

# Monoterpenoids and Their Synthesized Brominate Derivatives as Eco-Friendly Measures to Control Some Plant Pathogenic Fungi and Bacteria

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## ABSTRACT

**Background and Objective:** Phyto-pathogenic fungi or bacteria are one of the most important causes that decrease food and cash crops. Chemical pesticides cause damage to the environment. Thus, globally, there are attempts to provide eco-friendly products. Therefore, this work aimed to find alternative natural products for managing serious diseases (fungi and bacteria). **Materials and Methods:** Fungicidal and bactericidal of four monoterpenoids, as well as their synthesized brominated (Br) derivatives were investigated against six fungi and four bacteria *in vitro*. The synthesized brominated derivatives were confirmed by a mass spectrometer and GC/MS. **Results:** The soil and air-borne fungi were very sensitive to chlorothymol followed by thymol and carvacrol, respectively. However, the synthesized brominate derivatives such as bromocarvacrol were the most effective treatment against all the tested fungal strains, while, bromoegunol was a less effective treatment. Concerning the antibacterial activity, the tested monoterpenoids exhibited variable degrees of antibacterial against all of the tested bacterial strains and the conversion of monoterpenoids into bromo-derivatives enhanced the bactericidal activity. **Conclusion:** The chlorothymol (monoterpene) recorded the highest fungicidal activity against soil and air-born fungi. For bacterial activity, results exhibited that chlorothymol was the most effective monoterpene against bacteria. All the bromo-derivatives showed high activity against the most of tested fungi and bacteria.

## KEYWORDS

Fungicidal activity, bactericidal activity, monoterpenoids, brominate derivatives, soil-borne diseases, air-borne diseases

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## INTRODUCTION

Biopesticides are considered a unique solution to the problem of agrochemicals in crop protection. It reaches the target pest leaving the remaining 99.9% to enter the environment to cause hazards to non-target organisms including humans<sup>1</sup>. In the last two decades, scientists all over the world tried to minimize



the use of synthetic pesticides for the management of plant pathogens, insects, acari and weeds to avoid environmental pollution hazards. Besides, the targeted pathogen, pesticides may also kill various beneficial organisms. The increasing incidence of resistance among pathogens towards synthetic chemicals is also a cause of serious concern<sup>2</sup>. Major food and cash crops are attacked by certain pathogens especially fungal diseases which cause approximately 20% reductions in the yield<sup>3,4</sup>.

The discovery of antimicrobial compounds from plant sources is a safe route to a new generation characterized by eco-friendly, low toxicity, selectivity, highest efficacy and biodegradability<sup>5-7</sup>.

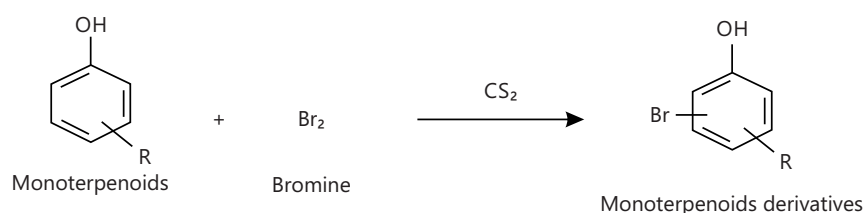
Search extensively for new biologically active terpenoids as a potential source for agrochemicals. Monoterpenes together with sesquiterpenes and diterpenes form the majority of essential oil. Monoterpenes are unsaturated hydrocarbons and some are oxygenated derivatives such as alcohol, ketones, carboxylic acid and phenol<sup>8,9</sup>. Chemical modification of natural monoterpenoids to various derivatives has been reported to result in modification of biological activity<sup>10-14</sup>. Therefore, this work was one of several attempts that have been conducted to find alternative natural products which are safe, biodegradable and eco-friendly for managing serious pests (fungi and bacteria) which attack crop plants.

## MATERIALS AND METHODS

In this investigation, the evaluated monoterpenoids and/or their synthesized brominated derivatives were implemented under laboratory conditions in 2021 at the Fungicide Bioassay Laboratory, Department of Pesticide Chemistry and Technology, Faculty of Agriculture, University of Alexandria, Alexandria, Egypt.

**Tested monoterpenoids:** The tested monoterpenoids were thymol, chlorothymol, eugenol and carvacrol. All monoterpenoids were purchased from Aldrich Chemicals Ltd., UK.

**General procedure for bromination of tested monoterpenoids:** The desired monoterpenoids (0.01 mol) thymol, eugenol, chlorothymol and carvacrol were dissolved in carbon disulfide (25 mL) in a round bottom flask fitted with a reflux condenser and pressure equivalent-dropping funnel. Bromine (0.01 mol) was dissolved in carbon disulfide (16 mL) and then added dropwise to a cooled flask (below 5°C) after the reaction mixture was stirred at room temperature for 1hr to complete (Scheme 1) and then monitored by thin layer chromatography (TLC with aluminum plate of 25, silica gel coated with fluorescent indicator F254, TLC size is 20×20 cm, MERCK, Germany). The reaction was quenched with water and extracted three times with methylene chloride (30 mL each). The organic layer was washed three times with water (100 mL) and dried over anhydrous sodium sulfate (50 g). The organic solvent was removed using a rotary evaporator under a high vacuum (brand name: Kryqcn, Model: RE501, rotating speed: 120 rpm, vacuum power: 0.098 mpa, China). The product was identified by a mass spectrometer (HP model, MS-5988) and GC/MS (Shimadzu QP2010) and Melting points were measured with capillary melting point apparatus model 1002-USA, UV spectra were recorded on a UV Spectrophotometer model UV-1601 SHIMADZU shown in Table 1 and 2.



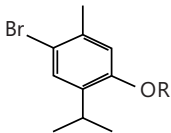
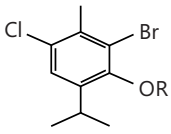
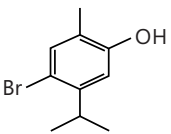
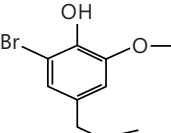
Scheme 1: Synthesis of the monoterpenoidal brominate derivatives

Table 1: Physical properties of the monoterpenoidal derivatives

Derivative	Purity (%)	M.P (°C )
Bromothymol	87	48
Bromochlorothymol	85	Oil
Bromocarvacrol	97	46
Bromoeugenol	95	Oil

M.P: Melting point

Table 2: Identification of the synthesized monoterpenoidal brominate derivatives

Compound	Empirical formula	Estimated M.W.	Found M.W.
Bromothymol	 <chem>C_{10}H_{13}OBr</chem>	229.11	229
Bromochlorothymol	 <chem>C_{10}H_{12}OClBr</chem>	263.56	263
Bromocarvacrol	 <chem>C_{10}H_{13}OBr</chem>	229.11	229
Bromoeugenol	 <chem>C_{10}H_{11}O_2Br</chem>	243.10	243

M.W.: Molecular weight

**Antifungal assay:** Six economic plant pathogenic fungi were chosen for this study. Fungi were obtained from the Fungicide Bioassay Laboratory, Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University. However, the studied fungi were as follows:

- **Soil borne fungi:** *Fusarium oxysporum*, *Rhizoctonia solani* and *Pythium debrinum*
- **Airborne fungi:** *Alternaria alternata*, *Helminthosporium sp.* and *Botrytis feba*

The antifungal activity of tested compounds was investigated by using the radial growth technique method<sup>15</sup>. Appropriate volumes of the stock solutions of the tested compounds either in dimethyl sulfoxide (DMSO) for solvent extracts or in distilled water for water extracts were added to molten nutrient agar (potato dextrose agar medium, PDA) to achieve the desired concentration immediately before pouring into the Petri dishes (9.0 cm in diameter) at 40-45°C.

Each concentration was tested in triplicate. Parallel controls were maintained with DMSO mixed with PDA. The discs of mycelial felt (0.5 cm diameter) of the plant pathogenic fungi, taken from 8-day-old cultures on PDA plates, were transferred aseptically to the center of Petri dishes. The treatments were incubated at 25°C in the dark. Colony growth diameter was measured after the fungal growth in the control treatments which completely covered the Petri dishes. Moreover, the percentage of mycelial growth inhibition was calculated according to the following formula<sup>16</sup>:

$$\text{Mycelial growth inhibition} = \frac{DC - DT}{DC} \times 100$$

where, DC and DT are the average diameters of the fungal colony of control and treatment, respectively.

**Bactericidal activity assay:** Four phytopathogenic bacteria, *Agrobacterium tumefaciens* (Crown gall bacteria), *Erwinia carotovra* subsp. *Carotovra* (soft rot bacteria), *Erwinia amylovora* (fire blight bacteria) and *Pseudomonas solanacearum* were provided by the Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Egypt. The bacterial strains were cultured in a glycerol agar medium. Streptomycin sulfate as a standard bactericide was supplied by El-Nile Company for Chemical Industry and Drugs, Egypt.

**Determination of minimum inhibitory concentrations (MICs):** Tested compounds were dissolved in dimethyl sulfoxide (DMSO). Appropriate volumes of the stock solution were added to nutrient agar and poured into Petri dishes (diameter 9 mm). After solidification, a bacterial culture has grown in a nutrient broth for 18 hrs (approximately  $10^8$  CFU mL<sup>-1</sup> was planted on four lines per each plate with three replicates from four bacteria species *Agrobacterium tumefaciens*, *Erwinia carotovra*, *Erwinia amylovora* and *Pseudomonas salnacearum* on the surface of the agar. The inoculum line was allowed to dry before inverting the plates for incubation at 27°C for 24 hrs. The control was nutrient agar with a maximum volume of dimethylsulfoxide added to the treatment. The antibiotic, streptomycin was used for comparison, The MIC was determined as the lowest concentration of the tested compound showing no visible bacterial growth in the agar plates. As recommended by the European Society of Clinical Microbiology and Infection Disease<sup>17</sup>.

**Statistical analysis:** The effective concentrations of evaluated compounds that inhibit the fungi mycelia or bacteria growth by 50% (EC<sub>50</sub>), were conducted by a linear regression method with probit analysis<sup>18</sup>.

## RESULTS AND DISCUSSION

**Identification of mono terpenoids derivatives:** The reaction of thymol, carvacrol, chlorothymol and eugenol with carbon disulfide and bromine gave bromothymol and bromocarvacrol as crystals, while bromochlorothymol and bromoeugenol as oily compounds. The percentage of the product yield obtained from the reaction was 80, 83, 92 and 90% for Bromothymol, Bromochlorothymol, Bromocarvacrol and Bromoeugenol, respectively. Physical properties and identification of the synthesized monoterpenoidal brominate derivatives showed in Table (1, 2) and Fig. (1-4).

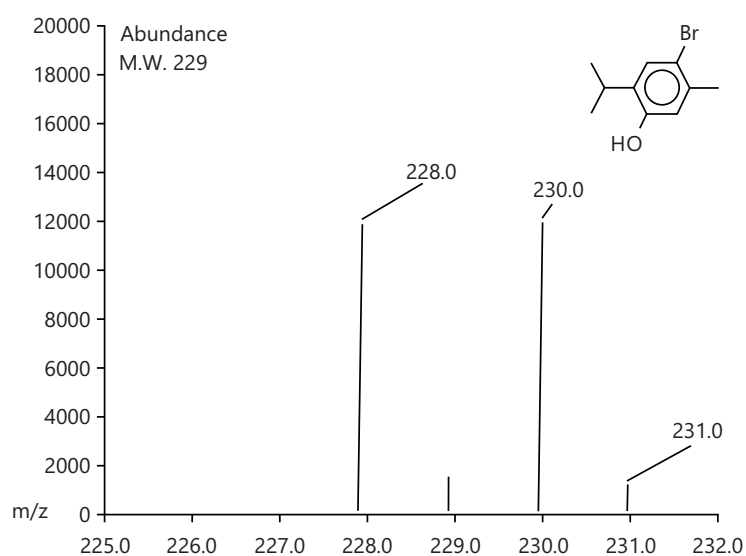


Fig. 1: MS spectra of bromothymol

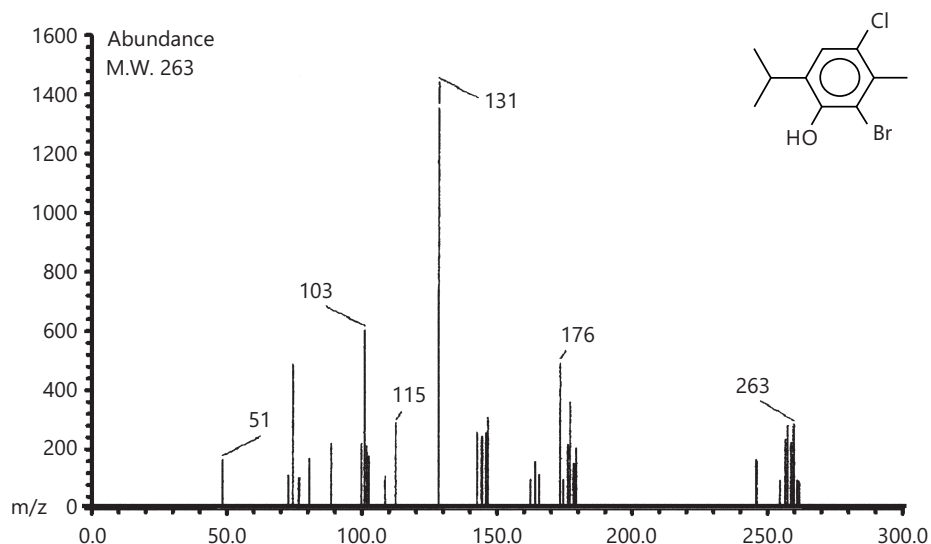


Fig. 2: MS spectra of bromochlorothymol

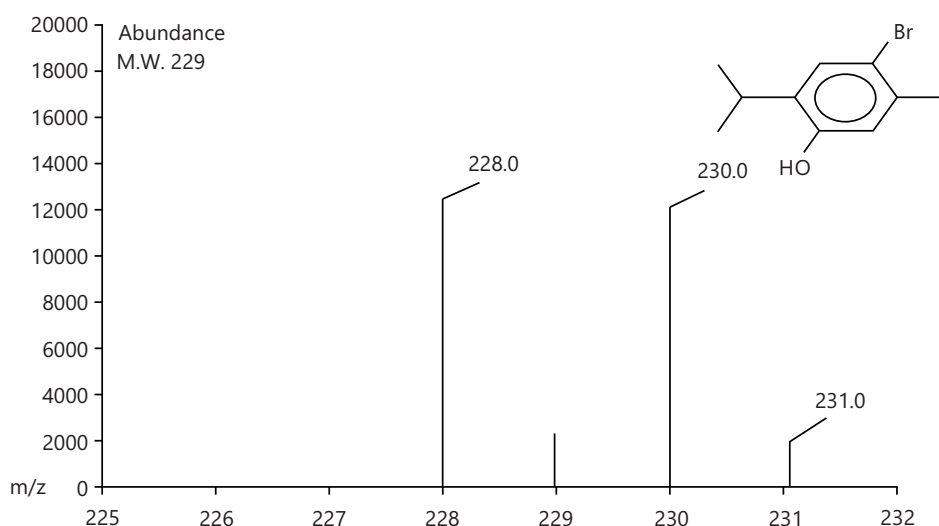


Fig. 3: MS spectra of bromocarvacrol

**Fungicidal activity of some monoterpenoids towards soil and air-borne fungi:** The fungicidal activity of four monoterpenoids namely, carvacrol, chlorothymol, eugenol and thymol were tested against soil-born fungi (*fusarium oxysprum*, *Rhizoctonia solani* and *Pythium debrinum*) and air-born fungi (*Botrytis feba*, *Helimanthosporum* sp. and *Alteranria alternate*) as shown in Table 3. The fungus *F. oxysprum* was very sensitive to chlorothymol, thymol, carvacrol and eugenol with  $EC_{50}$  values estimated by 13.40, 40.57, 140.34 and 271.73  $mg L^{-1}$ , respectively, while *Rhizoctonia solani* was sensitive to chlorothymol ( $EC_{50} = 21.00 mg L^{-1}$ ), carvacrol ( $EC_{50} = 21.88 mg L^{-1}$ ), thymol ( $EC_{50} = 30.90 mg L^{-1}$ ) and eugenol ( $EC_{50} = 140.33$ ). The soil-born fungus, *P. debrinum* recorded  $EC_{50}$  values estimated at 28.39, 43.00, 100.88 and 300  $mg L^{-1}$  with chlorothymol, thymol, carvacrol and eugenol, respectively. However, the standard fungicide (mancozeb) recorded  $EC_{50}$  values of 140.34, 154.59 and 91.70  $mg L^{-1}$  with *F. oxysprum*, *R. solani* and *P. debrinum*, respectively.

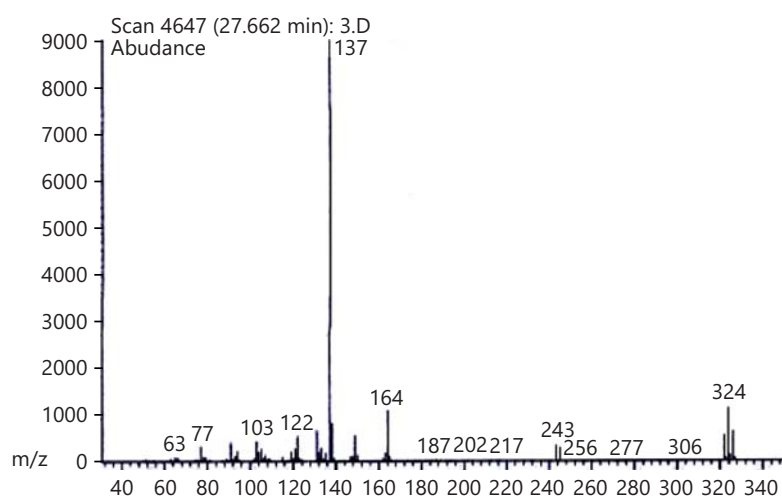


Fig. 4: MS spectra of bromoeugenol

Table 3: Fungicidal activity of tested monoterpenoids against phytopathogenic fungi

Fungus	EC <sub>50</sub> /ppm	95% confidence limits		Slope±SE
		Lower	Upper	
<b>Chlorothymol</b>				
<i>Fusarium oxysporum</i>	13.40	11.20	18.16	2.01±0.3
<i>Rhizoctonia solani</i>	21.00	15.50	31.22	2.00±0.19
<i>Pythium debrinum</i>	28.39	23.80	32.47	3.10±0.35
<i>Alteranria altranata</i>	07.58	05.31	8.44	1.10±0.18
<i>Helimanthosporum</i> sp.	04.49	00.21	7.09	2.60±0.35
<i>Botrytis febae</i>	03.80	00.84	5.12	1.40±0.16
<b>Thymol</b>				
<i>Fusarium oxysporum</i>	40.57	15.53	86.12	2.00±0.18
<i>Rhizoctonia solani</i>	30.90	27.14	34.84	2.60±0.24
<i>Pythium debrinum</i>	43.00	27.70	73.73	1.72±0.11
<i>Alteranria altranata</i>	27.82	15.61	42.70	2.80±0.26
<i>Helimanthosporum</i> sp.	17.38	09.66	100.14	2.30±0.29
<i>Botrytis febae</i>	22.97	08.85	40.88	2.40±0.3
<b>Carvacrol</b>				
<i>Fusarium oxysporum</i>	140.34	122.59	160.04	2.00±0.24
<i>Rhizoctonia solani</i>	21.85	16.59	27.44	1.60±0.19
<i>Pythium debrinum</i>	100.88	91.16	112.00	3.50±0.3
<i>Alteranria altranata</i>	18.80	12.40	25.80	0.78±0.13
<i>Helimanthosporum</i> sp.	53.96	22.92	93.62	1.90±0.2
<i>Botrytis febae</i>	23.99	14.98	30.97	1.60±0.32
<b>Eugenol</b>				
<i>Fusarium oxysporum</i>	271.73	217.01	403.75	2.68±0.4
<i>Rhizoctonia solani</i>	140.33	121.92	167.21	2.59±0.33
<i>Pythium debrinum</i>	>300	-	-	-
<i>Alteranria altranata</i>	>300	-	-	0.80±0.20
<i>Helimanthosporum</i> sp.	142.90	109.81	224.94	1.27±0.3
<i>Botrytis febae</i>	138.70	123.70	158.55	3.30±0.37
<b>Mancozeb</b>				
<i>Fusarium oxysporum</i>	140.34	122.59	160.04	2.00±0.24
<i>Rhizoctonia solani</i>	154.59	124.82	192.62	1.18±0.11
<i>Pythium debrinum</i>	91.70	61.93	294.80	0.90±0.28
<i>Alteranria altranata</i>	115.61	77.65	170.97	0.62±0.09
<i>Helimanthosporum</i> sp.	47.44	30.83	56.37	0.77±0.09
<i>Botrytis febae</i>	236.37	127.53	612.23	1.35±0.1

SE: Standard error

On the other hand, results showed that the air-born fungus *A. alternata* was very sensitive to chlorothymol ( $EC_{50} = 7.58 \text{ mg L}^{-1}$ ) followed by carvacrol ( $EC_{50} = 18.80 \text{ mg L}^{-1}$ ), thymol ( $EC_{50} = 27.82 \text{ mg L}^{-1}$ ) and eugenol ( $EC_{50} = 300 \text{ mg L}^{-1}$ ). *Helimanthosporum* sp., was sensitive towards chlorothymol, thymol, carvacrol and eugenol and recorded  $EC_{50}$  values estimated by 4.49, 17.38, 53.96 and  $142.90 \text{ mg L}^{-1}$ , respectively. The air-born fungus *B. fabae* was respectively very sensitive to chlorothymol, thymol, carvacrol and eugenol, with  $EC_{50}$  values of 3.80, 22.97, 23.99 and  $138.70 \text{ mg L}^{-1}$ . The estimated  $EC_{50}$  values of mancozeb against *A. alternata*, *Helimanthosporum* sp. and *B. fabae* were 115.61, 47.44 and  $236.37 \text{ mg L}^{-1}$ , respectively.

The obtained results indicated that carvacrol, thymol, chlorothymol and eugenol exhibited the highest antifungal activity against phytopathogenic fungi<sup>19-22</sup>. The activity of thymol, carvacrol, chlorothymol and eugenol against fungi may be attributed to containing the OH group in the aromatic ring. Also, the chlorine atom on the chlorothymol is playing an important role in the fungicidal activity against the tested fungal strains<sup>20,23</sup>. Therefore, it's possible to use these monoterpenoids as lead compounds to obtain new friendly fungicides for both humans and the environment.

#### **Fungicidal activity of synthesized monoterpenoidal brominate derivatives against soil and air-born fungi:**

The fungicidal activity of brominate monoterpenoid derivatives against soil-born fungi i.e., *F. oxysporum*, *R. solani* and *P. debrinum* was shown in Table (4). The gained results indicated that *F. oxysporum* was sensitive to treatments of bromocarvacrol ( $EC_{50} = 21.86 \text{ mg L}^{-1}$ ), followed by Bromothymol ( $EC_{50} = 26.54 \text{ mg L}^{-1}$ ), bromoegenol ( $EC_{50} = 56.22 \text{ mg L}^{-1}$ ) and bromochlorothymol ( $EC_{50} = 60.10 \text{ mg L}^{-1}$ ). While *Rhizoctonia solani* showed high sensitivity towards bromochlorothymol, bromocarvacrol, bromothymol and bromoegenol with  $EC_{50}$  values of 9.38, 10.46, 28.08 and  $40.51 \text{ mg L}^{-1}$ , consecutively. The fungus *P. debrinum* was very sensitive to the treatments of bromocarvacrol, bromochlorothymol, bromothymol and bromoegenol with  $EC_{50}$  values of 27.50, 40.93, 47.70 and  $165.20 \text{ mg L}^{-1}$ , respectively. The standard fungicide (mancozeb) recorded  $EC_{50}$  values of 12.02, 91.70 and  $154.60 \text{ mg L}^{-1}$  with *F. oxysporum*, *R. solani* and *P. debrinum*, respectively.

The air-born fungi results clarified that *A. alternata* was very sensitive to bromocarvacrol, bromothymol and bromochlorothymol with  $EC_{50}$  values of 16.34, 21.88 and  $21.94 \text{ mg L}^{-1}$ , respectively, while it was less sensitive to bromoegenol ( $EC_{50} = 163.69 \text{ mg L}^{-1}$ ). In the same context, *Helminthosporum* sp., recorded  $EC_{50}$  values estimated by 12.00, 15.47, 23.30 and  $28.82 \text{ mg L}^{-1}$  with bromocarvacrol, bromochlorothymol, bromothymol and bromoegenol, respectively. *Botrytis fabae* showed sensitivity towards bromothymol ( $EC_{50} = 14.80 \text{ mg L}^{-1}$ ), bromocarvacrol ( $EC_{50} = 15.36 \text{ mg L}^{-1}$ ), bromochlorothymol ( $EC_{50} = 28.11 \text{ mg L}^{-1}$ ) and bromoegenol ( $EC_{50} = 39.04 \text{ mg L}^{-1}$ ). The treatment of mancozeb recorded  $EC_{50}$  values of 47.44, 115.61 and  $236.37 \text{ mg L}^{-1}$  with *Helminthosporum* sp., *A. alternata* and *B. fabae*, respectively. These findings are in agreement with those obtained by Kaur *et al.*<sup>14</sup>, who found that halogenated thymol derivatives e.g. chlorothymol, dichlorothymo, monobromothymol and dibromothymol have strong antifungal and antibacterial activities. Also, Chauhan *et al.*<sup>24</sup>, found that thymol and its derivatives succeeded to inhibit the mycelia growth of *Rhizoctonia solani* during the *in vitro* study, while the soil application under greenhouse conditions thymol or its derivatives suppressed the damping-off in cucumber seedlings at a range of 26.67 to 100%.

**Bactericidal activity of tested monoterpenoids and their brominate derivatives:** In the current investigation, the bactericidal activity of four monoterpenoid compounds namely, carvacrol, thymol, chlorothymol and eugenol, in addition to, their brominated derivatives, Bromochlorothymol, Bromothymol, Bromocarvacrol and Bromoegenol were assessed against *A. tumefaciens*, *E. cartovora*, *E. amylovora* and *P. solanacearum* under laboratory conditions (Table 5). The bacterium of *A. tumefaciens* was very sensitive to standard bactericide streptomycin with  $MIC = 5 \text{ } \mu\text{g mL}^{-1}$ , followed by *E. cartovora*, *E. amylovora* and *P. solanacearum* with values estimated by  $10 \text{ } \mu\text{g mL}^{-1}$  of each.

Table 4: Fungicidal activity of tested monoterpenoid derivatives against phytopathogenic fungi

Fungus	EC <sub>50</sub> /ppm	95% confidence limits		Slope±SE
		Lower	Upper	
<b>Bromochlorothymol</b>				
<i>Fusarium oxysprum</i>	60.10	49.90	72.10	1.21±0.11
<i>Rhizoctonia solani</i>	9.38	7.80	10.90	2.30±0.24
<i>Pythium debrinum</i>	40.93	33.40	48.60	1.97±0.30
<i>Alteranria altranata</i>	21.94	1.25	40.30	2.00±0.24
<i>Helimanthosporum</i> sp.	15.47	9.40	46.80	2.50±0.29
<i>Botrytis febae</i>	28.11	15.77	50.11	1.00±0.10
<b>Bromothymol</b>				
<i>Fusarium oxysprum</i>	26.54	18.57	39.73	3.00±0.23
<i>Rhizoctonia solani</i>	28.08	11.54	150.56	2.30±0.27
<i>Pythium debrinum</i>	47.70	31.07	69.17	1.30±0.30
<i>Alteranria altranata</i>	21.88	17.17	33.35	1.40±0.26
<i>Helimanthosporum</i> sp.	23.30	20.26	29.50	2.80±0.15
<i>Botrytis febae</i>	14.80	9.47	23.36	2.80±0.23
<b>Bromocarvacrol</b>				
<i>Fusarium oxysprum</i>	21.86	16.59	27.44	1.63±0.15
<i>Rhizoctonia solani</i>	10.46	9.14	12.23	2.55±0.4
<i>Pythium debrinum</i>	27.50	23.29	33.626	1.82±0.19
<i>Alteranria altranata</i>	16.34	14.42	18.57	2.25±0.2
<i>Helimanthosporum</i> sp.	12.00	10.40	14.60	2.40±0.4
<i>Botrytis febae</i>	15.36	12.64	18.71	4.00±0.31
<b>Bromoeugenol</b>				
<i>Fusarium oxysprum</i>	56.22	28.70	70.20	2.70±0.25
<i>Rhizoctonia solani</i>	40.51	18.78	61.32	2.00±0.19
<i>Pythium debrinum</i>	165.2	139.00	200.2	1.70±0.23
<i>Alteranria altranata</i>	163.69	138.32	198.30	1.70±0.23
<i>Helimanthosporum</i> sp.	28.82	11.81	51.42	1.14±0.11
<i>Botrytis febae</i>	39.04	20.88	61.65	1.30±0.12
<b>Mancozeb</b>				
<i>Fusarium oxysprum</i>	12.02	4.59	25.82	1.38±0.06
<i>Rhizoctonia solani</i>	154.60	124.82	192.62	1.18±0.11
<i>Pythium debrinum</i>	91.70	61.93	294.80	0.90±0.20
<i>Alteranria altranata</i>	115.61	77.65	170.97	0.62±0.09
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<i>Botrytis febae</i>	236.37	127.53	612.23	1.35±0.1

SE: Standard error

Table 5: Bactericidal activity of some monoterpenoids and their derivatives by minimal inhibitory concentration (MIC)

Compound (µg mL <sup>-1</sup> )	<i>A. tumefaciens</i>	<i>E. amylovora</i>	<i>E. cartovora</i>	<i>P. solanacearm</i>
Chlorothymol	35	50	35	60
Thymol	100	90	300	>300
Eugenol	220	300	>300	300
Carvacrol	45	50	50	50
Bromochlorothymol	5	50	5	60
Bromothymol	9	200	10	3
Bromoeugenol	5	50	5	5
Bromocarvacrol	5	5	5	5
Sterptomycin	5	10	10	10

With respect to the *A. tumefaciens* strain it was sensitive to chlorothymol, carvacrol, thymol and eugenol with values of 35, 45, 100 and 220 µg mL<sup>-1</sup>, respectively. Meanwhile, *A. tumefaciens* showed great sensitivity towards bromochlorothymol, bromocarvacrol, bromoeugenol and bromothymol with MIC values of 5, 5, 5 and 9 µg mL<sup>-1</sup>, respectively. However, *E. amylovora* strain exhibited sensitivity to chlorothymol, carvacrol, thymol and eugenol with MIC values of 50, 50, 50 and 300 µg mL<sup>-1</sup>, respectively. Whilst, *E. amylovora* recorded sensitivity towards bromocarvacrol (5 µg mL<sup>-1</sup>), bromochlorothymol (50 µg mL<sup>-1</sup>), bromoeugenol (50 µg mL<sup>-1</sup>) and bromothymol (200 µg mL<sup>-1</sup>).



On the other hand, *E. cartovora* recorded sensitivity towards tested monoterpenoids such as chlorothymol, carvacrol, thymol and eugenol with values of 35, 50, 300 and  $>300 \mu\text{g mL}^{-1}$ , respectively. However, *E. cartovora* exhibited high sensitivity to bromochlorothymol ( $5 \mu\text{g mL}^{-1}$ ), bromocarvacrol ( $5 \mu\text{g mL}^{-1}$ ), bromoeugenol ( $5 \mu\text{g mL}^{-1}$ ) and bromothymol ( $10 \mu\text{g mL}^{-1}$ ). According to the obtained data, *P. solanacearum* showed sensitivity towards carvacrol, chlorothymol, eugenol and thymol with MIC values estimated by 50, 60, 300 and  $>300$ , consecutively. Furthermore, the transformed monoterpenoids such as bromothymol, bromocarvacrol, bromoeugenol and bromochlorothymol were very effective against *P. solanacearum* achieving 3, 5, 5 and  $60 \mu\text{g mL}^{-1}$ , respectively.

Several studies reported that the monoterpenes exhibited antifungal and antibacterial activities against a wide range of microorganisms<sup>25-28</sup>. The results of the current investigation are in agreement with El-Zemity *et al.*<sup>29</sup> who stated that chlorothymol was effective as a bactericide against *Agrobacterium tumefaciens* and *Erwinia carotovora var carotovora*. The activity of chlorothymol may be attributed to the chlorine atom which plays a good role in the bactericidal activity against the tested bacteria. Also, Mahboub and Memmou<sup>30</sup> found that synthesized 6-bromoeugenol or eugenol has antibacterial activity, especially in both positive and negative gram bacteria. Also, thymol derivatives had antibacterial activities with minimum inhibitory concentration values, 40 to  $80 \mu\text{g mL}^{-1}$ . Thymyl-4-nitrobenzoate recorded good inhibitory action towards plant pathogenic bacteria either as a major constituent or in combination with other antimicrobial agents<sup>32</sup>. Abd-El-Aziz *et al.*<sup>33</sup> indicated that using the essential oils of caraway (*Carum carvi* L.) and thyme (*Thymus vulgaris* L.) exhibited antibacterial efficacy against *Agrobacterium tumefaciens* in a laboratory study. Moreover, the application of caraway and thyme oils suppressed the gall formation by 63.16 and 89.47%, respectively, on apricot seedlings in pots.

## CONCLUSION

From the above results, it could be concluded that chlorothymol (monoterpene) recorded the highest fungicidal activity against the most of soil and air-born fungi. While, the brominated derivatives of monoterpenoids showed that bromocarvacrol was the most effective against all tested fungi, whereas, bromoeugenol was the least one. The same trend was recorded with bacteria, where chlorothymol (monoterpene) recorded the highest bactericidal activity. However, the brominated derivatives of monoterpenoids were more effective against all the tested bacteria. Therefore, this study proposed some eco-friendly solutions, but more experiments are needed in the future.

## SIGNIFICANCE STATEMENT

Fungi and bacteria are among the most common plant pathogens that lead to losses in agricultural crops, whether during planting or after harvest. Combating these diseases has become costly, especially recently, so it was necessary to search for effective alternatives that can be used safely without any adverse effects. This study was thus conducted to assess the impact of some monoterpenes and their brominate derivatives against soil and air fungi as well as bacteria. Also, results showed that some of the monoterpenes or their derivatives e.g., chlorothymol and bromocarvacrol were effective as fungicides and/or bactericides.

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