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Diisooctyl phthalate, the major secondary metabolite of *Bacillus subtilis*, could be a potent antifungal agent against *Rhizoctonia solani*: GC-MS and *in silico* molecular docking investigations

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Abstract

Bioactive secondary metabolite compounds produced by microorganisms, including bacteria, have certain functions, one of which is to act as antifungal agents. The current study examined the ability of seventeen *Bacillus* isolates to suppress the soilborne fungus *Rhizoctonia solani*, determining their potential as biocontrol agents. The KSAR2 isolate was the most effective at inhibiting *R. solani* mycelial growth, with a 61.2% inhibition rate achieved using the dual culture method. The analysis of the nucleotide sequencing of the 16S rRNA gene indicated that the isolate KSAR2 was identified as *Bacillus subtilis* and has been deposited in GenBank with the accession number PQ238901. The gas chromatography-mass spectrometry investigation revealed that the most abundant secondary metabolite compounds of KSAR2 were diisooctyl phthalate (38.18%), dibutyl phthalate (9.41%), tris(2,4-di-tert-butylphenyl) phosphate (7.19%), dotriacontane (6.67%), docosane (6.62%), and hexadecanoic acid (4.77%). A study of molecular docking interactions also found that diisooctyl phthalate had the highest binding energy with the fungal chitin synthase, measuring -7.90 kcal/mol. This indicates that interaction with this particular protein could elucidate the mechanism underlying the assessed antifungal activity. Finally, the present study showed that the rhizobacterium *B. subtilis* strain KSAR2 can inhibit the growth of *R. solani*. This makes it a promising biocontrol agent for protecting plants against fungal infestations. Furthermore, diisooctyl phthalate may function as a natural, bioactive fungicide by inhibiting chitin synthase in *R. solani* and potentially other fungi. This could consequently lead to important advancements in the development of novel and potent antifungal agents.

Keywords: GC-MS; molecular docking; diisooctyl phthalate; chitin synthase; *Rhizoctonia solani*; *Bacillus subtilis*.

1. Introduction

The secondary metabolites generated by various living microorganisms display an impressive diversity in their chemical structures and biological activities [1]. These secondary metabolites are made up of different compounds with low molecular weights that are produced during secondary metabolism. These include alkaloids, terpenoids, peptides, and polyketides, and they happen separately from the primary microbial growth phase. Each compound exhibits a range of biological activities. Several have already been recognized for their roles as antivirals, antimicrobials, antioxidants, and different biological activities. Some are utilized as colorants, pesticides, or growth enhancers for animals or plants [2–4]. A common soilborne necrotroph, *Rhizoctonia solani*, causes harm to numerous commercially significant crops. Members of this species differ greatly in terms of colony appearance, biochemical and molecular markers, pathogenicity, and aggressiveness. This diversity has allowed for the classification of the species into 14 somatically incompatible groups, also known as anastomosis groups. *R. solani* can infect a variety of hosts and cause several symptoms, like damping-off, seedling blight, stem canker, black scurf, hypocotyl rot, stem rot, crown rot, and limb rot [5]. The diseases produced by *R. solani*, which cause damping-off, wilt, and root rot of several crops, were managed using a variety of approaches, including breeding programs, agricultural methods, broad-spectrum

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fungicides, and biological control. Using chemical fungicides may be more effective than other methods in controlling diseases caused by *R. solani*, but it also creates an unevenness in the microbial community that is detrimental to the activity of beneficial organisms, pollutes the environment, and harms mammals and other beneficial living things [6]. Therefore, it is essential to conduct comparative studies between bioagents and conventional fungicides to identify the most effective microbe that can reduce the severity of plant diseases and assess its potential [7].

Biocontrol is regarded as one of the most efficient, secure, and beneficial methods for the environment [8,9]. Due to its high efficacy and environmental friendliness, the use of biocontrol agents is gaining attractiveness as an alternative approach for managing plant diseases [10]. Rhizobacteria that promote plant growth (PGPR) are well known for being the most efficacious biocontrol agents for controlling plant diseases. The *Bacillus* genus includes many endospore-forming, rod-shaped, and catalase-positive bacteria that can be isolated from a wide range of biological environments, such as soil, compost, the rhizosphere, fermented food, and clinical specimens [11]. Biocontrol of plant diseases has relied on *Bacillus* species and their related compounds for a long time because they release a wide range of helpful chemicals, can colonize roots better, and can make spores [12]. It has been found that many *Bacillus* species exhibit potent inhibitory activity against plant diseases and stimulate plant growth; as a result, over eighteen commercial products derived from *Bacillus* species have been sold all over the world [11].

Gas chromatography coupled with mass spectrometry (GC-MS) serves as a crucial instrument for finding new natural products because it can identify and analyze different chemical parts in microbial samples, such as organic acids, amino acids, alkaloids, and flavonoids [13–15]. Using GC-MS to look at extracts from different types of *Bacillus* makes it easier to find a wide range of metabolites, which can then be tested to see what biological effects they have [16]. This analytical approach provides essential insights into the metabolite composition of *Bacillus* spp., laying the groundwork for understanding their potential biological properties [17]. *In silico* predictions of pharmacological, pharmacokinetic, and toxicological outcomes are made using computational prediction models. These models significantly influence the selection of methods that propel advancements in both pharmaceuticals and technology [18]. Molecular docking is a cost-effective and efficient method for the development and evaluation of medicines. This method gives information about how drugs and receptors work together, which can be used to guess how the drug model will bind to target proteins, ensuring stable binding at the ligand binding sites [19,20]. Recently, it was utilized in the field of plant pathology to forecast the potential bioactive compounds that could combat plant pathogens [21].

In this context, the current study aimed to identify which of the isolated *Bacillus* isolates could serve as biocontrol agents against *R. solani*. The promising *Bacillus* isolate was further characterized at the molecular level by 16S rRNA analysis. Furthermore, bioactive secondary metabolite compounds present in bacterial culture filtrate were identified by GC-MS analysis. Subsequently, a computational molecular docking analysis was conducted to pinpoint bioactive compounds that may exhibit antifungal properties. This study seeks to predict the structures and formulas of bioactive compounds, which could be critical for the discovery and development of new antifungal formulations.

2. Materials and methods

2.1. Isolation experiments

In the Kingdom of Saudi Arabia (KSA), soil dilution from tomato, potato, pepper, and cucumber rhizospheres was used for isolation experiments. For fourteen minutes, one gram of soil was shaken in nine milliliters of sterile saline (0.85% NaCl) solution. 200 µL of the 10^{-5}, 10^{-6}, and 10^{-7} dilutions were applied to the glycerol nutrient agar media and the peptone nutrient agar medium [22]. After 48 hours of incubation at 30°C, many colonies of bacteria were visible. The observed colonies were separated and purified using the single colony isolation procedure. Bacterial cultures were kept in 50% glycerol broth at -80°C for further studies [23].

2.2. Antagonism test of *Bacillus* isolates

The antagonistic impact of *Bacillus* isolates as biocontrol agents against *R. solani*, a plant pathogenic fungus, was examined using an *in vitro* antagonist test. A streak line of the antagonistic bacterial isolates, cultured for two days, was inoculated onto potato dextrose agar (PDA) plates. This was done 48 hours before any testing fungi were inoculated. In the center of the Petri plate was a mycelial circle (5 mm in length) holding an energetically growing culture of the fungus under investigation, always at a constant space from the other edge, and incubated at 30°C for three to seven days. The method for calculating the percentage of inhibition was as outlined using Maurhofer et al. [24]. The formula growth inhibition rate (%) = [(R−r)/R] × 100 was used to calculate the percentage of inhibition in mycelium growth. In this formula, r represents the fungal development radius towards the bacterial treatment, while the value R represents the fungal colony's radius measurement on the control plate.

2.3. Molecular characterization of promising *Bacillus* isolate

The pure bacterial colony was injected into 5 mL of Luria-Bertani broth and incubated at 30 °C overnight with agitation. Following the manufacturer's guidelines, the Wizard Genomic DNA Purification Kit (Promega, USA) was employed to extract bacterial genomic DNA from a 1 mL bacterial pellet. The integrity and quality of the extracted DNA were assessed via 1.2% agarose gel electrophoresis, while purity (A260/A280) and concentration were measured using the SPECTRO Star Nano apparatus (BMG Labtech, Germany). The extracted genomic DNA was corrected to 50 ng/µL before being used as a template in PCR. To amplify the 16S rRNA gene, we used universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-CGGCTACCTTGTTACGACTT-3′). The 20-µL PCR reaction mixture had 10 µL of PCR master mix, 1 µL of DNA, 1 µL of each primer at a concentration of 10 pmol/µL, and 7 µL of double-sterile distilled water. The PCR protocol started with an initial denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 90 seconds. The last extension phase was carried out at 72°C for 5 minutes. Next, 5 µL of PCR products were separated using 2% agarose gel electrophoresis and photographed with a gel documentation system. The PCR product was sequenced immediately after purification using the QIAquick gel extraction kit (Qiagen Inc.). Sanger sequencing of PCR products was carried out using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) and the model 3130xl Genetic Analyzer (Applied Biosystems). The collected DNA nucleotide sequences were validated using NCBI-BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The annotated nucleotide sequence was analyzed and submitted to GenBank to obtain an accession number.

2.4. Gas chromatograph-mass spectrometer analysis of secondary metabolites

The GC-MS approach was utilized to identify the active biomolecules in the culture filtrate of isolate BR2. The culture filtrate was obtained by centrifuging the culture generated during the batch fermentation process. The culture filtrate was mixed with ethyl acetate in equal volumes (1:1 ratio) and stirred vigorously for 20 minutes. The ethyl acetate phase was isolated and concentrated with a rotary evaporator set at a temperature of 50 °C. The TRACE 1300 Series GC-MS (Thermo, USA) equipped with a split mode mass detector was utilized to identify secondary metabolite compounds. The transport was helium gas, and the flow rate was 1 mL/min. The column oven was initially maintained at a temperature of 45 °C. Then it was programmed to increase by 5 °C every minute until it reached 200 °C, and then it would stay there for 5 minutes. From there, it increased by 5 °C per minute until it reached 300 °C. The injector and MS transfer line maintained constant temperatures of 270 and 250 °C, respectively. Setting up a 3-minute solvent delay on the autosampler AS1310 in split mode, 1 µL portions of diluted samples were automatically fed into the GC machine. The electricity of 70 eV and an ion source at 200 °C were used to get electron impact (EI) mass spectra in full scan mode. The spectra ranged from 40 to 500 m/z. Parts were located by comparing mass spectra and retention durations to those in the WILEY 09 and NIST 11 databases [4].

2.5. Molecular docking assessment

The research collaboratory for structural bioinformatics protein data bank (RCSB PDB; https://www.rcsb.org/), AlphaFold (https://alphafold.ebi.ac.uk/), and the Molecular Operating Environment (MOE 2022.02, Chemical Computing Group, Montreal, QC, Canada) tools were utilized for the retrieval of proteins and ligands, as well as for molecular docking. The three-dimensional (3D) structures of secondary metabolite molecules (ligands) were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in structured data file (SDF) format and subsequently analyzed using molecular operating environment (MOE) software. The three-dimensional structures of fungal chitin synthase were obtained from the universal protein resource (UniProt) database (https://www.uniprot.org/). The target protein was prepped for docking using UCSF Chimera software by eliminating water and ligand molecules from the protein structures, in addition to performing energy minimization on the target protein. The target protein was docked with ligands by determining the binding site, employing the induced fit model for docking, and visualizing the results using the same program [25].

2.6. Statistical analysis

The results are presented as the average and the standard deviation (average ± SD), with the experiments being performed three times. Tukey's honest significant differences (H.S.D.) test was used to look for differences and find statistical significance. A significance level of $p \leq 0.05$ was set after the one-way analysis of variance (ANOVA) of the data using CoStat software (version 6.45; https://cohortsoftware.com/costat.html; 2022). We arranged the letters in descending order, with the relationship (a > b > c) indicating statistical significance. The letters appeared to be indistinguishable from one another.

3. Results

3.1. Fungal and bacterial isolates

The pathogenic *R. solani* strain R11 (Accession number OP456528) previously isolated was used in this study [26]. In the Kingdom of Saudi Arabia

(KSA), an isolation experiment was performed to isolate *Bacillus* isolates from the rhizosphere of tomato, potato, pepper, and cucumber. In the present investigation, seventeen *Bacillus* isolates were isolated and preserved. BR1, BR2, BR4, BR5, and BR6 *Bacillus* isolates were initially part of group 1, which was isolated from the tomato rhizosphere. Second, BR8, BR9, BR12, BR13, and BR14 were part of group 2, which was separated from the potato rhizosphere. Thirdly, BR15, BR16, BR17, and BR18 were extracted from the pepper rhizosphere by group 3. Finally, BR19, BR20, and BR21 were included in group 4, which was isolated from a cucumber rhizosphere.

3.2. Antagonism test of *Bacillus* isolates

The antagonistic influence of seventeen *Bacillus* isolates on the *in vitro* development of *R. solani* was investigated as a possible bioagent. There were notable variations between the *Bacillus* isolates, according to the findings from the current investigation (Figure 1). With the highest percentage of inhibition against the pathogen, 61.2%, isolate BR2 was shown to be more efficient than other isolates in stopping the fungal mycelia growth of *R. solani*. The next highest percentage of inhibition against the pathogen was reported by bacterial isolate BR6, which was 57.6%. The percentages of inhibition for the *Bacillus* isolates BR1, BR4, and BR9 were close to each other, which recorded 49%, 52.9%, and 50.2%, respectively. Isolate BR18 exhibited the lowest percentage of inhibition (5.9%) against *R. solani*. However, there were no antagonistic behaviors displayed by isolates BR8, BR14, BR19, BR20, and BR21 (Figure 2).

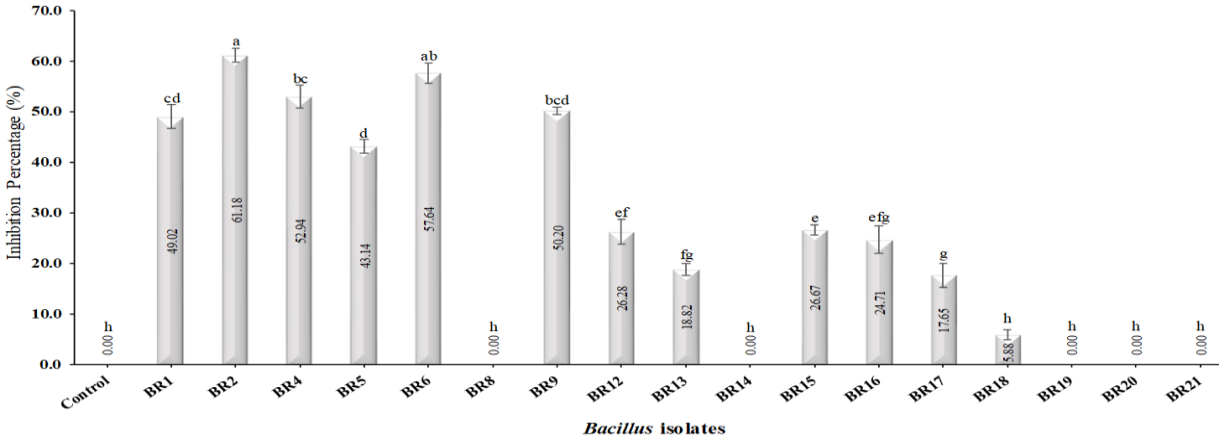


Fig. 1. Effect of seventeen *Bacillus* isolates on the mycelial development of *R. solani* utilizing the dual culture method. Each column displays the mean value of three duplicated measurements, while the bars represent the standard deviation. Tukey's HSD test, utilizing a significance threshold of 0.05, indicates that values within each column designated by the same letter (a/b/c/d/e) demonstrate no statistically significant differences.

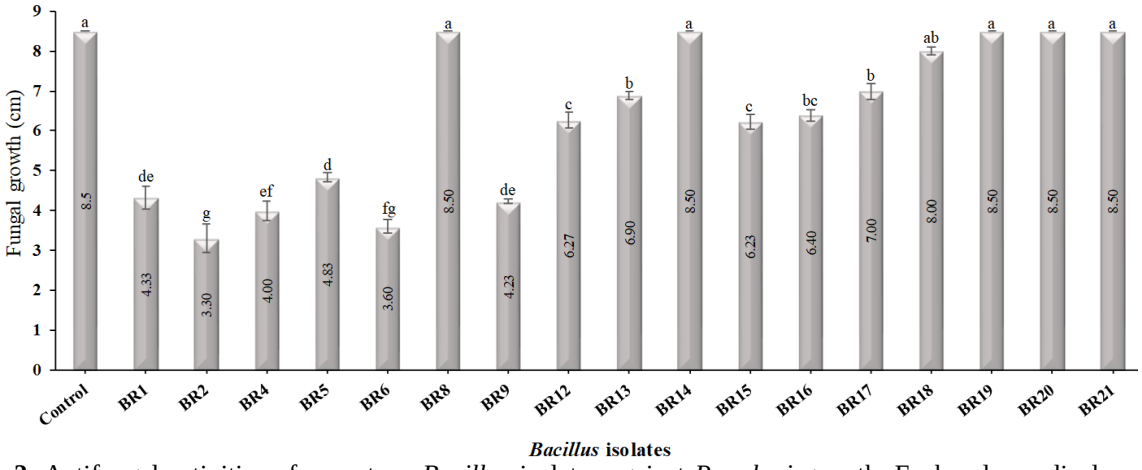


Fig. 2. Antifungal activities of seventeen *Bacillus* isolates against *R. solani* growth. Each column displays the mean value of three duplicated measurements of growth inhibition percentage, while the bars represent the standard deviation. Tukey's HSD test, utilizing a significance threshold of 0.05, indicates that values within each column designated by the same letter (a/b/c/d/e) demonstrate no statistically significant differences.

3.3. Molecular characterization of *Bacillus* isolate BR2

The morphological characteristics of *Bacillus* isolate BR2 were gram-positive and endospore-forming. On nutrient agar plates, colonies appeared creamy-white and rough, and their edges were slightly irregular. *Bacillus* isolate BR2 was molecularly characterized by sequencing the 16S rRNA gene. The PCR amplification yielded a product of approximately 1500 base pairs. Based on NCBI-BLAST alignment and phylogenetic tree analysis, the BR2 isolate has been determined to be *B. subtilis*. The annotated nucleotide sequence of 1448 bp has been submitted in the GenBank database under the name *B. subtilis* strain KSAR2, with the accession number PQ238901. The nucleotide sequence showed 100% sequence coverage with other *Bacillus* species that were deposited in GenBank. The KSAR2 was closely related to other *B. subtilis* strains, especially China isolate (Acc# OR335070) by 98.76% identity.

3.4. Identification of bioactive metabolites of *Bacillus subtilis*

Extract of KSAR2 culture filtrate, which was achieved by ethyl acetate, was analyzed using GC-MS analysis (Figure 3) to identify the specific compounds that could be responsible for its antagonistic effect against *R. solani*. The GC-MS tests showed that the KSAR2 culture filtrate contained 22 different compounds (Table 1). These compounds, including diisooctyl phthalate (38.18%), dibutyl phthalate (9.41%), tris(2,4-di-tert-butylphenyl) phosphate (7.19%), dotriacontane (6.67%), docosane (6.62%), hexadecanoic acid (4.77%), 7,9-ditertbutyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (3.06%), 2,5-Piperazinedione,3,6-bis(2-methylpropyl) (2.04%), hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester (2.04%), methanone,(1-hydroxycyclohexyl)phenyl (1.9%), octadecanoic acid (1.63%), 3,4-dihydro-2h-1,5-(3"-t-butyl) benzodioxepine (1.42%), hexadecane,2,6,10,14-tetramethyl (1.32%), octadecanoic acid, 2-hydroxyethyl ester (1.29%), and heptacosane (0.82%).

3.5. Molecular docking interaction investigation

The molecular docking analysis was performed to determine the potential binding interactions of the identified secondary metabolite compounds from *B. subtilis* strain KSAR2 with the chitin synthase of *R. solani* as the target protein. A total of sixteen metabolite compounds were successfully docked into the chitin synthase, with docking scores ranging from -4.20 to -7.90 kcal/mol (Table 2). Among them, diisooctyl phthalate and estra-1,3,5(10)-trien-17á-ol demonstrated a significant affinity for the binding site of chitin synthase, with docking scores of -7.90 and -7.20 kcal/mol, respectively (Figure 4). On the other hand, hexadecanoic acid and hexadecane showed the lowest binding affinity with the same docking score of -4.20 kcal/mol (Table 2). Figure 4 presents the three-dimensional visualizations of ligand-protein complex interactions between the two compounds and chitin synthase. The binding energy of diisooctyl phthalate predominantly arises from hydrophobic interactions with active site residue amino acids, including alkyl interactions with LEU136, LEU347, LEU351, ILE848, LEU852, and LEU853. Furthermore, estra-1,3,5(10)-trien-17á-ol exhibited interactions with VAL392 and ALA440 via hydrophobic interactions.

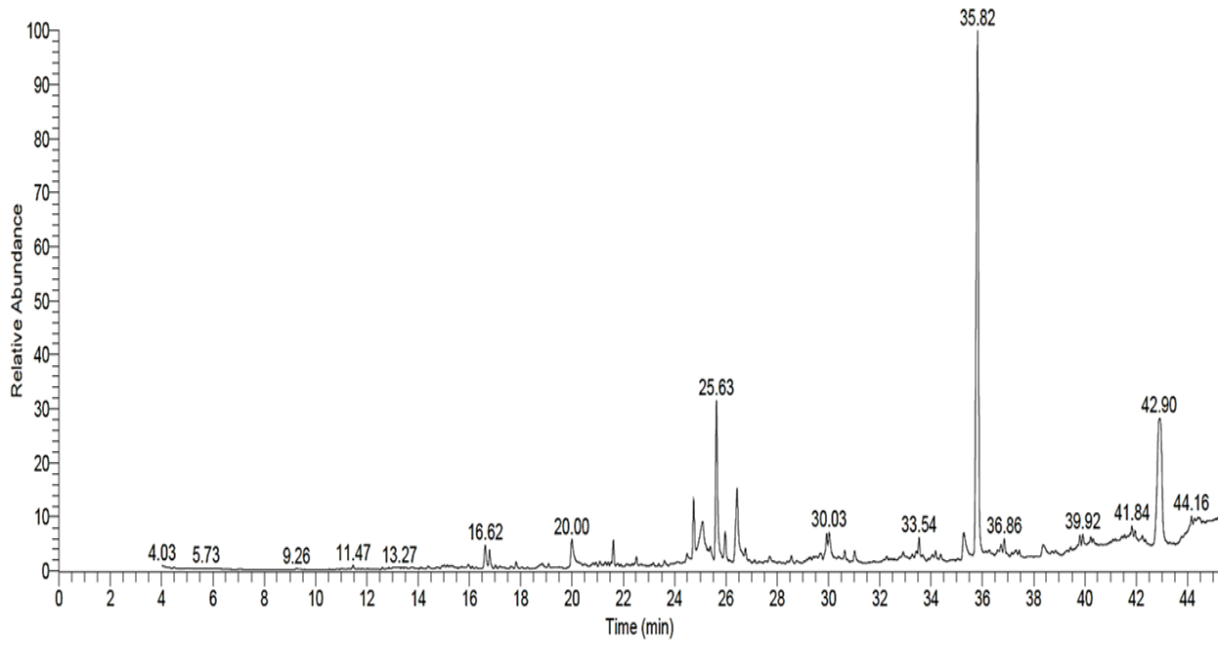
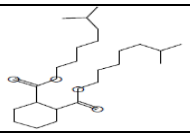
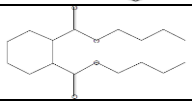
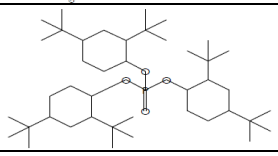
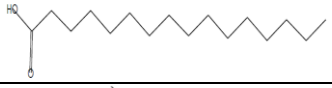
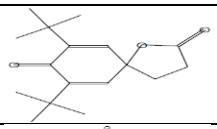
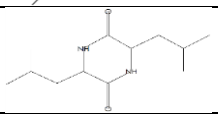
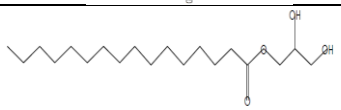
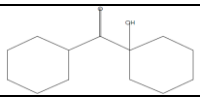
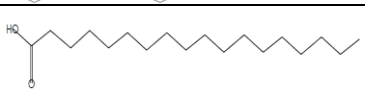
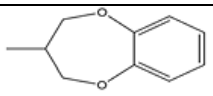
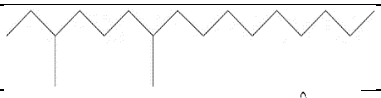
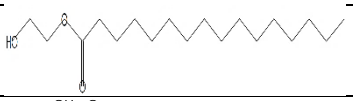
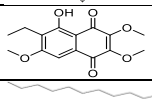


Fig. 3. GC-MS chromatography of ethyl acetate extract of the culture filtrate of *Bacillus subtilis* strain KSAR2.

Table 1. The list of detected compounds in the ethyl acetate extract of culture filtrate of *B. subtilis* strain KSAR2.

RT	Area %	Compound Name	Molecular Formula	Molecular Weight	Compound structure	CAS number
35.82	38.18	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390		131-20-4
25.63	9.41	Dibutyl phthalate	$C_{16}H_{22}O_4$	278		84-74-2
42.90	7.19	Tris(2,4-ditert-butylphenyl)phosphate	$C_{42}H_{63}O_4P$	662		95906-11-9
41.84	6.67	Dotriacontane	$C_{32}H_{66}$	450		544-85-4
33.54	6.62	Docosane	$C_{22}H_{46}$	310		629-97-0
26.44	4.77	Hexadecanoic acid	$C_{16}H_{32}O_2$	256		57-10-3
24.75	3.06	7,9-di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276		82304-66-3
25.10	2.04	2,5-Piperazinedione,3,6-bis(2-methylpropyl)-	$C_{12}H_{22}N_2O_2$	226		1436-27-7
35.27	2.04	Hexadecanoic acid,2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330		542-44-9
19.99	1.90	Methanone,(1-hydroxycyclohexyl)phenyl-	$C_{13}H_{16}O_2$	204		947-19-3
30.03	1.63	Octadecanoic acid	$C_{18}H_{36}O_2$	284		57-11-4
16.62	1.42	3,4-dihydro-2h-1,5-(3"-t-butyl) benzodioxepine	$C_{13}H_{18}O_2$	206		NA
21.62	1.32	Hexadecane,2,6,10,14-tetramethyl-	$C_{20}H_{42}$	282		638-36-8
38.36	1.29	Octadecanoic acid, 2-hydroxyethyl ester	$C_{20}H_{40}O_3$	328		111-60-4
36.86	0.82	Heptacosane	$C_{27}H_{56}$	380		593-49-7
31.02	0.75	Hexadecanoic acid, 2-hydroxyethylester	$C_{18}H_{36}O_3$	300		4219-49-2
25.40	0.56	6-ethyl-5-hydroxy-2,3,7-trimethoxynaphthoquinone	$C_{15}H_{16}O_6$	292		NA
24.49	0.48	Docosane, 11-decyl-	$C_{32}H_{66}$	450		55401-55-3

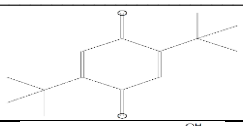
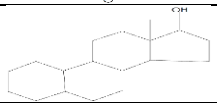
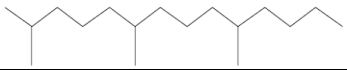
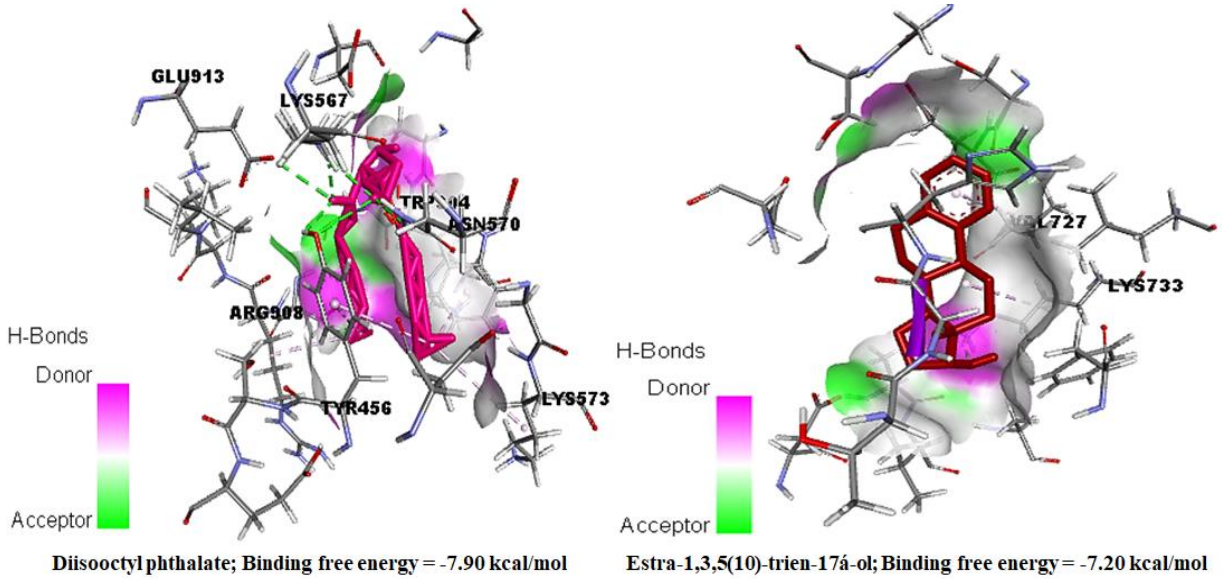
23.61	0.43	2,5-di-tert-butyl-1,4-benzoquinone	$C_{14}H_{20}O_2$	220		2460-77-7
27.70	0.40	Estra-1,3,5(10)-trien-17á-ol	$C_{18}H_{24}O$	256		2529-64-8
17.82	0.38	Hexadecane	$C_{16}H_{34}$	226		544-76-3
21.09	0.33	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240		14905-56-7

Table 2. Molecular docking score of secondary metabolites compounds of *B. subtilis* strain KSAR2 with chitin synthase of *R. solani*.

Compound Name	Docking score (kcal/mol)
Diisooctyl phthalate	-7.90
Estra-1,3,5(10)-trien-17á-ol	-7.20
Tris(2,4-ditert-butylphenyl)phosphate	-6.50
Docosane, 11-decyl-	-6.30
Dibutyl phthalate	-6.20
Heptacosane	-6.20
2,5-di-tert-butyl-1,4-benzoquinone	-6.10
Octadecanoic acid	-5.60
Octadecanoic acid, 2-hydroxyethyl ester	-5.50
Methanone,(1-hydroxycyclohexyl)phenyl-	-5.40
Docosane	-5.20
Dotriacontane	-5.10
Hexadecane,2,6,10,14-tetramethyl-	-4.50
Tetradecane, 2,6,10-trimethyl-	-4.50
Hexadecanoic acid	-4.20
Hexadecane	-4.20



Diisooctyl phthalate; Binding free energy = -7.90 kcal/mol **Estra-1,3,5(10)-trien-17á-ol; Binding free energy = -7.20 kcal/mol**

Fig. 4. The three-dimensional of molecular docking interactions of diisooctyl phthalate (left) and estra-1,3,5(10)-trien-17á-ol (right) with chitin synthase of *R. solani*.

4. Discussion

R. solani is a soil-borne fungus capable of producing sclerotia, allowing it to survive longer in the soil. It has a broad host range. As a result, managing *R. solani* is somewhat challenging. The majority of the pesticides employed to manage *R. solani* have harmful effects on non-targeted organisms as well as the environment, making them ineffective [27]. Because of growing consumer concerns about chemical pesticides remaining in food and environmental safety, there is a need to explore alternative approaches, such as biocontrol [26,28]. According to Huang et al. [29], *Bacillus* strains are crucial biocontrol agents against diseases caused by *R. solani* on pepper, potato, tomato, and other plants.

Bacillus strains possess not only the capability to impede the growth of fungal pathogens via contact-dependent antimicrobial metabolites but also the capability to submerge hydrolases such as protease, glucanase, chitinase, xylanase, and cellulase. These hydrolases target the glycosidic bonds of the core constituents of the fungal pathogen's cell wall, which include protein, glucan, and chitin. Additionally, they can secrete volatile organic compounds (VOCs), or antimicrobial metabolites, such as benzaldehyde, 1,2-benzothiazole-3(2H)-one, 1,3-butadiene, 2-nonanone, 2-decanone, and 2-propanone, which can distance inhibit target infections [30]. In this study, we assessed seventeen isolates of *Bacillus* as possible biocontrol agents against *R. solani*'s *in vitro* mycelial growth. With an inhibition percentage of 61.2%, KSAR2 outperformed the other *Bacillus* isolates in its inhibitory effectiveness against *R. solani* mycelia. The antagonistic impact of the bioagent, KSAR2 may be referred toward its excretion of enzymes and chemicals that have direct and indirect antifungal properties. Matar et al. [31] tested fourteen *B. subtilis* isolates for their ability to inhibit *R. solani* isolated from cotton. They showed four *B. subtilis* isolates had a more antagonistic effect against *R. solani*. *B. subtilis* strain B4 gave a greater inhibition percentage equal to 47.1% compared to the other strains against *R. solani* of peanut [32]. As stated by Huang et al. [29], *B. subtilis* effectively suppressed the mycelial development of *R. solani*, with a 42.3% inhibition percentage. Chen et al. [33] revealed that the antifungal substances in the filtrate of *B. subtilis* culture could cause more protoplasm leaks, cell-wall fragmentation, and cytoplasmic vacuoles in the *R. solani* mycelium.

Bioactive compound profiling revealed that there were 22 active compounds detected in *B. subtilis* strain KSAR2. However, there were six major bioactive compounds with the highest percentage of peak area. The most important compound found was diisooctyl phthalate. It was followed by dibutyl phthalate, tris(2,4-di-tert-butylphenyl) phosphate, dotriacontane, docosane, and hexadecanoic acid. Those bioactive compounds were previously described as antimicrobial and antifungal compounds. However, further purification and assays are required to confirm that those bioactive compounds were responsible for *B. subtilis* strain KSAR2 antifungal activity. Diisooctyl phthalate exhibits antibacterial and antifouling properties [34]. One of the most significant bioactive metabolites was formed by *Paecilomyces* sp. (AUMC 15510) [35] and *Trichoderma harzianum* TH7 [36]. It has properties that promote wound healing, as well as the ability to inhibit the growth of microorganisms [37,38]. Lykholat et al. [39] demonstrated that the diisooctyl phthalate, the main constituent of *Penicillium* sp.'s ethyl acetate extract, was responsible for its antifungal activity against *Alternaria alternata*. El-Enain et al. [34] have recently proposed that diisooctyl phthalate could potentially be used as a medicinal agent for the treatment of severe bacterial infections. Dibutyl phthalate had substantial inhibitory effects on the mycelial development of *Botrytis cinerea* [40]. Blazević et al. [41] demonstrated that dibutyl phthalate inhibited the development of gram-positive bacteria species *Staphylococcus aureus* and the yeast *Candida. albicans*. Furthermore, the antifungal activity of dibutyl phthalate, which is synthesized by *B. amyloliquefaciens* and *B. velezensis*, was shown [42]. Furthermore, *Streptomyces* has been found to exhibit antibacterial activity of dibutyl phthalate [43,44]. Dibutyl phthalate, a bioactive chemical synthesized by *Streptomyces albidoflavus* 321.2, exhibited significant antimicrobial action against both Gram-negative bacteria, and Gram-positive as well as unicellular and filamentous fungi. Dibutyl phthalate of *Streptomyces* strain BITDG-11 exhibits potent antifungal properties against *Fusarium oxysporum* f. sp. *cubense* [45]. Dibutyl phthalate has also been shown to be an antimicrobial bioactive compound in marine *Pseudomonas* strains [46,47] and other actinomycete strains [48,49]. Hexadecanoic acid is a fatty acid that has been found in many medicinal plants [50,51] as well as in *Bacillus* spp. metabolites [4,52]. It has been suggested that it may be antifungal, hypocholesterolemic, anticancer, antibacterial, antioxidant, nematicide, and pesticide [53]. The hexadecanoic acid synthesized by the *Bacillus* demonstrated significant efficacy in suppressing the growth of *Colletotrichum gloesporoides* [54]. Hexadecanoic acid can hinder the growth of fungi by creating intricate compounds by binding to the active components of fungal cell walls [55]. It has also been shown that the ethyl ester of octadecanoic acid, which was discovered in *B. atrophaeus* along with other chemicals, can effectively suppress *Verticillium* wilt [56]. Dotriacontane compounds exhibit antioxidant, antiplasmodic, and antimicrobial properties [57,58]. The essential oil extract contains 4.5% docosane and has been reported to have antibacterial properties [59]. Each of these compounds has previously been suggested to possess a specific antibacterial action [60]. Subsequent investigations will prioritize the examination of the precise antimicrobial mechanisms employed by these bioactive compounds.

The progress in computational science significantly influenced the identification of novel bioactive compounds [61]. Recently, the use of virtual screening methods in agriculture has become widespread and prevalent, significantly reducing the time and costs associated with the discovery and development of new antimicrobial agents [21,62]. Molecular docking is a way to find new ligands for protein structures. It is also a key part of using structure information to design antimicrobial agents

[63,64]. The development of novel antifungal agents is challenging due to the similarities in biological pathways between fungi and their hosts. Chitin synthases are enzymes associated with membranes that generate chitin, an essential structural element of fungal cell walls. It catalyzes chitin synthesis and transfer across the membrane, making it a target for antifungal agents. Blocking it leads to a targeted suppression of fungal growth or inhibition without adversely affecting vertebrates or plants [65,66]. In the context of plant infection, the degradation or prevention of pathogen chitin formation presents a promising approach to enhancing crop immunity against fungal infections [67]. In the current study, molecular docking interaction analyses were employed to investigate the binding capability of bioactive compounds from KSAR2 culture filtrate extract that could bind to fungal chitin synthase, which is a target for antifungal agents. A more negative docking score indicates a greater binding affinity between the receptor and ligand molecules, demonstrating the enhanced efficacy of bioactive substances [20]. This investigation identifies diisooctyl phthalate and estra-1,3,5(10)-trien-17á-ol as the primary compounds, exhibiting the highest docking scores of -7.90 and -7.20 kcal/mol, respectively, among the evaluated bioactive compounds. Interestingly, the docking analysis aligns with the GC-MS analysis, indicating that diisooctyl phthalate exhibited the highest peak area at 38.18%, along with the highest docking score of -7.90 kcal/mol. Therefore, diisooctyl phthalate may serve as bioactive compounds with the potential to act as antifungal agents against phytopathogenic fungi.

5. Conclusions

The current study demonstrated that the molecularly characterized *B. subtilis* strain KSAR2 (Accession number # PQ238901) exhibited notable antifungal activity against the soilborne fungus *Rhizoctonia solani*, achieving an inhibition rate of 61.2%. The GC-MS analysis of the ethyl acetate extract from the bacterial culture filtrate identified 22 unique compounds. The investigation detected a variety of secondary metabolite chemicals, with diisooctyl phthalate being the most common at 38.18%. Next, dibutyl phthalate was at 9.41%, tris(2,4-di-tert-butylphenyl) phosphate was at 7.19%, dotriacontane was at 6.67%, docosane was at 6.62%, and hexadecanoic acid was at 4.77%. The investigation of molecular docking interactions revealed that diisooctyl phthalate had the highest binding energy with fungal chitin synthase, quantified at -7.90 kcal/mol. Thus, the current study shows that the rhizobacterium *B. subtilis* strain KSAR2 may suppress the growth of *R. solani* and serve as an effective biocontrol agent for safeguarding plants from fungal infections. Additionally, diisooctyl phthalate could act as a natural, bioactive fungicide by inhibiting chitin synthase in *R. solani* and possibly other fungi. This may ultimately result in significant progress in creating new and effective antifungal agents. Nonetheless, additional isolation and purification of these compounds, along with more practical studies for open-field applications, are necessary.

Conflicts of Interest: The author declares no conflicts of interest.

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References

[1] Ofoedum AF, Uyanwa NC, Chikelu EC, Iroagba LN, Ugwoezuonu JN, Anaeke EJ, et al. Primary and Secondary Metabolites as Products of Microbial Metabolism: Uses and Application in Foods, Pharmaceutical and Allied Industries. A Review. Eur J Appl Sci Eng Technol 2024;2:4–16.

[2] Fouillaud M, Dufossé L. Microbial secondary metabolism and biotechnology. Microorganisms 2022;10:123.

[3] Ali SK, El-Masry SS, El-Adl K, Abdel-Mawgoud M, Okla MK, Abdel-Raheam HEF, et al. Assessment of antimicrobial activity and GC-MS using culture filtrate of local marine *Bacillus strains*. J Environ Sci Heal Part B 2024:1–18.

[4] Abdelkhalek A, Aseel DG, Király L, Künstler A, Moawad H, Al-Askar AA. Induction of Systemic Resistance to Tobacco mosaic virus in Tomato through Foliar Application of *Bacillus amyloliquefaciens* Strain TBorg1 Culture Filtrate. Viruses 2022;14:1830. https://doi.org/10.3390/v14081830.

[5] Ajayi-Oyetunde OO, Bradley CA, Ajayi-Oyetunde OO, Bradley CA, Ajayi-Oyetunde OO, Bradley CA. *Rhizoctonia solani*: taxonomy, population biology and management of rhizoctonia seedling disease of soybean. Plant Pathol 2018;67:3–17. https://doi.org/10.1111/ppa.12733.

[6] Senapati M, Tiwari A, Sharma N, Chandra P, Bashyal BM, Ellur RK, et al. *Rhizoctonia solani* Kühn pathophysiology: status and prospects of sheath blight disease management in rice. Front Plant Sci 2022;13:881116.

[7] Abdelrhim AS, Abdellatif YMR, Hossain MA, Alamri S, Pessarakli M, Lessy AMN, et al. Comparative study of three biological control agents and two conventional fungicides against coriander damping-off and root rot caused by *Rhizoctonia solani*. Plants 2023;12:1694.

[8] O'Brien PA. Biological control of plant diseases. Australas Plant Pathol 2017;46:293–304. https://doi.org/10.1007/s13313-017-0481-4.

[9] Collinge DB, Jensen DF, Rabiey M, Sarrocco S, Shaw MW, Shaw RH. Biological control of plant diseases–What has been achieved and what is the direction? Plant Pathol 2022;71:1024–47.

[10] Maurya RP, Koranga R, Samal I, Chaudhary D, Paschapur AU, Sreedhar M, et al. Biological control: a global perspective. Int J Trop Insect Sci 2022;42:3203–20. https://doi.org/10.1007/s42690-022-00881-9.

[11] Fira D, Dimkić I, Berić T, Lozo J, Stanković S. Biological control of plant pathogens by *Bacillus* species. J Biotechnol 2018;285:44–55.

[12] Soni R, Keharia H. Phytostimulation and biocontrol potential of Gram-positive endospore-forming Bacilli. Planta 2021;254:49.

[13] Abdelkhalek A, El-Gendi H, Al-Askar AA, Maresca V, Moawad H, Elsharkawy MM, et al. Enhancing systemic resistance in faba bean (*Vicia faba* L.) to Bean yellow mosaic virus via soil application and foliar spray of nitrogen-fixing *Rhizobium leguminosarum* bv. *viciae* strain 33504-Alex1. Front Plant Sci 2022;13:933498. https://doi.org/10.3389/fpls.2022.933498.

[14] Yassin Y, Aseel D, Khalil A, Abdel-Megeed A, Al-Askar A, Elbeaino T, et al. Foliar Application of *Rhizobium leguminosarum* bv. *viciae* Strain 33504-Borg201 Promotes Faba Bean Growth and Enhances Systemic Resistance Against Bean Yellow Mosaic Virus Infection. Curr Microbiol 2024;81:220. https://doi.org/10.1007/s00284-024-03733-6.

[15] Philip B, Behiry SI, Salem MZM, Amer MA, El-Samra IA, Abdelkhalek A, et al. *Trichoderma afroharzianum* TRI07 metabolites inhibit Alternaria alternata growth and induce tomato defense-related enzymes. Sci Rep 2024;14:1874. https://doi.org/10.1038/s41598-024-52301-2.

[16] Caulier S, Nannan C, Gillis A, Licciardi F, Bragard C, Mahillon J. Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. Front Microbiol 2019;10:302.

[17] Naveed M, Ishfaq H, Rehman SU, Javed A, Waseem M, Makhdoom SI, et al. GC–MS profiling of *Bacillus* spp. metabolites with an in vitro biological activity assessment and computational analysis of their impact on epithelial glioblastoma cancer genes. Front Chem 2023;11:1287599.

[18] Loza-Mejía MA, Salazar JR, Sánchez-Tejeda JF. *In silico* studies on compounds derived from calceolaria: Phenylethanoid glycosides as potential multitarget inhibitors for the development of pesticides. Biomolecules 2018;8:121.

[19] Lee K, Kim D. In-silico molecular binding prediction for human drug targets using deep neural multi-task learning. Genes (Basel) 2019;10:906.

[20] Ralte L, Khiangte L, Thangjam NM, Kumar A, Singh YT. GC–MS and molecular docking analyses of phytochemicals from the underutilized plant, Parkia timoriana revealed candidate anti-cancerous and anti-inflammatory agents. Sci Rep 2022;12:3395.

[21] Hamdy E, El-Gendi H, Al-Askar A, El-Far A, Kowalczewski P, Behiry S, et al. Copper oxide nanoparticles-mediated *Heliotropium bacciferum* leaf extract: Antifungal activity and molecular docking assays against strawberry pathogens. Open Chem 2024;22:20240028. https://doi.org/10.1515/chem-2024-0028.

[22] Al-Askar A, Al-Otibi FO, Abo-Zaid GA, Abdelkhalek A. Pyrrolo [1, 2-a] pyrazine-1, 4-dione, hexahydro-3-(2-methylpropyl), as the primary secondary metabolite of *Bacillus* spp., could be an effective antifungal agent against the soil-borne fungus, *Sclerotium bataticola*. Egypt J Chem 2024;67:1009-1022.

[23] Al-Askar A, Al-Otibi FO, Abo-Zaid GA, Abdelkhalek A. Gas chromatography-mass spectrometry comparative analysis of secondary metabolites produced by some *Bacillus* spp. as biocontrol agents against phytopathogenic fungi. Egypt J Chem 2024;67:997-1008.

[24] Maurhofer M, Keel C, Haas D, Défago G. Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHAO with enhanced antibiotic production. Plant Pathol 1995;44:40–50.

[25] Alotaibi BS, Abdel-Rahman Mohamed A, Abd-Elhakim YM, Noreldin AE, Elhamouly M, Khamis T, et al. Exploring the link between pyrethroids exposure and dopaminergic degeneration through morphometric, immunofluorescence, and in-silico approaches: the therapeutic role of chitosan-encapsulated curcumin nanoparticles. Front Pharmacol 2024;15:1388784.

[26] Behiry S, Soliman SA, Massoud MA, Abdelbary M, Kordy AM, Abdelkhalek A, et al. *Trichoderma pubescens* Elicit Induced Systemic Resistance in Tomato Challenged by *Rhizoctonia solani*. J Fungi 2023;9. https://doi.org/10.3390/jof9020167.

[27] Patil HJ, Srivastava AK, Singh DP, Chaudhari BL, Arora DK. Actinomycetes mediated biochemical responses in tomato (*Solanum lycopersicum*) enhances bioprotection against *Rhizoctonia solani*. Crop Prot 2011;30:1269–73.

[28] Spadaro D, Droby S. Development of biocontrol products for postharvest diseases of fruit: the importance of elucidating the mechanisms of action of yeast antagonists. Trends Food Sci Technol 2016;47:39–49.

[29] Huang Y, Wu Z, He Y, Ye B-C, Li C. Rhizospheric *Bacillus subtilis* exhibits biocontrol effect against *Rhizoctonia solani* in pepper (*Capsicum annuum*). Biomed Res Int 2017;2017:9397619.

[30] Zhang N, Wang Z, Shao J, Xu Z, Liu Y, Xun W, et al. Biocontrol mechanisms of *Bacillus*: Improving the efficiency of green agriculture. Microb Biotechnol 2023;16:2250–63.

[31] Moustafa HE, Abd-Elsalam HE, Abo-Zaid GA, Serour EA. as Biocontrol Agent, II. Biotechnology 2009;8:35–43.

[32] Ahmad A-GM, Abo-Zaid GA, Mohamed MS, Elsayed HE. Fermentation, formulation and evaluation of PGPR *Bacillus subtilis* isolate as a bioagent for reducing occurrence of peanut soil-borne diseases. J Integr Agric 2019;18:2080–92.

[33] Chen X, Zhang Y, Fu X, Li Y, Wang Q. Isolation and characterization of *Bacillus amyloliquefaciens* PG12 for the biological control of apple ring rot. Postharvest Biol Technol 2016;115:113–21.

[34] El-Enain A, Zeatar A, Zayed A, Elkhawaga M, Mahmoud Y. Diisooctyl Phthalate as A Secondary Metabolite from Actinomycete Inhabit Animal's Dung with Promising Antimicrobial Activity. Egypt J Chem 2023;66:261–77.

[35] Salem SH, El-Maraghy SS, Abdel-Mallek AY, Abdel-Rahman MAA, Hassanein EHM, Al-Bedak OA, et al. The antimicrobial, antibiofilm, and wound healing properties of ethyl acetate crude extract of an endophytic fungus *Paecilomyces* sp.(AUMC 15510) in earthworm model. Sci Rep 2022;12:19239.

[36] Khaledi N, Assareh MH. Evaluation of potential antifungal activity of secondary metabolites produced by *Trichoderma harzianum* against Alternaria blight disease of Cumin. J Crop Prot 2023;12:265–82.

[37] Amer MS, Barakat KM, Hassanein AEA. Phthalate derivatives from marine *Penicillium decumbens* and its synergetic effect against sepsis bacteria. Biointerface Res Appl Chem 2019;9:4070–6.

[38] Huang L, Zhu X, Zhou S, Cheng Z, Shi K, Zhang C, et al. Phthalic acid esters: Natural sources and biological activities. Toxins (Basel) 2021;13:495.

[39] Lykholat Y V, Khromykh NO, Didur OO, Drehval OA, Sklyar T V, Anishchenko AO. *Chaenomeles speciosa* fruit endophytic fungi isolation and characterization of their antimicrobial activity and the secondary metabolites composition. Beni-Suef Univ J Basic Appl Sci 2021;10:1–10.

[40] Li P, Feng B, Yao Z, Wei B, Zhao Y, Shi S. Antifungal activity of endophytic *Bacillus* K1 Against *Botrytis cinerea*. Front Microbiol 2022;13:935675.

[41] Blažević I, Radonić A, Mastelić J, Zekić M, Skočibušić M, Maravić A. Hedge mustard (*Sisymbrium officinale*): Chemical diversity of volatiles and their antimicrobial activity. Chem Biodivers 2010;7:2023–34.

[42] Soliman SA, Khaleil MM, Metwally RA. Evaluation of the antifungal activity of *Bacillus amyloliquefaciens* and *B. velezensis* and characterization of the bioactive secondary metabolites produced against plant pathogenic fungi. Biology (Basel) 2022;11:1390.

[43] El-Naggar MYM. Dibutyl phthalate and the antitumour agent F5A1, two metabolites produced by *Streptomyces nasri* submutant H35. Biomed Lett 1997:125–31.

[44] Lee D-S. Dibutyl phthalate, an α-glucosidase inhibitor from *Streptomyces melanosporofaciens*. J Biosci Bioeng 2000;89:271–3.

[45] Zhang L, Zhang H, Huang Y, Peng J, Xie J, Wang W. Isolation and evaluation of rhizosphere actinomycetes with potential application for biocontrolling *Fusarium* wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4. Front Microbiol 2021;12:763038.

[46] Hoang VLT, Li Y, Kim S-K. Cathepsin B inhibitory activities of phthalates isolated from a marine *Pseudomonas* strain. Bioorg Med Chem Lett 2008;18:2083–8.

[47] Isnansetyo A, Kamei Y. Bioactive substances produced by marine isolates of *Pseudomonas*. J Ind Microbiol Biotechnol 2009;36:1239–48.

[48] Roy RN, Laskar S, Sen SK. Dibutyl phthalate, the bioactive compound produced by *Streptomyces albidoflavus* 321.2. Microbiol Res 2006;161:121–6.

[49] Ahsan T, Chen J, Zhao X, Irfan M, Wu Y. Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG-3 strain KX852461 to control target spot disease in tobacco leaf. AMB Express 2017;7. https://doi.org/10.1186/s13568-017-0351-z.

[50] Youssef NH, Qari SH, Behiry SI, Dessoky

ES, El-Hallous EI, Elshaer MM, et al. Antimycotoxigenic activity of beetroot extracts against *altenaria alternata* mycotoxins on potato crop. Appl Sci 2021;11:4239. https://doi.org/10.3390/app11094239.

[51] Umaiyambigai D, Saravanakumar K, Raj AG. Phytochemical profile and antifungal activity of leaves methanol extract from the *Psydrax dicoccos* (Gaertn) teys. & binn. rubiaceae family. Int J Pharma Phytochem Ethnomed 2017;7:53–6.

[52] Awan ZA, Shoaib A, Schenk PM, Ahmad A, Alansi S, Paray BA. Antifungal potential of volatiles produced by *Bacillus subtilis* BS-01 against *Alternaria solani* in *Solanum lycopersicum.* Front Plant Sci 2023;13:1089562.

[53] Okwu DE. Improving the nutritive value of cassava tapioca meal with local spices. J Nutraceuticals, Funct Med Foods 2001;3:43–50.

[54] Grahovac J, Pajčin I, Vlajkov V. *Bacillus* VOCs in the context of biological control. Antibiotics 2023;12:581.

[55] Idris N. Potential of Hexadecanoic Acid as Antimicrobials in Bacteria and Fungi that Cause Decay in Mustard Greens *Brassica juncea* L. Int J Appl Biol 2022;6:36–42.

[56] Mohamad OAA, Li L, Ma J-B, Hatab S, Xu L, Guo J-W, et al. Evaluation of the antimicrobial activity of endophytic bacterial populations from Chinese traditional medicinal plant licorice and characterization of the bioactive secondary metabolites produced by *Bacillus atrophaeus* against *Verticillium dahliae*. Front Microbiol 2018;9:924.

[57] Bertorello A, Aperia A. Short-term regulation of Na+, K+-ATPase activity by dopamine. Am J Hypertens 1990;3:51S-54S.

[58] Balch JF, Balch PA. Prescription for Nutritional Healing. Avery Publishing Group Inc., New York. 1990; pp. 12–39.

[59] Uma B, Parvathavarthini R. Antibacterial effect of hexane extract of sea urchin, *Temnopleurus alexandri* (Bell, 1884). Int J PharmTech Res 2010;2:1677–80.

[60] El Shafay SM, Ali SS, El-Sheekh MM. Antimicrobial activity of some seaweeds species from Red sea, against multidrug resistant bacteria. Egypt J Aquat Res 2016;42:65–74.

[61] Azeez YH, Othman KA, Omer RA, Qader IN. Comprehensive Computational Analysis of Molecular Properties and Interactions in Bioactive Compounds. Russ J Phys Chem A 2024;98:1434–60.

[62] Omar AZ, Hamdy E, Hamed EA, Hafez E, Abdelkhalek A. The curative activity of some arylidene dihydropyrimidine hydrazone against Tobacco mosaic virus infestation. J Saudi Chem Soc 2022:101504.

[63] Alimi D, Hraoui M, Hajri A, Taamalli W, Selmi S, Sebai H. Bioactivity and molecular docking studies of selected plant compounds. J Sci Food Agric 2024;104:4391–9.

[64] Rashmi, Tandon R, Kalia A, Raju B, Silakari O. Bioactive molecules isolated from *Cymbopogon flexuosus* and *Monarda citriodora* essential oils: Chemical profiling, antimycotic potential, and molecular docking studies against green and blue molds of Kinnow. J Essent Oil Bear Plants 2024;27:1130–47.

[65] Ren Z, Chhetri A, Guan Z, Suo Y, Yokoyama K, Lee S-Y. Structural basis for inhibition and regulation of a chitin synthase from *Candida albicans*. Nat Struct Mol Biol 2022;29:653–64.

[66] Barthel L, Cairns T, Duda S, Müller H, Dobbert B, Jung S, et al. Breaking down barriers: comprehensive functional analysis of the *Aspergillus niger* chitin synthase repertoire. Fungal Biol Biotechnol 2024;11:3.

[67] Zeng T, Rodriguez-Moreno L, Mansurkhodzaev A, Wang P, van den Berg W, Gasciolli V, et al. A lysin motif effector subverts chitin-triggered immunity to facilitate arbuscular mycorrhizal symbiosis. New Phytol 2020;225:448–60.