MedCrave
Step into the World of Research

Horticulture International Journal

Research Article

Open Access

CrossMark

Transcriptomic analysis of *Botrytis cinerea* in response to *Tagetes remotiflora* derived essential oil, hydrolate, and homeopathic oil

Abstract

Essential oils, hydrolates and some homeopathic substances derived from essential oils are alternatives for the control of pathogenic fungi. The molecular effect of several essential oils is still unknown and there is no research on the effect of hydrolates and homeopathic substances. The objective was to evaluate *in vitro* the effect of essential oil, hydrolate, and homeopathic oil from *Tagetes remotiflora* and Tween 20 (TW) against *B. cinerea*; as well as to determine the expression of *B. cinerea* genes due to the effect of hydrolate and Tween 20. The *in vitro* inhibition effect of these substances was evaluated in culture media. Essential oil and hydrolate were the most effective treatments in inhibiting the growth of *B. cinerea*, while Tween 20 and homeopathic oil had a lesser effect. RNA-seq was used to identify differentially expressed genes (DEGs) in response to the treatments evaluated *in vitro*. In response to hydrolate, 1,662 DEGs were found, 1,314 down-regulated and 348 up-regulated. With Tween 20, 791 DEGs were found, 456 down-regulated and 335 up-regulated. Due to the low effect of the homeopathic essential oil on *B. cinerea*, no DEGs were found. The high inhibition of the essential oil on the fungus did not allow its sequencing because RNA of sufficient quantity and quality was not obtained. The hydrolate affected pathways related to amino acids and carbohydrates; while Tween 20 affected the peroxisome and fatty acid metabolism. *In vitro* evaluation and transcriptomic analysis confirmed that the essential oil and hydrolate of *T. remotiflora* have potential application against *B. cinerea*, without detracting from the importance of the surfactant.

Keywords: essential oil, gray mold, homeopathic oil, inhibition, transcriptomic analysis, tween 20

Volume 9 Issue 3 - 2025

MA Ruiz-Gonzalez,[1] MA Serrato-Cruz,[1] R Solano-Vidal,[2] E Valadez-Moctezuma[1]

[1]Department of phytotechnics, Universidad Autónoma Chapingo, México

[2]Agricultural Parasitology, Universidad Autónoma Chapingo, México

Correspondence: E.Valadez-Moctezuma, Department of phytotechnics, Universidad Autónoma Chapingo. km 38.5 Carretera México-Texcoco. 56230, Chapingo, Estado de México, Email evaladezm@chapingo.mx

Received: July 11, 2025 | **Published:** August 01, 2025

Introduction

The "gray mold" disease, caused by *Botrytis cinerea* (Ascomycota-Sclerotiniaceae), affects different crops and is the second fungal pathogen of scientific and economic importance.[1] Its complete genome was sequenced during 2016, estimated at about 42,630,066 assembled nucleotides.[2] This fungus infects more than 400 economically important plant species, including vegetables, fruits and flowers.[3] For crops such as strawberry (*Fragaria* × *ananassa* Duch.) with global importance, "gray mold" causes fruit losses of up to 40%.[4] Therefore, the development of fungicides has been necessary; however, chemicals are a global concern because of their toxic effects on humans and the environment.[5] Continuous applications of chemicals have generated resistance in *B. cinerea* leading to an increase in the applied dose and the development of more potent chemicals and further aggravating the negative effects against the environment and human health.[6]

Some current control alternatives promote the use of plant-derived natural products and biocontrol agents,[7] of which essential oils and hydrolates have emerged as a biological alternative for their protective and preventive activity against *B. cinerea*.[8] Both essential oils and hydrolates are safe and environmentally friendly alternatives.[9] The essential oils are recognized as safe by the Food and Drug Administration (FDA).[10] Moreover, hydrolats have uses in the food sector due to their antimicrobial or antioxidant properties, suggesting that they are also safe substances.[11]

The genus *Tagetes* (Asteraceae) includes several species that have been studied for their biological activity against fungi.[12] Uniquely, essential oils of *Tagetes lemmonii* and *T. patula* have partially or completely inhibited *B. cinerea in vitro*.[13,14] Recently, hydrolates of *T. coronopifolia*, *T. terniflora*, *T. minuta* and *T. parryi* inhibited this fungus *in vitro*.[15]

The inhibition effects observed on *B. cinerea* and in other fungi are related to the mechanism of action associated with the diversity of chemical compounds present in essential oils.[16] Recently, omics sciences have been employed to understand the effect these compounds have on fungal cells.[17] Particularly, transcriptomics is useful to investigate genes related to pathogenesis and response to stimuli.[18] For example, by transcriptomic analysis, the change in gene expression in response to essential oil applications against fungi has been investigated.[19,20] Also, the effect that essential oils can have on specific fungal pathways has been shown, for example, causing damage to secondary metabolite biosynthesis, amino acid metabolism, energy metabolism, carbohydrate, membrane, ribosome, cell wall, transport, lipid and genetic information processing.[21,22]

Although there is a history of transcriptomic analyses on the effect of essential oils against fungi, for hydrolats no information is yet generated. It is only known that their effects could be attributed to the presence of hydrocarbons, alcohols, ketones, aldehydes, esters, acids or to phenolic compounds.[23,24]

On the other hand, homeopathy applied to plants is another alternative to combat and control fungal diseases.[25] For example, it has been possible to inhibit the germination of *Alternaria solani* and *Corynespora cassiicola* treated with homeopathic essential oils 6, 12, 30, 30, 60, 60, 100 and 200 CH derived from *Eucalyptus citriodora*, *Cymbopogon citratus*.[26] It has also been possible to partially inhibit *B. cinerea* with nosode 7 CH and arsenic 6 CH.[14]

On the mechanism of action of homeopathic substances, it has been confirmed that they work through the regulation of gene expression.[27] High dilutions could affect some subtle and early levels of signal transduction or gene expression.[28]

Surfactants (Tween 20, Tween 40, Tween 80) are used to prepare emulsions with essential oils.[29] However, they have been found to have inhibitory effects or are growth stimulants of *Beauveria bassiana* and *Cordyceps sinensis*.[30,31]

Although research has been conducted on the control of pathogens through the application of homeopathic substances, there is little information on the molecular mechanisms and metabolisms affected by homeopathics, as well as on the effect of hydrolats. Therefore, the objective of the study was to evaluate *in vitro* the effect of essential oil, hydrolate, and homeopathic oil from *T. remotiflora* and Tween 20 against *B. cinerea*; as well as to determine the transcriptomic profile of *B. cinerea* in response to the antifungal activity of the substances evaluated.

Material and methods

In vitro inhibition assays against *B. cinerea*

Evaluation of PDA medium: A strain of *B. cinerea* stored in the Genetic Resistance Laboratory of the Universidad Autónoma Chapingo (accession PP401673.1) was used. The fungus was reactivated in Potato Dextrose Agar (PDA) medium (BD Bioxon®) and incubated in dark conditions at 20 ± 2 °C for five days. In five Erlenmeyer flasks, 100 mL of PDA medium was prepared and sterilized at 120 °C for 15 min (FelisaR Autoclave, Mexico). The PDA medium was allowed to cool to room temperature (17 °C). The treatment substances (v:v) were added: essential oil (EO) of T. remotiflora (500 µL), 100 mL of hydrolate (H), 100 µL of Tween 20 (TW), 40 µL of homeopathic essential oil (HO) and one treatment was used as control (CO).

From a five-day-old colony of *B. cinerea*, inoculum was obtained and transferred to the center of the Petri dishes. Incubation was as indicated above. To estimate the percentage of inhibition, radial growth was measured with a digital vernier every 24 h.[32] Each treatment had five replicates. A completely randomized design was used. Analysis of variance was performed in SAS® OnDemand for Academics.[33]

Evaluation in NYDA liquid medium: Nutrient yeast-glucose agar (NYDA) medium was prepared with 1.2 g nutrient broth, 0.75 g yeast extract, 1.5 g glucose and 150 mL distilled water. In 50 mL Falcon tubes, 10 mL of sterilized medium was added followed by the treatment substances (EO, H, HO and TW). The control treatment consisted of NYDA medium alone. Tubes were inoculated from a 1×10^4 μL^{-1} spore suspension. Three replicates were applied for each treatment. The inoculated tubes were incubated at 20 ± 2 °C and constant agitation at 100 rpm in an orbit shaker (ORBIT, Labline instruments) for 96 h. Every 24 h, fungal growth was estimated by changes in optical density with an ND-1000 v3.5 spectrophotometer (NanoDrop Technologies, Inc. USA).

RNA extraction: 48 h cultures from liquid medium were used for RNA extraction, the culture was recovered by cold centrifugation at 3,800 rpm for 17 min (eppendorfR 5810R, Germany). TRIzol™ Reagent kit (Invitrogen) was used for extraction. RNA quality and quantity were determined with the RNA Pico Sensitivity Reagent Kit and LabChipGX Touch PE (PerkinElmer, USA). Twenty-four libraries were synthesized with TruSeq Stranded mRNA LT Sample Prep Kit. On an Agilent 2100 Bioanalyzer with DNA 1000 chip, the size distribution of PCR-enriched fragments was analyzed. Sequencing of 101 bp paired-end reads was performed at Macrogen (Seoul, South Korea) with NovaSeq6000 (Illumina, USA). Sequence files were registered at the National Center for Biotechnology Information, accession numbers: BioProject PRJNA1143533 (SRR30421781-SRR30421792).

Sequencing, mapping and annotation: Bioinformatics analysis was performed in Galaxy Europe (https://usegalaxy.eu//). FastQC (v0.74+galaxy0) tools were used to create a quality report of the reads. Trimmomatic (v0.39+galaxy2) with the parameters Slidingwindow:5:25, Avgqual:30 and Minlen:60 was used to improve the quality of the readings by trimming and filtering. Mapping was performed with the RNA STAR tool (v2.7.11a+galaxy0) to map the trimmed reads to the reference genome of *Botrytis cinerea* B05.10. obtained from https://genome.ucsc.edu/index.html. The FeatureCounts tool (v2.0.3+galaxy2) was used for counting reads per gene. The Limma-Voom v3.58.1+galaxy0 tool was used to determine the differentially expressed genes (DEGs) for hydrolate *vs* control, homeopathic oil *vs* control and Tween 20 *vs* control comparisons. A |logFC|>1 and adjusted *p*-value <0.05 were used for DEGs extraction. The heatmap2 tool (v3.1.3.1+galaxy0) was used to visualize the data.

Gene ontology (GO) analysis and KEGG pathways: For enrichment of DEGs in KEGG pathways, KOBAS 3.0[34] was used. In the GO analysis, g:Profiler[35] was used. In both programs, significance threshold: Benjamini-Hochberg FDR=0.05 and the reference genome *B. cinerea* B05.10 were used.

Results

In vitro inhibition assays against *B. cinerea*

Evaluation of PDA medium: Inhibition results with the treatments essential oil, hydrolate, homeopathic oil, Tween 20 and control were reported at five days. Essential oil inhibited mycelial growth by 91% and hydrolate by 86% ($p < 0.05$). Tween 20 inhibition was 28% ($p < 0.05$). The homeopathic essential oil inhibited fungal growth by 19%, but with no statistical difference compared to the control. Essential oil and hydrolate inhibited mycelial growth four times more than homeopathic essential oil and three times more than Tween 20 (Figure 1).

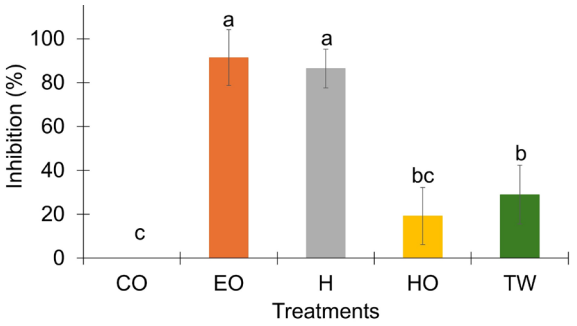


Figure 1 Inhibition of mycelial growth of *B. cinerea* at five days due to the effect of the treatments essential oil 0.5% (EO), hydrolate 100% (H), homeopathic oil 6 CH (HO), Tween 20 0.1% (TW) compared to control (CO). Means ± standard deviation (n=5) with different letters are statistically different ($p < 0.05$).

Evaluation of NYDA liquid medium: The mycelial growth observed on NYDA liquid medium was similar to that on PDA medium. The essential oil caused the least growth of *B. cinerea* at the 24 and 48 h evaluation times ($p < 0.05$), whereas, the hydrolate reduced the growth of the fungus at both times, although the differences were only significant at 24 h. Mycelial growth in the homeopathic oil and Tween 20 treatments was lower than that of the control, but there were no significant statistical differences ($p < 0.05$) (Figure 2).

Citation: Ruiz-Gonzalez MA, Serrato-Cruz MA, Solano-Vidal R, et al. Transcriptomic analysis of *Botrytis cinerea* in response to *Tagetes remotiflora* derived essential oil, hydrolate, and homeopathic oil. *Horticult Int J.* 2025;9(3):120–128. DOI: 10.15406/hij.2025.09.00333

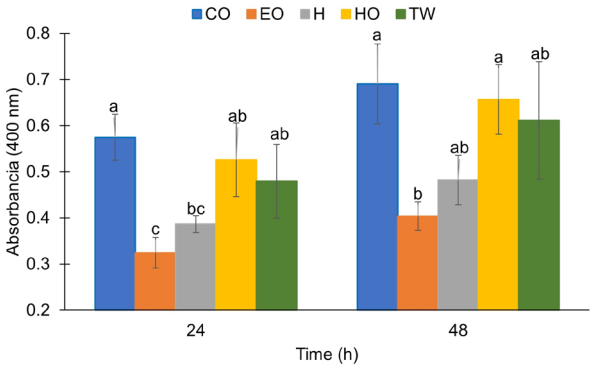


Figure 2 Growth of *B. cinerea* in NYDA liquid medium with the treatments of 0.5% essential oil (EO), 100% hydrolate (H), 6 CH homeopathic oil (HO) and 0.1% Tween 20 (TW) compared to control (CO). Means ± standard deviation (n=5) with different letters are statistically different ($p < 0.05$).

Sequencing and mapping: Total RNA was sequenced from four treatments: hydrolate, homeopathic oil, Tween 20 and control. Due to severe inhibition of the fungus with the essential oil treatment (Figures 1 and 2), RNA of sufficient quantity and quality was not obtained for sequencing. The total number of reads from the twelve libraries was 293,555,820 and ranged from 20,435,149 (TW3) to 31,220,838 (H2) with an average of 24,462,985 reads (Table S1). After quality control, 261,319,701 clean reads were retained, a maximum of 27,696,340 (H2) and a minimum of 18,294,462 (TW3), with an average of 21,776,641 reads. About 89% of readings were retained (Table S1).

The total number of clean reads mapped onto the *Botrytis cinerea* B05.10. reference genome was 252,138,043 which represented 96.5%; and this percentage ranged from 95 (H3) to 98.4% (TO2). Similarly, the percentage of uniquely mapped reads ranged from 94.8% (H3) to 98.1% (TO2) (Table S1).

Differential gene expression: The Limma-Voom method was used to extract DEGs (|logFC|>1 and adjusted $p < 0.05$) and adjusted $p < 0.05$) from comparisons between the three treatments and the control. The MDS plot showed that the first three axes collected most of the gene expression variation (86%). The first axis collected the largest percentage of the variation (58%) and on which the hydrolate treatment was separated from the other three treatments, while, on axis 2 (23%), the Tween 20 treatment was separated from the other treatments (Figure 3). The transcriptomic profile under homeopathic oil treatment was indistinguishable from the control (Figure 3).

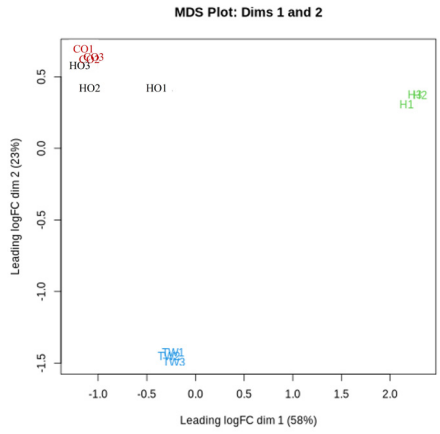


Figure 3 MDS plot showing gene expression variation of *B. cinerea* obtained from RNA-seq data at 48 h in response to 100% hydrolate (H1, H2, H3), 6 CH homeopathic oil (HO1, HO2, HO3), 0.1% Tween 20 (TW1, TW2, TW3) and control (CO1, CO2, CO3) treatments.

In the hydrolate vs control comparison, 1,662 DEGs were identified (|logFC|>1 and adjusted $p < 0.05$), of which 1,314 were down-regulated and 348 up-regulated. In the Tween 20 vs control comparison, 791 DEGs were found, of which 456 were down-regulated and 335 up-regulated. However, in the homeopathic essential oil vs control comparison, no DEGs were found (|logFC|>1 and adjusted p<0.05) (Figure 4). A total of 469 DEGs were common between the hydrolate treatment and the Tween 20 treatment. While 1,193 and 322 DEGs were unique to both treatments (Figure S1).

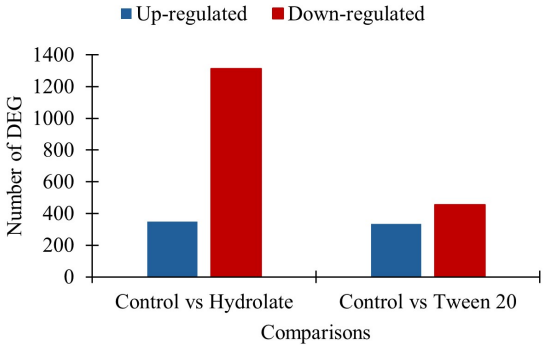
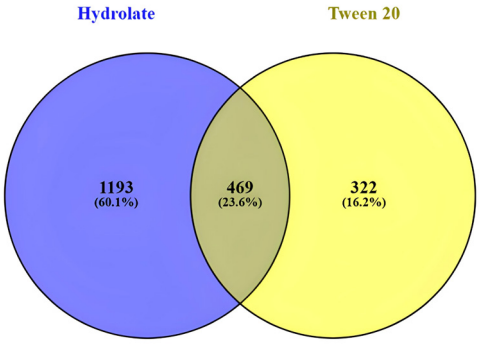


Figure 4 Number of differentially expressed genes (DEGs; |logFC|>1 and adjusted *p*-value <0.05) in *B. cinerea* because of hydrolate and Tween 20 compared to control.



Supplementary Figure 1. Venn diagram showing unique and shared DEGs of *B. cinerea* due to hydrolate and Tween 20 treatments.

Annotation of differentially expressed genes

Enrichment of KEGG pathways

The 1,662 DEGs extracted from the hydrolate *vs* control comparison allowed enrichment of 3 KEGG pathways ($p < 0.05$), namely "valine, leucine and isoleucine degradation", "propionate metabolism" and "sulfur metabolism". For the pathway: "valine, leucine and isoleucine degradation", thirteen genes were found, of which only gene *BCIN_01g09170* was up-regulated due to hydrolate, whereas genes *BCIN_13g03020*, *BCIN_01g03020*, *BCIN_08g05100*, *BCIN_04g04810*, *BCIN_02g07690*, *BCIN_11g04250*, *BCIN_07g02750*, *BCIN_08g05050*, *BCIN_08g05040*, *BCIN_13g01430*, *BCIN_03g05840* and *BCIN_02g06840* were down-regulated (Figure 5A). In the pathway: "propionate metabolism", nine genes (BCIN_13g03020, *BCIN_15g05660*, *BCIN_01g03020*, *BCIN_11g04250*, *BCIN_03g05840*, *Bcpdh1*, *BCIN_04g03150*, *BCIN_13g01430*, *BCIN_07g03110*) were found, all were down-

regulated due to hydrolate (Figure 5B). In the "sulfur metabolism" pathway, seven genes were found, of which six were up-regulated (*Bcmet5*, *Bcmet3*, *BCIN_15g02880*, *Bcmet10*, *Bcmet16*, *Bcsox1*) due to hydrolate and *BCIN_12g05950* gene was down-regulated (Figure 5C).

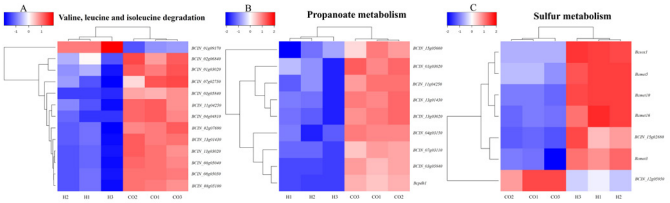


Figure 5 Heat maps of the expression profile of genes related to KEGG pathways enriched in *B. cinerea* in response to 100% hydrolate (H1, H2, H3) compared to control (CO1, CO2, CO3). A: valine, leucine and isoleucine degradation, B: propionate metabolism, C: sulfur metabolism. Heat maps were constructed based on Z-score. Red color indicates up-regulated genes, and blue color indicates down-regulated genes, the intensity of the colors represents the level of expression.

The 791 DEGs extracted from the Tween 20 *vs* control comparison allowed enrichment of five KEGG pathways ($p < 0.05$): "peroxisome", "fatty acid degradation", "fatty acid metabolism", "unsaturated fatty acid biosynthesis" and "glyoxylate and dicarboxylate metabolism". For the pathway: "unsaturated fatty acid biosynthesis", the four identified genes (*BCIN_14g02030*, *BCIN_03g03940*, *BCIN_04g06330*, *BCIN_13g00280*) were up-regulated (Figure 6A). For the pathway: "fatty acid metabolism", seven genes (*BCIN_14g02030*, *BCIN_03g03940*, *Bcfaa2*, *BCIN_04g06330*, *BCIN_16g03180*, *BCIN_11g01810*, *BCIN_13g00280*) were found, all were up-regulated due to Tween 20 treatment (Figure 6B). For the pathway: "fatty acid degradation", seven DEGs were found; *BCIN_02g04910*, *BCIN_14g02030*, *Bcfaa2*, *BCIN_16g03900*, *BCIN_16g03180* and *BCIN_03g03940* genes were up-regulated due to Tween 20 and *BCIN_01g02880* gene was down-regulated (Figure 6C).

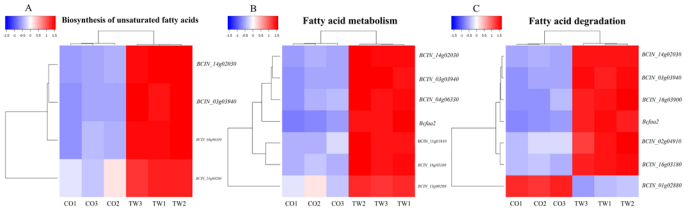


Figure 6 Heat maps of the expression profile of genes related to KEGG pathways enriched in *B. cinerea* in response to Tween 20 (TW1, TW2, TW3) compared to control (CO1, CO2, CO3). A: unsaturated fatty acid biosynthesis, B: fatty acid metabolism, C: fatty acid degradation. Heat maps were constructed based on Z-score. Red color indicates up-regulated genes, and blue color indicates down-regulated genes, the intensity of the colors represents the level of expression.

For the pathway: "glyoxylate and dicarboxylate metabolism", five genes were found, of which two (*BCIN_09g01320* and *BCIN_09g06110*) were up-regulated and three (*Bccat7*, *Bccat5* and *Bcfdh1*) were down-regulated because of Tween 20 (Figure 7A). For the pathway: "peroxisome", 19 genes were found, 17 of them (*BCIN_05g06540*, *BCIN_14g02960*, *BCIN_14g02030*, *BCIN_03g03940*, *Bcpex11*, *Bcsps19*, *Bcfaa2*, *BCIN_04g06330*, *BCIN_15g01560*, *BCIN_16g03900*, *Bcpex6*, *Bcpxa1*, *BCIN_07g03000*, *BCIN_04g03710*, *Bcpex13*, *Bcpex10*, *Bcpex14*) were up-regulated with Tween 20 and only two genes (*Bccat7*, *Bccat5*) were down-regulated (Figure 7B).

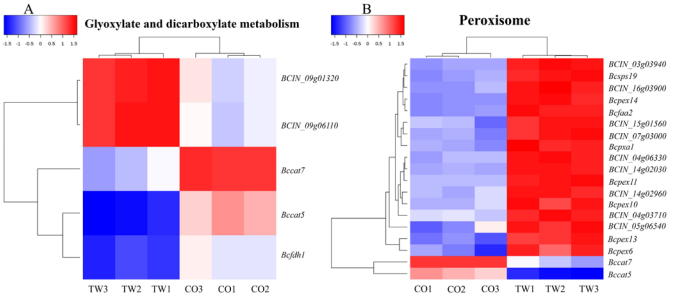


Figure 7 Heat maps of the expression profile of genes related to KEGG pathways enriched in *B. cinerea* in response to Tween 20 (TW1, TW2, TW3) compared to control (CO1, CO2, CO3). A: glyoxylate and dicarboxylate metabolism, B: peroxisome. Heat maps were constructed based on Z-score. Red color indicates up-regulated genes, and blue color indicates down-regulated genes, the intensity of the colors represents the level of expression.

Gene ontology (GO) analysis: The 1,662 DEGs from the hydrolate *vs* control comparison allowed the enrichment of 62 GO terms ($p < 0.05$). For the category "molecular function" (MF), 33 GO terms were enriched, the three most significant were "oxireductase activity", "catalytic activity" and "monooxygenase activity". For the category "biological process" (BP), 27 GO terms were enriched, the three most significant were "transmembrane transport", "carbohydrate metabolic process" and "carbohydrate catabolic process". In addition, other carbohydrate-related terms such as "polysaccharide catabolic process", "polysaccharide metabolic process" and "maltose metabolic process" were enriched. For the category "cellular component" (CC), two GO terms were enriched, these were "membrane" and "extracellular region" (Table S2).

In the Tween 20 *vs* control comparison, the 791 DEGs allowed the enrichment of 77 GO terms. For the MF category, 23 GO terms were enriched, the three most significant were "oxidoreductase activity", "catalytic activity" and "heme binding". For the BP category, 47 GO terms were enriched, the most significant were "fatty acid metabolic process", "peroxisomal membrane transport" and "peroxisome organization". Also, other terms related to "fatty acid beta oxidation", "fatty acid oxidation", "fatty acid catabolic process" and "fatty acid transport" were enriched. For the CC category, seven GO terms were enriched, the three most significant were "microbodies", "peroxisome" and "membrane" (Table S3).

Notably, several enriched GO terms were related to the enriched KEGG pathways. Specifically, GO terms related to carbohydrate metabolism and transport in the hydrolate *vs* control comparison and GO terms related to fatty acid metabolism in the Tween 20 *vs* control comparison. In addition, GO terms related to detoxification were enriched due to the two treatments hydrolate and Tween 20 which were "response to toxic substances", "cellular response to toxic substances", "detoxification", "cellular detoxification" and "cellular oxidative detoxification" (Tables S2 and S3). For the comparison hydrolate *vs* control, 13 DEGs were found in these four GO terms, except for the term "cellular oxidative detoxification" where 12 genes were identified (Figure 8A). For the Tween 20 treatment, the same nine DEGs were found in the five GO terms related to detoxification (Figure 8B). The *Bccat6*, *Bcprd4*, *Bcprx9*, *Bcglr2*, *Bcgst8*, *Bcprx2* and *Bcprd10* genes were identified only in the hydrolate treatment and the *Bccat2*, *Bcgst12*, *Bccat5* and *Bcprd11* genes were found only in the Tween 20 treatment. While *Bccat7*, *BccatA*, *BcppoA90*, *Bcprd3* and *Bcccp2* genes were common in both treatments (Figure 8 A and B). In terms of expression, *Bccat6*, *Bcccp2*, *Bcprd3* and *Bcprx2* genes

Citation: Ruiz-Gonzalez MA, Serrato-Cruz MA, Solano-Vidal R, et al. Transcriptomic analysis of *Botrytis cinerea* in response to *Tagetes remotiflora* derived essential oil, hydrolate, and homeopathic oil. *Horticult Int J.* 2025;9(3):120–128. DOI: 10.15406/hij.2025.09.00333

were up-regulated and *Bccat7*, *Bcprd4*, *Bcprx9*, *BcppoA90*, *BccatA*, *Bcglr2*, *Bcgst8*, *Bcprd10* genes were down-regulated in the hydrolate *vs* control comparison. In the Tween 20 *vs* control comparison, *Bccat2*, *Bcccp2*, *Bcprd3* and *Bcgst12* genes were up-regulated and *Bccat5*, *Bccat7*, *BccatA*, *BcppoA90*, *Bcprd11* genes were under-expressed. In both treatments: hydrolate and Tween 20, *Bccat7*, *BcppoA90* and *BccatA* genes were down-regulated while *Bcccp2* gene was up-regulated (Figure 8 A and B).

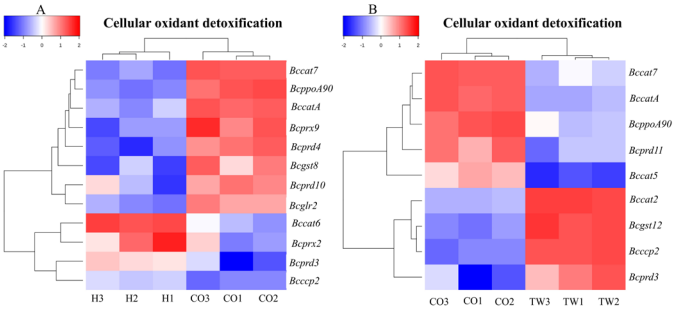


Figure 8 Heat maps of the expression profile of genes related to "cellular oxidative detoxification" in *B. cinerea* in response to hydrolate (H1, H2, H3) and Tween 20 (TW1, TW2, TW3) compared to control (CO1, CO2, CO3). Heat maps were constructed based on Z-score. Red color indicates up-regulated genes, and blue color indicates down-regulated genes, the intensity of the colors represents the level of expression.

Discussion

Essential oils, hydrolats and some homeopathic substances are alternative antifungal substances to chemical fungicides. Essential oils have more diverse uses, although the use of hydrolates and homeopathic substances for fungal control is still underexplored. Although the effect of oils on fungi has already been studied by transcriptomics, no information has been found on the effect of hydrolates and homeopathic substances derived from essential oils.

In vitro inhibition of *B. cinerea*

In this experiment, *T. remotiflora*, a species with abundant chemical compounds, was studied.[36] Of the treatments evaluated, essential oil, hydrolate, homeopathic oil and Tween 20, the 0.5% essential oil of *T. remotiflora* was the most effective in controlling the growth of *B. cinerea* under *in vitro* conditions, which coincides with results observed with the oils of *T. tenuifolia* (2 µL) and *T. lemmonii* (1%).[14,37] In this study, 100% hydrolate of *T. remotiflora* also significantly reduced the growth of *B. cinerea*. Likewise, hydrolates obtained from *Tagetes* species (*T. coronopifolia*, *T. minuta*, *T. parryi* and *T. terniflora*) as well as *Daucus carota* hydrolates also inhibited fungal growth.[8,15]

The amount, diversity and abundance of compounds present in the oils and in the hydrolates affect the fungal and bacterial inhibition processes differently.[16,38] The inhibitory effect of the essential oil can be attributed to the majority compounds (trans-β-ocimene, 2-carene, cyclohexene, 1-methyl-4-(1-methylethylidene)- and 5,7-octadien-4-one, 2,6-dimethyl-, (E)-) of the essential oil of *T. remotiflora*.[36] The hydrolates contain traces of essential oils,[38] therefore, some common compounds are found with essential oils, however, the abundance is different, and this could influence fungal inhibition.[15] *T. parryi* hydrolate was effective in controlling *B. cinerea* on flowering and fruiting strawberry plants.[15] Therefore, the next task will be to validate the effect of *T. remotiflora* hydrolate under *in vivo* conditions.

In the present study, Tween 20 at 0.1% reduced to 28% the growth of *B. cinerea*. The inhibitory effect of Tween 20 has already been observed on *Beauveria bassiana*.[31] However, *Rhizopus oryzae* was not inhibited by a high concentration (1.5%) of Tween 20.[39] This indicated that surfactants do not inhibit all fungi, they may even stimulate the growth and germination of *Beauveria bassiana* conidia.[31] Also, the type of surfactant influences the inhibition response of fungi.[40] Therefore, it is recommended to use low concentrations (<0.2%) or standardize concentrations according to the organism.

The 6 CH homeopathic oil treatment of *T. remotiflora* had the lowest inhibition (19%) against *B. cinerea*. Different results have been mentioned in other studies; homeopathic 6 CH of *Eucalyptus citriodora* and *Cymbopogon citratus* inhibited the germination of *Alternaria solani* and *Corynespora cassiicola* conidia from 26 to 34%.[26] Also, homeopathic oils of *Psidium cattleyanum* (7 CH and 12 CH) reduced the incidence of *Aspergillus flavus* and *Penicillium* sp. on bean seeds.[41] Therefore, evaluation of other dynamizations (>6 CH) of *T. remotiflora* could enhance the inhibition of *B. cinerea*.

Transcriptomic profiling of *B. cinerea* in response to hydrolate and Tween 20

Although the severe inhibition of the fungus with the essential oil treatment did not allow obtaining RNA of sufficient quantity and quality for sequencing, a shorter sampling time (6, 12 and 24 h) could allow the recovery of RNA for future analysis.

To evaluate the effect of the hydrolate and Tween 20 treatments at the molecular level, gene expression profiles obtained from RNA-seq data at 48 h were compared. The number of clean sequences obtained in *B. cinerea* was 18,294,462 to 27,696,340 (average 21,776,641, 89% of the total) and was similar to that reported by Li et al.[21] where they obtained 22,130,161 to 31,530,791 reads (average 24,950,141) from 72 h *in vitro B. cinerea* cultures treated with *Melaleuca alternifolia* essential oil.[21] Similarly, Haile et al.[42] obtained 18,245,810 to 55,880,939 reads (average 27,363,951) in samples of *Vitis vinifera* fruits inoculated with *B. cinerea* and collected after four weeks. Variations among RNA-Seq reads could be due to the fungal strain used, sampling time, experimental conditions, sequencing strategies and bioinformatic analysis flow. The results are directly related to the design, execution and analysis of the experiment.[43]

As for the mapping performed in this study, about 96.4% of the clean reads were mapped onto the *Botrytis cinerea* B05.10 reference genome. In other studies, the mapping onto the same genome has been 67 to 78%,[42] while Srivastava et al.[44] mentioned that the mapping was 97% in samples obtained from *Solanum lycopersicum* infected by *B. cinerea*. The sequencing data and mapping obtained in this study indicated the suitability of this information for further analysis.

In the DEGs extracted from the hydrolate, 1,662 DEGs were found, of these, 1,314 were down-regulated and 348 were up-regulated. Li et al.,[21] in cultures of *B. cinerea* treated with *Melaleuca alternifolia* oil, identified 280 DEGs, of which 173 were up-regulated and 107 down-regulated. Due to the effect of *Perilla frutescens* oil against *Aspergillus flavus*, 5,914 DEGs were identified, 3,025 were up-regulated and 2,889 were down-regulated.[22] In *Alternaria alternata*, 1,334 DEGs were found, 621 up-regulated and 713 down-regulated in response to citral.[45] In *Sclerotinia sclerotiorum* treated with *Anethum graveolens* essential oil, 3,793 DEGs were identified, of which 2,334 were up-regulated and 1,459 down-regulated.[19] In addition to the experimental and analytical factors mentioned above, another factor that could influence the differences between DEGs is the diversity of compounds present in the essential oils evaluated. For

example, *Melaleuca alternifolia* oil contains two major compounds (1,8-cineole and terpinen-4-ol).[21] Whereas that of *Perilla frutescens* contains at least 13 compounds[46] and in the work of Wang et al.[45] only one compound (citral) was used. *Anethum graveolens* contains 26 compounds.[22] The essential oil of *T. remotiflora* evaluated in this study contains 31 chemical compounds.[36]

Regarding the effect of Tween 20, 791 DEGs were found, 456 were down-regulated and 335 were up-regulated. In *Rhizopus oryzae*, 2,935 DEGs were identified, 1,094 up-regulated and 1,841 down-regulated due to the effect of triethanolamine.[39] Surfactants can influence the surface of fungi through the adsorption process and change their physicochemical characteristics.[47] In addition, each surfactant may have different effects on different fungal species.[40]

Enrichment of KEGG pathways due to hydrolate

DEGs due to hydrolate allowed the enrichment of pathways related to amino acid and carbohydrate metabolism. Specifically, the pathways "valine, leucine and isoleucine degradation", "sulfur metabolism" and "propionate metabolism" were activated. "Sulfur metabolism" was also enriched with *Melaleuca alternifolia* oil in *B. cinerea*.[40] In *Aspergillus flavus*, the pathways: "valine, leucine and isoleucine degradation" and "sulfur metabolism" were enriched due to *Perilla frutescens* oil.[22] The pathways: "valine, leucine and isoleucine degradation" and "sulfur metabolism" were enriched due to citral in *Alternaria alternata*.[48]

For the pathway: "valine, leucine and isoleucine degradation", there were 12 genes down-regulated due to hydrolate. The down-regulated genes *BCIN_13g03020*, *BCIN_01g03020*, *BCIN_08g05040*, *BCIN_08g05050*, *BCIN_13g01430*, *BCIN_11g04250* and the up-regulated gene (*BCIN_01g09170*) affected the catabolic process of branched-chain amino acids (valine, leucine and isoleucine). These amino acids are important components of proteins and precursors of secondary metabolites in fungi.[48] The genes *BCIN_08g05100*, *BCIN_07g02750*, *BCIN_04g04810*, *BCIN_02g07690* and *BCIN_03g05840* were involved in the inhibition of fatty acid beta-oxidation (3-methylbut-2-enoyl-CoA). This process (β-oxidation of fatty acids) is required for cell development.[31] The *BCIN_07g02750* and *BCIN_02g06840* genes inhibited the synthesis of acetoacetyl-CoA, a metabolic intermediate important for the synthesis of ergosterol which is a component of membranes and is important in antifungal activity.[49]

Inhibition of the "propionyl-CoA metabolism" pathway was due to the down-regulated of nine genes. The *BCIN_13g03020*, *BCIN_01g03020*, *BCIN_13g01430* and *BCIN_11g04250* genes inhibited propionyl-CoA synthesis, whereas the *BCIN_04g03150* gene inhibited propionate synthesis. Blocking propionyl-CoA or propionate utilization has been reported to be related to fungal inhibition.[50] *BCIN_01g03020* and *BCIN_03g05840* genes inhibited acetyl-CoA, an essential molecule of metabolism and its high production is an indicator of good metabolic status of the organism.[51] *Bcpdh1* and *BCIN_07g03110* genes influenced the synthesis of methylisocitrate and propionyl-CoA. Zhu et al.[52] mention that the accumulation of intermediates such as propionyl-CoA and methylisocitrate, which inhibit the pyruvate dehydrogenase complex and succinyl-CoA synthetase in primary metabolism, has an impact on the inhibition of filamentous fungi.

In the "sulfur metabolism" pathway, six genes were found to be up-regulated and one gene down-regulated due to hydrolate. The only down-regulated gene (*BCIN_12g05950*) was involved in sulfide oxidation by quinone: oxidoreductase. Affecting this process could generate the accumulation of hydrogen sulfide which is usually toxic in the organism.[53] The up-regulated genes, *Bcmet5*, *Bcmet3* and *BCIN_15g02880* are involved in the biosynthesis of amino acids such as cysteine, this sulfur amino acid is part of proteins and is a precursor of several metabolites.[54] The genes *Bcmet3*, *Bcmet5* and *Bcmet16* are involved in sulfate assimilation. This process is essential as sulfate assimilation is required for its incorporation into cysteine and homocysteine.[55] The genes *Bcmet10* (sulfite reductase activity) and *Bcsox1* (oxidoreductase activity) are important for the oxidation of sulfite to sulfate[56] and in the degradation or detoxification of pollutants through oxidation.[57] The gene overexpression observed in *B. cinerea* could be due to the fact that sulfur availability is essential for defense against oxidative stress and is involved in several metabolic processes in fungi.[55]

Enrichment of KEGG pathways by Tween 20

DEGs in response to Tween 20 enriched four peroxisome and fatty acid related pathways ("peroxisome", "fatty acid degradation", "fatty acid metabolism", "unsaturated fatty acid biosynthesis") and one carbohydrate pathway ("glyoxylate and dicarboxylate metabolism"). With the application of triethanolamine, Wu et al.[39] did not find the same enriched pathways, however, they reported the "lipid metabolism" pathway which is akin to fatty acid metabolism. In this study, most of the genes found in the "peroxisome" pathway were up-regulated (*BCIN_04g03710*, *BCIN_14g02030*, *BCIN_04g06330*, *BCIN_03g03940*, *Bcpxa1*, *Bcsps19*, *BCIN_14g02960*, *Bcfaa2*, *BCIN_16g03900*) and act in fatty acid oxidation. As mentioned above, β-oxidation of fatty acids is essential to utilize acetyl-CoA as a carbon and energy source for growth.[58] The genes *Bcpex6*, *BCIN_05g06540*, *Bcpex10*, *Bcpex13*, *Bcpex14* and *Bcpex11* influenced membrane proteins (PEX2, PEX6, PEX10, PEX11, PEX13, PEX14). These proteins (peroxins) are of importance in membrane assembly and import of matrix proteins.[59] While the two down-regulated genes (*Bccat7* and *Bccat5*) are in response to hydrogen peroxide. The under-expression of these genes could affect cellular compounds in the fungus.[60]

Peroxisome impairment impacted fatty acid synthesis, oxidation and degradation.[58] In addition, peroxisomes are part of fungal membranes,[61] necessary to sustain fruiting body formation, maturation and germination of sexual spores.[62] In filamentous fungi, including *B. cinerea*, peroxisomes are key to produce secondary metabolites necessary for their development and pathogenesis.[63]

In the enriched and fatty acid-related pathways, they were mostly up-regulated genes involved in β-oxidation of fatty acids, for example, in the "fatty acid degradation" pathway *BCIN_02g04910* and *BCIN_16g03900* genes were up-regulated. Also, the *BCIN_14g02030* and *BCIN_03g03940* genes that enable β-oxidation of fatty acids were up-regulated in all three pathways ("fatty acid degradation", "fatty acid metabolism", "unsaturated fatty acid biosynthesis"). The genes *Bcfaa2* and *BCIN_16g03180* ("fatty acid degradation", "fatty acid metabolism") act in fatty acid biosynthesis.[64] Likewise, the *BCIN_04g06330* and *BCIN_13g00280* genes, common in the "fatty acid metabolism" and "unsaturated fatty acid biosynthesis" pathways, were associated with β-oxidation and omega-6 fatty acids. These are structural constituents, are part of lipids and are signaling compounds in several pathways.[65]

As for the "glyoxylate and dicarboxylate metabolism" pathway, the down-regulated genes *Bccat7* and *Bccat5* act in response to hydrogen peroxide and the *Bcfdh1* gene (down-regulated) catalyzes the NAD-dependent oxidation of formate to carbon dioxide, which has a role in the detoxification of exogenous formate in non-methylotrophic

organisms.[64] The *BCIN_09g01320* gene (up-regulated) enables isocitrate lyase enzyme activity and the *BCIN_09g06110* gene enables malate synthase activity. Isocitrate lyase is a glyoxylate cycle enzyme that converts isocitrate to succinate during glyoxalate derivatization, which aids the growth of the organism,[66] while malate synthase is important in the pathogenicity and virulence of various fungi.[67] The glyoxylate cycle may act in stress defense in fungal cells and pathogenesis.[68]

Although in this study Tween 20 showed 28% inhibition and significant impact on *B. cinerea* gene expression, it is important to consider possible neutral effects since organisms respond differently (inhibition, stimulation of growth and germination) as indicated by previous studies.

Enrichment of GO categories by hydrolate and Tween 20

As described above, hydrolate and Tween 20 affected *B. cinerea* differently. For each treatment, several GO terms related to the enriched KEGG pathways were enriched. Functional annotation of DEGs from the hydrolate treatment allowed the enrichment of 62 GO terms. For the category "molecular function" (MF) 33 GO terms were enriched, 27 for "biological process" (BP) and 2 for "cellular component" (CC). In another investigation, where essential oils were applied in *B. cinerea*, 42 GO terms were enriched, 15 for MF, 18 in BP and 13 in CC.[21] In other fungi (*Alternaria alternata*, *Sclerotinia sclerotiorum* and *Aspergillus flavus*) treated with essential oils, 40 GO terms were enriched, 12 for MF, 25 in BP and 3 in CC.[45] Chen et al.[19] mentions the enrichment of 56 GO terms, 19 for MF, 19 in BP and 18 in CC. While, Hu et al.[22] indicated the enrichment of 34 GO terms, in each GO category 10 GO terms were enriched. Some GO terms enriched in this research were also enriched in some studies mentioned above. For example, in the MF category the pathway "catalytic activity"[21,45] and "oxireductase activity"[19,45] were enriched. For the BP category, the GO terms "transmembrane transport", "carbohydrate metabolic process",[19] "polysaccharide catabolic process" and "carbohydrate catabolic process"[45] are also affected. For the CC category, the categories "extracellular region" and "membrane" were enriched.[21]

Tween 20 DEGs enriched 77 GO terms, of these, 23 GO terms were enriched for the MF category, 47 in BP and seven for the CC category. In response to triethanolamine against *R. oryzae* there was enrichment of 20 GO terms, of which six were enriched for the MF category, six in BP and eight for the CC category.[39] Both treatments (Tween 20 and triethanolamine) affected GO terms of "catalytic activity" and "membrane". Although Tween 20 affected more processes (77) in *B. cinerea*, this could be because Tween 20 dissolves cell membrane lipids and makes them permeable.[69] While triethanolamine, in addition to its use as a surfactant, is also a pH regulator.[70]

In both treatments (hydrolate and Tween 20), several GO terms related to detoxification were enriched. All enriched GO terms ("response to toxic substances", "cellular response to toxic substances", "detoxification", "cellular detoxification" and "cellular oxidative detoxification") are activated when the organism is in contact with toxic substances, thus triggering a transport process to move the toxic substance away from sensitive areas or to reduce or eliminate the toxicity of superoxide radicals or hydrogen peroxide.[71] In this study, due to hydrolate, genes that enable peroxidase and catalase activity were down-regulated (*Bcprd10*, *BcppoA90*, *Bcprd4*, *Bcprx9* and *Bccat7*) and there were also up-regulated genes (*Bcprd3*, *Bcccp2* and *Bccat6*). These enzymes are oxidoreductases that act against reactive oxygen species present within cells.[72] The *Bcprx2* gene, up-regulated in *B. cinerea*, enables thioredoxin peroxidase activity and thioredoxin peroxidase has been shown to be useful for detoxification.[73] Likewise, the enzymes glutathione transferase and glutathione reductase are essential for detoxification and free radical scavenging.[74] In the present study, *Bcgst8* (glutathione transferase) and *Bcglr2* (glutathione reductase) genes were down-regulated due to hydrolate. Also, the *BccatA* gene, which catalyzes hydrogen peroxide degradation, was down-regulated. In response to Tween 20, also peroxidase activity genes were down-regulated (*Bcprd11* and *BcppoA90*) and up-regulated (*Bcprd3* and *Bcccp2*). Likewise, the catalase activity genes, *Bccat7* (down-regulated) and *Bcprd3* (up-regulated). As in the hydrolate treatment, genes that aid in hydrogen peroxide degradation were down-regulated (*BccatA* and *Bccat5*). Most organisms exert two mechanisms for detoxification; the first is the use of vacuoles[75] and the second is based on chemical modifications (inactivation, hydrolysis, oxidation or conjugation.[76] For example, *Beauveria bassiana* uses single vesicles to direct toxic compounds into detoxifying vacuoles.[75] Other fungi perform direct detoxification of toxic compounds through catalysis and enzymatic modification.[77]

Conclusions

In the laboratory conditions, *T. remotiflora* hydrolate and essential oil are more effective in controlling *B. cinerea* than Tween 20 and homeopathic oil. At the molecular level, *B. cinerea* shows a different gene response to hydrolate and Tween 20. Hydrolate inhibited the mycelial growth of *B. cinerea* by affecting amino acid and carbohydrate-related pathways. While Tween 20 affected peroxisome and fatty acid metabolism. Although hydrolate and Tween 20 affected detoxification-related functions, the gene profile was different for each treatment. Hydrolate could be used as a safe antifungal input against *B. cinerea* once its *in vivo* effect is verified.

Acknowledgments

None

Funding statement

No funding was received for this study.

Conflicts of interest

The authors declare no conflict of interest.

References

1. Dean RA, Van Kan JA, Pretorius ZA, et al. The Top 10 fungal pathogens in molecular plant pathology. *Mol Plant Pathol.* 2012;13(4):414–430.
2. Van Kan JAL, Stassen JHM, Mosbach A, et al. A Gapless genome sequence of Botrytis cinerea. *Mol Plant Pathol.* 2016;18(1):75–89.
3. Kuroyanagi T, Bulasag AS, Fukushima K, et al. Botrytis cinerea identifies host plants via capsidiol recognition. *PNAS Nexus.* 2022;1(5):pgac274.
4. Cano M, Darghan A, Cuervo J. Temporal distribution of Botrytis cinerea and its relationship to strawberry production subjected to microbial treatments. *Rev Colomb Cienc Hortic.* 2023;17:e15284.
5. Gikas GD, Parlakidis P, Mavropoulos T, et al. Particularities of fungicides and factors affecting their fate and removal efficacy: a review. *Sustainability.* 2022;14(7):4056.
6. Shao W, Zhao Y, Ma Z. Advances in fungicide resistance in Botrytis cinerea in China. *Phytopathology.* 2020;111(3):455–463.
7. Haq IU, Rahim K, Yahya G, et al. Eco–smart biocontrol strategies utilizing potent microbes for sustainable management of phytopathogenic diseases. *Biotechnol Rep.* 2024;44:e00859.
8. Zatla AT, Dib MEA, Djabou N, et al. Antifungal activities of Daucus carota oils for strawberry gray rot control. *J Essent Oil Res.* 2017;29:391–399.

9. Almeida HHS, Crugeira PJL, Amaral JS, et al. Disclosing the potential of Cupressus leylandii, Eucalyptus globulus, Aloysia citrodora, and Melissa officinalis hydrosols as eco–friendly antimicrobial agents. *Nat Prod Bioprospect.* 2024;14(1):1.

10. Jackson–Davis A, White S, Kassama LS, et al. A Review of Regulatory Standards and Advances in Essential Oils as Antimicrobials in Foods. *J Food Prot.* 2022;86(2):100025.

11. Shafie MH, Kamal ML, Razak NAA, et al. Antioxidant and antimicrobial activity of plant hydrosols for cosmeceuticals. *Jundishapur J Nat Pharm Prod.* 2022;17:e12401.

12. Salehi B, Valussi M, Morais–Braga MFB, et al. Tagetes spp. essential oils: chemical characterization and biological activity. *Molecules.* 2018;23(11):2847.

13. Șesan TE, Enache E, Iacomi BM, et al. Antifungal activity of plant extracts against Botrytis cinerea in blackcurrant crop. *Acta Sci Pol Hortorum Cultus.* 2015;14:29–43.

14. Larios-Palacios OE, Amador-González MJ, Martínez-González JC, et al. *In vitro* evaluation of methods against Botrytis cinerea. *Rev Mex Cienc Agríc.* 2020;11(3):593-606.

15. Ruíz–González MA, Serrato–Cruz MA, Valadez–Moctezuma E, et al. Destilados y aceite homeopático de Tagetes remotiflora: efecto en Botrytis cinerea. Rev Mex Cienc Agric. 2024.

16. Nazzaro F, Fratianni F, Coppola R, et al. Essential oils and antifungal activity. Pharmaceuticals. 2017;10(4):86.

17. Dai X, Shen L. Advances and trends in omics technology development. *Front Med*. 2022;9:911861.

18. Hazra A, Ghosh S, Naskar S, et al. Global transcriptome analysis reveals fungal disease–responsive core gene landscape in tea. *Sci Rep.* 2023;13(1):17186.

19. Chen Y, Li W, Zeng H, et al. Transcriptome analysis reveals the mechanism of dill seed essential oil against *Sclerotinia sclerotiorum*. *Nat Prod Commun.* 2022;17.

20. Yuan T, Hua Y, Zhang D, et al. Efficacy and Antifungal Mechanism of Rosemary Essential Oil against *Colletotrichum gloeosporioides.* Forests. 2024;15(2):377.

21. Li Z, Shao X, Wei Y, et al. Transcriptome of Botrytis cinerea response to tea tree oil. *Appl Microbiol Biotechnol*. 2020;104(5):2163–2178.

22. Hu Z, Yuan K, Zhou Q, et al. Mechanism of antifungal activity of *Perilla frutescens* essential oil against Aspergillus flavus by transcriptomic analysis. *Food Control.* 2021;123:107703.

23. Shen X, Chen W, Zheng Y, et al. Chemical composition and antioxidant activity of hydrosols from Areca catechu and Cocos nucifera. Ind Crops Prod. 2017;96:110–119.

24. Hu W, Li C, Dai J, et al. Antibacterial activity and mechanism of *Litsea cubeba* essential oil against methicillin–resistant Staphylococcus aureus (MRSA). *Ind Crops Prod*. 2019;130:34–41.

25. Moreno NM. Agrohomeopatía como alternativa a los agroquímicos. *Rev Med Homeopatía.* 2017;10:9–13.

26. Oliveira JSB, Schwan–Estrada KRF, Bonato CM, *et al.* Homeopathy with essential oils for spore germination and phytoalexin induction. *Cienc Agron.* 2017;48.

27. De A, Das D, Dutta S, et al. Potentized homeopathic drug Arsenicum Album 30C inhibits intracellular ROS generation and upregulates arsenic resistance gene expression in *E. coli. Chin J Integr Med.* 2012;10(2):210–227.

28. Bellavite P, Betti L. Homeopathy and the science of high dilutions: when to believe the unbelievable. *Int J High Dilution Res*. 2021;11(40):107–109.

29. Gorjian H, Mihankhah P, Khaligh NG. Influence of tween nature and type on physicochemical properties and stability of spearmint essential oil (*Mentha spicata* L.) stabilized with basil seed mucilage nanoemulsion. *J Mol Liq*. 2022;359:119379.

30. Liu Y, Wu J. Effects of Tween 80 and pH on fungal pellets and EPS production. *J Ind Microbiol Biotechnol*. 2012;39(4):623–628.

31. Mwamburi LA, Laing M, Miller R. Effect of surfactants and temperature on germination and vegetative growth of *Beauveria bassiana*. *Braz J Microbiol.* 2015;46(1):67–74.

32. Fokkema NJ. The rôle of saprophytic fungi in antagonism against *Drechslera sorokiniana* (*Helminthosporium sativum*) on agar plates and on rye leaves with pollen. *Physiol Plant Pathol*. 1973;3(2):195–202.

33. SAS OnDemand for Academics. SAS. 2024.

34. Bu D, Luo H, Huo P, et al. KOBAS—intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis. *Nucleic Acids Res.* 2021;49(W1):W317–W325.

35. Kolberg L, Raudvere U, Kuzmin I, et al. g:Profiler—web service for functional enrichment and ID mapping. *Nucleic Acids Res.* 2023;51:W207–W212.

36. Ruiz–González MA, Serrato–Cruz MA, Valadez–Moctezuma E, et al. Chemical composition of *Tagetes hydrolates* and evaluation against fungi in strawberry. Rev Mex Fitopatol. 2024;42:26.

37. Christova PK, Dobreva AM, Dzhurmanski AG, et al. Impact of plant essential oils on the growth of pathogens *Botrytis cinerea*, *Fusarium solani,* and *Phytophthora pseudocryptogea. Life.* 2024;14(7):817.

38. D'Amato S, Serio A, López CC, et al. Hydrosols: biological activity and potential as antimicrobials for food applications. *Food Control.* 2018;86:126–137.

39. Wu N, Zhang J, Ou W, et al. Transcriptome of Rhizopus oryzae pellet formation using triethanolamine. *Biotechnol Biofuels.* 2021;14:230.

40. Li R, ZiWei Z, Chao Y, et al. Effects of Tween–20 and Tween–80 on Magnaporthe oryzae spores. *Xi Nan Nong Ye Xue Bao*. 2016;29(10):2379–2382.

41. Rocha CH, Agostinetto L, Boff P, et al. Psidium cattleyanum essential oil controls seed pathogens. *Rev Verde*. 2019;15:14–19.

42. Haile ZM, Malacarne G, Pilati S, et al. Dual transcriptome and metabolic analysis of *Vitis vinifera* cv. pinot noir berry and botrytis cinerea during quiescence and egressed infection. *Front Plant Sci.* 2020;10:1704.

43. Bruning O, Rauwerda H, Dekker RJ, et al. Valuable lessons–learned in transcriptomics experimentation. *Transcription.* 2015;6(3):51–55.

44. Srivastava DA, Arya GC, Pandaranayaka EP, et al. Transcriptome profiling of Botrytis cinerea infection in Solanum lycopersicum. *Mol Plant Microbe Interact.* 2020;33(9):1103–1107.

45. Wang L, Jiang N, Wang D, Wang M. Effects of Essential Oil Citral on the Growth, Mycotoxin Biosynthesis and Transcriptomic Profile of *Alternaria alternata*. *Toxins.* 2019;11(10):553.

46. Zeng Q, Wang L, Long S, et al. Inhibitory Effects and Mechanisms of Perilla essential oil inhibits Botryosphaeria dothidea: effects and mechanism. J Fungi. 2024;10(8):526.

47. Hamzah N, Singhal N, Padhye L, et al. Effect of surfactants on Aspergillus brasiliensis ATCC 16404 physicochemical properties. *J Environ Chem Eng.* 2018;6(2):3392–3398.

48. Steyer JT, Todd RB. Branched–chain amino acid biosynthesis in fungi. *Essays Biochem*. 2023;67(5):865–876.

49. Zhang Y, Wei W, Fan J, *et al.* Mitochondrial Acetyl CoA acetyltransferase as antifungal target in *Aspergillus fumigatus. Appl Environ Microbiol.* 2020;86:e02986–e02919.

50. Maerker C, Rohde M, Brakhage AA. Methylcitrate synthase from Aspergillus fumigatus. FEBS J. 2005;272(14):3615–3630.

51. Shi L, Tu BP. Acetyl–CoA and metabolic regulation: mechanisms and consequences. *Curr Opin Cell Biol.* 2015;33:125–131.

52. Zhu C, Tang Y, Ren D, et al. Propionate regulates metabolism and pathogenicity in Botrytis cinerea. *Food Chem*. 2023;410:135443.

53. Landry AP, Ballou DP, Banerjee R. Hydrogen sulfide oxidation by sulfide quinone oxidoreductase. *Chembioche*m. 2020;22(6):949–960.

54. Brzywczy J, Natorff R, Sieńko M, et al. Multiple fungal enzymes possess cysteine synthase activity *in vitro*. *Res Microbiol.* 2007;158(5):428–436.

55. Traynor AM, Sheridan KJ, Jones GW, et al. Involvement of Sulfur in the Biosynthesis of Essential Metabolites in Pathogenic Fungi of Animals, Particularly Aspergillus spp.: Molecular and Therapeutic Implications. *Front Microbiol.* 2019;10:2859.

56. Simon J, Kroneck PM. Advances in microbial physiology. In: Poole RK, editor. Microbial Sulfite Respiration. Elsevier; 2013.

57. Singh SK, Singh MK, Singh VK, et al. Microbe–mediated remediation of environmental contaminants. In: Kumar A, et al., editors. Microbial Enzymes in Remediation. Elsevier Inc.; 2021.

58. Zhang T, Wang X, Li X, et al. MoLrp1–MoMsn2 signaling supports fatty acid oxidation for fungal growth. Plant Commun. 2023;4(4):100561.

59. Kato H. *Peroxisomes: biogenesis, function, and role in human disease.* In: Imanaka T, Shimozawa N, eds. Structural Biology of Peroxisomal Proteins, Peroxins. Springer; 2019.

60. Qin G, Liu J, Cao B, et al. Hydrogen peroxide acts on sensitive mitochondrial proteins to induce death of a fungal pathogen revealed by proteomic analysis. PLoS ONE. 2011;6:e21945.

61. Cassilly CD, Maddox MM, Cherian PT, et al. SB–224289 antagonizes the antifungal mechanism of the marine depsipeptide papuamide A. *PLoS ONE.* 2016;11(5):e0154932.

62. Peraza–Reyes L, Berteaux–Lecellier V. Peroxisomes and sexual development in fungi. Front Physiol. 2013;4:244.

63. Van Der Klei IJ, Veenhuis M. The Versatility of peroxisome function in filamentous fungi. Subcell Biochem. 2013;69:135–152.

64. The UniProt Consortium. UniProt: the universal protein knowledgebase in 2023. Nucleic Acids Res. 2022;51(D1):D523–D531.

65. Guimarães A, Venâncio A. The potential of fatty acids and their derivatives as antifungal agents: a review. *Toxins.* 2022;14(3):188.

66. Mishra SK, Tripathi G, Kishore N, et al. Drug development against tuberculosis: Impact of alkaloids. Eur J Med Chem Rep. 2017;137:504–544.

67. Zhao X, Wang Y, Zhao Y, *et al. AoMls* gene in *Arthrobotrys oligospora* influences conidiation, trap formation, and pathogenicity. *Appl Microbiol Biotechnol.* 2013;98:2555–2563.

68. Zeng N, Zhang N, Ma X, et al. TranscriptomicsIntegrating transcriptomics and metabolomics in Aureobasidium pullulans differentiation in *Aureobasidium pullulans* NG. J Fungi. 2022;8 (8):882.

69. Jamur MC, Oliver C. Permeabilization of cell membranes. *Methods Mol Biol.* 2009;588:63–66.

70. Musiał W, Kubis A. Effect of some anionic polymers on pH of triethanolamine aqueous solutions. *Polim Med.* 2004;34:21–29.

71. Embl–Ebi. QuickGO. 2024.

72. Poljovka A, Musil M, Bednář D, et al. Comparison of fungal thermophilic and mesophilic catalase–peroxidases for their antioxidative properties. Antioxidants. 2023;12(7):1382.

73. Mir AA, Park S, Sadat MA, et al. Systematic characterization of the peroxidase gene family provides new insights into fungal pathogenicity in Magnaporthe oryzae. Sci Rep. 2015;5:11831.

74. Allocati N, Masulli M, Di Ilio C*,* et al. Glutathione transferases: substrates, inhibitors and pro–drugs in cancer and neurodegenerative diseases. *Oncogenesis*. 2018;7(1):8.

75. Li Y, Ren H, Wang F, et al. P4–ATPase–mediated vesicle transport detoxifies secondary metabolites. eLife. 2023;12:e79179.

76. Dwivedi P, Paolella L. Tick-off the gender diversity box: examining the cross-level effects of women's representation in senior management. *Acad Manage J.* 2023.

77. Westrick NM, Smith DL, Kabbage M. Detoxification of plant defense compounds during fungal necrotrophy. Front Plant Sci. 2021;12:651716.