ENHANCED THIAMETHOXAM DEGRADATION IN WATER AND SOIL BY *Phanerochaete* sp. Th1 AND *Ensifer* sp. Th2

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Abstract

Thiamethoxam is widely applied to control pest insects, but it causes serious environmental contamination. In this study, the degradation processes of the compound in soil and water collected from a rice field by native microorganisms, by mixed culture of Phanerochaete sp. Th1 and Ensifer sp. Th2, and by both the mixed pure culture and native microorganisms were conducted. The result showed that the mixed culture inoculation increased thiamethoxam in all media. In liquid media, the degradation percentage of the substrate in collected water was 21.8±4.4%, collected water + inoculation was 44.2±5.0%, and mineral medium + inoculation was 98.0±0.4%. Degradation determination in media with 70% collected water and 30% dry soil showed that the main degradation occurred in the liquid phase. The degradation rates in the media with 50% collected water and 50% dry soil were higher in the surface layer, and lower in the bottom. Indigenous microorganisms also played an important role in the degradation process. This study provided valuable information on the thiamethoxam degradation in simulated media of a rice field and the role of Phanerochaete sp. Th1 and Ensifer sp. Th2 to enhance the degradation.

Keywords: *Degradation, inoculation, microorganism, soil, thiamethoxam.*

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TĂNG CƯỜNG PHÂN HỦY THIAMETHOXAM TRONG NƯỚC VÀ TRONG ĐẤT BỞI P*hanerochaete* sp. Th1 VÀ *Ensifer* sp. Th2

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Tóm tắt

Thiamethoxam là thuốc trừ sâu được sử dụng rộng rãi, điều này gây ô nhiễm môi trường nghiêm trọng. Trong nghiên cứu này, sự phân hủy thiamethoxam trong đất và nước được thu thập từ ruộng lúa bởi các vi sinh vật bản địa, hay tăng cường bằng vi sinh vật Phanerochaete sp. Th1 và Ensifer sp. Th2, và bằng cả vi sinh vật bản địa cùng với vi sinh vật tăng cường đã được đánh giá. Kết quả cho thấy rằng việc bổ sung hỗn hợp Phanerochaete sp. Th1 và Ensifer sp. Th2 làm tăng cường sự phân huỷ thiamethoxam trên tất cả các môi trường. Trong môi trường lỏng, tỷ lệ phân hủy trong nước thu được là 21,8±4,4%, nước thu được có bổ sung vi sinh vật là 44,2±5,0%, và môi trường khoáng có bổ sung vi sinh vật là 98,0±0,4%. Đối với ngiệm thức có 70% nước và 30% đất thì sự phân hủy chủ yếu xảy ra ở trong nước. Tốc độ phân hủy ở nghiệm thức 50% nước thu được và 50% đất diễn ra cao hơn ở lớp bề mặt và thấp hơn ở đất dưới đáy. Các vi sinh vật bản địa cũng đóng một vai trò quan trọng trong quá trình phân hủy. Nghiên cứu này cung cấp thông tin có giá trị về sự phân hủy thiamethoxam trong môi trường mô phỏng ruộng lúa nơi có đất vào nước, cũng như vai trò của Phanerochaete sp. Th1 và Ensifer sp. Th2 trong sự tăng cường sự phân huỷ.

Từ khoá: *Bổ sung, đất, phân huỷ, thiamethoxam, vi sinh vật.*

1. Introduction

Thiamethoxam is a neonicotinoid insecticide widely used in the agricultural sector. It is a broad-spectrum insecticide, belonging to the neonicotinoid class. This active ingredient has the ability to kill sucking insects such as aphids, thrips, beetles, stink bugs, etc. This pesticide is used on crops including rice, corn, mango, orange and others. The compound causes serious toxicity to many species, including birds, fish and non-target insects (Finnegan et al., 2017), vertebrates (Zhao et al., 2020) and invertebrates (Saraiva et al., 2017). In addition, the effects of thiamethoxam on native microbial diversity and changes the bacterial community structure have been reported (Yu et al., 2020; Wu et al., 2021; Zhang et al., 2021).

Thiamethoxam is highly soluble in water, so it has a potential to contaminate water. Indeed, the compound has been detected at 20.1–225 µg/L in freshwater (Saraiva et al., 2017) and 67% of groundwater samples (Bradford et al., 2018). Moreover, it was found in soil (Zhang et al., 2016) and in sediments (Kuechle et al., 2019). Thiamethoxam is so persistent in environments with the half-time values from months to years (Goulson & Kleijn, 2013). However, the dissipation of the compound depended on soil properties and native microorganisms. The insecticide may accumulate in both water and soil phases, especially in rice fields. Therefore, the determination on its degradation in both media should be conducted.

In a previous report, a fungal strain *Phanerochaete* sp. Th1 and bacterial isolate *Ensifer* sp. Th2 were obtained from soil utilizing thiamethoxam as a sole carbon, nitrogen and sulfur source (Oanh & Duc, 2023). In this study, degradation in water and soil collected from a rice field was determined. The degradation by indigenous microorganisms, by mixture of *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2, and by both the mixed culture and indigenous microorganisms in water and soil were compared.

2. Materials and methods

2.1. Thiamethoxam degradation in mineral medium

Mineral medium was prepared following Oanh and Duc (2023) with the components of 2.79 g/L Na_2HPO_4, 1.00 g/L KH_2PO_4, 0.20 g/L $MgCl_2 \cdot 6H_2O$, 0.5 g/L $(NH_4)_2SO_4$, and 1.0 mL of trace solution. The trace solution consisted of 0.30 g/L H_3BO_3, 0.20 g/L $FeCl_2 \cdot 6H_2O$, 0.10 g/L $ZnCl_2 \cdot 7H_2O$, 0.03 g/L $Na_2MoO_4 \cdot 2H_2O$, 0.03 g/L $MnCl_2 \cdot 4H_2O$, and 0.01 g/L $CuCl_2 \cdot 2H_2O$. The pH was adjusted to 7.0±0.1 using HCl and NaOH. The solid medium was obtained by adding 2.0% agar. All media were autoclaved at 121 °C for 15 min. Thiamethoxam was dissolved in absolute ethanol at 0.1 M used as a stock solution.

Thiamethoxam degradation was carried out using plastic containers as described below. Each container was added with 1.0 L mineral medium. The incubation was conducted at the room temperature diffuse light and static condition within 5 days.

2.2. Inoculation with *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2

Phanerochaete sp. Th1 and *Ensifer* sp. Th2 were individually cultured in 250 mL-flask containing 100 mL mineral medium supplemented with 5 g/L thiamethoxam. The flask was shaken at 150 rpm for 5 days at room temperature (~30 ^oC). Microorganisms were collected by centrifuging at 10.000 rpm for 5 min, and then suspended in fresh MM medium at 10^8 CFUs/mL used for inoculation. In all experiments in liquid and soil media with augmentation, each microbial strain was inoculated at 0.5×10^6 CFU/L.

2.3. Water and soil collection

Water and soil samples were collected at a same site from a rice field in Cao Lanh city, Dong Thap province. The samples were collected two months after rice cultivated. Soil was collected at the surface layer at a depth of 0-15 cm. The soil texture included 24.5±2.2% sand, 53.8±4.4% silt and 21.6±2.2% clay. The pH of soil was 6.6±0.4. Main chemical components included 4.1±0.3% total C, 0.32±0.0% total N, 17.4±1.2 ppm P_2O_5, and 10.8±0.8 ppm K_2O of dry soil. Soil and water were transferred into plastic containers (length × width × depth = 15 × 10 × 25 cm), with 1.0 L/container.

2.4. Thiamethoxam degradation in collected water and soil

The experiments included 100% water, 70% water and 30% dry soil, 50% water and 50% dry soil. Thiamethoxam was supplemented at 5 g/L. All containers were incubated at the room temperature and diffuse light for 5 days. Water and soil were collected one time per days with 5 mL each time. For the experiment with 70% water + 30% dry soil, the first sample was collected after 6 hours so that all soil settled in the bottom. Water was transferred into a

sterile bottle before collecting soil, and then re-added into the container. Soil was collected using a spoon and collected after filtering using filter paper before chemical extraction.

Thiamethoxam in soil was extracted twice with acetonitrile. For soil submerged under water in the container containing 70% water and 30% dry soil, soil was added with two volumes of acetonitrile, vortexed for 5 min and kept in a static condition for 10 min. The liquid media were used to determine the substrate concentrations.

2.5. Effects of thiamethoxam on fungi and bacteria number in soil

Soil collected from the container with 50% water and 50% dry soil was used in this experiment. Soil sample (1.0 g) was collected on the surface and bottom layers, crushed with a sterile spoon and transferred into a mineral medium. The solution was shaken at 200 rpm for 10 min, diluted and spread on agar plate containing the mineral medium. The plates were incubated at room temperature for 2 days to enumerate merged colonies.

2.6. Chemical and statistical analysis

Thiamethoxam concentrations were determined by HPLC Model 600E, spherisorb C18 5 UV, 4.5×250 mm column. The mobile phase was methanol controlled at the flow rate of 0.5 mL/min. The Waters UV detector model 2487 was used at 254 nm. The variance and the significant differences were calculated using Duncan's test in SPSS software program version 22.0.

3. Results and discussion

3.1. Thiamethoxam degradation in mineral water and in water collected from a rice field

Thiamethoxam degradation by indigenous microorganisms in water collected from the rice field was about 21.8±4.4% (Figure 1a). The degradation by a mixed culture of *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2 was determined in both the mineral medium and collected water. Compound degradation with the inoculation was 44.2±5.0% in collected water (Figure 1b), and 98.0±0.4% after 5 days in the mineral medium (Figure 1c). These results showed that the degradation rates in the media were in the

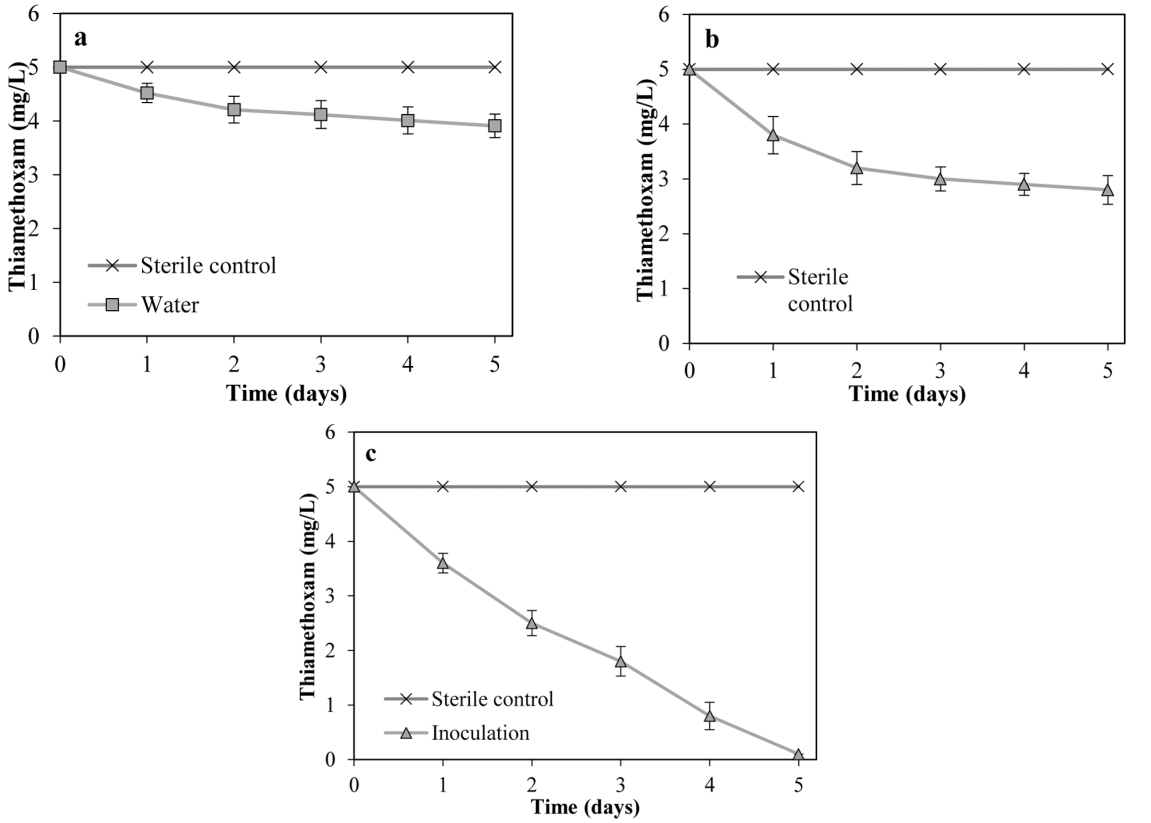

Figure 1. Thiamethoxam degradation by (a) indigenous microorganisms, by (b) mixed culture of *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2 in water collected from the rice field and by (c) mixed culture of *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2 in mineral medium.

descending order: mineral medium with inoculation > collected water with inoculation > collected water. The shortage of mineral molecules and poor degradation by indigenous microorganisms in the collected water might be the reason of the lower degradation process. Meanwhile, all abiotic controls showed negligible degradation. Previous study also presented that thiamethoxam quickly degraded in the mineral medium (Oanh & Duc, 2023).

Some thiamethoxam-degrading microorganisms have been isolated and determined for their degradation in previous studies. For instance, the addition of nitrogen and sulfur sources in media increased thiamethoxam degradation by *Labrys portucalensis* F11 (Boufercha et al., 2022) and *Ensifer adhaerens* TMX-23 (Zhou et al., 2013). Other microorganisms degrade the compound as a sole carbon source. *Enterobacter cloacae* TMX-6 utilized 20% of thiamethoxam in liquid media as the sole carbon source at 10 mg/L after 15 days (Zhan et al., 2021). Bacteria isolated from agricultural soils degraded from 20.88% to 45.28% of the substrate after 15 days in a liquid medium (Rana et al., 2015). *P. chrysosporium* degraded 27% of the compound at 10 mg/L for 25 days, but the degradation was decreased at higher thiamethoxam concentrations in a liquid culture medium (Chen et al., 2019).

3.2. Thiamethoxam degradation in phases of liquid and soil collected from a rice field

In this experiment, the degradation was analyzed in both liquid and soil phases. Thiamethoxam was mostly contained in liquid phase with the concentration were 5.9 mM/L, and only 2.2 mM/L of the substrate accumulated in soil. The high water solubility of the compound (4100 mg/L) could be explained the phenomenon. The degradation percentages in water with and without bacteria inoculation were about 58.7% and 83.6% on average, respectively (Figure 2a). In soil, these corresponding data were 49.1% and 76.4%. The degradation in the liquid phase in this experiment was significantly higher than that in water without soil as described above. The result showed that indigenous soil microorganisms played an important role in the degradation. Moreover, the degradation in augmentation treatment using the mixed culture was higher than natural process, indicating that *Phanerochaete* sp. Th1

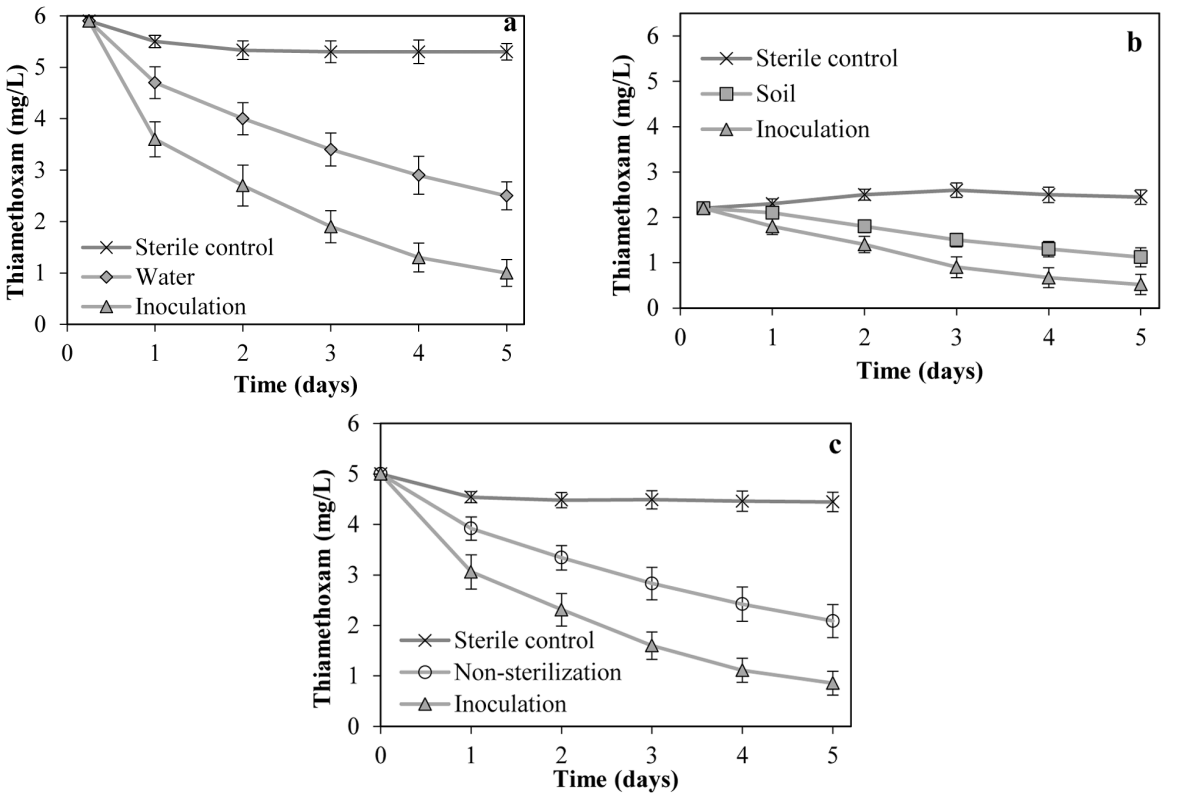

Figure 2. Thiamethoxam degradation in (a) water, (b) soil and (c) total liquid and soil. The experiment was conducted in the media with water (70%) and soil (30%), with and without inoculation with *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2

and *Ensifer* sp. Th2 co-operated well with indigenous microorganisms. However, the chemical remediation in soil and water could not be separated because thiamethoxam is high mobility (organic carbon partition coefficient (K_{oc}) = 68.4).

Thiamethoxam reduction in the control of liquid media was significant (Figure 2a), while thiamethoxam concentration in control of soil increased (Figure 2b). This result could be attributed to thiamethoxam deposited in the soil during the incubation. To summarize the degradation in both liquid and soil phases, the degradation with and without bacteria inoculation was about 57.8% and 82.6% on average, respectively, while the reduction in the sterile control was around 9.8% (Figure 2c).

3.3. Thiamethoxam effects on bacteria and fungi and the gradation of the compound in soil

Thiamethoxam effects on bacterial numbers were determined on the surface and bottom layers of soil in the plastic containers. In the sterile soil, living bacteria and fungi were not found at the beginning. However, they were presented after 5 days; probably they entered from air into soil. Bacteria numbers exceeded fungi in all treatments. In non-sterile soil, the numbers of bacteria and fungi in the surface layer significantly increased, while the numbers were not changed in the bottom layer. The increase in upper soil layer was probably due to the incubation in suitable condition such as in a diffuse light and room temperature. The densities of bacteria and fungi in upper layer were higher than those in the bottom layer because of higher available oxygen.

The numbers of bacteria and fungi in soil spiked with thiamethoxam were not statistically different compared to soil without the insecticide, indicating that the compound did not cause negative effects on bacteria. The inoculation in sterile soil significantly increased bacteria and fungi in soil, both upper and bottom layers. This result showed that *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2 grew well in soil. In a previous study, the inoculation of these bacterial strains co-operated with microorganisms in soil well (Oanh & Duc, 2023).

Table 1. Bacteria and fungi in surface and bottom layers of soil collected from a rice field for 5 days

Thiamethoxam	Treatment	Bacteria and fungi (×10^6 CFU/g dry soil)(*)					
		At the beginning		After 5 days			
				Surface		Bottom	
		Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
Without thiamethoxam addition	Sterile control	0	0	0.06±0.01^a	0.002±0.00^a	0.03±0.01^a	0.001±0.00^a
	Non-sterile soil	55.6±0.21	0.62±0.07	68.2±6.5^c	0.74±0.08^b	53.5±4.5^c	0.60±0.06^b
Thiamethoxam addition	Sterile control	0	0	0.05±0.01^a	0.002±0.00^a	0.04±0.01^a	0.001±0.00^a
	Non-sterile soil	55.6±5.21	0.62±0.07	68.6±8.2^c	0.77±0.064^b	55.5±6.8^c	0.58±0.03^b
	Sterile, inoculation	1.0	1.0	15.4±4.8^b	2.21±0.44^c	11.3±3.6^b	1.73±0.11^c
	Non-sterile, inoculation	56.6±0.21	1.62±0.07	75.2±9.1^{cd}	3.05±0.21^d	60.5±7.2^{cd}	2.26±0.23^d

(*) The lowercase superscript letters show statistically significant differences within a column ($p < 0.05$).

3.4. Thiamethoxam degradation in soil

In this experiment, thiamethoxam degradation was conducted in sterile soil, non-sterile soil, soil with and without inoculation of *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2. Figure 3 showed that the degradation rates were in the order: sterile control < non-sterile soil ≤ sterile soil and inoculation ≤ non-sterile soil and inoculation. The inoculation of *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2 augmented the degradation, indicating that the bacterial and fungal strains could adapt to a new environment.

The degradation in upper soil layer was significantly higher than those in the bottom soil. The degradation in non-sterile soil and sterile soil + the inoculation were not statistically different (Figure 3). The degradation in surface layer with

the inoculation was nearly 50% on average, which was higher about 10% than that in the bottom soil. Bacteria and fungi in upper soil had a higher density and available oxygen resulting in higher degradation. Moreover, the reduction of the compound in upper soil was somewhat smaller than in bottom layer might be because of the evaporation of thiamethoxam. In addition, thiamethoxam was dissipated in sterile soil probably due to the irreversible adsorption in soil.

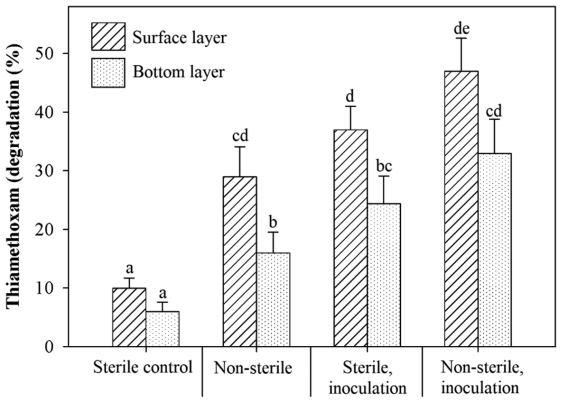

Figure 3. Thiamethoxam degradation in surface and bottom layers of soil in the experiment with 50% soil and 50% water collected from a rice field for 5 days.

The different letters above the bar indicate the statistical difference among treatments ($p < 0.05$).

In a previous report, thiamethoxam was also dissipated in sterile soil, and the process depended on its concentrations (Oanh & Duc, 2023). The inoculation of *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2 in soil collected from a maize field with a moisture content of 50% resulting in 85.1±4.8% and 78.2±6.2% of thiamethoxam were degraded for 25 days, at 2 and 10 mg/kg dry soil, respectively (Oanh & Duc, 2023). Obviously, the degradation in soil depended on chemical concentrations, physical and chemical properties, indigenous microorganisms and other conditions.

Thiamethoxam biodegradations in soil depended on the insecticide concentrations (Yu et al., 2020), both its concentrations and soil types (Wu et al., 2021). The half-lives of thiamethoxam in silty loam soil at 1.8, 18.0, and 180.0 mg/kg were 6.2, 9.5, and 76.2 days, respectively (Wu et al., 2021). The degradation of the compound at 2 mg thiamethoxam/kg soil was 30.28% and 91.20% in the sterilized and nonsterilized soils for 60 days, respectively (Zhang et al., 2021). Moreover, the bioaugmentation to enhance the degradation in soil has been studied. For example, the inoculation of *Bacillus aerophilus* IMBL 4.1 in clay loam soil increased thiamethoxam degradation (Rana & Gupta, 2019). Li et al. (2022) presented that the amendment of *P. chrysosporium* increased thiamethoxam degradation in both sterilized and non-sterilized wetland soils.

4. Conclusion

This study was to simulate the thiamethoxam degradation in a rice field where the insecticide is usually applied to control insect pests. In non-augmentation media, the degradation rates were in the order: 100% water < 50% soil + 50% water < 70% water + 30% soil. The inoculation with the mixture of *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2 increased the degradation in all these media. The results showed that both indigenous microorganisms and *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2 played an important role in thiamethoxam degradation.

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