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Biodegradation of Dimethoate 40% by *Bacillus subtilis* OQ347968 Isolated from Polluted soils

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PESTICIDE residues are decomposed by a number of microorganisms and used as a source of nitrogen and carbon. The discovery of new strains capable of tolerating and degrading toxic pesticides can increase the flexibility of strategic planning for environmental decontamination initiatives. Our goal was to isolate bacteria tolerant to different concentrations of 40% dimethoate (0-80 µg L^{-1}) from soil samples, determine the optimization of cultural conditions (pH, temperature and incubation time), and examine the inhibitory/inducing effect on the plant growth enhancing traits. The most effective bacterial isolate was identified by biochemistry and phylogenetics, and was also tested for HPLC biodegradation. Data showed that only one strain (SH1) had the greatest tolerance to dimethoate after 72 hours at the highest concentrations (80 µg L^{-1}), despite the fact that all isolates (SH1–SH7) showed some degree of tolerance. Furthermore, at the greatest concentration of dimethoate, the highest growth was observed by the SH1 isolate, which recorded 0.64 for optimum pH 7, 0.69 for optimum temperature 30 °C, and 0.79 for optimum incubation time 72 h. In the same context, isolate (SH1) recorded positive results in producing IAA, NH_3, P solubilization, and siderophores at 60 µg L^{-1} compared to other tested isolates and different concentrations of dimethoate. Therefore, according to the biochemical characteristics and 16S rRNA sequence, SH1 isolate belonged to *Bacillus subtilis* that had GenBank accession number OQ347968. According to the results of HPLC biodegradation, after 7 days, *B. subtilis* OQ347968 had the highest rates of dimethoate elimination (80 mg L^{-1}). These findings imply that *B. subtilis* OQ347968 has a great deal of ability to degrade dimethoate 40% in a variety of environmental conditions.

Keywords: *Bacillus subtilis*; Dimethoate 40%; Cultural conditions, Plant growth promoting traits; HPLC biodegradation.

1. Introduction

The manufacture of numerous agrochemicals, synthetic pesticides, fertilizers, and other chemicals has been facilitated by the modern era's fast industrialization. Among them, pesticides are a modern agriculture sector miracle since they improve production and lower labor costs by getting rid of pests that would otherwise destroy the crops (Simmons, 2017; Abdalla et al.2023). Pesticides are organic substances used to control pests; nevertheless, when their constituents are released into the environment, they become pollutants (Chapalamadugu and Chaudhry, 1992). These releases can take the form of inadvertent spills, direct application, container-cleaning waste, and other different scenarios. They damage the ecosystem and people's health as they are released into the land, water, and air (Gomah, 2015; Morillo and Villaverde, 2017).

Some of the pesticides used to protect crops can harm microorganisms and have negative effects. Dimethoate concentrations have been shown to negatively impact plant growth regulators such IAA and P (solubilization), as well as viability, growth, N_2 fixation, and nif genes (Askar and Kudhur, 2013; Madhaiyan et al., 2006). Dimethoate is a well-known

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systemic organophosphorus insecticide and acaricide. It is also considered a carbamate insecticide. It has been effectively used since 1956 to manage a wide range of insects, including as woolly aphids, red spider mites, pea midges, thrips, wheat bulby, sawy, and suckers (Deshpande, et al., 2004). Dimethoate kills insects by contact and stomach action because it disrupts the activity of acetylcholinesterase (AChE), which is essential for the proper functioning of both human and insect nervous systems (Hassal, 1990). Dimethoate is stable in aqueous circumstances between pH 2 and 7, according to the World Health Organisation (WHO), and it is classified as a moderately harmful chemical. Dimethoate poisoning can occur orally, however skin absorption is the most common route of absorption (Al-Jaghbir et al., 1992). Soil, vegetables, and even cow milk have been shown to contain dimethoate residues and their oxidized counterparts (Srivastava and Raizada, 1996). With a half-life (DT50) of 12 days, dimethoate can dissolve in an alkaline solution at pH 9 (Deshpande et al., 2004) at higher temperatures than 96 °C (Andreozzi et al., 1999). Furthermore, in the absence of biodegradation, dimethoate can have a half-life in soil of up to 206 days at 25 °C (Hassal, 1990). Biodegradation is gaining popularity as a dependable and reasonably priced biotechnological technique for clearing contaminated areas. According to Torstensson (2000), the biotic (microbial degradation) and abiotic (adsorption, chemical degradation) processes occurring in the biomixture determine how effective biobeds are. New approaches to treating pesticides have been made possible by research on the importance of abiotic factors in biobed systems (Saez et al., 2018; Tortella et al., 2020). Studies involving bioaugmentation in real or pilot systems (Saez et al., 2018; Pinto et al., 2020) or isolated strains in pesticide bioremediation (Umar Mustapha et al., 2020) give us possibilities for decontamination procedures and pesticide bioremediation. It has been reported that microbial activity occurs during the treatment of high pesticide concentrations (Góngora-Echeverra et al., 2019). The possibility of using these bacteria to detoxify severely contaminated areas in situ has made them particularly interesting in recent years. These bacteria are capable of breaking down organophosphorus chemicals. According to Ortiz-Hernández and Sánchez-Salinas (2010), these bacteria are the result of local microbial communities' evolutionary process whereby they have adapted to these pollutants. Researchers have looked at the bacteria that are utilized in bioremediation procedures to remove contaminants like pesticides (Góngora-Echeverría et al., 2020). Numerous microorganisms have been identified as having degrading activity in the specific case of the pesticides used in this investigation. Among these microorganisms, those belonging to the genus *Bacillus* are interesting due to their ability to degradation of dimethoate by the breakdown products of dimethoate metabolism (DebMandal et al., 2008), or production of intra and extra organophosphorus phosphatase enzymes (Ambreen, et al., 2020). A number of bacteria have been shown in earlier studies to be able to biodegrade dimethoate (Jiang et al., 2007; Abd El-Hafez, 2014; Shahid et al., 2019). In this context, DebMandal et al. (2008) showed that *B. licheniformis* culture extract may biodegrade 100% dimethoate within 3 days, while *Xanthomonas campestris* pv. *Translucens* can biodegrade 97.8% of dimethoate within 32 days (Derbalah et al., 2021).

Therefore, Our investigation was intended to- (i) isolate and screening the dimethoate 40% tolerant bacterial isolates (ii) evaluate the influence of different concentrations of dimethoate 40% on growth pattern of bacterial isolates (iii) determine the optimization of cultural conditions (iv) examine the inhibitory/stimulating effect of dimethoate 40% on plant growth promoting traits, (v) identification of the most potent bacterial isolate by biochemical and phylogenetically, and (vi) determination for biodegradation of dimethoate by HPLC.

2. Materials and Methods

2.1. Dimethoate 40% -resistant bacteria isolation and purification from soil samples

Samples of soil were taken at the El-Mahla El-Kobra region, which is situated between 30°34' N and 30°45' E longitude. 7 samples, ranging in depth from 0 to 20 cm, were collected in June 2020 from 7 soil sites that had been watered for more than ten years using drainage water from drains No. 5. In order to isolate bacteria, the soil samples were brought to the lab, air dried at room temperature (25°C), and sieved through a 2 mm screen. The method involved serially diluting a 10 g soil sample in sterile normal saline solution (NSS) and shaking it well with a vortex. Then, 0.5 ml of the soil suspension was inoculated with 100 ml of liquid Mineral Salt Medium (MSM) (Shaw and Burns 2004). The only carbon and energy source used for this was 50 μg L^{-1} of dimethoate 40% ($C_5H_{12}NO_3PS_2$). An orbital shaking incubator operating at 150 rpm was used to incubate the infected media at 30 °C. Two days later, the subculturing was done again (three rounds total), and 100 μl of the final culture was surface plated on solidified MSM-agar plates with 40% dimethoate. The plates were then incubated for two days at 30 °C. To verify the purity of the cultures, a single colony of 7 distinct bacterial isolates was chosen, streaked 5 times on the same medium, and kept on the same medium at 4 °C until needed.

2.2. Screening of dimethoate 40% resistant bacteria by enrichment culture method (*In Vitro*)

Abraham and Silambarasan (2016) report that the minimum inhibitory concentration (MIC) method was first used to screen 7 distinct bacterial isolates for dimethoate 40% tolerance. Using this method, the bacterial isolates were individually exposed to 0, 10, 20, 40, 60, and 80 µg L^{-1} of pesticide in MSM medium, resulting in dimethoate 40% breakdown. To estimate the remaining 40% dimethoate concentration, the infected medium was cultured for 15 days at 30 °C on an orbital shaker (150 rpm). Using a UV/Visible spectrophotometer (540 nm, Jenway model 6705, UK) set to measure turbidity at certain intervals of 3, 8, and 15 days, promising dimethoate 40% degrading bacterial species were selected.

2.3. Optimization of cultural conditions (incubation times, pH and temperature) for optimum biodegradation of different concentration of dimethoate 40%.

The Nutrient Broth (NB) medium was created in conical flasks (500 ml) containing 100 ml of medium that was sterilized at 121 °C for 20 min. The medium was then inoculated with 1 ml of inoculum containing 10^7 cfu / ml of various isolates, in triplicate, from each concentration of dimethoate 40%. The inoculated flasks were incubated at 150 rpm for 24, 48, and 72 hours at varying temperatures (25, 30, and 35 °C), as well as at varying pH levels (6, 7, and 8). A UV/Visible spectrophotometer (Jenway model 6705, UK) was used to measure the growth based on the turbidity of the culture at 600 nm in order to determine the ideal incubation duration, pH, and temperature (Kamaraj et al., 2022).

2.4. Assessment of plant growth promoting activities under different concentration of dimethoate 40%

Indole acetic acid production

The tested isolates' production of indole-3-acetic acid (IAA) was measured using a modified version of Brick et al.'s method (Brick et al., 1991). The assay involved growing the bacterial isolate in 100 mL of NB medium with 100 µg mL^{-1} of tryptophan and supplementing it with 0, 10, 20, 40, 60, and 80 mg L^{-1} of dimethoate 40% concentrations. The inoculum, which contained 10^7 cfu / ml of different isolates, was then added. The inoculum was then incubated at 30 °C for 5 days while being shaken at 120 r/min. After full incubation, the culture (5 mL) was centrifuged at 8000 rpm for 10 minutes, and 2 ml of the supernatant were combined with 100 µl of orthophosphoric acid and 4 ml of Salkowsky reagent (2% 0.5M $FeCl_3$ produced in 35% $HClO_4$). The mixture was then incubated for an hour at 30 °C in the dark to develop colour. At 530 nm, the generated pink color's absorbance was measured.

Phosphate solubilization

By cultivating the bacterial isolates in Pikovskaya (PVK) medium and tribasic calcium phosphate in the presence and absence of 10, 20, 40, 60, and 80 µg L^{-1} of dimethoate 40% concentrations, the phosphate solubilization capability was assessed. Additionally, the infected broth was cultured for ten days at 30 °C and 120 rpm. After centrifuging liquid cultures at 5000×g for 15 minutes, the available P level in the various treatments was evaluated in the culture supernatants after 10 days of growth (Olsen and Sommers, 1982). As a function of phosphate content, the blue colour created was measured for absorbance at 610 nm.

Ammonia production

According to Cappuccino and Sherman's (1992) description, a quantitative assay was used to assess the ammonia generation by the tested isolates. In summary, examined isolates were inoculated into peptone water broth medium supplemented with 0, 10, 20, 40, 60, and 80 µg L^{-1} of dimethoate 40% concentrations. The medium was then incubated at 30 °C for 4 days while being shaken at 200 rpm. Following the incubation period, 5 mL of liquid broth was centrifuged for 10 minutes at 5000×g. The supernatant was then collected, combined with 1 mL of Nessler's reagent, and topped off with 10 mL of sterile distilled water that was free of NH_3. The standard used to test absorbance at 450 nm was NH_3Cl.

Siderophore production

Using various concentrations of dimethoate 40%, the Chrome Azurol S (CAS) technique (Alexander and Zuberer, 1991) was used to quantitatively evaluate the siderophore secretion by the investigated isolates. Using NB liquid medium injected with 100 µL (107 cfu / ml) of bacterial culture, siderophores were quantitatively quantified after 72 h of incubation at 30 °C. The growth medium was centrifuged at 3000×g for 30 minutes to extract the supernatant, which was then combined 1:1 with the CAS solution. After 20 min, at 630 nm, the optical density of the produced orange color was measured.

2.5. Identification of the most potent bacterial isolate

Of the 7 bacterial isolates, strain SH1 exhibited a comparatively high rate of growth and the production of activities that promote plant development at varying concentrations of 40% dimethoate. The chosen isolate was identified phylogenetically by

16S ribosomal RNA (16S rRNA) sequence analysis and biochemically utilizing a VITEK 2 compact system for bacterial identification.

2.6. Molecular identification of the selected isolate

Using the GeneJet Bacterial Genomic DNA Extraction Kit (Fermentas), the genomic DNA of the test bacterial isolates cultured on nutritional broth was extracted. At Sigma Scientific Services Co., Giza, Egypt, the isolate's 16 S rRNA gene was amplified using polymerase chain reaction (PCR) using universal primers. Maxima Hot Start PCR Master Mix (Fermentas) and a hybrid thermal reactor thermocycler were utilized to generate a PCR product of about 1.5 kbp utilizing forward and reverse primers (F, 5′-AGA GTT TGA TCC TGG CTC AG-'3 and R, 5’-GGT TAC CTT GTT ACGACT T-3′). The cycle parameters included an initial 10 min of 95 °C denaturation (enzyme activation) for one cycle, followed by 30 seconds of denaturation at 95 °C, 1 minute of annealing at 65 °C, and 1 min of extension at 72 °C for 30 cycles. A final elongation phase lasting 10 min at 72 °C came next. Following analysis on 1% (w/v) agarose gels, the PCR products were forwarded to GATC Biotech DNA Sequencing (Germany) for ABI 3730xl DNA Sequencing. The BioEdit version 5.0.9 sequence editor was used to input the sequence data. After analysing base-calling, a continuous sequence was produced. The RDP Sequence Aligner programme (https://rdp.cme.msu.edu/) was used to align the entire sequence. The RDP and GenBank databases provided the sequences that were used in the phylogenetic analysis. The neighbor-joining method was used to create a dendrogram, and the degree of confidence in the topology of the trees was assessed.

2.7. Determination of *B. subtilis* OQ347968 biodegradation of dimethoate

According to (Zhu et al. 2010), purification was guaranteed by inspecting the preparation under a microscope and then keeping the nutrient slits open. The strain was grow on a mineral salt medium (MSM) containing dimethoate (80 ppm) as the only carbon sources and cultured at 28 °C for 7 days. Every 24 h, 1 ml subsamples were extracted from each tube for the determination of dimethoate. Equal amounts of ethyl acetate were used as the extracting reagent during the two extractions of these subsamples. Vortexing was used to homogenise the mixture for three minutes (Abdelrazek et al. 2023a). After filtering and drying with anhydrous sodium sulphate, the ethyl acetate containing leftover dimethoate was filtered through glass-fibre paper (Whatman GF/B). The filtrates were combined during this successive process. After the filtrate was completely evaporated, 50 µL of HPLC-grade dichloromethane was used to dissolve it for analysis. Using an HPLC, the quantity of dimethoate was measured (Abdelrazek et al. 2013). HPLC was used to evaluate the samples directly. In the isocratic elution method, a 60:40 ratio of acetonitrile to distilled water was utilized as the mobile phase for the dimethoate assay. Wakosil-II 5 C18-100 (Wako, Japan) was used to fill the column (i.d. 4.6 mm; length 250 mm) while the mobile phase flow rate was kept constant at 1 mL min^{-1}. The samples were injected at 3.84 minutes after the retention period of 3.30 minutes using a UV detector operating at 210 nm (Abdelrazek et al. 2023b).

2.8. Statistical analyses

To measure PGP features, one-way analysis of variance using the statistical software program "COSTAT", with ten replicates was used for the experimental setup, the data are presented as the mean ± SD, and the Excel program, Office 2016 was used to prepare the figures for the manuscript (Gomez and Gomez, 1984). The relations between cultural conditions and different concentration of dimethoate were determined by the Pearson correlation coefficients (R).

3. Results

3.1. Dimethoate 40% -resistant bacteria isolation and purification from soil samples

For over a decade, the studied area was irrigated with drainage water which the soil samples yielded seven bacterial isolates, designated SH 1–7. All isolates were grown on nutrient broth and/or agar medium that had been boosted with increased concentrations of dimethoate 40% (0-80 µg L^{-1}) in order to test for tolerance. Even though every isolate exhibited some tolerance to dimethoate 40%, only one isolate (SH1) had the highest tolerance, which was measured as 0.64 by optical density at 600 nm using a spectrophotometer after 72 hours of growth on NB medium (Figure 1). Consequently, at the highest concentrations of dimethoate 40% (80 µg L^{-1}), the results of the tested isolates were in the descending order of SH1 > SH2 > SH5 > SH7 > SH4 > SH6 > SH3 (Figure 1).

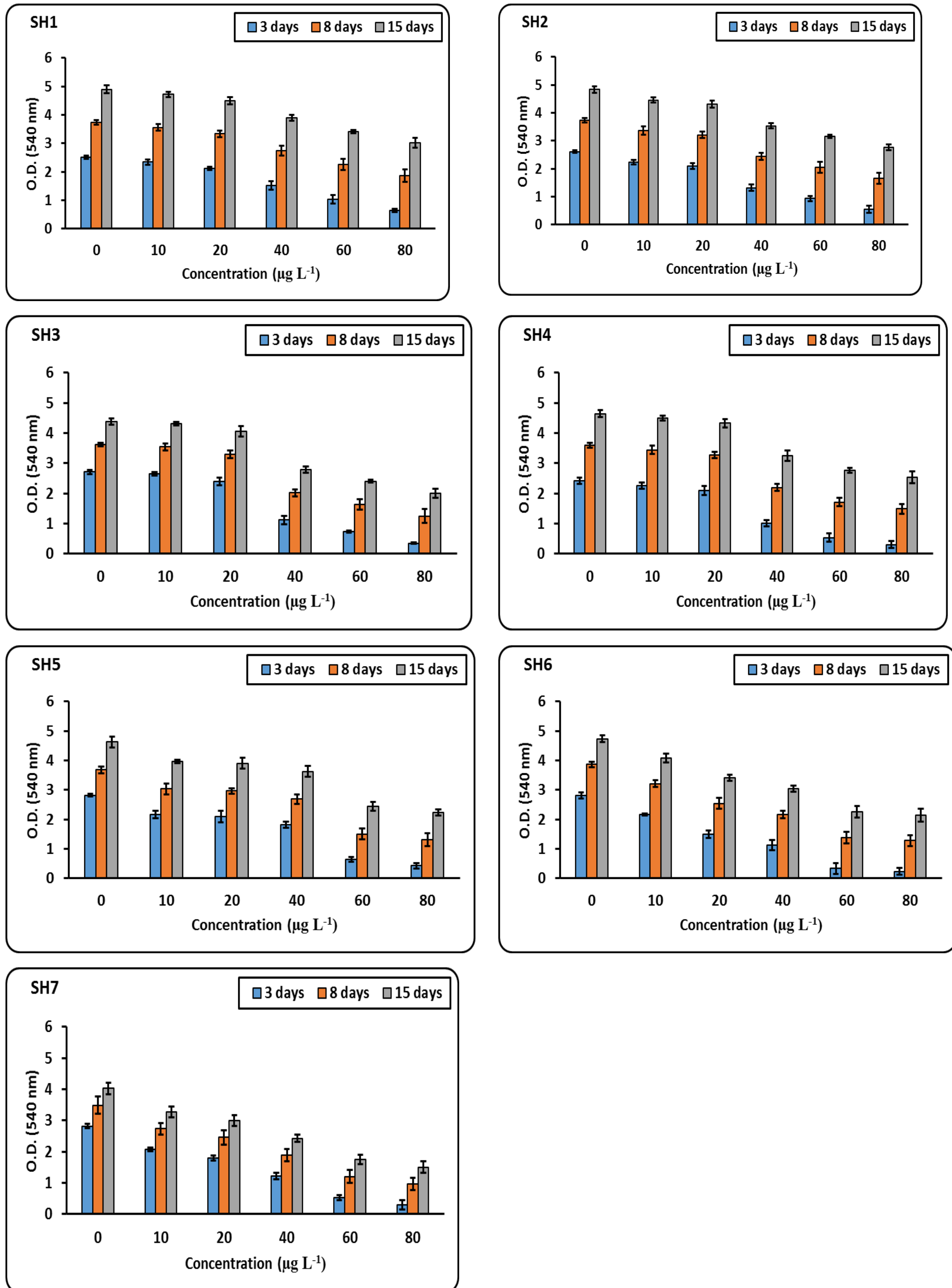

Fig. 1. Screening the growth of tested isolates on MSM medium supplemented with 0, 10, 20, 40, 60 and 80 mg L^{-1} of dimethoate 40% at 3, 8 and 15 days.

3.2. Optimization of cultural conditions

The ideal nutrient broth medium was made, the medium was inoculated with the tested isolates, and the medium was incubated at 150 rpm with pH values adjusted to 6, 7, and 8, temperatures adjusted to 25, 30, and 35 °C, and incubation times adjusted to 24, 48, and 72 hours under various concentrations of dimethoate 40% (Figure 2). Results show that the optimum growth of the investigated isolates at pH 7 ranged between 0.39 and 0.64, from 0.33 to 0.69 at 30 °C, and from 0.54 to 0.79 after 72 hours of incubation under the highest dosage of dimethoate 40% (80 µg L^{-1}).

Herein, the highest growth was observed by the tested isolates followed the descending order of SH1 (0.64) > SH2 (0.59) > SH3 (0.54) > SH4 (0.52) > SH5 (0.48) > SH6 (0.44) > SH7 (0.39) for optimum pH (7), and SH1 (0.69) > SH3 (0.64) > SH2 (0.63) > SH4 (0.52) > SH5 (0.44) > SH6 (0.41) > SH7 (0.33) for optimum temperature (30 °C), and SH1 (0.79) > SH3 (0.74) > SH2 (0.73) > SH7 (0.73) > SH4 (0.62) > SH6 (0.61) > SH5 (0.54) for optimum incubation time (72h), at the highest concentration of dimethoate 40% (80 mg L^{-1}), (Figure 2).

3.3. Assessment of plant growth promoting activities under different concentration of dimethoate 40%

The bacterial isolates (SH1-SH7) were screened for later evaluation of their plant growth-promoting properties, including IAA, NH_3, P solubilization, and siderophores production, under various concentrations of dimethoate 40% (0, 10, 20, 40, 60, and 80 µg L^{-1}). When cultivated on higher dimethoate 40% concentrations up to 60 mg L^{-1} as opposed to the control (no dimethoate 40%), tested isolates produced more IAA over time. However, at the higher dimethoate 40% concentrations (80 µg L^{-1}), IAA synthesis reduced. In both the control and the highest dimethoate 40% concentration (60 µg L^{-1}), bacterial isolate (SH1) produced the most IAA among all tested bacterial isolates.

When dimethoate 40% concentration in the growing medium was increased up to 60 µg L^{-1} compared to control, ammonia production by the examined bacterial isolates was marginally enhanced; however, raising dimethoate 40% above 60 µg L^{-1} dramatically lowered the ammonia production (Table 2). Comparing SH1 isolate to the other tested isolates, the maximum value of ammonia production at 60 µg L^{-1} treatment was 63.57 µg mL^{-1} (Table 1).

In the growing medium supplemented with various doses of dimethoate 40%, the two bacterial isolates (SH1 and SH2) demonstrated the greatest solubilized phosphate content (Table 1). These isolates performed better at solubilizing phosphate when the growth medium's dimethoate 40% concentration was increased to 60 µg L^{-1}. When growth medium was supplemented with 80 µg L^{-1} of dimethoate 40%, the SH1 and SH2 isolates both recorded 5.08 and 3.95 µg mL^{-1} of solubilized phosphate, respectively (Table 2). This indicates that increasing dimethoate 40% concentration above 60 µg L^{-1} significantly reduced the ability of bacterial isolates to solubilize phosphate. Even though SH1 bacterial isolate produced more siderophores under increasing dimethoate 40% (up to 60 µg L^{-1}), and all isolates had similar levels of siderophores, there were only very slight changes between isolates and treatments. In comparison to the control and dimethoate 40% treatments below 60 µg L^{-1}, differences between treatments above this concentration and bacterial isolates were more pronounced and values were lower (Table 1). In addition, when compared to other isolates, the SH1 isolate performed the best under all dimethoate 40% treatments, with average siderophore production of 0.079, 0.175, 0.281, 0.422, 0.605, and 0.048 µM L^{-1} at the control, 10, 20, 40, 60, and 80 mg L^{-1} of dimethoate 40%, respectively.

3.4. Identification of the most potent bacterial isolate

Biochemical characterization

A single bacterial isolate (SH1) was identified phylogenetically by 16S ribosomal RNA (16S rRNA) sequencing analysis and biochemically utilizing a VITEK 2 compact system for bacterial identification (Table 2 and Figure 3). Biochemical identification, showed negative results for L-Lysine Arylamidase, L- Asparate Arylamidase, L-Proline Arylamidase, Beta-N-Acetyle-Glucosaminidase, Ala-phe-Pro- Arylamidase, D-Galactose, Methyl-D-Xyloside, Alpha-Manosidase, Glycine Arylamidase, D-Melezitose, N-Acetyl- Glucosamine, Urease, L-Rhaminose, Phosphoryle Choline and Esculin hydrolysis tests and other biochemical testes showed positive tests (Table 3). On the other hand, sequence information was imported into the BioEdit sequence editor version 5.0.9. A continuous sequence was produced when base-calling was examined. The RDP Sequence Aligner program (https://rdp.cme.msu.edu/) was used to align the entire sequence. The RDP and GenBank databases provided the sequences used in the phylogenetic study. The neighbor-joining technique was used to create a dendrogram. It was also decided how confident to be in tree topology. It is generally customary to refer to Bacillus subtilis SH1 by its GenBank entry number, such as Bacillus subtilis OQ347968 (Figure 3).

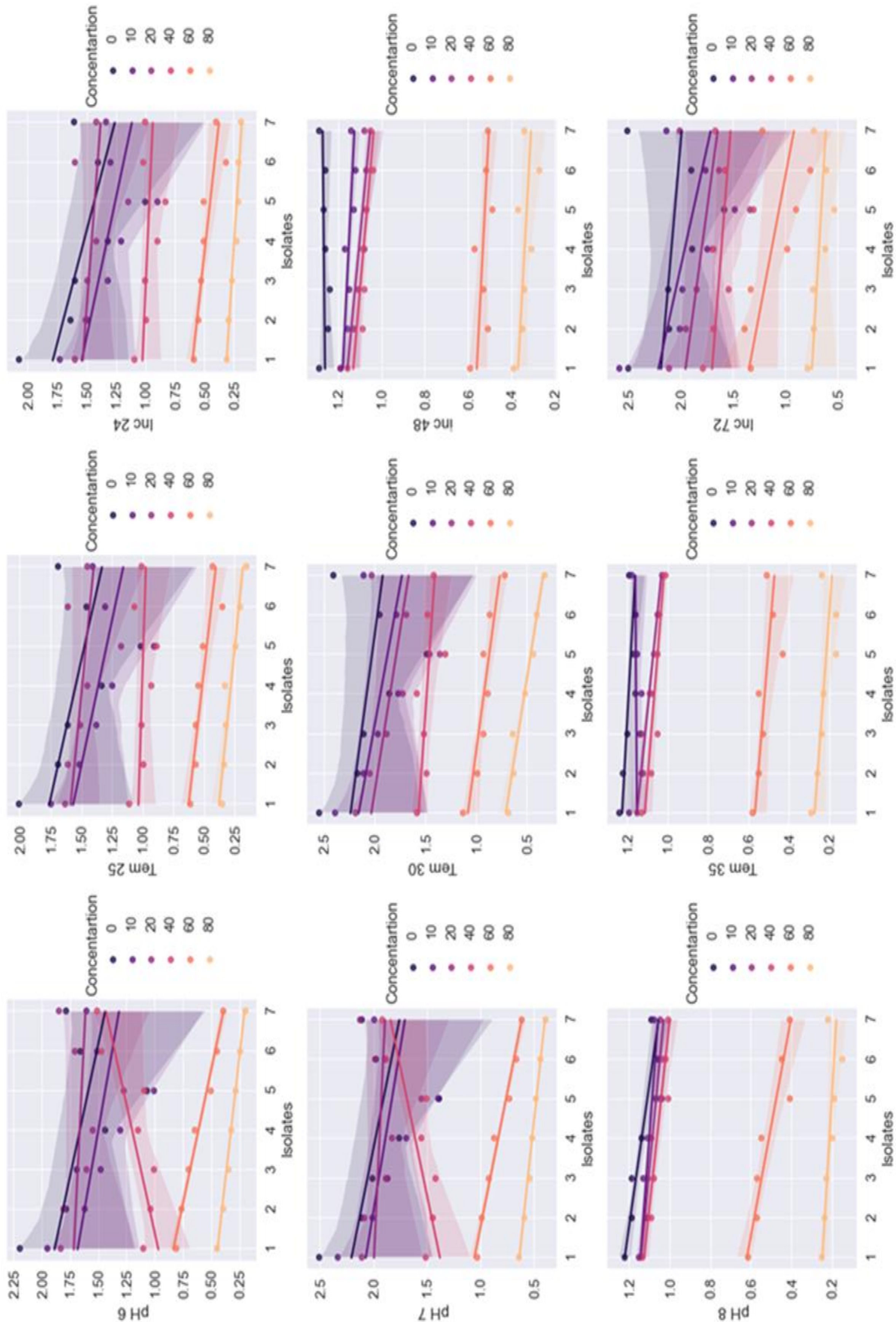

Fig. 2. Effect of different pH (6, 7 and 8), different temperatures (25 , 30 and 35 °C), and different incubation times (24, 48, and 72h), on the growth of tested isolates on NB medium supplemented with 0, 10, 20, 40, 60 and 80 µg L^{-1} of dimethoate 40%. 1: SH1; 2: SH2; 3: SH3; 4:SH4; 5:SH5; 6:SH6 and 7: SH7.

Table 1. Plant-growth-promoting traits of tested isolates under increasing of dimethoate 40% concentrations (µg L^{-1}).

Parameter	Isolates	Dimethoate 40% (µg L^{-1})					
		0	**10**	**20**	**40**	**60**	**80**
IAA (µg mL^{-1})	**1**	8.77 ± 0.36 a	12.01 ± 0.80 a	15.56 ± 1.26 a	17.95 ± 0.40 a	20.51± 0.40 a	7.19 ± 0.71 a
	2	7.18 ± 0.07 ab	10.45 ± 0.70 ab	13.33 ± 1.70 ab	14.72 ± 1.78 b	17.28 ± 0.40 b	5.60 ± 0.56 ab
	3	6.86 ± 0.48 ab	9.64 ± 0.79 ab	11.85 ± 0.91 ab	13.57 ± 0.48 bc	16.13 ± 0.48 c	5.29 ± 0.34 ab
	4	6.18 ± 0.17 bc	8.96 ± 1.44 b	10.51 ± 1.85 bc	11.56 ± 1.44 c	13.79 ± 0.28 d	4.94 ± 0.62 bc
	5	4.38 ± 0.41 c	5.83 ± 0.45 c	7.71 ± 1.20 c	8.43 ± 0.45 d	10.99 ± 0.45 e	3.14 ± 0.89 c
	6	7.46 ± 0.40 ab	10.57 ± 1.40 ab	12.45 ± 2.40 ab	13.84 ± 1.94 bc	15.73 ± 0.41 c	5.55 ± 1.40 ab
	7	6.62 ± 0.91 ab	9.40 ± 1.16 b	11.61 ± 1.09 b	14.00 ± 1.16 bc	16.56 ± 0.42 bc	5.05 ± 0.59 bc
Ammonia production (µg mL^{-1})	**1**	18.42 ± 0.76 a	26.43 ± 1.76 a	40.46 ± 3.28 a	50.25 ± 1.13 a	63.57 ± 1.25 a	12.95 ± 1.28 a
	2	15.07 ± 2.25 ab	23.00 ± 1.55 ab	34.67 ± 4.42 ab	41.22 ± 4.98 b	53.57 ± 1.24 b	10.08 ± 1.00 ab
	3	14.41 ± 1.01 ab	21.21 ± 1.73 ab	30.82 ± 2.37 ab	38.01 ± 1.35 bc	50.01 ± 1.49 c	9.52 ± 0.60 ab
	4	12.99 ± 2.45 bc	19.71 ± 3.16 b	27.32 ± 4.82 bc	32.37 ± 4.02 c	42.74 ± 0.87 d	8.89 ± 1.12 bc
	5	9.21 ± 0.86 c	12.82 ± 1.00 c	20.04 ± 3.13 c	23.59 ± 1.27 d	34.06 ± 1.41 e	5.65 ± 1.60 c
	6	15.67 ± 2.95 ab	23.26 ± 3.09 ab	32.38 ± 6.24 ab	38.75 ± 5.43 bc	48.77 ± 1.27 c	10.00 ± 2.53 ab
	7	13.91 ± 1.92 ab	20.68 ± 2.55 b	30.19 ± 2.84 b	39.20 ± 3.24 bc	51.34 ± 1.31 bc	9.08 ± 1.07 bc
Phosphate solubilizing (µg mL^{-1})	**1**	7.08 ± 0.29 a	9.44 ± 0.63 a	12.64 ± 1.02 a	14.78 ± 0.33 a	18.16 ± 0.36 a	5.08 ± 0.50 a
	2	5.80 ± 0.86 ab	8.21 ± 0.55 ab	10.83 ± 1.38 ab	12.12 ± 1.46 b	15.31 ± 0.35 b	3.95 ± 0.39 ab
	3	5.54 ± 0.39 ab	7.57 ± 0.62 ab	9.63 ± 0.74 ab	11.18 ± 0.40 bc	14.29 ± 0.43 c	3.73 ± 0.24 ab
	4	4.99 ± 0.94 bc	7.04 ± 1.13 b	8.54 ± 1.51 bc	9.52 ± 1.18 c	12.21 ± 0.25 d	3.49 ± 0.44 bc
	5	3.54 ± 0.33 c	4.58 ± 0.36 c	6.26 ± 0.98 c	6.94 ± 0.37 d	9.73 ± 0.40 e	2.22 ± 0.63 c
	6	6.03 ± 1.13 ab	8.31 ± 1.10 ab	10.12 ± 1.95 ab	11.40 ± 1.60 bc	13.94 ± 0.36 c	3.92 ± 0.99 ab
	7	5.35 ± 0.74 ab	7.39 ± 0.91 b	9.44 ± 0.89 b	11.53 ± 0.95 bc	14.67 ± 0.37 bc	3.56 ± 0.42 bc
Siderophores (µM L^{-1})	**1**	0.079 ± 0.003 a	0.175 ± 0.012 a	0.281 ± 0.023 a	0.422 ± 0.009 a	0.605 ± 0.012 a	0.048 ± 0.005 a
	2	0.064 ± 0.010 ab	0.152 ± 0.010 ab	0.241 ± 0.031 ab	0.346 ± 0.042 b	0.510 ± 0.012 b	0.038 ± 0.004 ab
	3	0.062 ± 0.004 ab	0.140 ± 0.011 ab	0.214 ± 0.016 ab	0.319 ± 0.011 bc	0.476 ± 0.014 c	0.036 ± 0.002 b
	4	0.055 ± 0.010 bc	0.130 ± 0.021 b	0.190 ± 0.033 bc	0.272 ± 0.034 c	0.407 ± 0.008 d	0.033 ± 0.004 bc
	5	0.039 ± 0.004 c	0.085 ± 0.007 c	0.139 ± 0.022 c	0.198 ± 0.011 d	0.324 ± 0.013 e	0.021 ± 0.006 c
	6	0.067 ± 0.013 ab	0.154 ± 0.020 ab	0.225 ± 0.043 ab	0.326 ± 0.046 bc	0.465 ± 0.012 c	0.037 ± 0.009 ab
	7	0.059 ± 0.008 abc	0.137 ± 0.017 b	0.210 ± 0.020 b	0.329 ± 0.027 bc	0.489 ± 0.012 bc	0.034 ± 0.004 b

In a column means followed by a common letter are not significantly different at 5% level by DMRT. [a-e]: Duncan's Letters.

Table 3. Biochemical characteristics of isolate No. SH1 using Biomerieux VITEK 2 system on BCL card.

Test	Mnemonic	Result	Test	Mnemonic	Result
Beta-Xylosidase	BXYL	+	D-Mannitol	dMAN	+
L-Lysine Arylamidase	Lys.A	-	D-Mannose	D.MNE	+
L- Asparate Arylamidase	AspA	-	D-Melezitose	D.MLZ	-
Leucine Arylamidase	Leu.A	+	N-Acetyl- Glucosamine	NAG	-
Phenylalanine Arylamidase	Phe A	+	Palatinose	PLE	+
L-Proline Arylamidase	Pro A	-	Urease	URE	-
Bata-Galactosidase	BGAL	+	L-Rhaminose	IRHA	-
L- Arylamidase	Pyr.A	+	Beta- glucosidase	BGLU	+
Alpha-Galactosidase	AGAL	+	Beta-Mannosidase	BMAN	+
Alanine Arylamidase	Ala.A	+	Phosphoryle Choline	PHC	-
Tyrosine Arylamidase	Tyr A	+	Pyruvate	PVATE	+
Beta-N-Acetyle-Glucosaminidase	BNAG	-	Alpha-Glucosidase	AGLU	+
Ala-phe-Pro- Arylamidase	APPA	-	D-Tagatose	D TAG	+
Cyclodextrin	CDEX	+	D-Trehalose	D TRE	+
D-Galactose	D.GAL	-	Inulin	INU	+
Glycogen	GLYG	+	D-Glucose	D.GLU	+
Methyl-D-Xyloside	MdX	-	Growth in 6.5% NaCl	NaCl 6.5%	+
Alpha-Manosidase	AMAN	-	Polymixin-B Resistance	POLYB R	+
Maltotriose	MTE	+	D-Ribose	D.RIB	+
Glycine Arylamidase	Gly A	-	Esculin hydrolysis	ESC	-
Ellman	ELLM	+	Kanamycin Resistance	KAN	+

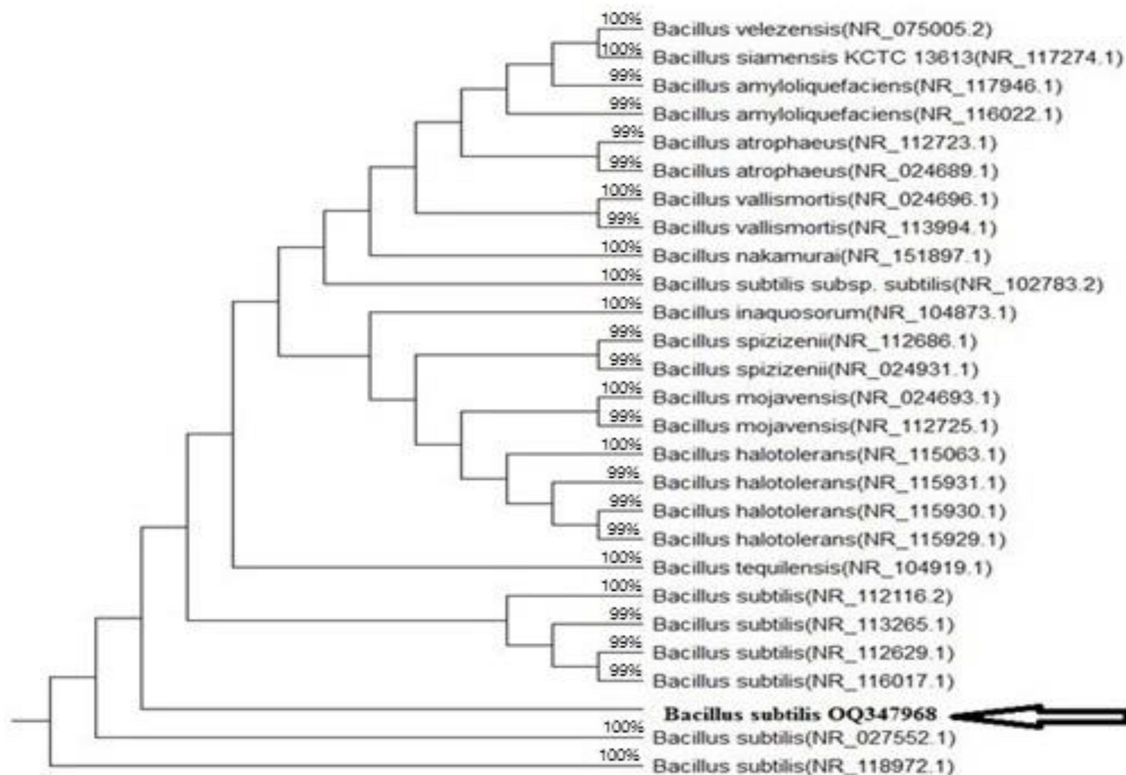

Fig. 3. Neighbour-joining phylogenetic tree reconstructed on the basis of a 1.5 kpb 16 S rRNA gene sequence showing the phylogenetic position of *Bacillus subtilis* OQ347968.

3.5. Determination of *B. subtilis* OQ347968 biodegradation of dimethoate

Over seven days, the biodegradation of 80 µg L^{-1} of dimethoate by the bacterial strain *B. subtilis* OQ347968 was assessed with removal kinetics, demonstrating the outcomes of the various treatments that were carried out. The *B. subtilis* OQ347968 control strain and the control (culture media plus dimethoate without bacterial cells) were used to measure the kinetics of degradation. This bacterial strain exhibited the lowest levels of 100% dimethoate clearance, as would be predicted. While the results in the treatments (with the chemical concentration of dimethoate) showed a significant difference in their kinetics, as the removal rates reached 100%. However, when comparing the removal of pesticide between the control and the treatments, there are significant differences. Therefore, the results showed that the strain *B. subtilis* OQ347968 has higher rates of removal of dimethoate after 7 days.

4. Discussion

For the decomposition of pesticides in polluted environments, biodegradation is a realistic, economical, and sustainable method. Due to the fact that microorganisms may metabolize a number of xenobiotic substances, including pesticides, and subsequently detoxify or mineralize them, the biological elimination of chemical contaminants becomes the preferred method.

In our investigation, isolate SH1 demonstrated the maximum tolerance to the pesticide chosen (dimethoate 40%), which is employed in agronomic practices to eliminate the many dangerous pests of insects to safeguard the crops and, consequently, to increase food production. However, unwise and dangerous pesticide use led to their deposition in the soil, which reduced crop production and increased pathogen resistance to these pesticides. For agronomists, these are extremely upsetting characteristics that require attention and an immediate fix to overcome such a troublesome situation. We therefore sought to identify soil-dwelling bacteria that are pesticide resistant in order to improve crop yield even in pesticide-polluted soil and these bacteria may be useful in reducing pesticide toxicity. When SH1 isolate was exposed to varied pesticide concentrations, it was discovered that it had an oddly higher tolerance to them. It is assumed that the isolate SH1 may have decomposed the test pesticide and used it as a nutrition source for their growth that it was cultured on NB medium supplemented with varying doses of dimethoate 40%. In addition, the isolate SH1 displayed greater

resistance to pesticide applied to solid medium as a result. A fascinating complex process that is regulated by microorganisms at the genetic and physiological level is the tolerance/resistance and degradation of pesticides among soil bacteria (Jahn et al., 2017). Physiological changes that cause microbial metabolism to create a new metabolic pathway to avoid a biochemical response that is hindered by a specific pesticide are supported as temporary resistance to pesticides. On the other hand, genetic changes that are passed down through succeeding generations of microorganisms are what provide permanent resistance. Therefore, biodegradation is a capability of microorganisms that have evolved pesticide resistance (Ishag et al., 2016). The solubility and mobility of these chemicals in an aquatic environment as opposed to those observed on solid medium may have contributed to the harmful and growth-inhibiting effects of some pesticides on bacterial cells cultured in liquid culture medium. Bacterial cytoplasmic membrane transport of inorganic ions of chemical pesticides may be the cause of the reduction in their growth pattern after exposure to pesticides. Similar to this, respiratory arrest following pesticide uptake by the surface of bacterial cells eventually results in bacterial death while developing under the stress of pesticides. Pesticides also interfere with internal metabolism after absorption by attaching to amino and sulphide groups, which reduces the metabolic activity of bacterial cells. These occurrences cause alterations in the soil's oxido-reduction potential, and depending on the type and quantity of pesticides present, the affected microorganism may eventually be eradicated from polluted areas or die. However, bacterial degradations of the dimethoate 40% pesticide depends not only on the culture conditions but also on the isolates and species of bacteria (Singh and Walker, 2006). Ahemad and Khan (2012) and Shahid et al. (2018) showed similar lethal/damaging and inhibitory effects of pesticides on the development patterns of *Mesorhizobium* strain MRC4 and *Azotobacter* and *Bacillus* sp. cultivated on minimal salt medium modified with varied concentrations of pesticides. Also, a number of chemical substances, including pesticides and petroleum products, have been found to be degraded by B. cereus, *Bacillus mycoides*, and *Pseudomonas aeruginosa* (Shaer et al., 2013; Abdurruhman et al., 2015).

In our investigation, isolate SH1 performed exceptionally well among the tested isolates at pH 7, 30 °C, and 72 h of incubation time with the highest dosage of dimethoate 40% (80 µg L^{-1}). According to several studies (Kamaraj et al., 2022; Fahmy et al., 2022), the ideal pH, temperature, and incubation time are important factors in the microbial biotransformation of pesticides. Microbial enzymes typically mediate this process, which is active under neutral or slightly acidic/alkaline conditions (Jin et al. 2012). Therefore, the main element controlling microbial growth is the pH of the medium. Sidal and Yilmaz (2012) found that Pseudomonas sp. produced the maximum concentration of biosurfactant and rhamnolipid at an initial pH of 7.0 and 30° C. P. aeruginosa and *B. subtilis* 181 produced biosurfactants most efficiently at pH 7.0 and 37 °C (Saikia et al., 2012; Fahmy et al., 2022). Furthermore, Abo-Amer (2011) contended that diazinon degradation was inhibited by both acidic and alkaline pH. In addition, Bacillus sp. and *Micrococcus* sp. showed 71.6% disintegration after 10 days of incubation at 0.1% v/v chlorpyrifos. According to Doolotkeldieva et al. (2018), active *B. polymyxa* and *P. fluorescens* bacterial strains were reportedly used in consortiums and individual cultures, on the pesticide Aldrin, which they have shown high rates of degradation activity in just 12 days.

Different types of growth-promoting bioactive molecules, such as auxins, cytokinins, gibberellins, ethylene, and abscisic acids, can be produced by microbial communities living in different rhizosphere soils (Cohen et al., 2009; Shahida et al., 2019). Phytohormones are one of these plant growth regulators and are crucial for the entire growth and development of plants (Pramanik and Mohapatra, 2017). According to Teotia et al. (2016), auxin, the primary substance made by most plants, is the analogue of indole acetic acid (IAA), which is made directly from tryptophan. Auxin controls many crucial physiological processes, including cell growth and division, tissue differentiation, root initiation, faster growth, phototropism, geotropism, and apical dominance. Both in the absence and presence of pesticides, isolate SH1 in this investigation produced a sizable amount of IAA in NB broth supplemented with 100 µg mL^{-1} tryptophan. In earlier research (Khan et al., 2007; Miransari and Smith, 2009), who also discussed the similar continuous secretion and decrease in the production of IAA by *Burkholderia cepacia, Azotobacter* sp., and *Bacillus* sp. in herbicide-stressed circumstances. One very astounding property of isolate SH1 that may be useful in enhancing plant growth in pesticide-stressed environments is the sustained production of

phytohormone even at increasing pesticide concentrations. It is expected that the IAA produced by isolate SH1 in this discovery and by many more beneficial soil microorganisms (PGPR) in both normal (non-stressed) and stressed environments would aid in plant development and general performance. However, the gradual increase in pesticide concentration accompanied by a consistent decline in phytohormone secretion is undoubtedly a troubling issue that necessitates research into how the release of IAA can be protected in harsher environments.

Another metabolite produced by bacteria is ammonia, which is created through the hydrolytic breakdown of urea by urease, the decarboxylation of amino acids, and nitrite ammonification. Some plants' root exudates have been found to contain NH_3, and *Burkholderia, Aeromonas, Pseudomonas*, and *Bacillus* are just a few of the PGPR strains known to produce it (Rani and Kumar, 2017). Similar to this, Azarmi et al. (2016) observed NH_3 restriction by rhizosphere bacteria in polluted soil.

The inorganic phosphate in the present findings was significantly solubilized by isolate SH1, changing the medium's pH in the process. In contrast, isolate SH1 was able to solubilize inorganic phosphate despite the presence of various pesticide dosages as long as the broth's pH was raised. In general, it is hypothesized that soil-dwelling bacteria (PGPR) are important members of soil ecosystems and play a significant role in the growth and improvement of plants through a variety of methods. One of the crucial roles that soil microorganisms play, for instance, is the solubilization of mineral P in the rhizosphere region and the subsequent provision of soluble P to the plant system (Ditta and Khalid, 2016).

By producing siderophores, soil bacteria are also known to indirectly promote the development and growth of plants (Saha et al., 2016). In order to combat this, we assessed the tested isolates' capacity to produce siderophore under pesticide stress. As can be seen from the results, isolate SH1 was capable of producing siderophores up to 60 μg L^{-1}, which gradually decreased with rising dimethoate 40% concentrations. Iron is supplied to plants with the mechanisms for absorbing it in iron-deficient situations through siderophores made by the soil microbial population (Joshi et al., 2006). According to Wilson et al. (2016), in aerobic conditions, Fe mostly exists as Fe^{3+} and may also form insoluble hydroxides and oxyhydroxides, making it accessible to microorganisms. Under the influence of pesticides, Isolate SH1 produced a sizable amount of siderophore, demonstrating that this isolate has the ability to suppress illnesses caused by phytopathogens and might therefore be employed as a biocontrol agent. In addition, siderophores may have a role in local and systemic host resistance in plants (Rijavec and Lapanje, 2016) and may directly encourage the manufacture of additional antimicrobial compounds by increasing the availability of these elements to the bacteria.

On the other hand, our data offers *B. subtilis* OQ347968 had the potential to grow in a wide range of environmental circumstances and may be utilized as an effective instrument of degradation of dimethoate (Derbalah et al. 2021). *B. subtilis* OQ347968 showed high potential for the biodegradation of the tested insecticide, in agreement with many researchers. The final results of the bioremediation of dimethoate by the bacterial strain in this study are consistent with those of Abdelrazek et al. (2023b) who indicated the high ability of bacterial isolates to bioremediate dimethoate and other pesticides and completely mineralized insecticides in aqueous media after incubation. The results for dimethoate in this study are in agreement with those of Lone and Wani (2012), Yin and Lian (2012) , and Pandey et al.(2011), who reported the high ability of fungal isolates to biore- mediate dimethoate and other organophosphorus and completely mineralized insecticides in aqueous media after incubation.

5. Conclusion

Many microorganisms biodegrae pesticide residues and use them as a source of carbon and nitrogen which that the flexibility of strategic planning for environmental decontamination.

SH1–SH7 isolates had some degree of tolerance to dimethoate after 72 hours at the highest concentrations (80 µg L^{-1}), however, the data revealed that just one strain (SH1) exhibited the most tolerance. Moreover, the SH1 isolate showed the maximum development at the highest concentration of dimethoate in pH 7, temperature of 30 °C, and incubation period of 72 hours. On the other hand, at various dimethoate concentrations, isolate (SH1) showed positive results in producing IAA, NH_3, P solubilization, and siderophores at 60 µg L^{-1}. Therefore, the 16S rRNA sequence and biochemical traits indicated that the SH1 isolate belonged to Bacillus subtilis, which had the GenBank accession number OQ347968. After seven days, *B. subtilis* OQ347968 exhibited the highest rates of dimethoate elimination (80 mg L^{-1}) based on the results of HPLC biodegradation. These results suggest that *B. subtilis* OQ347968 is highly capable of degrading 40%

dimethoate under a range of environmental conditions.

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