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Tables: 3, Figs : 9

Screening of antifungal activities of 21 oxygenated monoterpenes *in-vitro* as plant disease control agents

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ABSTRACT

This study was designed to evaluate the antifungal activities of pure oxygenated monoterpenes (borneol, borneol acetate, camphor, carvone, 1,8-cineole, citronellal, β-citronellol, dihydrocarvone, fenchol, fenchone, geraniol acetate, isomenthol, limonene oxide, linalool, linalool acetate, menthol, menthone, nerol, nerol acetate, terpinen-4-ol and α-terpineol) against 31 plant pathogen fungi in *in vitro* mycelial growth assays. Among the tested compounds, β-citronellol, nerol, menthol, terpinen-4-ol, α-terpineol, carvone, borneol and commercial benomyl had potent inhibitory effects against most of the tested fungal species. In particular, β-citronellol and nerol completely inhibited the growth of assayed fungi. Their inhibitory effects were also more stronger than commercial benomyl. Based on these results, β-citronellol, nerol as well as menthol, α-terpineol and terpinen-4-ol may be used as new antifungal compounds against plant pathogenic fungal species in agriculture.

Key words: Antifungal, borneol, benomyl, β-citronellol, essential oil, menthol, nerol, oxygenated monoterpenes, terpinen-4-ol, α-terpineol.

INTRODUCTION

Insects and plant diseases (caused by fungi, bacteria and viruses) cause major losses in crop yields. The microorganisms also have unfavourable effects on quality, safety and food storage. Synthetic chemicals are widely used to control the plant diseases, but they have undesirable effects, (i) leave toxic residues in food products (3,12) (ii) cause environmental pollution owing to their slow biodegradation (3) (iii) risk of microbial resistance and (iv) high cost-benefit ratio (4,22). Therefore, alternative pesticides including the plant extracts and essential oils, less damaging to the mammalian health and environment are being studied as fungicides (12,16,20).

Plant essential oils contain numerous compounds: monoterpenes (hydrocarbons and oxygenated derivatives), sesquiterpenes (hydrocarbons and oxygenated derivatives) and aliphatic compounds (alkanes, alkenes, ketones, aldehydes, acids and alcohols). Oxygenated monoterpenes are major components of plant essential oils (31) and many plants essential oils contain relatively high amount of oxygenated monoterpenes (3,5,9,12,18,21,24,25). Antifungal activities of several essential oils and their major

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components are known (2,7,11,14,15,16,18,19,20,24,25,27,29,30,32). However, the antifungal activities of essential oils and pure essential oil components were tested for a limited number of microorganisms (2,7,11,14,15,16,17,19,20,24,27,29,30,32). Recently, we reported the antifungal activity of plant essential oils and pure components against some plant pathogenic fungi (7,8,10,18). There is need for new research to discover the relationships between the chemical composition and antifungal activity of essential oils and to discover new antifungal reagent(s) to protect plants against fungal disases. Thus, the aim of the present study was to assess the antifungal activities of 21 pure oxygenated monoterpenes against 31 plant and postharvest pathogenic fungi.

MATERIALS AND METHODS

The pure compounds (Fig. 1) were purchased from Fluka, Sigma, Merck, Aldrich and Alfa. The compounds tested for antifungal activities were borneol (Fluka), borneol acetate (Sigma), camphor (Fluka), carvone (Fluka), 1,8-cineole (Sigma), citronellal (Sigma), β-citronellol (Fluka), dihydrocarvone (Alfa), fenchol (Fluka), fenchone (Fluka), geraniol acetate (Alfa), isomenthol (Alfa), limonene oxide (Aldrich), linalool (Fluka), linalool acetate (Fluka), menthol (Fluka), menthone (Fluka), nerol (Sigma), nerol acetate (Alfa), terpinen-4-ol (Aldrich), α-terpineol (Merck) and benomyl (Dupont).

Fungal species and antifungal activity assays

The agricultural pathogenic fungi were obtained from the culture collection at Faculty of Agriculture, Department of Plant Protection, Atatürk University, Erzurum, Turkey. All fungi cultures were maintained on potato dextrose agar (PDA) and stored at 4 ^{0}C. The fungal species used in the experiments are *Alternaria alternata* (Fries: Fries) von Keissler, *Alternaria solani* (Ellis & Martin) Jones & Gront, *Botrytis* sp., *Drechslera* sp., *Fusarium acuminatum* Ellis & Everhart, *Fusarium chlamydosporum* Woll. & Reink., *Fusarium culmorum* (W. G. Smith) Sacc., *Fusarium equiseti* (Corda) Sacc., *Fusarium graminearum* Schw., *Fusarium incarnatum* (Rob.) Sacc., *Fusarium nivale* Ces. Ex Sacc., *Fusarium oxysporum* Schlecht., *Fusarium proliferatum* (Mats.) Nirenberg, *Fusarium sambucinum* Funk, *Fusarium scirpi* Lamb. & Fautr., *Fusarium semitectum* Berk. & Rav., *Fusarium solani* (Mart.) Sacc., *Fusarium tabacinum* (Beyma) W. Gams & Garlagh, *Fusarium verticillioides* (Sacc.) Nirenberg, *Nigrospora* sp., *Penicillium jensenii* Zaleski, *Penicillium* sp., *Phoma* sp., *Pythium ultimum* Trow., *Rhizoctonia solani* Kuehn, *Sclerotinia minor* Jagger, *Sclerotinia sclerotiorum* (Libert) de Bary, *Sclerotinia* sp., *Verticillium albo-atrum* Reinke & Berthold, *Verticillium dahliae* Klebahn, *Verticillium tenerum* (Nees ex Fr.) Link.

Antifungal activity was studied through the *in vitro* contact assay which produce hyphal growth inhibition (7,18). Potato dextrose agar (PDA) plates were prepared using 9 cm dia glass Petri dishes. The compounds were dissolved in methanol (1/1;w/v) and added to each of PDA plates containing 20 ml of agar at 50 ^{0}C at 20 µl/Petri dish concentration for liquid compounds and at 12.0 mg/Petri dish for solid compounds. Benomyl (12.0 mg/Petri dish) was used as positive control. Discs of 5 mm dia of each fungi culture tested was cut from 1 week-old cultures on PDA plates and then mycelial surface of the disc was placed

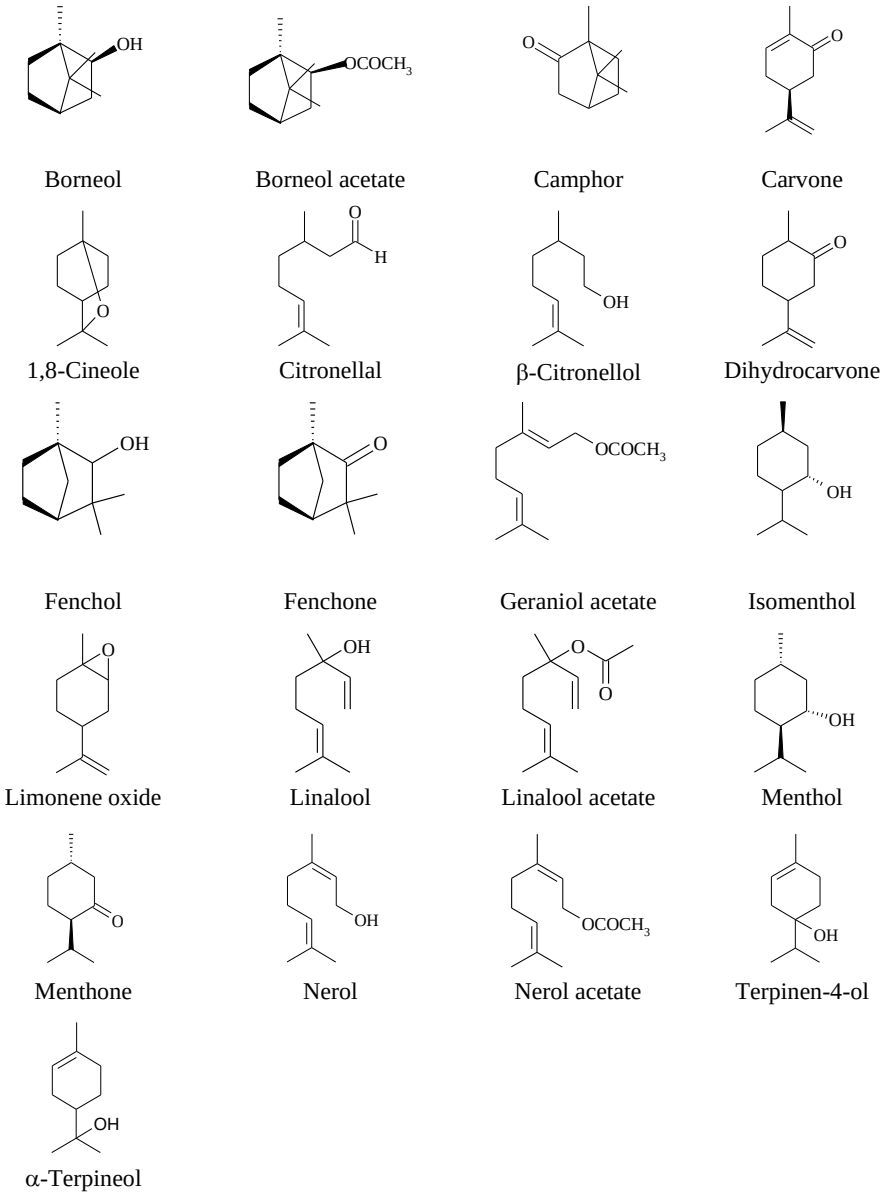


Figure 1. Chemical structures of oxygenated monoterpenes, tested for antifungal activity.

upside down on the centre of the dish. Thus, fungus was brought in direct contact with growth medium in dish. Then, the plates were incubated in dark at 22±2 ºC. After 6 days of inoculation, the hyphae extension dia (mm) was measured at 24 h intervals. Mean of growth measurements was calculated from three replicates of each fungal species. PDA plates

containing methanol (20µl/Petri dish) without compound solutions were used as negative control. In addition, PDA plates treated with the benomyl (12.0 mg/ Petri dish) were used as positive controls.

Mycelial growth inhibition (GI) was calculated as a percentage from the difference between growth of treated and control mycelium using the following equation:

$$GI(\%)=\frac{C-T}{C} \times 100$$

Where, C is mean of hyphal extension (mm) of negative controls and T is mean of hyphal extension (mm) of plates treated with the tested compounds.

Statistical analysis

To determine the statistical significance of obtained results against fungi, one-way variance analses (ANOVA) were done using SPSS 9.0 software package. Differences between means were tested through LSD and values of $P< 0.05$, 0.01 and 0.001 were considered significantly different.

RESULTS AND DISCUSSION

In general, most of the tested compounds inhibited the fungi growth. β-citronellol, nerol, terpinen-4-ol, α-terpineol, menthol, borneol, carvone and commercial benomyl showed broader antifungal spectrum and stronger toxicity to mycelial growth. In particular, β-citronellol, nerol and menthol were most inhibitory and β-citronellol and nerol completely inhibited the growth of the tested fungi (Table 1; Figs. 3,4). The inhibitory effects of these compounds were also higher than commercial benomyl (Table 1). These compounds were more toxic against *Alternaria alternata*, *A. solani* and *Sclerotinia* sp., whereas benomyl did not have activity against these fungal species. However, menthone, 1,8-cineole, limonene oxide, borneol acetate and camphor inhibited the growth of limitted number of the assayed fungi (Figs. 6,8,9). These compounds were less effective against fungal species than other tested compounds. Among the tested compounds, menthone and 1,8-cineole had weakest antifungal activity and inhibited only the growth of *Penicillium* sp. (Figs. 6,9). However, 1,8-cineole, camphor, fenchone, fenchol, linalool acetate and menthone stimulated the growth of some test fungal species (Figs. 6,7,8,9). Among the tested fungal species, the growth of *A. alternate* was significantly stimulated by majority of test compounds (Figs. 6,7,8,9).

These results also show that alcohol derivatives of oxygenated monoterpenes had greater fungitoxic effects as compared with their ketone derivatives. For instance, while menthone was effective on the growth of only *Penicillium* sp., menthol was a potent growth inhibitor against all tested fungi than negative control (Figs. 4,9). Similar results were also found for fenchol and fenchone (Fig. 7). Our results show that alcohol derivatives of oxygenated monoterpenes were more active than acetate derivatives (Tables 1-3). Nerol, linalol and borneol were more inhibitory to fungi growth over their acetates, nerol acetate, linalol acetate and borneol acetate (Figs. 2,4,8,9). Relatively high

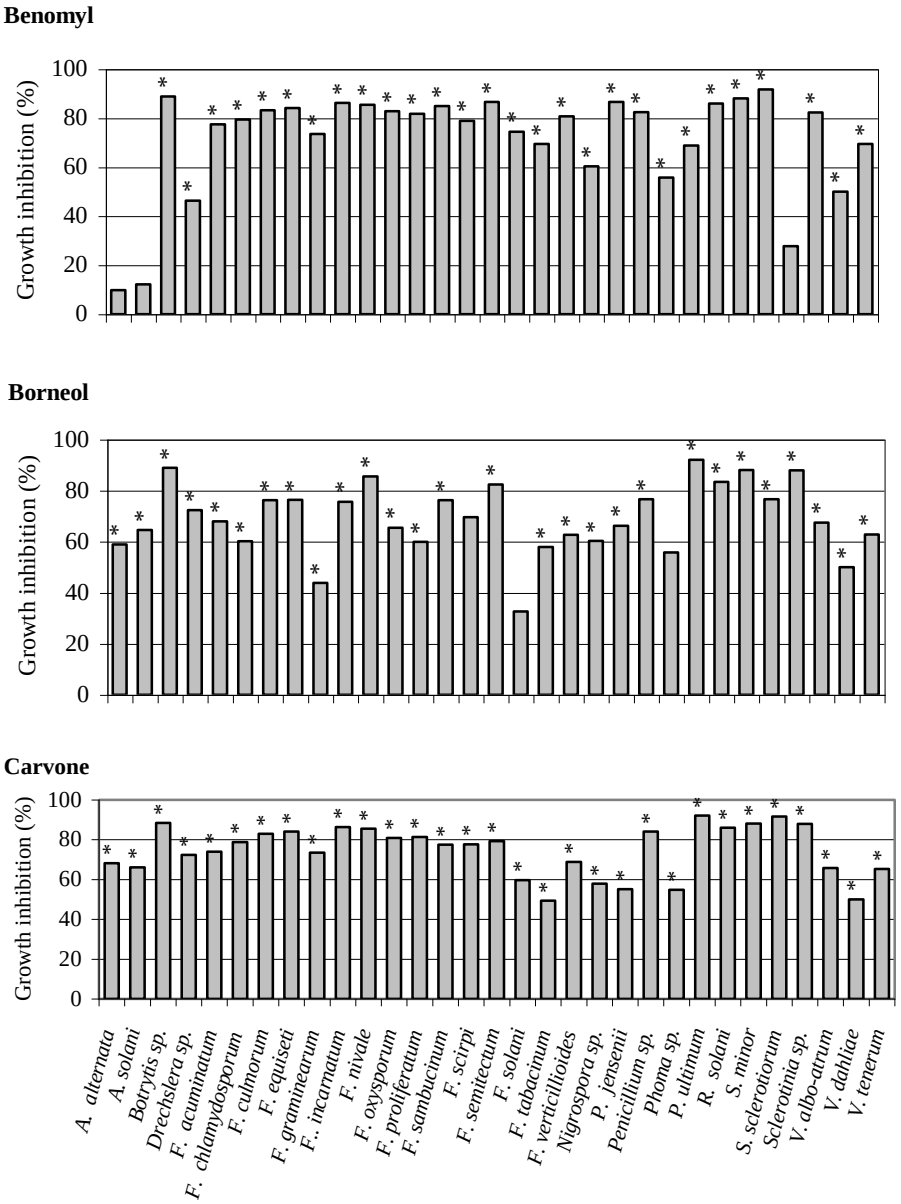
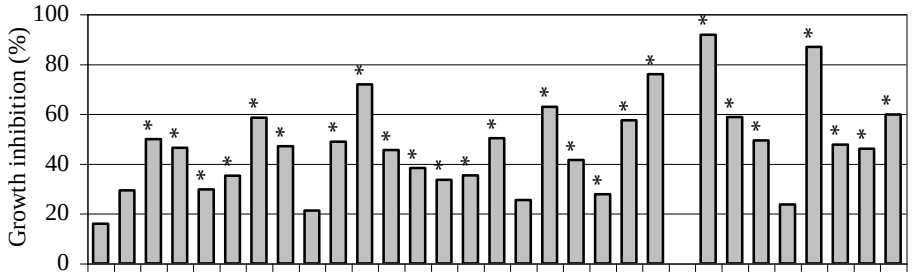
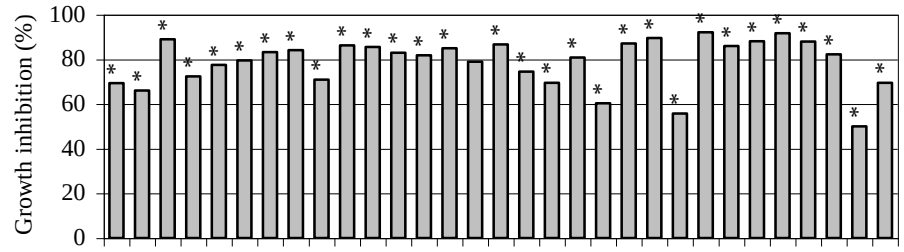


Figure 2. Growth inhibitory effects of benomyl, borneol and carvone. *: Significantly different ($p< 0.05$).

Citronellal



β-Citronellol



Isomenthol

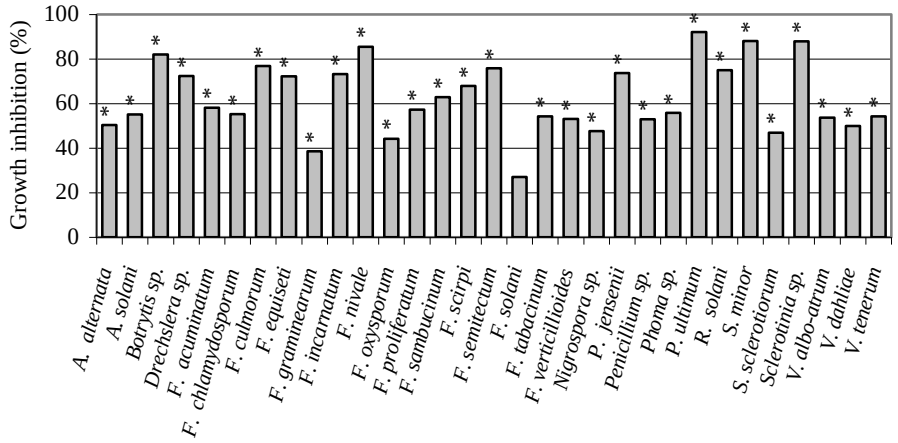


Figure 3. Growth inhibitory effects of citronellal, ß-citronellol and isomenthol. *: Significantly different ($p < 0.05$).

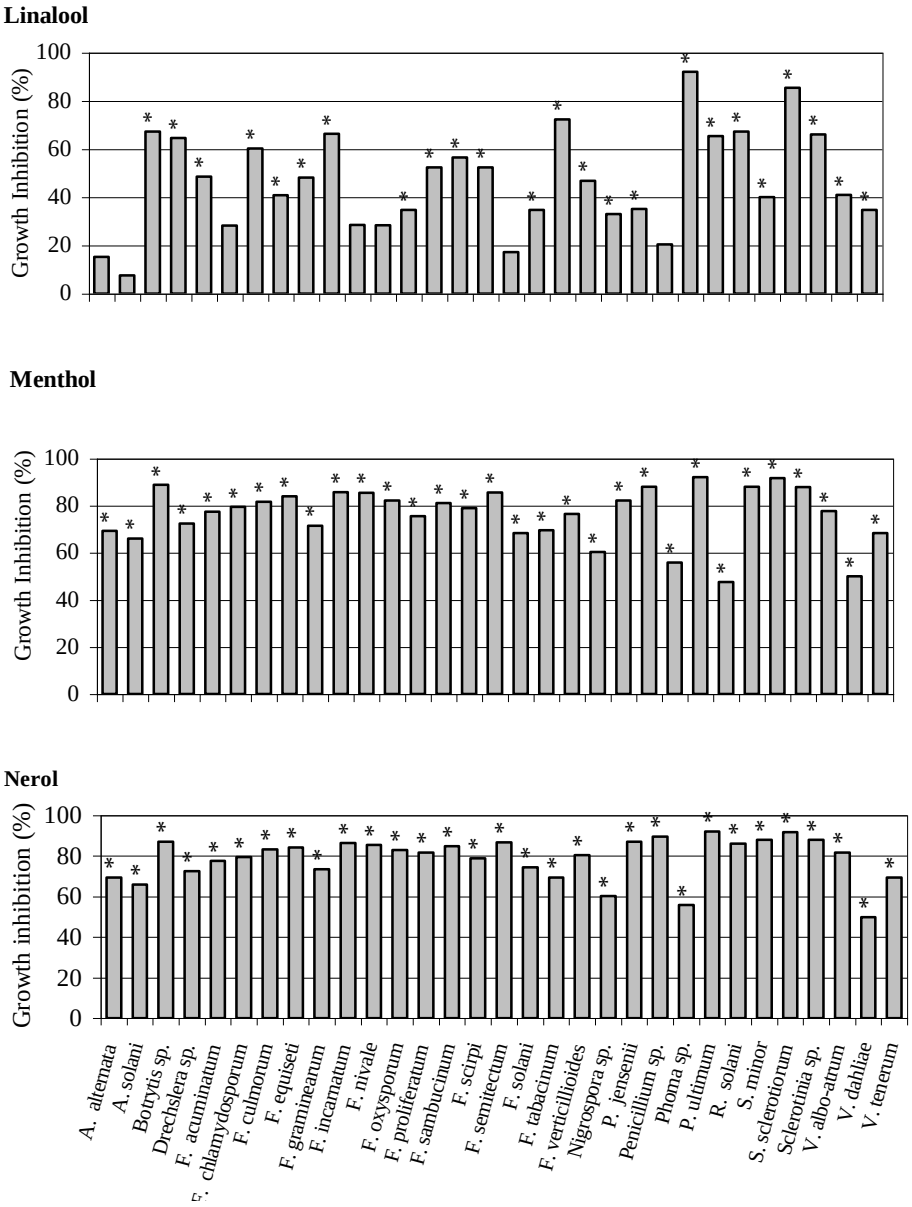
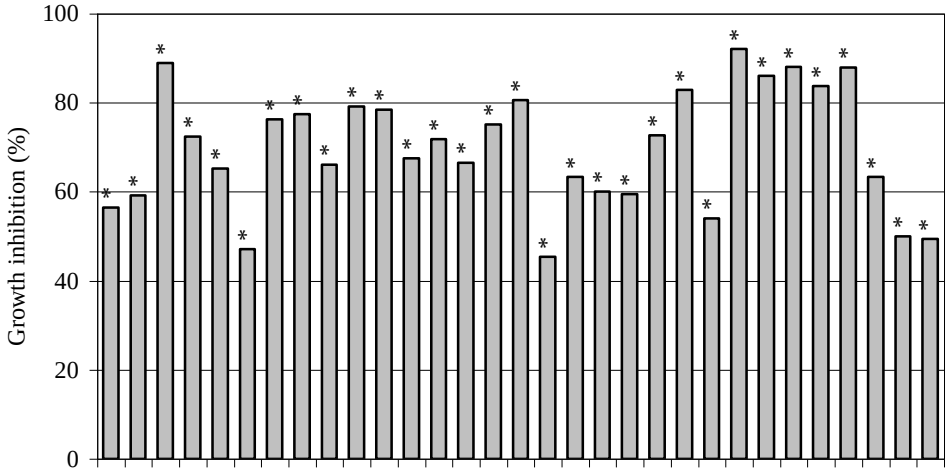


Figure 4. Growth inhibitory effects of linalool, menthol and nerol. *: Significantly different ($p< 0.05$).

Terpinen-4-ol



α-Terpineol

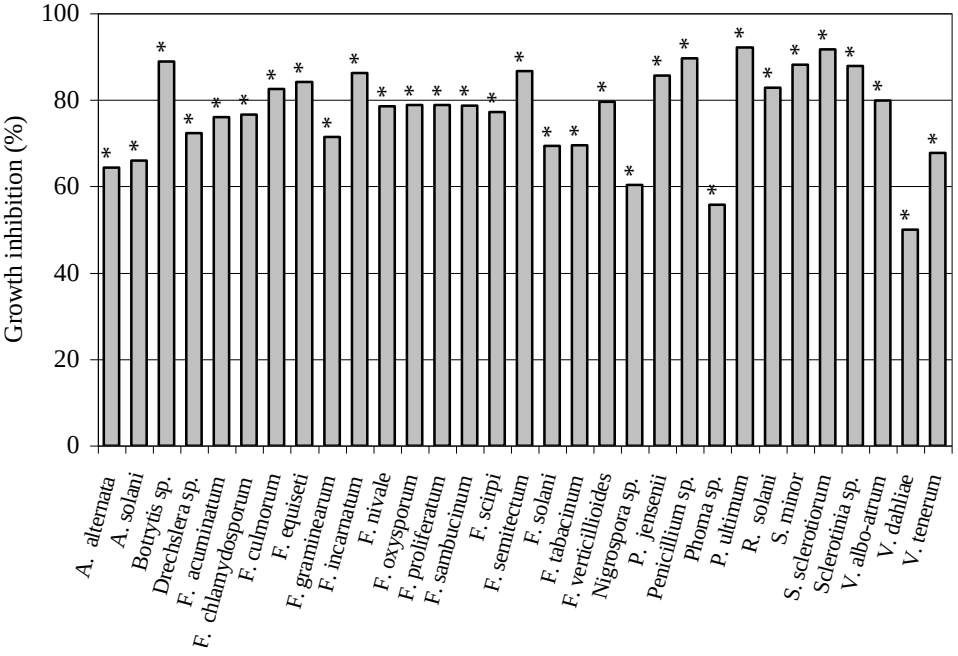
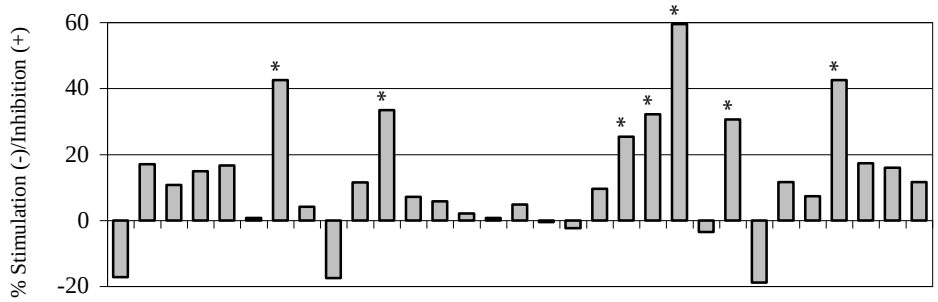
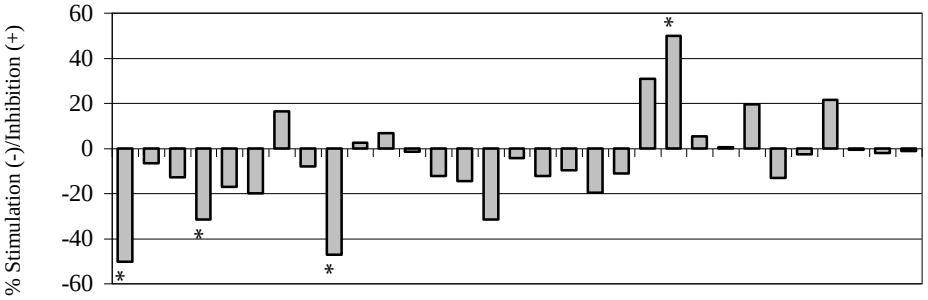


Figure 5. Growth inhibitory effects of terpinen-4-ol and α-terpineol. *: Significantly different ($p < 0.05$).

Borneol acetate



1,8-Cineole



Camphor

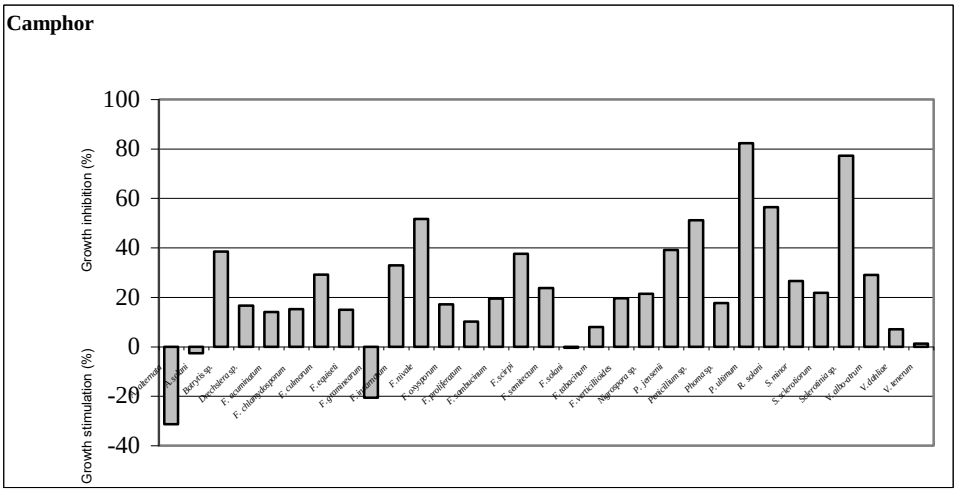
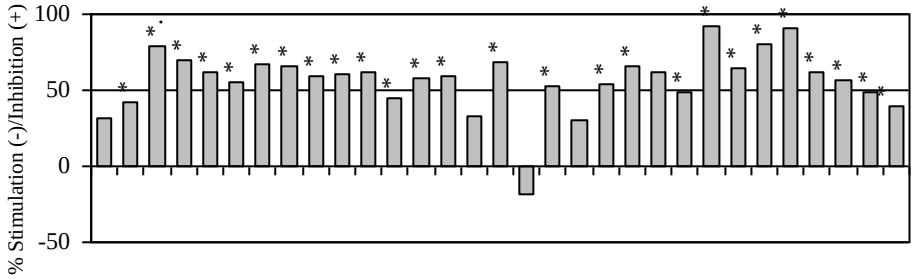
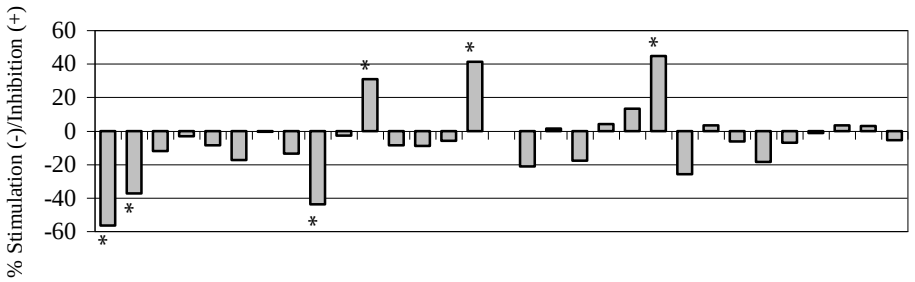


Figure 6. Growth inhibitory and stimulatory effects of borneol acetate, camphor and 1,8-cineole. *: Significantly different ($p < 0.05$).

Dihydrocarvone



Fenchone



Fenchol

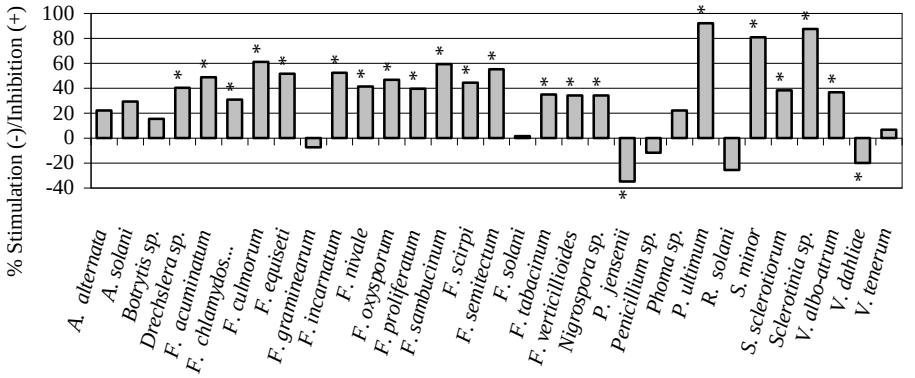
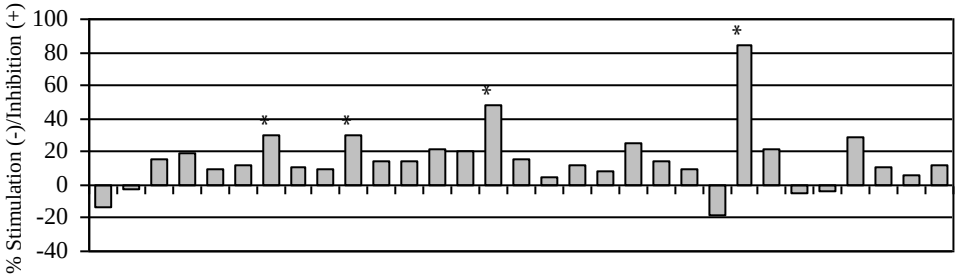
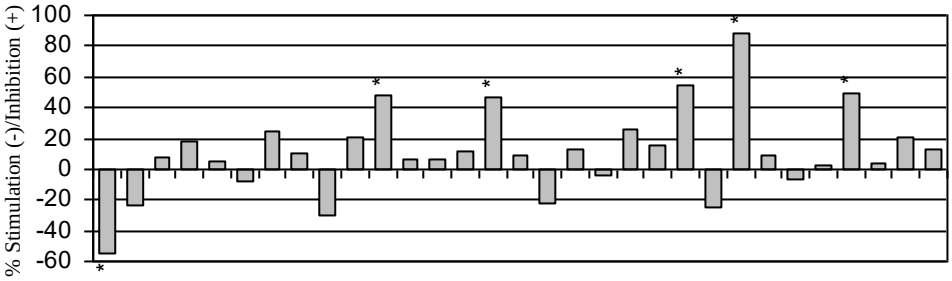


Figure 7. Growth inhibitory and stimulatory effects of dihydrocarvone, fenchone and fenchol. *: Significantly different ($p < 0.05$).

Limonene oxide



Linalool acetate



Geraniol acetate

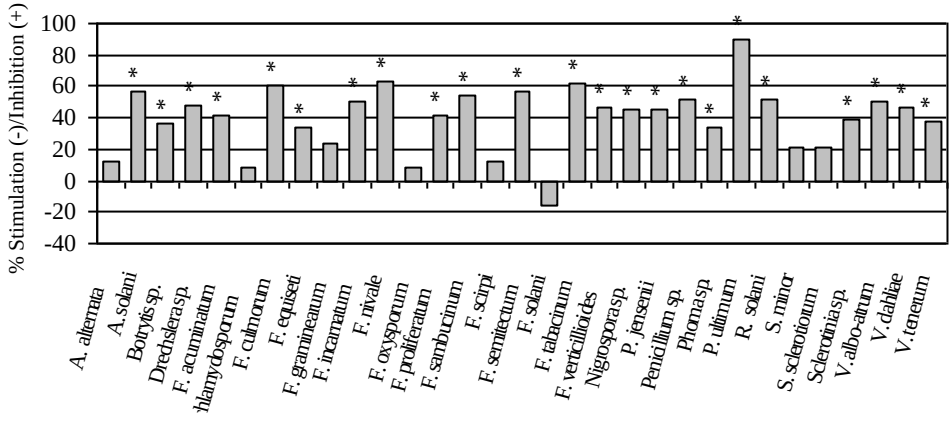
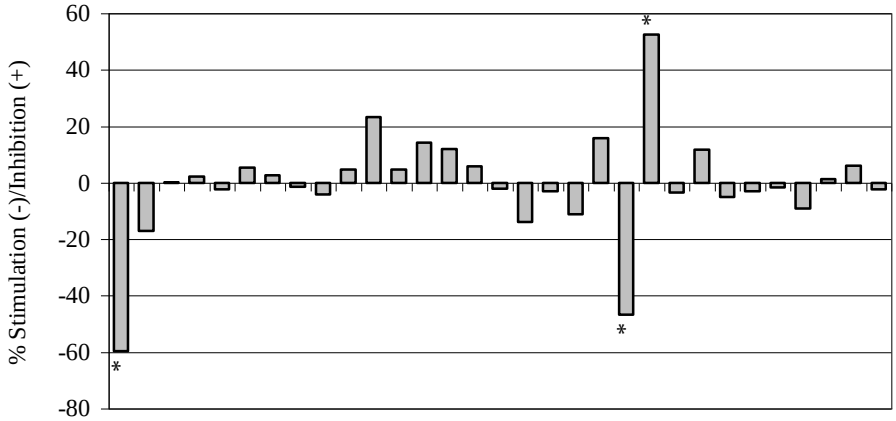


Figure 8. Growth inhibitory and stimulatory effects of limonene oxide, linalool acetate and geraniol acetate. *: Significantly different ($p < 0.05$).

Menthone



Nerol acetate

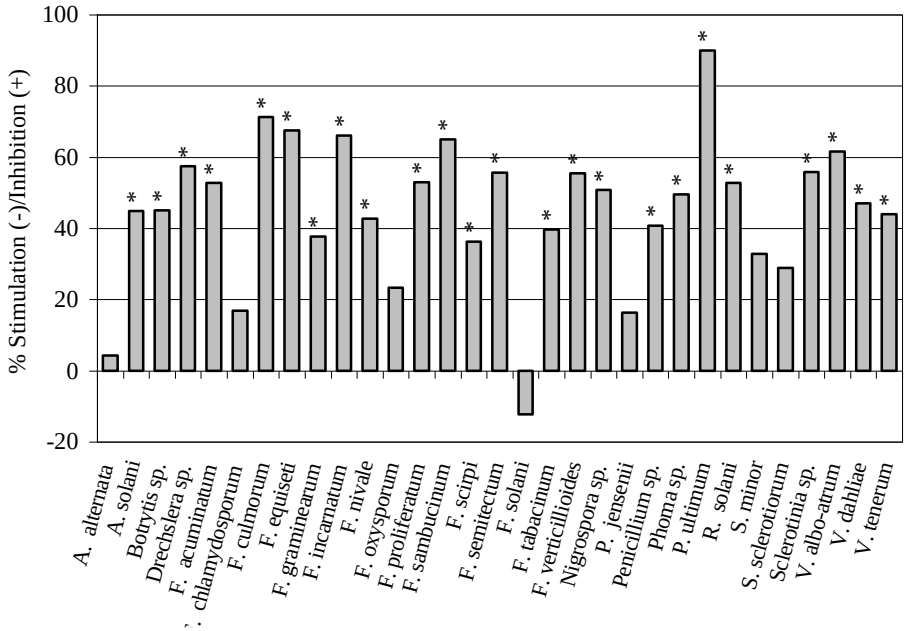


Figure 9. Growth inhibitory and stimulatory effects of menthone and nerol acetate. *: Significantly different ($p< 0.05$).

antifungal activity of alcohol derivatives than ketone and ester derivatives can be attributed to their high solubility in test medium. Previously, it has been shown that alcohol derivatives of oxygenated monoterpenes were more soluble in water than ketone and ester derivatives (13,33).

Several studies have reported the analysis and antifungal activity of the essential oils from various genus (2, 7,8,10,15,18,19,20,23,24,25,27,32). The antifungal properties of some pure monoterpenes and naturally occuring have been also evaluated (7,11,15,16,19,24,25,27,30,32). However, in previous reports, limited number of pure monoterpenes and/or fungal species were used for antifungal activity assays (7,11,15,16,19,24,25,27,30,32). Furthermore, oxygenated monoterpenes showed selective antifungal activity. In previous research, the antifungal activity of 22 monoterpenes was screened against the postharvest pathogens, *Botrytis cinerea* and *Monilinia fructicola* (32). Monoterpenes (citronellal, citronellol, menthol and geraniol) are good growth inhibitors (32). According to our results, citronellal, β-citronellol and nerol were fungitoxic against *Botrytis* sp. Terpinen-4-ol, α-terpineol, linalool and 1,8-cineole are inhibitory to the growth of *Penicillium* sp. (15). We found similar results on the antifungal properties of these monoterpenes against *Penicillium* sp. (Tables 1-3). In view of our results, the growth of *Penicillium* sp. was strongly inhibited by α-terpineol and terpinen-4-ol, but linalool and 1,8-cineole exhibited weaker antifungal activity against this pathogen (Figs. 4, 5, 6). Furthermore, linalool and linalol acetate were tested for antifungal activity against *Sclerotinia sclerotiorum*, *S. cepivorum* and *Fusarium oxysporum* and found that linalool was effective on the growth of *S. sclerotiorum*, whereas it was not active against *F. oxysporum* and *S. cepivorum* (24). Similarly, linalool exhibited moderate inhibitory effects on the growth of *S. sclerotiorum*, whereas, it was not active against *F. oxysporum* (Table 1; Fig. 4). On the other hand, our results also showed that 1,8-cineole and camphor were slightly effective against the tested fungi and are in agreement with other researchers who found that 1,8-cineole and camphor have slight or non-inhibitory effect on plant pathogens (25,27). In addition to these reports, carvone and menthol are more toxic against various fungal species (11,16,30). Similarly, in our study, menthol and carvone were more fungitoxic against all tested fungi (Table 2; Figs. 2, 4).

Our results have demonstrated that β-citronellol, nerol, menthol as well as α-terpineol, terpinen-4-ol and borneol were more fungitoxic against all tested fungi. Besides, nerol and β-citronellol completely inhibited the growth of assayed pathogenic fungi and in many cases, their inhibitory effects were also greater than commercial benomyl fungicide. Hence, we suggest that β-citronellol, nerol, menthol and α-terpineol may be used as new antifungal reagents against plant pathogens in agriculture. Further studies are needed to evaluate the cost, efficacy, safety and antifungal spectrum of these compounds.

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