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Diazotroph-mediated Thiamethoxam Degradation: A Sustainable Approach to Reduce CO_2 Emission from Contaminated Soil

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ABSTRACT

Xenobiotic compounds such as utilization of pesticides are a major cause of greenhouse gases influx in atmosphere. Persistent pesticide accumulation in soil poses serious threats to microbial diversity, ecosystem stability and food safety. The present study aimed at evaluating the pesticide-degrading efficiency of nitrogen-fixing bacterial isolates obtained from cereal-crop rhizospheres. Sixteen diazotrophic isolates were screened for their ability to tolerate and degrade commonly used neonicotinoid pesticide thiamethoxam, under laboratory conditions. Primary and secondary screening identified several isolates exhibiting high tolerance and potential for xenobiotic degradation. Primary screening involved qualitative analysis using MSM agar plate enriched with various concentrations of thiamethoxam (10, 50 and 100 ppm) to evaluate the tolerance by growing nitrogen fixing bacterial isolates on it. Tolerant isolates further subjected to secondary screening using MSM broth having varying concentrations of Thiamethoxam (10, 50, 100 ppm). This was analyzed qualitatively using UV- spectrophotometer. The results confirm that selected six nitrogen-fixing isolates (CY18, CY11, CY13, CA10, CKA23 and CKU40) possessed dual functionality, maintaining nitrogen fixation while degrading pesticide residues. These isolates showed tolerance in primary screening and increase in bacterial OD during secondary screening which indicated some stochastic hydrolytic and oxidative reactions occurred to degrade thiamethoxam. Thus, study underscores the potential of native diazotrophs as sustainable bioremediation agents capable of mitigating pesticide contamination and could help in climate resilience by reducing greenhouse gases emission.

Key words: Thiamethoxam, greenhouse gas, pesticide degradation, nitrogen fixing bacteria, soil

INTRODUCTION

The total amount of grain produced worldwide has grown from five hundred million tonnes to seven hundred million tonnes since the end of the 20th century. Cereals make about 80% of the food consumed by humans. Pesticide usage enhances crop productivity in India. During the green revolution, more chemical fertilizers and pesticides were used to boost crop production in order to feed the world's expanding population (Penuelas *et al*., 2023). However, a lot of environmental problems were brought about by the green revolution, such as an increase in soil fertility loss, acidity, nitrate leaching, resistance to weed species, and a decrease in biodiversity. Even though they are crucial for controlling pests, their extensive use contributes to the biomagnification phenomenon, which affects human health and the environment (Zhou *et al*., 2024). With sales of US$ 627 million in 2009, thiamethoxam, (EZ)-3-(2-chloro-1,3-thiazol-5-methyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene (nitro) amine, is now the second-largest neonicotinoid. Thiamethoxam is now registered for 115 agricultural applications in more than 64 nations. It serves as one of the most popular neonicotinoid insecticides and one of the best-selling pesticides in the world, used extensively for seed, soil, and foliar treatments. It is a novel neonicotinoid having contact, stomach and systemic action that is produced from nitromethylene. It is active against sucking and eating insects including aphids, white flies, plant hoppers, thrips and beetles that target diverse crops such as rice, maize, cotton, vegetables and mango. However, it may contaminate surface and subsurface waterways due to its unique qualities, such as poor soil sorption and high leaching capabilities. Depending on the conditions of the soil, THIA has been demonstrated to have a half-life of up to 300 days. An extremely critical environmental problem results from these long half-lives as well as the fact that only 5% of the active neonicotinoid chemicals are absorbed by the crops, with the rest of them dispersing into the surrounding environment. In addition to surviving in agricultural soils, the commonly used neonicotinoid thiamethoxam has been shown to increase CO_2 emissions and stimulate soil microbial respiration (Ge *et al*., 2025). This suggests that THIA contamination may accelerate soil organic carbon loss and increase greenhouse gas fluxes from treated fields. Additionally, exposure to THIA alters soil microbial populations and metabolic processes, which has an impact on carbon cycling and pollutant persistence (Shukla *et al*., 2025). Researchers are concentrating on getting rid of THIA due to growing worries about the dangers it poses to non-target creatures and the quality of water resources. Advanced Oxidation Processes (AOPs), ultraviolet radiation (UV), ozonation (O_3) and hybrid processes UV/O_3, electrochemical oxidation, Fenton oxidation, and ultrasound oxidation are mentioned in most publications describing the degradation of THIA from water. However, all of these chemical and physical degrading techniques are costly, time-consuming and impractical in some distant areas. To address the issue of neonicotinoid persistence in the environment, bioremediation is a potentially affordable, ecologically sustainable option. In soil, microorganisms are crucial to the breakdown of manmade pollutants. Their exceptional capacity to fully mineralize a wide range of aliphatic, aromatic and heterocyclic chemicals offers an environmentally beneficial *in situ* detoxifying technique. Through the process of biodegradation, bacteria and other organisms transform and break down organic molecules into chemicals that ultimately produce carbon dioxide, water, or methane. They can use almost any naturally occurring or artificially created molecule as their only source of carbon and energy (Wend *et al*., 2024). Thus, an effort was made to investigate 16 nitrogen-fixing bacteria isolated from cereal soil system that could be able to degrade thiamethoxam from soil.

MATERIALS AND METHODS

The nitrogen-fixing bacterial isolates used in this study were pre-isolated and maintained by the laboratory (Department of Bio-Sciences and Technology, MMEC, MMDU). These isolates were previously collected from cereal-crop rhizospheres. For maintenance, the isolates were cultivated on Burk's nitrogen-free medium and kept at -120 ºC. Burk's medium (Hi media) contained 0.800 g K_2HPO_4, 0.2 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.130 g $CaCl_2$, 0.000253 g Na_2MoO_4, 0.00145 g $FeCl_3$ and 20.00 g sucrose per liter. Before autoclaving, Burk's medium was adjusted to a pH of 7. Meanwhile, the pesticide degradation assay was screened using a mineral salts medium (MSM) broth that contained 1.6 g K_2HPO_4, 0.4 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.02 g $CaCl_2$ and 1 ml salt stock solution (1.8 g $MnSO_4.H_2O$, 0.2 g $ZnSO_4$, 0.1 g $CuSO_4$, and 0.25 g Na_2MoO_4, in 1000 ml distilled water). Before being autoclaved at 121°C for 20 min, the MSM medium's starting pH was brought down to 7.0. Additionally, every chemical and solvent utilized in this investigation was of analytical reagent quality.

97% analytical grade thiamethoxam (THIA) used in the present study was procured from a local pesticide distributor. 0.1 g of THIA was dissolved in 100 ml of acetone (Hi media) to create the stock solution (1000 ppm) of thiamethoxam. Syringe filtration through membranes with a pore size of 0.22 ìm was used to sterilize the soluble solution, which was then kept at 4°C.

Thiamethoxam was added to MSM agar plates that lack carbon and nitrogen sources at several doses (10, 50 and 100 ppm) for primary screening. The chosen nitrogen-fixing bacterial isolates were streaked on MSM agar plates to assess tolerance, and growth was observed on the plates over 24- to 72-h incubation period at 30ºC (Gangola *et al*., 2018; Attya, 2020). MSM broth was used to further select tolerant isolates for secondary screening. Six bacterial isolates that showed tolerance on solid medium were grown in 100 ml MSM broth with various pesticide concentrations during the secondary screening from 10 and 100 ppm, and incubated for 10 days at 30ºC on a rotary shaker running at 120 rpm. Growth was measured quantitatively using a UV-Vis Spectrophotometer and subjectively using visual.

Every two days, bacterial growth was measured at 600 nm using a UV-Vis spectrophotometer (Malla *et al*., 2022). After that, 10 ml of each culture's sample was taken out, spun for 10 min at 8000 rpm, and utilized for biodegradation studies. The pesticide concentration was determined at each absorbance after the supernatant was diluted 10 times. A standard thiamethoxam solution was used to create a standard curve. Absorbance at 600 nm for bacterial growth and 253 nm for thiamethoxam was measured to monitor degradation. To account for contamination and compare growth, two controls were kept: uninoculated MSM+pesticide and MSM+bacterial strain. The following formula was used to get the % degradation.

% of Thiamethoxam degradation = [(Initial conc. – Final conc.)/Initial conc.] × 100

RESULTS AND DISCUSSION

Only six of the sixteen nitrogen-fixing bacterial isolates that were chosen for the current study were able to grow on MSM plates enriched with 10 ppm thiamethoxam; the remaining isolates were unable to do so, indicating a considerable degree of variation in pesticide tolerance within the bacterial community (Fig. 1). The isolates were CY18, CY11, CY13, CA10, CKA23 and CKU40, chosen on the basis of their strong efficiency towards seed germination and seedling growth. These findings are consistent with earlier reports in literature that pesticide tolerance and degradation potential are highly isolate-dependent (Cycon *et al*., 2017). The use of MSM devoid of additional carbon and nitrogen sources provided a stringent selection pressure, thereby ensuring that only those isolates capable of consuming thiamethoxam as a potential source of carbon and nitrogen would survive. It has been seen that OD of bacteria at different concentrations of THIA (10 ppm (Fig. 1), 50 ppm (Fig. 2) and 100 ppm (Fig. 3) found to be increasing with each passing day and OD of thiamethoxam found to be decreasing simultaneously (Table 1) which was clear indication that bacteria utilized the thiamethoxam as a nutrition and showed growth in MSM media.

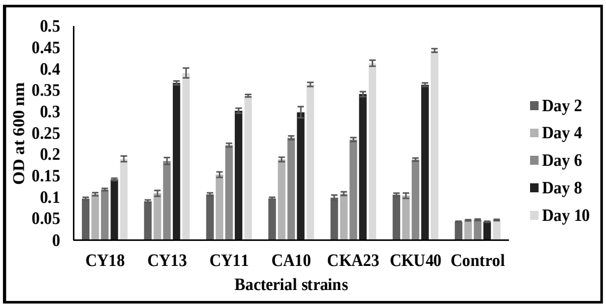

Fig. 2. Bacterial growth curve at 10 ppm of thiamethoxam.

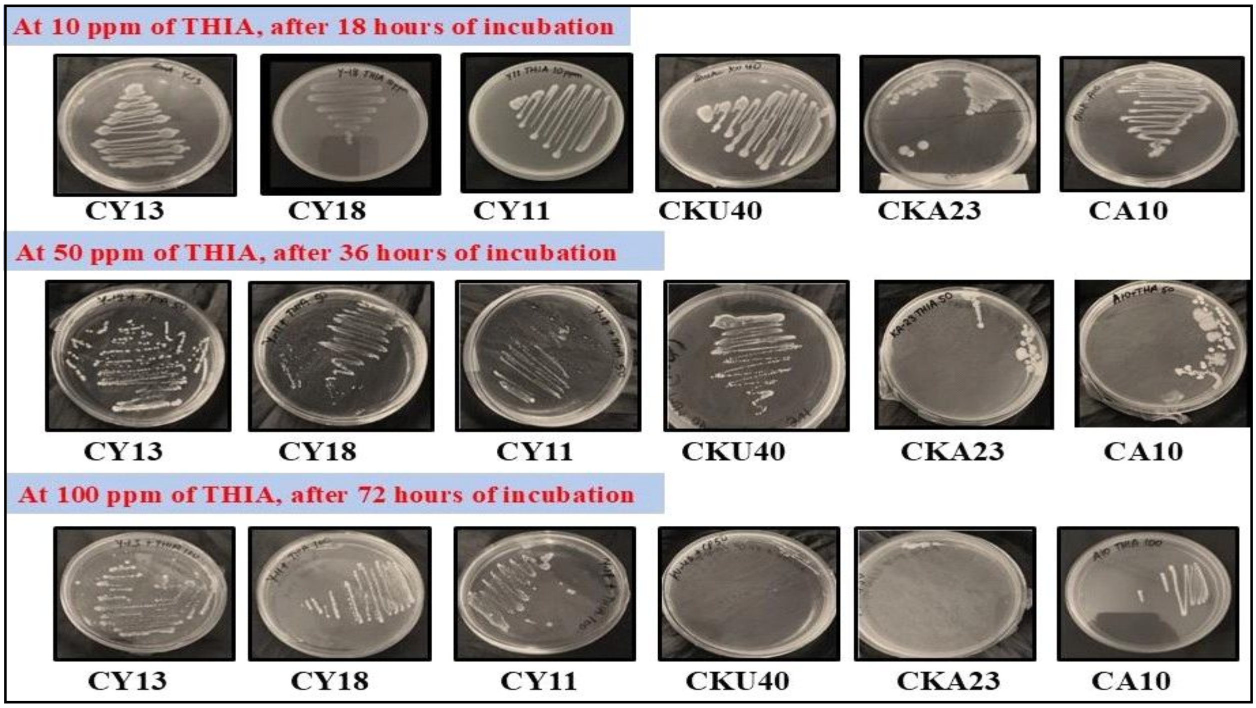

Fig. 1. Differential behaviour of selected bacterial isolates at different conc. of THIA on MSM agar plate.

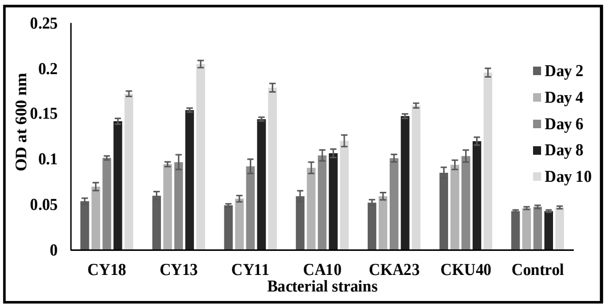

Fig. 3. Bacterial growth curve at 50 ppm of thiamethoxam.

Table 1. Percentage of degradation of 100 ppm of THIA in MSM

Isolates	Day 2 (%)	Day 4 (%)	Day 6 (%)	Day 8 (%)	Day 10 (%)
CY18	9.03	28.61	43.18	45.07	47.88
CY13	12.41	29.00	45.93	67.74	76.00
CY11	8.79	32.12	40.64	54.15	66.21
CA10	10.20	25.01	30.80	44.08	50.57
CA10	10.20	25.01	30.80	44.08	50.57
CKA23	9.81	20.13	37.66	50.46	60.03
CKU40	10.57	30.92	48.17	58.00	70.97

This approach is particularly important because most of studies have relied on nutrient enriched media and may not accurately check *in situ* degradation potential of bacteria. Furthermore, the study confirms that nitrogen-fixing bacteria are able to adapt in pesticide induced stress and metabolize pesticides under minimal nutrient conditions, thereby demonstrating their dual role in bioremediation and soil fertility improvement. The primary screening also revealed that bacterial isolates exhibited varying lag times before visible growth when exposed to increasing concentrations of thiamethoxam (Fig. 1). Specifically, as pesticide concentration increased, different isolates required more time to adapt and initiate growth. This observation aligns with the classical microbial growth theory, where higher concentrations of toxic compounds exert stress, leading to an extended lag phase as cells activate detoxification mechanisms or up regulate stress-response genes. Similar results have been reported for Pseudomonas putida and Bacillus spp., where exposure to pyrethroids and neonicotinoids delayed growth initiation due to membrane stress and need for induction of specific degradative enzymes (Bhatt *et al.*, 2023). Thus, the progressive increase in adaptation time observed in this study demonstrates the dose-dependent inhibitory effect of pesticides on microbial physiology, but also highlights the resilience of nitrogen-fixing bacteria to overcome this stress.

The secondary screening further validated the degradation potential using MSM broth enriched with thiamethoxam at varying concentrations, where bacterial growth was monitored using OD from 600 nm (Figs. 2, 3 and 4), while pesticide degradation was tracked at 253 nm (thiamethoxam) on the basis of which, per cent of degradation was calculated (Table 1). Growth kinetics indicated that the selected isolates could sustain growth up to 10 days in the presence of pesticides, with a gradual increase in optical density reflecting bacterial adaptation and metabolic activity (Figs. 2, 3 and 4).

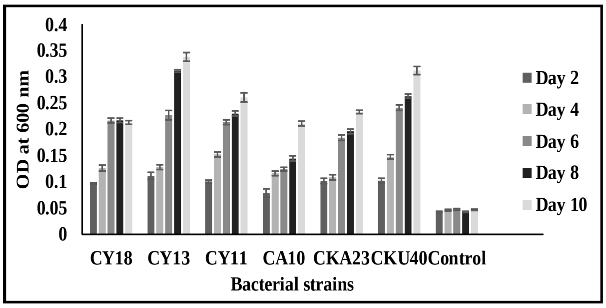

Fig. 4. Bacterial growth curve at 100 ppm of thiamethoxam.

Similar study has been examined in *Bacillus, Pseudomonas, Serratia* and *Azotobacter* species when exposed to synthetic pyrethroids and neonicotinoids (Conde Avila *et al.*, 2025, Liu *et al.*, 2025). Pesticide tolerance study under carbon and nitrogen-deficient conditions would highlight the superiority of these isolates for field-level application. In the secondary screening, growth kinetics and pesticide degradation were monitored in MSM culture. The result of bacterial growth curves and per cent of degradation in thiamethoxam demonstrated a strong positive correlation between biomass increase and pesticide breakdown. This suggests that active pesticide degradation primarily occurred during exponential and early stationary phases, when enzyme activity and metabolic demand were the highest. Beyond this stage, the rate of degradation plateaued may be due to substrate depletion or accumulation of intermediate metabolites. This confirmed that bacterial growth was directly linked to the pesticide availability as a substrate.

Collectively, the present study provided evidence that nitrogen-fixing bacteria are capable of adapting to the pesticide stress at varying concentrations and subsequently utilize pesticides as carbon/nitrogen source in a growth-dependent manner. The novelty of this study lies in demonstrating this growth-degradation synchrony under MSM conditions lacking external nutrients, which strengthens the claim that these isolates are promising candidates for bioremediation in nutrient-limited agricultural soils. However, microbial degradation strategies are more potent to degrade pesticide and enhance climate resilience by reducing greenhouse gas emission from toxic soil (Lopes *et al*., 2022; Renganathan and Gaysina, 2025).

CONCLUSION

Pesticide usage enhances crop productivity, during the green revolution, more chemical fertilizer and pesticides were used to boost crop production in order to feed the world's expanding population. However, a lot of environmental problems were brought about by the green revolution, such as an increase in soil fertility loss, acidity, nitrate leaching, resistance to weed species, and a decrease in biodiversity. Thiamethoxam is one of the most popular neonicotinoid insecticides. In addition to surviving in agricultural soils, it has been shown to increase CO_2 emissions and stimulate soil microbial respiration. This study provided evidence that degradation of thiamethoxam by nitrogen-fixing bacteria has a strong positive correlation between biomass increase, pesticide breakdown and reduction in CO_2 emissions.

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