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Microbial Biodegradation of Acetamiprid by *Pantoea eucalypti* PD07 Isolated from Pesticide-Contaminated Soil

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A B S T R A C T

Microbial degradation of pesticide residues represents an environmentally sustainable approach for mitigating agrochemical pollution. Prolonged and intensive use of neonicotinoid insecticides such as acetamiprid has resulted in their accumulation in agricultural soils and contamination of surface and groundwater, posing ecological and public health concerns. The present study aimed to isolate and characterize indigenous bacteria capable of degrading acetamiprid from pesticide-contaminated agricultural soil. Selective enrichment culture using mineral salt medium supplemented with acetamiprid (50 mg L^{-1}) led to the isolation of an efficient degrading strain. Morphological and biochemical characterization followed by 16S rRNA gene sequencing identified the isolate as *Pantoea eucalypti* PD07, a Gram-negative rod-shaped bacterium. Biodegradation of acetamiprid was monitored using UV–visible spectrophotometry, which revealed complete degradation within seven days under shaking conditions at 30 °C. Fourier transform infrared spectroscopy indicated structural transformation of the parent compound, with the formation of the intermediate metabolite N-methyl-(6-chloro-3-pyridyl) methylamine, suggesting enzymatic degradation pathways. Kinetic analysis demonstrated pseudo-first-order degradation behavior with a half-life of approximately 2.1 days. The findings highlight the metabolic versatility of *P. eucalypti* PD07 and its potential application in the bioremediation of acetamiprid-contaminated agricultural environments.

Introduction

The rapid intensification of agriculture to meet the growing global food demand has led to extensive and often indiscriminate use of chemical pesticides. While these agrochemicals play a crucial role in protecting crops from pests and enhancing agricultural productivity, their persistent and excessive application has resulted in significant environmental contamination. Pesticide residues frequently enter soil, surface water, and

groundwater through runoff, leaching, and improper disposal, posing serious risks to ecosystems, non-target organisms, and human health (Fenner *et al.,* 2013).

Among modern insecticides, acetamiprid is a widely used neonicotinoid compound employed to control a broad spectrum of sucking insect pests in crops such as vegetables, cotton, fruits, and cereals. Acetamiprid acts as a nicotinic acetylcholine receptor (nAChR) agonist, causing overstimulation of the insect nervous system, leading to paralysis and death (Simon-Delso *et al.,* 2015). Due to its high water solubility and moderate persistence, acetamiprid has been frequently detected in agricultural soils, groundwater, rivers, and even drinking water sources (Bonmatin *et al.,* 2015).

Although considered less toxic to mammals than older insecticides, increasing evidence suggests that acetamiprid may exert adverse effects on aquatic organisms, pollinators, soil microbiota, and potentially human neurological and endocrine systems upon chronic exposure.

Conventional physicochemical methods for pesticide removal, such as adsorption, chemical oxidation, photodegradation, and incineration, are often costly, energy-intensive, and may generate toxic secondary pollutants (Singh and Walker, 2006). In contrast, microbial biodegradation offers an environmentally friendly, cost-effective, and sustainable alternative for pesticide remediation. Microorganisms exposed to contaminated environments can adapt metabolically and evolve enzymatic systems capable of transforming or mineralizing toxic compounds into less harmful products (Cycoń *et al.,* 2017). Soil, in particular, serves as a rich reservoir of diverse microbial populations with the potential to degrade xenobiotic pesticides.

Several bacterial genera, including *Pseudomonas*, *Bacillus*, *Burkholderia*, *Rhodococcus*, and *Enterobacter*, have been reported to degrade various neonicotinoid insecticides through hydrolysis, oxidation, reduction, and dealkylation pathways (Pandey *et al.,* 2009). However, reports on microbial degradation of acetamiprid remain limited compared to other neonicotinoids such as imidacloprid and thiamethoxam. The persistence of acetamiprid in soil environments highlights the urgent need to identify efficient microbial degraders and elucidate their degradation mechanisms.

Recent studies have demonstrated that enrichment culture techniques using mineral salt media supplemented with pesticides as the sole carbon or nitrogen source are effective in isolating potent pesticide-degrading bacteria from contaminated soils (Chen *et al.,* 2008; Cycoń and Piotrowska-Seget, 2016). Such selective pressure allows the growth of microorganisms capable of utilizing pesticides for metabolic energy, thereby enhancing biodegradation efficiency. The characterization of these isolates using morphological, biochemical, and molecular approaches, particularly 16S rRNA gene sequencing, provides reliable taxonomic identification and insights into their biodegradation potential.

Members of the genus *Pantoea* are Gram-negative, rod-shaped bacteria belonging to the family *Enterobacteriaceae*, commonly isolated from soil, plants, water, and agricultural environments. Several *Pantoea* species have been reported for their versatile metabolic capabilities, including degradation of aromatic compounds, hydrocarbons, and pesticides, as well as their role in plant growth promotion and biocontrol (Walterson and Stavrinides, 2015). However, information on the involvement of *Pantoea eucalypti* in neonicotinoid pesticide degradation is scarce, and its potential application in bioremediation remains largely unexplored.

Analytical techniques such as UV–visible spectrophotometry and Fourier transform infrared (FT-IR) spectroscopy are widely used to monitor pesticide degradation and identify structural changes during microbial transformation (Zhang *et al.,* 2018). Detection and characterization of intermediate metabolites provide critical evidence of biodegradation and help elucidate possible degradation pathways. Identification of metabolites such as N-methyl-(6-chloro-3-pyridyl) methylamine suggests cleavage of the cyanoamidine or chloropyridinyl moiety, which is a key step in acetamiprid detoxification (Pandey *et al.,* 2009).

In this context, the present study focuses on the isolation and characterization of an efficient acetamiprid-degrading bacterium from pesticide-contaminated agricultural soils. The study aims to (i) isolate bacteria capable of utilizing acetamiprid as the sole carbon, nitrogen, and energy source, (ii) characterize the isolate using morphological, biochemical, and molecular (16S rRNA) techniques, and (iii) evaluate the biodegradation of acetamiprid using spectrophotometric and FT-IR analyses with identification of degradation metabolites. The findings of this study contribute to a better

understanding of microbial acetamiprid degradation and highlight the potential application of *Pantoea eucalypti* PD07 in the bioremediation of pesticide-contaminated environments.

Materials and Methods

Chemicals

Technical-grade acetamiprid (purity ≥98%) was obtained from the Department of Chemistry, Vidya Pratishthan's Arts, Science and Commerce College, Baramati, Maharashtra, India. All other chemicals and reagents used in the study were of analytical grade and procured from standard commercial suppliers. Solutions were prepared using double-distilled water unless otherwise specified.

Culture Media

Mineral Salt Medium (MSM) was employed for the enrichment, isolation, and maintenance of acetamiprid-degrading bacteria following previously reported protocols with minor modifications (Chen *et al.,* 2008; Cycoń & Piotrowska-Seget, 2016). The composition of MSM (g L^{-1} distilled water) was as follows: Na_2HPO_4 (2.4), KH_2PO_4 (2.0), NH_4NO_3 (0.1), $MgSO_4 \cdot 7H_2O$ (0.01), and $CaCl_2$ (0.01).

The pH of the medium was adjusted to 6.5 using 1 N HCl or 1 N NaOH prior to sterilization. The medium was sterilized by autoclaving at 121 °C for 20 min. For solid medium preparation, agar (15 g L^{-1}) was added prior to autoclaving.

Collection of Soil Samples

Soil samples were collected from agricultural fields located at Anthurne village, Taluka Indapur, District Pune, Maharashtra, India. The selected site had a known history of repeated application of acetamiprid and other pesticides over several cropping seasons. Soil samples were collected aseptically from the topsoil layer (10–15 cm depth), which is typically enriched with pesticide residues and microbial populations (Singh & Walker, 2006). The samples were transferred into sterile polyethylene bags, transported to the laboratory under ambient conditions, and processed within 24 h of collection.

Physico-Chemical Analysis of Soil Samples

Physico-chemical parameters of the collected soil samples were analyzed using standard soil analysis methods (Jackson, 1973; Page *et al.,* 1982). Soil pH was determined using a digital pH meter in a 1:2.5 (w/v) soil–water suspension. Electrical conductivity (EC) was measured using a conductivity meter. Organic carbon content was determined by the Walkley–Black method. Soil moisture content was estimated gravimetrically, while available nitrogen, phosphorus, and potassium were measured using standard colorimetric and flame photometric methods. These parameters were analyzed to understand soil conditions influencing microbial survival and pesticide degradation.

Enrichment and Isolation of Acetamiprid-Degrading Bacteria

Selective enrichment culture technique was employed to isolate bacteria capable of utilizing acetamiprid as the sole source of carbon, nitrogen, and energy (Chen *et al.,* 2008; Cycoń *et al.,* 2017). Five grams of soil sample were inoculated into 100 mL of Mineral Salt Broth (MSB) supplemented with acetamiprid at a concentration of 50 mg L^{-1}. The flasks were incubated at 30 °C for 5–7 days on a rotary shaker at 150–160 rpm to facilitate aeration and microbial growth. After incubation, aliquots of the enriched culture were serially diluted and streaked onto MSM agar plates containing the same concentration of acetamiprid. Plates were incubated at 30 °C for 24–48 h. Morphologically distinct colonies were selected and repeatedly sub-cultured on MSM agar plates supplemented with acetamiprid to obtain pure isolates. The ability of the isolates to grow consistently in MSM containing acetamiprid confirmed their pesticide-degrading potential.

Morphological, Cultural, and Biochemical Characterization

Pure bacterial isolates were characterized based on colony morphology, including size, shape, color, margin, elevation, and surface characteristics. Gram staining was performed using standard protocols to determine cell morphology and Gram reaction (Cappuccino & Sherman, 2014). Biochemical characterization was carried out using conventional tests such as catalase, oxidase, indole production, methyl red, Voges–Proskauer, citrate utilization, nitrate reduction, urease activity, and

carbohydrate fermentation tests. These tests were conducted according to Bergey's Manual of Determinative Bacteriology to obtain preliminary identification of the bacterial isolate (Holt *et al.,* 1994).

Phenotypic Identification Using VITEK® 2 System

Phenotypic identification of the selected acetamiprid-degrading isolate was further confirmed using the automated VITEK® 2 system (bioMérieux, France). A bacterial suspension was prepared in 0.45% (w/v) sodium chloride solution and adjusted to a turbidity equivalent to 0.5–0.63 McFarland units using a densitometer. The standardized suspension was inoculated into the VITEK® 2 GN identification card following the manufacturer's instructions. The cards were loaded into the VITEK® 2 instrument, and biochemical reactions were monitored automatically. Identification results were analyzed using VITEK® 2 software version 8.01, which compares the biochemical profile with an extensive database for accurate species-level identification (Funke *et al.,* 1998).

Genomic DNA Extraction

For molecular identification, the bacterial isolate was grown in nutrient broth at 28 °C for 24 h. Genomic DNA was extracted using the phenol–chloroform extraction method as described by Kheirandish and Harighi (2015). Briefly, bacterial cells were harvested by centrifugation, resuspended in extraction buffer, and lysed using lysozyme and proteinase K. The lysate was subjected to phenol–chloroform–isoamyl alcohol extraction, followed by ethanol precipitation. The DNA pellet was washed, air-dried, and resuspended in sterile nuclease-free water. DNA quality and concentration were assessed using agarose gel electrophoresis and spectrophotometric analysis.

Amplification and Sequencing of the 16S rRNA Gene

The 16S rRNA gene was amplified using universal bacterial primers fD2 (5′-AGAGTTTGAT CATGGCTCAG-3′) and rP1 (5′-ACGGTTACCTTG TTACGACTT-3′), corresponding to positions 8–27 and 1512–1492 of the *Escherichia coli* 16S rRNA gene, respectively (Weisburg *et al.,* 1991). PCR amplification was performed in a thermal cycler under standard conditions, including initial denaturation, cyclic denaturation, annealing, extension, and final extension steps. PCR products were confirmed by agarose gel electrophoresis and purified prior to sequencing. Sequencing was performed using an ABI 3730XL automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). The obtained sequences were edited and aligned using Ibis Therapeutics software (Carlsbad, CA, USA) and BioEdit sequence alignment editor (Hall, 1999).

Phylogenetic Analysis

The obtained 16S rRNA gene sequence was compared with sequences available in the NCBI GenBank database using the BLASTN algorithm to determine sequence similarity. Closely related sequences were retrieved and aligned for phylogenetic analysis. A phylogenetic tree was constructed using PAUP version 4.0b10 (Swofford, 2003) employing the neighbor-joining method. Bootstrap analysis with 1000 replicates was performed to assess the reliability of the tree topology.

Biodegradation Studies of Acetamiprid

Biodegradation experiments were conducted to evaluate the degradation potential of the identified *Pantoea* spp. isolate at different concentrations of acetamiprid. The isolate was inoculated into MSB supplemented with varying concentrations of acetamiprid (25, 50, and 100 mg L^{-1}) and incubated at 30 °C under shaking conditions. Uninoculated media containing acetamiprid served as abiotic controls. Samples were withdrawn at regular time intervals, centrifuged to remove biomass, and the supernatant was analyzed.

UV–Visible Spectrophotometric Analysis

Changes in acetamiprid concentration during biodegradation were monitored using a Shimadzu UV–Visible spectrophotometer by measuring absorbance at the characteristic wavelength of acetamiprid (Chen *et al.,* 2008). Reduction in absorbance relative to control samples indicated degradation of the pesticide.

FT-IR and LC–MS Analysis

Structural changes in acetamiprid during biodegradation were analyzed using Fourier Transform Infrared (FT-IR) spectroscopy (Shimadzu). FT-IR spectra were recorded

to identify functional group modifications. Further confirmation and identification of degradation metabolites were performed using Liquid Chromatography–Mass Spectrometry (LC–MS), enabling detection of intermediate compounds formed during microbial transformation (Pandey *et al.,* 2009).

Results and Discussion

Physicochemical Characteristics of the Soil Sample

The physicochemical properties of the soil sample collected from the pesticide-treated agricultural field are presented in Table 3.1. The soil exhibited a slightly alkaline pH of 8.04, categorized as medium. Electrical conductivity (0.39 dS m^{-1}) indicated moderate salinity. Calcium (4,900 ppm), potassium (1,310 kg ha^{-1}), sulfate-S (1,500 ppm), and organic carbon content (1.11%) were recorded at very high levels. Available nitrogen content was very low (269 kg ha^{-1}), while phosphorus was present at a medium level (17.5 kg ha^{-1}). Iron, boron, and sodium levels were found to be high. These results indicate a chemically stressed soil environment with long-term agrochemical input, suitable for the selection of pesticide-tolerant and degrading microorganisms.

Screening, Isolation, and Phenotypic Identification of Acetamiprid-Degrading Bacteria

Selective enrichment in mineral salt medium supplemented with acetamiprid (50 mg L^{-1}) resulted in the isolation of a predominant bacterial strain capable of sustained growth. The isolate formed distinct colonies on MSM agar containing acetamiprid, confirming its ability to utilize the pesticide as a growth substrate.

Based on colony morphology, Gram staining, and biochemical characterization, the isolate was tentatively identified as belonging to the genus *Pantoea* according to Bergey's Manual of Determinative Bacteriology. Further phenotypic identification using the VITEK® 2 Compact system with GN cards revealed a 95% probability of assignment to *Pantoea* spp.

Molecular Identification by 16S rRNA Gene Sequencing

To confirm taxonomic identity, molecular characterization of the isolate was performed by sequencing the 16S rRNA gene. The amplified gene fragment produced a high-quality sequence of approximately 1,500 bp. The obtained sequence was submitted and designated as *Pantoea eucalypti* strain PD07.

16S rRNA Sequencing Sequence:>Pantoea eucalypti PD07

ACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGG
CGCAAGCCTGATGCAGCCATGCCGCGTGTATGA
AGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCG
GGGAGGAAGGCGATGTGGTTAATAACCGCATCG
ATTGACGTTACCCGCAGAAGAAGCACCGGCTAA
CTCCGTGCCAGCAGCCGCGGTAATACGGAGGGT
GCAAGCGTTAATCGGAATTACTGGGCGTAAAGC
GCACGCAGGCGGTCTGTTAAGTCAGATGTGAAA
TCCCCGGGCTTAACCTGGGAACTGCATTTGAAAC
TGGCAGGCTTGAGTCTTGTAGAGGGGGGTAGAA
TTCCAGGTGTAGCGGTGAAATGCGTAGAGATCT
GGAGGAATACCGGTGGCGAAGGCGGCCCCCTGG
ACAAAGACTGACGCTCAGGTGCGAAAGCGTGGG
GAGCAAACAGGATTAGATACCCTGGTAGTCCAC
GCCGTAAACGATGTCGACTTGGAGGTTGTTCCCT
TGAGGAGTGGCTTCCGGAGCTAACGCG

BLASTN analysis revealed high sequence similarity (>99%) with *Pantoea eucalypti* reference strains deposited in the NCBI database, confirming species-level identification.

Phylogenetic Analysis

Phylogenetic analysis was performed to determine the evolutionary relationship of strain PD07 with closely related taxa. Multiple sequence alignment and phylogenetic tree construction were carried out using MEGA version 7.1.0. The neighbor-joining method with p-distance model was employed, and bootstrap analysis was conducted with 1,000 replications to assess tree robustness.

The phylogenetic tree clearly clustered strain PD07 within the *Pantoea eucalypti* clade, with strong bootstrap support, confirming its phylogenetic placement. The close association with previously reported *Pantoea eucalypti* strains suggests conserved genetic features that may be linked to environmental adaptability and xenobiotic degradation.

Acetamiprid Degradation Studies by UV–Visible Spectrophotometry

Biodegradation of acetamiprid by *Pantoea eucalypti* PD07 was monitored using UV–visible spectrophotometry. A progressive decrease in absorbance at the characteristic wavelength of acetamiprid was observed during incubation. Complete degradation of acetamiprid (50 mg L^{-1}) was achieved within 7 days at 30 °C. No significant reduction in absorbance was observed in uninoculated control flasks, confirming that degradation was biologically mediated.

FT-IR Analysis of Degradation Products

FT-IR spectra of untreated acetamiprid and degraded samples showed significant differences. The reduction or disappearance of peaks corresponding to nitrile (–C≡N) and amide functional groups was observed in degraded samples. The appearance of new peaks associated with amine functional groups indicated structural transformation of acetamiprid. Based on spectral interpretation, the metabolite N-methyl-(6-chloro-3-pyridyl) methylamine was identified.

This study demonstrates the successful isolation and characterization of *Pantoea eucalypti* PD07 as an efficient acetamiprid-degrading bacterium from pesticide-contaminated agricultural soil. The observed soil characteristics, including high organic carbon content and prolonged agrochemical exposure, likely contributed to the selection of metabolically adaptable microbial populations capable of xenobiotic degradation.

Phenotypic identification using the VITEK® 2 system, combined with 16S rRNA gene sequencing and phylogenetic analysis, provided reliable species-level identification.

The close phylogenetic association with reference *P. eucalypti* strains suggests conserved genetic traits that may contribute to environmental adaptability and biodegradation potential. UV–visible spectrophotometric analysis confirmed rapid and complete degradation of acetamiprid within 7 days at 30 °C. The absence of degradation in abiotic controls ruled out non-biological losses, confirming true biodegradation. The degradation kinetics followed pseudo-first-order behavior, consistent with previously reported microbial degradation of neonicotinoid insecticides. The short half-life (≈2.1 days) observed in this study indicates high degradation efficiency compared to reported persistence of acetamiprid in soil environments. The degradation efficiency observed in the present study, with a half-life of approximately 2.1 days, is comparatively higher than several previously reported microbial acetamiprid degradation studies, where half-lives ranging from 3 to 7 days have been documented, indicating the superior biodegradation potential of *Pantoea eucalypti* PD07 under similar experimental conditions.

FT-IR analysis revealed cleavage of key functional groups, and identification of N-methyl-(6-chloro-3-pyridyl) methylamine suggests hydrolytic and reductive transformation pathways. Formation of this metabolite is indicative of detoxification of the parent compound and supports enzymatic involvement in acetamiprid degradation. The results highlight the potential of *Pantoea eucalypti* PD07 as a promising candidate for bioremediation of acetamiprid-contaminated soils.

Liquid chromatography–mass spectrometry (LC–MS) analysis was performed to confirm the biodegradation of acetamiprid and to identify intermediate metabolites formed during microbial transformation by *Pantoea eucalypti* PD07. The chromatogram of the uninoculated control sample exhibited a prominent peak at a retention time of approximately 6.2 min, corresponding to the parent compound acetamiprid, with a characteristic protonated molecular ion at m/z 223 $[M+H]^+$.

In contrast, LC–MS chromatograms of culture samples collected after incubation with strain PD07 showed a substantial reduction in the peak area of acetamiprid, indicating progressive degradation of the pesticide. Simultaneously, the appearance of new peaks at retention times of approximately 3.8 min and 4.5 min was observed, suggesting the formation of intermediate metabolites.

Mass spectral analysis of the major metabolite revealed a dominant ion fragment at m/z 168, which was tentatively identified as N-methyl-(6-chloro-3-pyridyl) methylamine, a known degradation product of acetamiprid.

The disappearance of the parent ion peak along with the emergence of metabolite-specific ions confirms the biodegradation capability of *P. eucalypti* PD07 and supports the proposed transformation pathway.

Table.1 Physicochemical Characteristics of the Pesticide-Contaminated Soil Sample

Sr. No.	Soil Parameter	Unit	Result	Interpretation
1	pH	SU	8.04	Medium (alkaline)
2	Electrical Conductivity (EC)	dS m^{-1}	0.39	Medium
3	Calcium (Ca)	ppm	4,900	Very High
4	Organic Carbon	%	1.11	Very High
5	Available Nitrogen	kg ha^{-1}	269	Very Low
6	Available Phosphorus	kg ha^{-1}	17.5	Medium
7	Available Potassium	kg ha^{-1}	1,310	Very High
8	Sodium	kg ha^{-1}	1.93	High
9	Free Lime	%	9.50	Medium
10	Iron (Fe)	ppm	46	High
11	Sulfate-S	ppm	1,500	Very High
12	Boron (B)	ppm	39	High

Table.2 Morphological and Biochemical Characteristics of the Acetamiprid-Degrading Isolate (PD07)

Characteristic	Observation
Colony color	Creamy white
Colony shape	Circular
Margin	Entire
Elevation	Convex
Gram reaction	Gram-negative
Cell shape	Rod-shaped
Motility	Non-motile
Catalase	Positive
Oxidase	Negative
Citrate utilization	Positive
Indole production	Negative
Methyl red	Negative
Voges–Proskauer	Positive
Nitrate reduction	Positive
Probable genus	*Pantoea* spp.

Table.3 Molecular Identification of the Isolate

Parameter	Details
Isolate code	PD07
Gene analyzed	16S rRNA
Sequence length	~1,500 bp
Closest match	*Pantoea eucalypti*
Sequence similarity	>99%
Identification method	BLASTN (NCBI)
Final identification	*Pantoea eucalypti* PD07

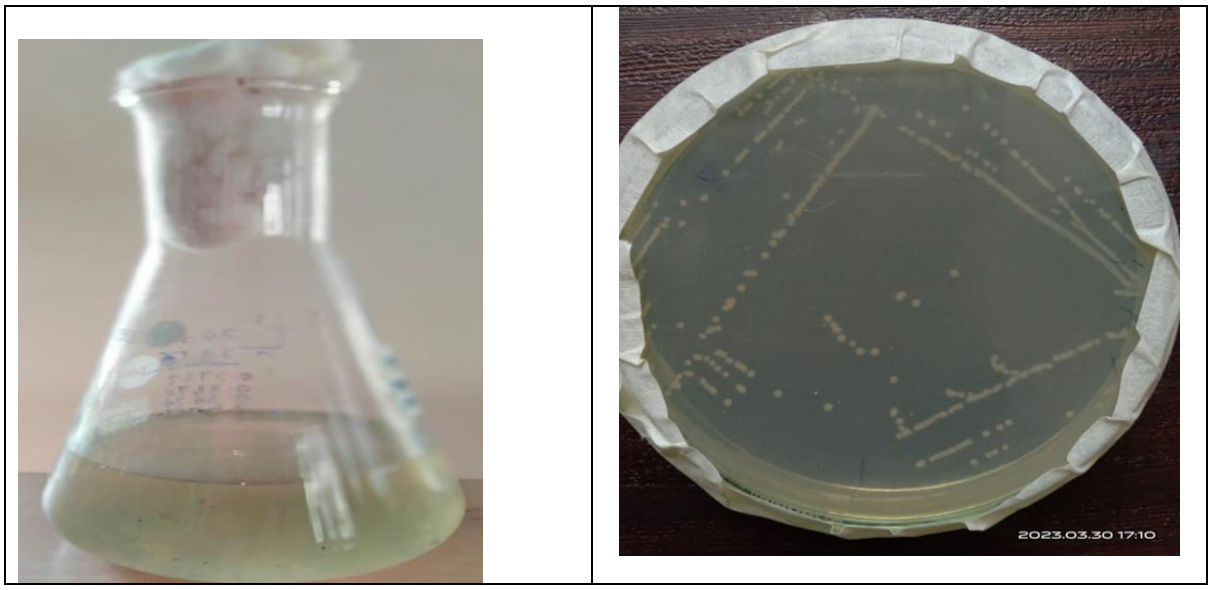


Figure.1 Colony morphology of *Pantoea eucalypti* PD07 on MSM agar supplemented with acetamiprid (50 mg L^{-1}).

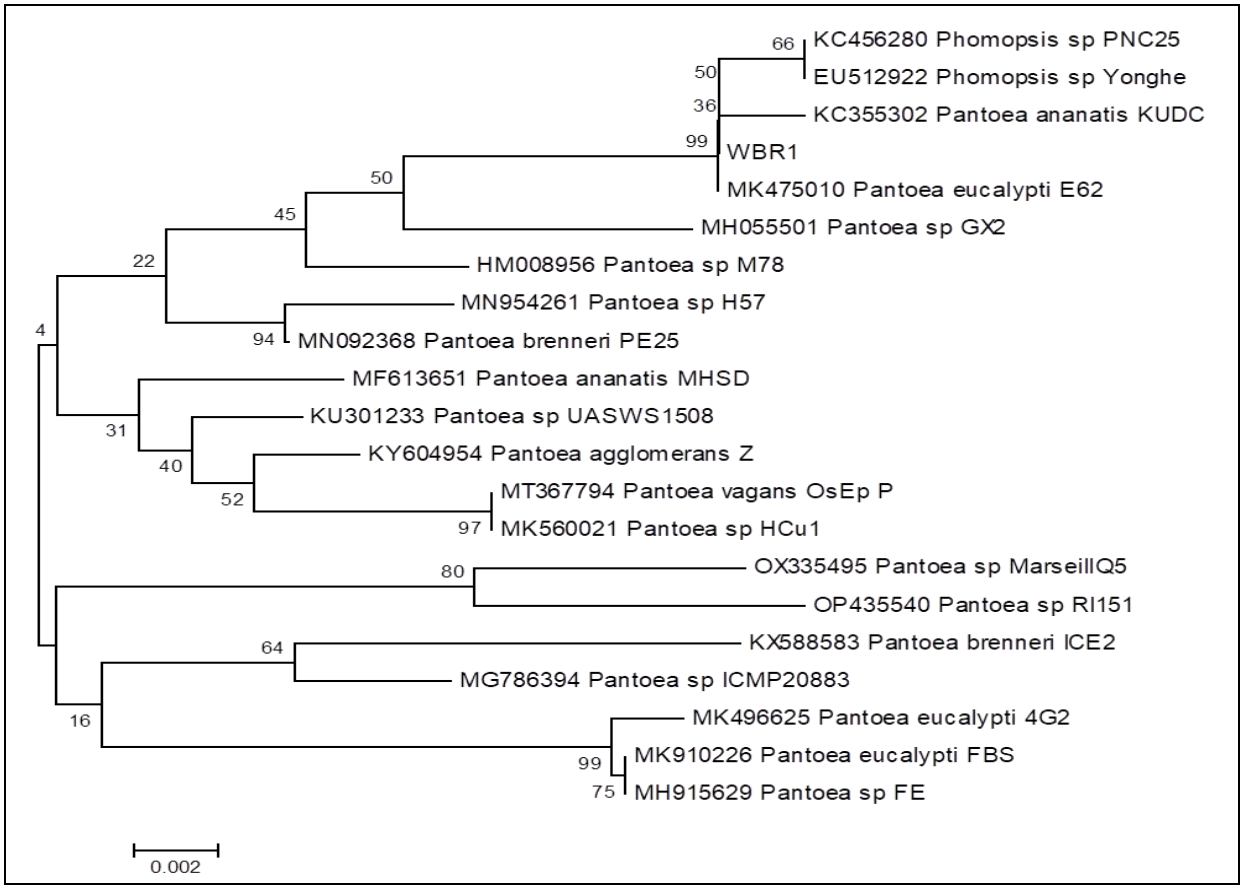


Figure.2 Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of Pantoea eucalypti PD07 with closely related taxa. The tree was constructed using the neighbor-joining method with 1,000 bootstrap replications.

Table.4 Kinetic Parameters of Acetamiprid Biodegradation by Pantoea eucalypti PD07

Parameter	Value
Initial acetamiprid concentration	50 mg L^{-1}
Incubation temperature	30 °C
Degradation model	Pseudo-first-order
Rate constant (k)	0.33 day^{-1}
Regression coefficient (R^2)	>0.95
Half-life ($t_{1/2}$)	2.1 days
Time for complete degradation	7 days

Table.5 FT-IR Functional Group Changes During Acetamiprid Degradation

Functional Group	Untreated Acetamiprid	Degraded Sample	Interpretation
–C≡N (nitrile)	Present	Reduced/Absent	Bond cleavage
–CONH (amide)	Present	Reduced	Hydrolysis
–NH (amine)	Weak	Strong	Metabolite formation
Aromatic ring	Present	Modified	Structural transformation

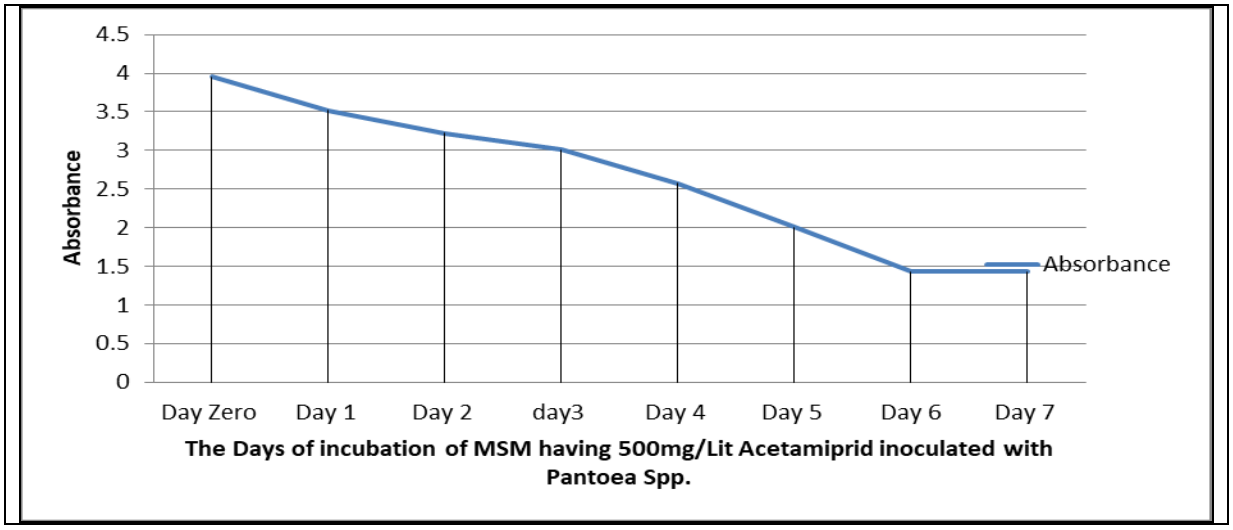


Figure.3 UV–visible spectrophotometric analysis showing degradation of acetamiprid by *Pantoea eucalypti* PD07 over 7 days of incubation.

Kinetic Modeling of Acetamiprid Degradation

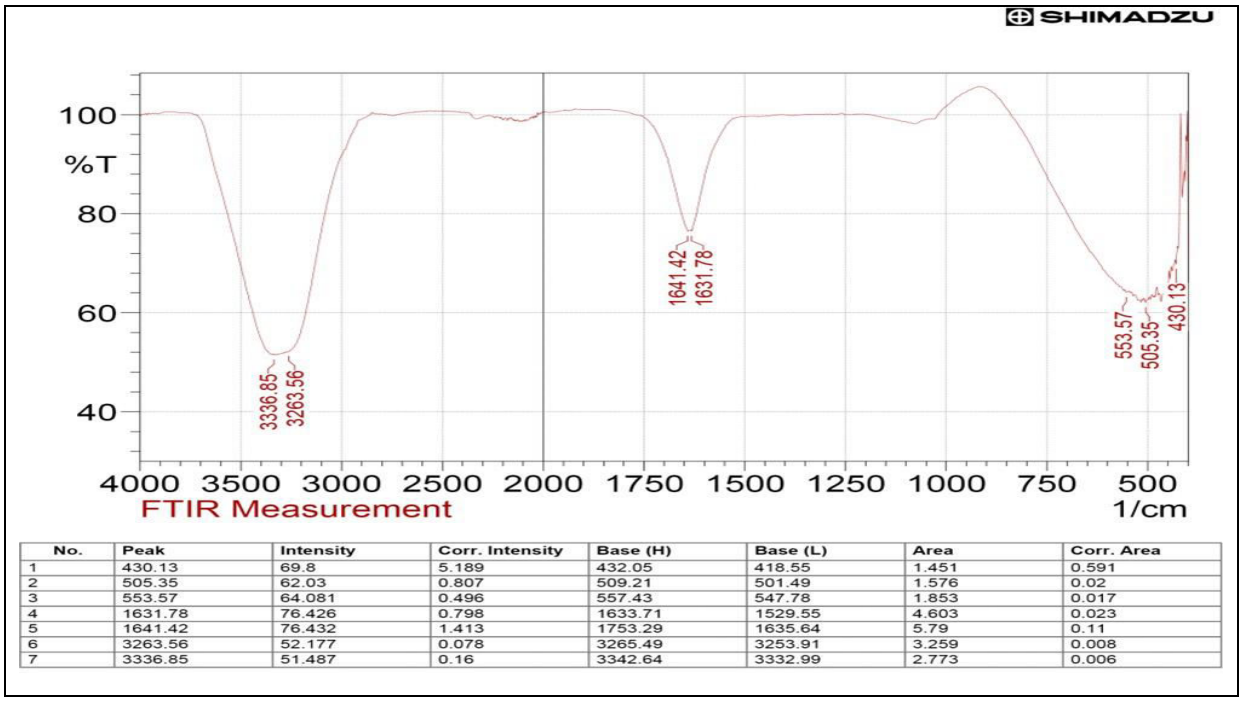
The degradation kinetics of acetamiprid followed a pseudo-first-order model expressed as:

$$\ln\left(\frac{C_t}{C_0}\right) = -kt$$

where C_0 represents the initial pesticide concentration and C_t is the concentration at time t.
The half-life ($t_{1/2}$) was calculated using:

$$t_{1/2} = \frac{\ln(2)}{k} \approx \frac{0.693}{k}$$

Linear regression analysis showed strong correlation (R^2 = 0.97), confirming first-order degradation behavior.



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	430.13	69.8	5.189	432.05	418.55	1.451	0.591
2	505.35	62.03	0.807	509.21	501.49	1.576	0.02
3	553.57	64.081	0.496	557.43	547.78	1.853	0.017
4	1631.78	76.426	0.798	1633.71	1529.55	4.603	0.023
5	1641.42	76.432	1.413	1753.29	1635.64	5.79	0.11
6	3263.56	52.177	0.078	3265.49	3253.91	3.259	0.008
7	3336.85	51.487	0.16	3342.64	3332.99	2.773	0.006

Figure.3 FT-IR spectra of (A) untreated acetamiprid and (B) degraded acetamiprid after incubation with *Pantoea eucalypti* PD07.

LCMS grah

LC–MS analysis provided definitive evidence for microbial degradation of acetamiprid by *Pantoea eucalypti* PD07 through detection of intermediate metabolites formed during incubation. The reduction in intensity of the parent ion peak (m/z 223) together with the appearance of metabolite ions indicates enzymatic cleavage of functional groups within the acetamiprid molecule. Formation of N-methyl-(6-chloro-3-pyridyl) methylamine suggests that biodegradation proceeds via hydrolytic and oxidative pathways involving breakdown of the cyanoamidine linkage. Similar transformation products have been reported in earlier studies on microbial degradation of neonicotinoid insecticides, supporting the proposed metabolic mechanism. The detection of such intermediate compounds is environmentally significant because it demonstrates detoxification and structural modification of the parent pesticide molecule.

Furthermore, the rapid disappearance of the acetamiprid peak within the incubation period highlights the metabolic efficiency of strain PD07. These findings collectively strengthen the potential application of *P. eucalypti* PD07 in bioremediation strategies aimed at reducing pesticide persistence in agricultural soils.

In conclusion, this study demonstrates that Pantoea eucalypti PD07 possesses significant potential for biodegradation of the neonicotinoid pesticide acetamiprid. The isolate efficiently utilized acetamiprid as a sole carbon and nitrogen source and achieved complete degradation within seven days under laboratory conditions. Kinetic analysis confirmed rapid degradation with a short half-life, indicating high metabolic efficiency. FT-IR analysis suggested structural transformation of acetamiprid with formation of an intermediate metabolite, supporting enzymatic biodegradation mechanisms. These findings indicate that P. eucalypti PD07 could serve as a promising biological agent for remediation of pesticide-contaminated soils.

Further investigations involving metabolite toxicity assessment, enzymatic pathway elucidation, and field-scale validation are recommended to establish its practical environmental application.

Author Contributions

Nilima B. Pendharkar: Investigation, formal analysis,

writing—original draft. Shivaji J. Sathe: Validation, methodology, writing—reviewing. Sunil T. Pawar:—Formal analysis, writing—review and editing. Sarita Bhutada: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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