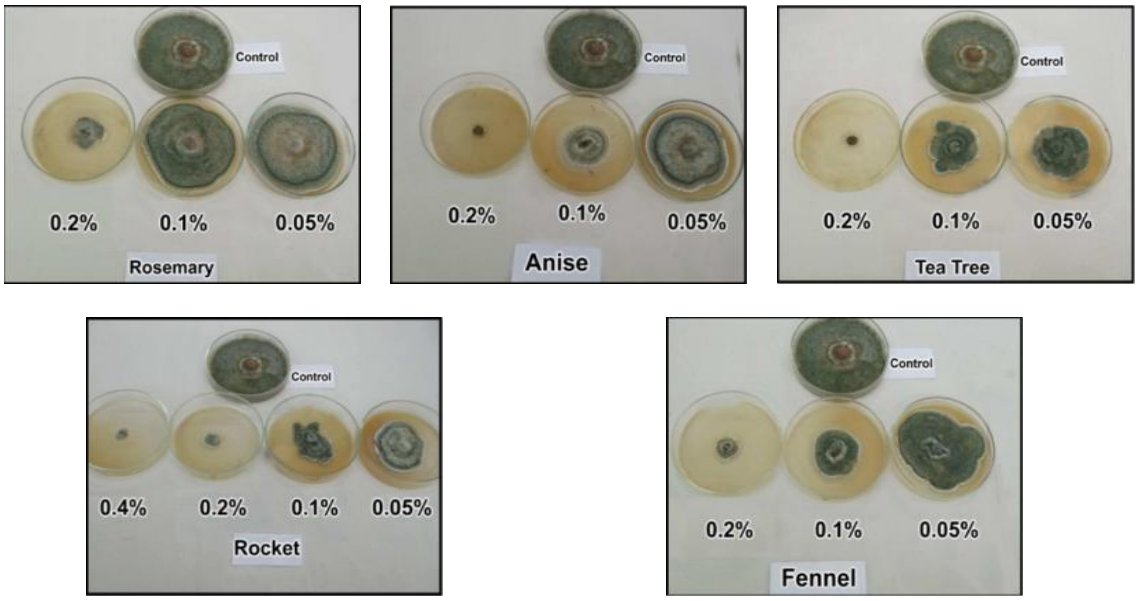

Plate 2. Effect of essential oils on the linear growth (mm) of *T. viride*

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 monoterpens 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 $(CH3COOH)_2Pb$, 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.

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