Journal of Agricultural Chemistry and Environment, 2017, 6, 175-185
http://www.scirp.org/journal/jacen
ISSN Online: 2325-744X
ISSN Print: 2325-7458

Phyto-Fungicides: Structure Activity Relationships of the Thymol Derivatives against *Rhizoctonia solani*

Kamlesh R. Chauhan[1*], Thanh C. Le[1], Praveen Kumar Chintakunta[1], Dilip K. Lakshman[2*]

[1]Invasive Insects Biocontrol & Behavior Laboratory, USDA-ARS, BARC-West, Beltsville, MD, USA
[2]Sustainable Agricultural Systems Laboratory, USDA-ARS, BARC-West, Beltsville, MD, USA
Email: *kamal.chauhan@ars.usda.gov, *dilip.lakshman@ars.usda.gov

How to cite this paper: Chauhan, K.R., Le, T.C., Chintakunta, P.K. and Lakshman, D.K. (2017) Phyto-Fungicides: Structure Activity Relationships of the Thymol Derivatives against *Rhizoctonia solani*. *Journal of Agricultural Chemistry and Environment*, **6**, 175-185.
https://doi.org/10.4236/jacen.2017.64012

Received: September 29, 2017
Accepted: November 6, 2017
Published: November 9, 2017

Copyright © 2017 by authors and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/
Open Access

Abstract

Thymol, the key component of the thyme oil and its derivatives were evaluated for their structure activity relationship as fungicide against *Rhizoctonia solani*. Since plant-based chemicals are considered as "Generally Recognized as Safe" (GRAS) chemicals, there is great potential to use those and synthetic derivatives against *R. solani* and other fungal pathogens, *in vitro*, and in the greenhouse or field conditions. Among the six thymol derivatives evaluated, thymol acetate was considered as the most suitable commercially viable plant-based fungicide due to its superior efficacy as well as lowest residue.

Keywords

Fungicide, Thymol Derivatives, *Rhizoctonia solani*

1. Introduction

Agricultural plants are susceptible to several economically important soilborne fungal diseases. Fungi are considered the foremost important plant pathogen and *Rhizoctonia solani* is a ubiquitous soilborne fungal pathogen causing damping off, root rot and aerial blights of economically important crops, forest trees, ornamentals and turf grasses, as well as decay of postharvest products. Soilborne fungal pathogens, including *R. solani* have been traditionally controlled using chemicals, some of which deplete natural resources, inconsistent in efficacy and toxic to the environment. Thus, eco-friendly management using safer chemicals (biorationals) are being increasingly sought to control plant diseases.

Management of fungal diseases with conventional fungicides has been erratic due to high genetic variability of pathogens. Also, persistence of many fungicides following use has resulted in pathogen resistance to several fungicides, pollution of the environment, and dangerous accumulation of toxic chemicals in the food chain. In fact, the 1996 "Food and Quality Protection Act" of the United States has dramatically restricted the use of many conventional pesticides upon which the American farmers have long depended. Thus, eco-friendly management using safer chemicals (biorationals) are being increasingly sought to control plant diseases.

Plants are inexhaustible reservoirs of secondary metabolites, some of which are antimicrobial in nature and are believed to function as a natural defense mechanism against pests and pathogens. Several of these chemicals are less phytotoxic and easily biodegradable. Many chemicals derived from plants are widely consumed as food additives or are well-known in folk medicine and are traditionally used to protect stored grains to inhibit toxicogenic pathogens and insect pests as well as repellents of insects in household. Since plant chemicals are considered as "Generally Recognized as Safe" (GRAS) chemicals, there is great potential to use those and synthetic derivatives against *R. solani* both *in vitro* and in greenhouse conditions.

Thymol (5-methyl-2-(1-methylethyl) phenol, PubChem CID: 6989) is a natural monoterpene phenol found primarily in thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), and tangerine (*Citrus tangerina*) peel [1]. As a component of naturally occurring essential oil, thymol has been demonstrated as potent antifungal agent [2]. The chemical induces lipid peroxidation and disrupts fungal ergosterol biosynthesis and cell membrane integrity [1] [3] [4]. Thymol dissipates from soil within 5 days of treatment, also degrades faster; those features make it an ideal fumigant to control soilborne pathogens [5]. Per the Environmental Protection Agency (EPA), thymol has minimal potential toxicity and poses minimal risk, thus thymol has the potential to be developed as a novel botanical pesticide [1]. Recently submicron emulsion spray formulation of thymol was found to effectively control head blight of wheat caused by *Fusarium graminearum* [2]. Moreover, thymol was found to act synergistically with nystatin against *Candida* species [4].

The present study investigated the antifungal effectiveness of thymol and its six synthetic derivatives against the common soilborne plant pathogenic fungus *R. solani* with respect to *in vitro* growth inhibition and biocontrol efficiencies in the greenhouse to suppress cucumber seedling damping off caused by the pathogen. The objective was to evaluate which structural elements of thymol molecule are relatively important for their antifungal properties. The structural analogs consisted of the derivatization of hydroxyl group with either ether or ester linkage on the basic benzene ring structure. Ultimately, we want to evaluate thymol with decreasing polarity, increasing vapor pressure and pH elevation from acidic to almost neutral. Understanding the structure-activity relationship

(SAR) of thymol derivatives is important for development of more effective antifungal formulations for the control of soilborne plant pathogens.

2. Materials and Methodology

2.1. Fungal Isolate

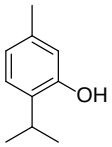
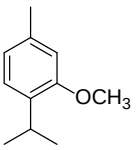
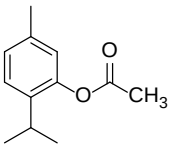
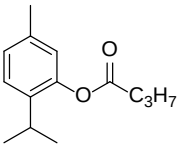
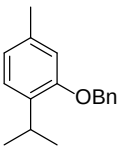
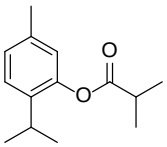
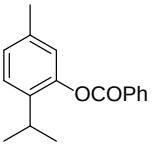
A highly virulent and damping-off causing soilborne fungus *R. solani* anastomosis group (AG) 4, isolate Rs 23A [6] was used for antifungal bioassay thymol derivatives. The fungus is known to infect many species of vegetables, cereals, ornamentals, turfgrasses, and forest trees [7]. *R. solani* was maintained on potato dextrose agar (PDA) in sterile disposable plastic Petri plates at 22˚C incubator in dark. *R. solani* inoculum was prepared for greenhouse bioassay by growing it on sterile cracked wheat as described earlier [8]. A dose response of the *R. solani* inoculum was conducted by mixing using 0.25, 0.5, 0.75, 1.0, 1.25, and 1.50 g inoculums per kg dry weight potting soil plus sterile water to maintain 25% - 30% moisture. The mixture was incubated at room temperature for 2 weeks and the infested soil was used for sowing cucumber (*Cucumis sativus* L. cv muncher) seeds. The suppression of germination of cucumber seedlings and post-emergence damping-off caused by *R. solani* off was recorded. The lowest inoculum dose that induced 100% post-emergence damping-off was estimated to be 1 - 1.25 g/kg soil (data not shown). For soil fumigation with thymol derivatives, the minimum inoculum dose giving rise to 100% damping-off was used (**Table 4**).

Thymol and its derivatives: All the derivatives of thymol (**Figure 1**) were prepared by slightly modified literature methods [9] [10]. Ester derivatives of thymol (acetate, butyrate, isobutyrate, and benzoate) were obtained simply by treating 1.0 equivalent of thymol with 1.1 equivalents of respective acid chlorides in presence of 3-5 equivalents of pyridine. Thymol ethers (methyl and benzyl) were synthesized by treating 1.0 equivalent of thymol with 1.2 equivalents of sodium hydride (60% in oil) and 1.2 equivalents respective halide (methyl iodide and benzyl bromide) in DMF solvent. All the synthesized molecules were purified by column chromatography on silica gel. Except thymol and thymol acetate all other derivatives were significantly different in their critical pressure and log P values (**Table 1**).

2.2. *In vitro* Poisoned Food Bioassays

The *in vitro* poisoned food technique was used to investigate the mycelial growth inhibitory potentials of thymol and its derivatives [8] [11]. In brief, 15, 30, 45, 60, 75, and 150 µL of test chemicals were mixed per 20 mL of autoclaved PDA maintained at 50˚C water bath, and each mixture was poured on a sterile disposable plastic Petri plate (100 mm × 15 mm, Thermo Fisher Waltham, MA) to solidify [11]. The concentrations of the chemicals in respective treatments were calculated as 0.075, 0.15, 0.225, 0.3, 0.375, and 0.75%. Since 0.3% soybean oil in PDA has no effect on the *in vitro* growth of *R. solani* (Lakshman *et al.*, 2017), 0.3% soybean oil was used as a negative control in the experiment. A

Table 1. Comparative chemical and physical properties of thymol and its six derivatives used for evaluating relative fungicidal potentials.

Chemical	Structure	Properties			
		M. Wt	Rt[a]	Critical pressure[b] (bar)	(Log P)[b]
Thymol		150	15.801	34.4	3.37
Thymol methyl ether		164	11.527	25.46	3.63
Thymol acetate		192	14.948	23.66	3.34
Thymol butyrate		220	14.648	19.79	4.41
Thymol benzyl ether		240	17.828	20.16	5.36
Thymol isobutyrate		220	14.182	19.93	4.56
Thymol benzoate		254	18.660	20.57	5.24

[a]Determined on Agilent GC-MS 6890N, MSD 5973, Column: Agilent 122-5032, carrier gas: Helium, flow rate: 1.2 mL/min; [b]Calculated by Chembiodraw 13.

broad-spectrum soil fungicide Banrot® 40 WP (The Scotts Company, Ohio) was used as a positive control in the *in vitro* bioassay, following manufacturer's recommendation. A 5 mm diameter agar disk of 5 days old *Rhizoctonia* culture was placed in the center of Petri plate containing 20 ml solidified PDA and sealed with stretched Parafilm® M (Thermo Fisher Waltham, MA). The plates were incubated at room temperature in the dark until the pathogen reached to the edge of the plates in the soybean oil control treatment. Radial measurement of fungal growth was recorded by taking three measurements in millimeter at

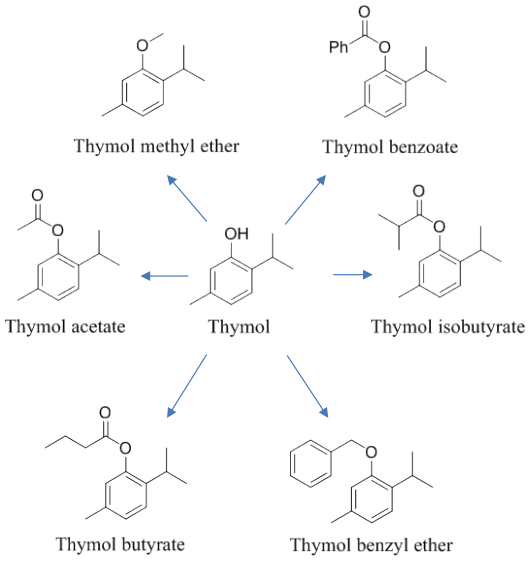


Figure 1. Thymol and derivatives.

equal distance from each plate, and the average radial mycelia growth from the three plates was calculated. The percent mycelial growth inhibition (MGI) due to plant extract was calculated using the following formula: MGI = (rc − rt)/rc × 100 where rc = avg. radial growth in millimeter of pathogen in control plate; rt = avg. radial growth in millimeter of pathogen in treated plate [12]. Each plant chemical experiment was done in three Petri dishes, and the experiment was performed twice. The concentration of chemical at which the pathogen shown complete inhibition of mycelial growth was taken as minimum inhibitory concentration (MIC) to the pathogen.

2.3. *In Plate* Bioassay for Fungicidal and Fungistatic Actions

For differentiating between the fungistatic (no new growth) versus fungicidal (death of fungus) properties of test chemicals, three 5 mm diameter plugs were taken from the peripheral mycelial growth from each plant extract treated plate and placed in a fresh PDA plate [13]. Observations for mycelial growth (fungistatic action) or no growth (fungicidal action) were recorded 7 days after incubation in dark at room temperature. The lowest concentration of each chemical showing complete inhibition of fungal growth (*i.e.*, fungistatic) is occasionally termed as the minimal inhibitory concentration (MIC). Minimal lethal concentration (MLC) is attributed as the minimal concentration of chemical showing fungicidal action [14]. As control, peripheral mycelial plugs from soybean oil amended PDA plates were checked for fresh mycelial growth in fresh PDA plates.

2.4. Suppression of *Rhizoctonia* Damping-off in Cucumber Seedlings in Greenhouse

Procedure as described earlier [15] was followed for testing the effectiveness of

chemicals to control *Rhizoctonia* damping-off from cucumber. In brief, 300 g of dry greenhouse soil (Pro-mix, Premier Horticulture LTD, Red Hill, PA) was mixed with *Rhizoctonia* inoculum and incubated for 15 days as described above. The infested soil was subsequently mixed with 100 mL of selected chemical emulsified in a carrier solution containing 0.5% (w/v) L-α-lecithin (Sigma-Aldrich Corporation, St. Louis, MO), 0.1% (v/v) Triton X-114 (Rohm and Haas, Philadelphia, PA) and sterile water to obtain a final concentration of 0.3% of chemical (0.3 mL of chemical per 100 g of soil, or 3000 ppm). The infested soil treated with only the carrier solution served as an untreated control. As positive control, Banrot® 40 WP was used following manufacturer's recommendation to control the disease. Following the treatment, the soil was thoroughly stirred to mix the contents and incubated in a sealed double polythene bag, in the dark at room temperature for 4 days. Following the incubation, the bag was opened to allow the chemical to dissipate. Treated soil was distributed to 4-inch sterile plastic pots into which 12 to 16 seeds of cucumbers (80% - 100% germination) were sown and lightly watered to keep soil moist. The treatments were color coded, and pots were randomized across treatment. All plants were kept in a greenhouse between 20˚C (night) to 26˚C (day) with 14 h of light. There were three pots per treatment in each experiment, and the experiment was repeated once more. A total number of seedlings without any sign of damping-off in each pot were recorded, averaged, and the percentage of disease suppression was calculated 4 weeks after sowing cucumber seeds.

3. Results

3.1. *In vitro* Antifungal Properties of Thymol Derivatives

All the tested thymol derivatives, including the thymol showed antifungal properties in terms of inhibition of fungal growth in the poisoned food bioassay.

Amongst the chemical tested, thymol acetate, thymol benzylether, and thymol showed the highest antifungal activities, demonstrating complete (*i.e.*, 100%) inhibition of fungal growth at the lowest tested concentration (0.075%) of the chemicals (Table 2, Figure 2). On the other hand, thymol butyrate, thymol methyl ether and thymol benzoate demonstrated 97.00%, 83.75% and 59.17% inhibition, respectively, at the highest tested concentration (0.75%). Amongst the three antagonistically best chemicals, thymol acetate and thymol showed MLC (*i.e.*, fungicidal activity) at 0.075% concentration, the thymol benzyl ether showed MIC (*i.e.*, fungistatic action) at 0.075% concentration, but MLC at 0.150% concentration. Thus, both thymol acetate and thymol were found to be more potent antifungal chemicals than the thymol benzylether. The thymol derivative, thymol isobutyrate reached MIC only at 0.750%. Banrot® 40 WP at 150 µg/ml concentration in PDA medium gave complete suppression (*i.e.*, 100%) of *R. solani*, and was fungistatic in action (Figure 2).

The relative antifungal potentials of thymol acetate and thymol were compared by using lower concentrations of the respective chemicals in the poisoned

Table 2. Comparison of antagonistic potentials of thymol and its six chemical derivatives.

Conc. % (µl/plate)	% Inhibition of mycelial growth of *Rhizoctonia solani*						
	Thymol methyl ether	Thymol acetate	Thymol butyrate	Thymol benzylether	Thymol isobutyrate	Thymol benzoate	Thymol
0.075% (15)	15.83 (S)	100 (C)	79.47 (S)	100 (S)	66.12 (S)	24.17	100 (C)
0.15% (30)	57.08 (S)	100 (C)	92.80 (S)	100 (C)	78.90 (S)	53.90	100 (C)
0.225% (45)	72.92 (S)	100 (C)	96.67 (S)	100 (C)	80.57 (S)	53.62	100 (C)
0.300% (60)	73.00 (S)	100 (C)	99.97 (S)	100 (C)	82.50 (s)	55.85	100 (C)
0.375% (75)	80.00 (S)	100 (C)	99.72 (S)	100 (C)	87.50 (S^a)	51.40	100 (C)
0.75% (150)	83.75 (S)	100 (C)	97.00 (S^a)	100 (C)	98.62 (C)	59.17	100 (C)

C = Fungicidal; S = Fungistatic; S^a = 2/3 germinated.

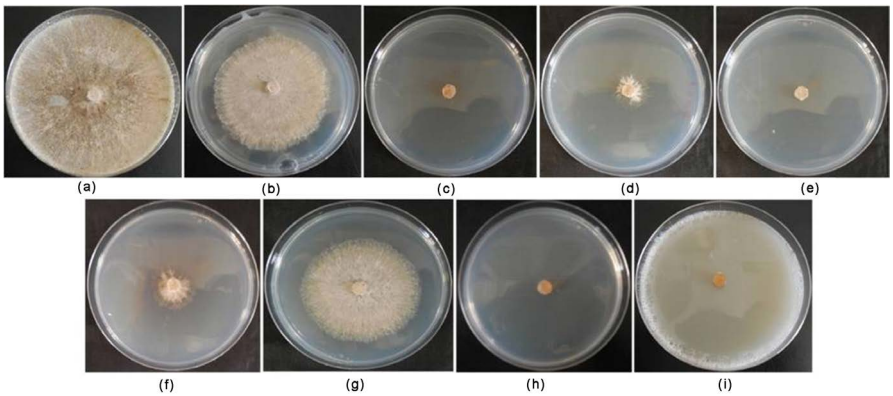


Figure 2. *In plate* antagonism of thymol and its synthetic derivatives at the rate of 0.075% (15 ppm) on the plant pathogenic fungus *Rhizoctonia solani* in the poisoned food bioassay on potato dextrose agar (PDA) medium. Banrot® 40 WP, a known chemical fungicide used following manufacturer's recommended dose served as a positive control. Plates show *R. solani* growth on PDA (a); on thymol methyl ether (b); thymol acetate (c); thymol butyrate (d); thymol benzyl ether (e); thymol isobutyrate (f); thymol benzoate (g); thymol (h); and Banrot (i).

food bioassay. Both the chemicals completely inhibited fungal growth at 0.021% concentration (*i.e.*, MIC). However, at 0.012% concentration, thymol acetate was 96.25% inhibitory to *R. solani*; thymol was only 58.25% inhibitory to the fungus at the same concentration (Table 3). Besides, thymol acetate was fungicidal at 0.041% concentration (*i.e.*, MLC), whereas thymol showed MLCat 0.058% concentration. Thus, thymol acetate was found to be slightly more potent antifungal chemical than the thymol itself.

3.2. Control of *Rhizoctonia* Damping-off with Thymol Derivatives

Thymol and the six derivatives were compared at the rate of 0.3% (v/w) as soil amendment to evaluate effectiveness for controlling damping-off from cucumber seedlings by *Rhizoctonia* pathogen in the greenhouse (Table 4). In presence of

Table 3. Relative antagonistic potentials of thymol and thymol acetate in the poisoned food assay.

Concentration % (µl/plate)	% Inhibition of mycelial growth of *Rhizoctonia solani*	
	Thymol acetate	Thymol
0.058% (11.67)	100 (C)	100 (C)
0.041% (8.3)	100 (C)	100(S)
0.025% (5.00)	100 (S)	100 (S)
0.021% (4.25)	100 (S)	100 (S)
0.012% (2.50)	96.25 (S)	58.25 (S)
0.008% (1.67)	83.35 (S)	26.67 (S)
0.004% (0.83)	8.00 (S)	0.00 (S)

Table 4. Greenhouse bioassay of plant chemicals to suppress damping-off of cucumber seedlings caused by *Rhizoctonia solani.*

Treatments	% Seedling emergence
Rhizoctonia only	20.62
Control (NO *Rhizoctonia*)	100
Thymol methyl ether+ *Rhizoctonia*	62.50
Thymol acetate + *Rhizoctonia*	100
Thymol butyrate + *Rhizoctonia*	95.81
Thymol isobutyrate + *Rhizoctonia*	97.87
Thymol benzyl ether + *Rhizoctonia*	54.12
Thymol benzoate + *Rhizoctonia*	47.87
Thymol + *Rhizoctonia*	100
Rhizoctonia + Banrot® 40 WP	100

Note: 0.3% of the chemical was used for soil fumigation.

Rhizoctonia pathogen alone, seedling emergence was only 20.62% compared to absence of the pathogen. On the other hand, soil amendment in a *Rhizoctonia* infested soil with thymol acetate, thymol butyrate, thymolbenzyl ether, and thymol resulted in 100%, 95.81%, 97.87%, and 100% seedling emergence, respectively. As a positive control, Banrot® 40 WP treatment of *Rhizoctonia* inoculated soil at manufacturer's recommended dose gave rise to 100% seedling emergence. Although slightly less efficient control of damping-off were noted, thymol methyl ether, thymol isobutyrate, and thymol benzoate gave 62.50%, 54.12%, and 47.87% emergence of cucumber seedlings, respectively. Subsequently, lower doses of both thymol acetate and thymol were compared as soil treatment (Table 5). At 0.075% (v/w) dose, thymol acetate as soil amendment permitted higher emergence of cucumber seedlings than amendment with thymol.

Table 5. Comparison of *Rhizoctonia* damping-off management potentials of thymol and thymol acetate as soil treatment in the greenhouse.

Treatments	% Seedling emergence	
No *Rhizoctonia*	100	100
Rhizoctonia only	0	0
	Thymol acetate	Thymol
Rhizoctonia + 0.225% Chemical	100	96.60
Rhizoctonia + 0.15% Chemical	73.00	86.60
Rhizoctonia + 0.075% Chemical	50.00	36.60

4. Discussion

Investigations on the structure-function relationship of antifungal chemicals are important to identify and develop economically viable and effective fungicides with minimum phytotoxicity, low half-life, and reduced environmental hazards [16].

In this investigation six derivatives of thymol were tested along with thymol, both *in vitro* for antagonistic potential, and in the greenhouse as soil amendment to control damping-off caused by *R. solani*. All the derivatives of thymol were prepared by slightly modified literature methods [9] [10].

Among the tested derivatives thymol acetate was identified slightly more effective than thymol itself in the *in vitro* assay, although thymol was equally effective for the control of damping-off in the greenhouse. While, thymol butyrate was not as antifungal as thymol acetate or thymol in the poisoned food bioassay, it matched with the disease control abilities of thymol and thymol acetate. From these results, it is possible to conclude that compared to thymol, thymol-esters are more or equally efficient than thymol-ethers. However, it is evident that as the alkyl chain in ester become larger the molecule is becoming less effective. In other words, ester with shorter alkyl chain (thymol acetate) is more efficient than that of esters with long/large alkyl chain (butyrate and isoutyrate) or ester with aromatic ring (benzoate). It remains to be seen what other characteristics of thymol butyrate (e.g., physical properties, stability and interaction with the soil environment) play role in management of the disease.

Thymol acetate with higher critical pressure has lowest half-life among all the derivatives we have evaluated. Thymol acetate, as a volatile liquid exhibits critical physiochemical properties compared to Thymol (**Table 1**). Deviation from log P value and critical pressure reduces fungicidal activity. Based on simple and economically viable synthetic conversion of thymol to thymol acetate, it becomes choice of soil fungicide. Our research represents the first formal report of antifungal properties of several derivatives of thymol against the soilborne plant pathogenic fungus *R. solani*. Understanding the influence of various side groups on hydroxy functionality is critical to the development of more effective and broad-spectrum fungicides. A cost comparison and ease of industrial synthesis

of the respective derivatives have not been attempted. However, their relative phytotoxic effects, compatibility with commercial pesticides, and antagonistic effects against a broad group of pathogenic fungi and bacteria, insects, and weeds should be investigated to narrow down the most effective thymol derivative(s), to enhance soil fumigation potential, and to develop formulations for foliar spray.

Acknowledgements

We thank Ruchi Pandey for technical help and Sharkina Parker for volunteer assistance for part of the project. Financial support from Chemveda Life Sciences is gratefully acknowledged.

References

[1] Gao, T., Zhou, H., Zhou, W., Hu, L., Chen, J. and Shi, Z. (2016) The Fungicidal Activity of Thymol against *Fusarium graminearum* via Inducing Lipid Peroxidation and Disrupting Ergosterol Biosynthesis. *Molecules*, **21**, 770. https://doi.org/10.3390/molecules21060770

[2] Gill, T.A., Li, J., Doppler, M. and Scofield, S.R. (2016) Thymol-Based Sub-Micron Emulsions Exhibit Antifungal Activity against *Fusarium graminearum* and Inhibit *Fusarium* Head Blight (FHB) in Wheat. *Journal of Applied Microbiology*, **121**, 1103-1116. https://doi.org/10.1111/jam.13195

[3] Ahmad, A., Khan, A., Akhtar, F., Yousuf, S., Xess, I., Khan, L.A. and Manzoor, N. (2011) Fungicidal Activity of Thymol and Carvacrol by Disrupting Ergosterol Biosynthesis and Membrane Integrity against *Candida*. *European Journal of Clinical Microbiology & Infectious Diseases*, **30**, 41-50. https://link.springer.com/content/pdf/10.1007%2Fs10096-010-1050-8.pdf https://doi.org/10.1007/s10096-010-1050-8

[4] Castro, R.D., Souza, T.M.P.A., Bezerra, L.M.D., Ferreira, G.L.S., Costa, E.M.M.B. and Cavalcanti, A.L. (2015) Antifungal Activity and Mode of Action of Thymol and Its Synergism with Nystatin against *Candida* Species Involved with Infections in the Oral Cavity: An *in vitro* Study. *BMC Complementary and Alternative Medicine*, **15**, 417. https://doi.org/10.1186/s12906-015-0947-2

[5] Hu, D. and Coats, J. (2008) Evaluation of the Environmental Fate of Thymol and Phenethyl Propionate in the Laboratory. *Pest Management Science*, **64**, 775-779. http://onlinelibrary.wiley.com/doi/10.1002/ps.1555/epdf

[6] Lakshman, D.K., Alkharouf, N., Roberts, D.P., Mitra, A. and Natarajan, S.S. (2012) Gene Expression Profiling of the Plant Pathogenic Basidiomycetous Fungus *Rhizoctonia solani* AG 4 Reveals Putative Virulence Factors. *Mycologia*, **104**, 1020-1035. https://doi.org/10.3852/11-226

[7] Papavizas, G.C. and Lewis, J.A. (1986) Isolating, Identifying, and Producing Inoculum of *Rhizoctonia solani*. In: Hickey, K.D., Ed., *Methods for Evaluating Pesticides for Control of Plant Pathogens*, The American Phytopathological Society Press, St. Paul, MN, 50-53.

[8] Lakshman, D.K., Chauhan, K.R., Pandey, R. and Choudhury, B. (2017) Evaluation of Plant-Based Antifungal Chemicals and Control of Damping-Off Caused by *Rhizoctonia solani*. *Biopesticides International*, **13**, 21-34.

[9] Guadalupe, A.-L., Araceli, P.-V., Francisco, H.-L., Myrna, D.-C., Robert, B., Edel-

mira, L. and Rachel, M. (2010) Antinociceptive Effect of Extracts and Compounds from *Hofmeisteria schaffneri*. *Journal of Ethnopharmacology*, **131**, 425-432. https://doi.org/10.1016/j.jep.2010.07.009

[10] Hyoungsu, K., Ceshea, M.W., Yongho, P. and Jiyong, H (2007) Stereoselective Synthesis of Tetrahydrofuran Lignans via BF_3·OEt_2-Promoted Reductive Deoxygenation/Epimerization of Cyclic Hemiketal: Synthesis of (−)-Odoratisol C, (−)-Futokadsurin A, (−)-Veraguensin, (+)-Fragransin A_2, (+)-Galbelgin, and (+)-Talaumidin. *Organic Letters,* **9**, 3965-3968. https://doi.org/10.1021/ol7016388

[11] Sinclair, J.B. and Dhingra O.D. (1995) Basic Plant Pathology Methods. CRC Press, Boca Raton, Fl, 448 p.

[12] Vincent, J.M. (1947) Distortion of Fungal Hyphae in the Presence of Certain Inhibitors. *Nature*, **159**, 850. https://doi.org/10.1038/159850b0

[13] Garber, R.H. and Houston, B.R. (1959) An Inhibitor of *Verticillium albo-atrum* in Cotton Seed. *Phytopathology*, **49**, 449-450.

[14] Nana, W.L., Eke, P., Atogho, T.B., Toghueo, K.R.M., Chatue, C.G., Pokaa, N.A., Ekounda, T.V., Etoa, F.X. and Fekam, B.F. (2015) *In-vitro* Fungitoxic Effect of Cold and Hot Water Extracts of three *Ocimum* Species on Fungi Causing Common Bean (*Phaseolus vulgaris* L.) Root Rot in Cameroon. *International Journal of Current Microbiology and Applied Sciences*, **4**, 596-606.

[15] Huang, Q. and Lakshman, D.K. (2010) Effect of Clove Oil on Plant Pathogenic Bacteria and Bacterial Wilt of Tomato and Geranium. *Journal of Plant Pathology,* **92**, 701-707.

[16] USEPA/IRIS (2014) Integrated Risk Information System. http://www.epa.gov/iris/