BILINGUAL PUBLISHING GROUP

Journal of Environmental & Earth Sciences

https://journals.bilpubgroup.com/index.php/jees

ARTICLE

Earthworms and Cellulase Activity in Agricultural Soils of Nakhon Pathom, Thailand

***Rungroj Kraisittipanit* [1], *Arnat Tancho* [2,3,4], *Supranee Watpathomsub* [1], *Ranida Tuanudom* [1], *Patcharee Panraksa* [1], *Narawadee Prathum* [5], *Kanaporn Sujarit* [6], *Waraphas Nontati* [2], *Tamkan Junyangdikul* [1], *Dhanes Rangsrikajee* [1], *Titiya Meechai* [5*]**

[1]*Faculty of Medicine, Bangkokthonburi University, Bangkok 10170, Thailand*

[2]*Natural Agriculture Research and Development Center, Maejo University, Chiang Mai 50290, Thailand*

[3]*The Royal Society of Thailand, Bangkok 10300, Thailand*

[4]*Faculty of Agricultural Production, Maejo University, Chiang Mai 50290, Thailand*

[5]*Faculty of Dentistry, Bangkokthonburi University, Bangkok 10170, Thailand*

[6]*Division of Biology, Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Pathum Thani 12110, Thailand*

ABSTRACT

Earthworms play a vital role in enhancing soil quality and structure in agricultural ecosystems. This study investigated the diversity of earthworm species found in banana and guava orchards in Nakhon Pathom, Thailand, where 166 samples were collected. The results showed that 56.63% of earthworms were in the adult stage. In the banana orchard, researchers identified two families and four species: from the family Megascolecidae, they found Amynthas alexandri, Metaphire posthuma, and Polypheretima elongata, along with one species from the family Moniligastridae, Drawida sp. In the guava orchard, two families and two species were identified: from the family Megascolecidae, Metaphire posthuma, and from the family Moniligastridae, *Drawida* sp. Fungi isolated from the intestines of earthworms, precisely the strains EW2, EW3, EW6, EW13, EW16, EW23, EW25, EW26, EW28, EW38, EW39, EW40, EW41, EW43, and EW44, have demonstrated the ability to produce cellulase. Among these, the fungus EW41 exhibited the highest cellulase activity, measuring 32.97 units per milliliter at an optimal temperature of 60 °C and a pH of 5.0. This study high-

*CORRESPONDING AUTHOR:

Titiya Meechai, Faculty of Dentistry, Bangkokthonburi University, Bangkok 10170, Thailand; Email: titiya.mee@bkkthon.ac.th

ARTICLE INFO

Received: 14 May 2025 | Revised: 29 May 2025 | Accepted: 30 May 2025 | Published Online: 13 June 2025

DOI: https://doi.org/10.30564/jees.v7i6.10027

CITATION

Kraisittipanit, R., Tancho, A., Watpathomsub, S., et al., 2025. Earthworms and Cellulase Activity in Agricultural Soils of Nakhon Pathom, Thailand. Journal of Environmental & Earth Sciences. 7(6): 439–450. DOI: https://doi.org/10.30564/jees.v7i6.10027

lights that most earthworm species in agricultural soils belong to the family Megascolecidae. Furthermore, earthworms play a crucial role in enhancing the chemical properties of the soil. The cellulolytic fungi present in earthworm intestines contribute to the natural decomposition of organic matter, thereby promoting soil health by converting plant residues into nutrient-rich compost. These findings suggest promising applications of earthworm-associated fungi in sustainable agriculture and organic waste management.

Keywords: Earthworm; Agricultural Soils; Potent Cellulase; Megascolecidae

1. Introduction

Nakhon Pathom Province, located in central Thailand, covers an area of approximately 2,168.33 square kilometers. The province is divided into 7 districts and 106 sub-districts, with a population of around 921,882 people. Agriculture plays a central role in the province's economy. Rice farming accounts for 41.5%, followed by freshwater aquaculture (26.1%) and horticulture (23.5%). Despite the province's fertile soil, the extensive use of chemical fertilizers and pesticides in farming practices has raised concerns about soil health and sustainability. Consequently, researchers are exploring ways to reduce dependency on chemicals and promote more sustainable farming methods. Earthworms play a crucial role in enhancing soil quality and fertility within agricultural systems. They improve soil structure by burrowing and creating channels, which enhances aeration and water infiltration, making the soil more conducive to plant growth. Additionally, earthworms accelerate the decomposition of organic matter, thereby releasing nutrients in forms readily available to plants. Furthermore, they help control pathogens and weeds, promoting healthier soil and boosting crop productivity [1–4]. Therefore, understanding the role of earthworms in soil enrichment is essential for sustainable agricultural practices [5].

Soil integrity is influenced by several factors, including nutrient content, pH, soil structure, and cation exchange capacity (CEC). A crucial indicator of soil health is the carbon-to-nitrogen (C/N) ratio, which significantly impacts earthworm growth and the ability to decompose organic matter [2]. Various species of earthworms inhabit Thailand's agricultural soils, each with specific habitats and dietary preferences. For instance, "*Eisenia fetida*" thrives in soils with a C/N ratio ranging from 20:1 to 30:1, while "*Lumbricus terrestris*" prefers soils with a higher C/N ratio of 30:1 or more [6]. Understanding the soil conditions and the specific needs of different earthworm species can help farmers select the most suitable species to improve soil health. Moreover, earthworms harbor various microorganisms in their intestines that play a vital role in breaking down organic matter and releasing nutrients into the soil. These microorganisms produce enzymes such as hydrolytic enzymes, which decompose organic compounds; oxidases, which facilitate oxidation; cellulases, which break down cellulose fibers; and phosphatases, which release plant-available phosphorus [7,8]. These enzymes contribute to nutrient cycling and support plant growth by making nutrients accessible to crops.

This study aims to investigate the earthworm species found in agricultural areas, specifically in banana and guava orchards in Nakhon Pathom. The goal is to assess how this knowledge can improve soil quality and reduce the reliance on chemical fertilizers. Additionally, the research will explore the cellulase enzyme production capabilities of fungi present in the earthworm gut. These insights will enhance our understanding of the biological processes that contribute to soil fertility. The findings from this research will support sustainable agricultural development in Thailand, providing farmers with practical solutions for maintaining soil health while minimizing chemical inputs. Through this approach, the study seeks to promote eco-friendly farming practices that ensure long-term agricultural productivity and environmental sustainability.

2. Methodology

2.1. Site Description and Sampling Procedures

Earthworm samples were collected in July 2024 from two agricultural areas: guava orchards and banana plantations (**Figure 1**). The sampling was conducted using the hand-sorting method, which involved excavating soil to a depth of approximately 30 centimeters. Samples were ran-

domly collected from five locations within each orchard, with each sampling area measuring 0.5 meters by 0.5 meters. All excavated soil was carefully sifted to extract earthworms of all sizes, which were then classified into developmental stages, including juvenile, sub-adult, and adult. The adult earthworms were subsequently identified following the methodology outlined by Gate (1939) [9].

(a)

(b)

Figure 1. The sampling areas. (**a**) banana plantation and (**b**) guava orchard.

2.2. Soil Analysis

Soil samples collected from the study area were analyzed at the Research and Development Center for Natural Agriculture, Maejo University. The following factors were assessed: pH level, electrical conductivity (EC), organic matter content, carbon-to-nitrogen (C/N) ratio, nitrogen content, phosphorus content, potassium content, calcium content, and magnesium content. The procedures were as follows:

2.2.1. pH Measurement

The 20-gram soil sample was weighed and placed in a 50-milliliter beaker. Distilled water (20 milliliters) was added, and the mixture was stirred using a glass rod to ensure thorough mixing. The solution was allowed to stand for 15 minutes, followed by two additional repetitions of the same process. The pH was measured using a pH meter, with the electrode rinsed with distilled water before each measurement, and the data were recorded.

2.2.2. Electrical Conductivity (EC) Measurement

The 6-gram soil sample was placed in a 50-milliliter plastic centrifuge tube, to which 30 milliliters of distilled water was added. The mixture was shaken for 30 minutes and allowed to stand for 5 minutes to facilitate sedimentation. The supernatant was centrifuged at 4000-5000 RPM for 7 minutes, and the resulting clear liquid was used to measure electrical conductivity. The EC meter was calibrated with a standard solution of 0.01 M KCl before measurement, with the probe rinsed and dried before use. The measured values were recorded for further calculations.

2.2.3. Organic Matter (OM) Measurement

A 0.1 gram soil sample, passed through a 0.5 mm sieve, was placed in a 250-milliliter conical flask, and the exact weight was recorded. A 10-milliliter aliquot of $K_2Cr_2O_7$ solution was pipetted into the flask, and the mixture was swirled to ensure thorough mixing. A blank was prepared by adding 10 milliliters of $K_2Cr_2O_7$ to an empty flask containing 10 milliliters of concentrated H_2SO_4, which was allowed to react for approximately 30 minutes. After adding 50 milliliters of distilled water, the mixture was titrated with $FeSO_4$, using 4-5 drops of indicator. The solution's color gradually shifted from green to reddish-brown at the endpoint, and the volume of $FeSO_4$ used was recorded.

2.2.4. Carbon-to-Nitrogen (C/N) Ratio Measurement

0.1 grams of a soil sample, passed through a 0.5 mm sieve, were placed in a foil cup with the edges folded into a spherical shape, and a standard EDTA sample (0.15 g) was added. The samples were analyzed using a combustion

analyzer, and the results were recorded.

2.2.5. Nitrogen (N) Content Analysis

A 0.2-gram soil sample, passed through a 0.5 mm sieve, was placed in a digestion tube, and the exact weight was recorded. A blank tube was also prepared. A mixture of 5 milliliters of digestion acid was added to a fume hood and allowed to digest overnight. The digested solution was cooled, transferred to a 50-milliliter volumetric flask, and adjusted to volume with distilled water. A 10-milliliter aliquot of the digested sample was placed in a distillation apparatus, with 5 milliliters of 40% NaOH added. An indicator flask was prepared with 5 milliliters of 4% boric acid and 100 µL of N indicator in a 125-milliliter flask. The sample was distilled, and the indicator's color change was noted; the final titration using 0.05 M HCl was recorded.

2.2.6. Phosphorus (P) Content Analysis

The 1-gram soil sample was placed in a centrifuge tube, and the exact weight was recorded. Bray II solution (25 milliliters) was added, and the mixture was shaken for 30 minutes. The solution was filtered using No. 5 filter paper and prepared for absorbance measurement in a 25-milliliter volumetric flask. Five milliliters of a mixture of ascorbic acid and ammonium molybdate were added, along with a sufficient amount of 1% boric acid to fill half the flask. The volume was adjusted, and the mixture was shaken thoroughly. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 882 nm, starting with standard solutions before measuring the samples. The results were recorded for standard curve construction and phosphorus content calculation.

2.2.7. Analysis of Potassium, Calcium, and Magnesium (K, Ca, and Mg) Content

A 0.2-gram soil sample, passed through a 0.5 mm sieve, was placed in a digestion tube, and the exact weight was recorded. A mixture of nitric acid and perchloric acid (20 milliliters) was added and left overnight in a fume hood. The solution was digested until a clear or opaque white color was observed, then cooled and transferred to a 100-milliliter volumetric flask, adjusting to volume with distilled water. The sample was filtered through No. 5 filter paper for subsequent potassium, calcium, and magnesium concentration analysis. For potassium, the sample was diluted 20 times by adding 500 µL of the solution to a 15-milliliter centrifuge tube and adjusting to 10 milliliters with distilled water. For calcium and magnesium, the samples were diluted 40 times. Potassium, calcium, and magnesium concentrations were measured using Flame Atomic Absorption Spectroscopy, starting with standard solutions before measuring the samples, and the results were recorded for further calculations.

2.3. Earthworm Identification

After collecting earthworm samples, the identification process was carried out following the methodology outlined by Gate (1939) [9]. The earthworms were examined based on their morphological features, such as body length, clitellum position, male and female pore locations, spermathecal pore, genital markings, prostate gland, copulatory pouch, and body color. These characteristics were observed under a stereomicroscope for detailed and accurate identification. The earthworms were classified into developmental stages, including juvenile, sub-adult, and adult. Adult earthworms were identified to the species level based on these morphological traits, ensuring accurate classification for further analysis. The use of stereomicroscopy helped in distinguishing species based on subtle anatomical differences.

2.4. Fungal Isolation and Cultivation

Minimal agar medium was prepared, consisting of glucose (1 gram), monopotassium phosphate (9 grams), sodium citrate (0.5 grams), magnesium sulfate (0.1 grams), ammonium sulfate (1 gram), and agar powder (20 grams). All ingredients were dissolved in 500 milliliters of distilled water. Once homogeneous, 5 grams of sodium carboxymethyl cellulose were added and thoroughly mixed. The final volume was adjusted to 1,000 milliliters, and the medium was sterilized using an autoclave at 121.5 degrees Celsius and 15 psi for 15 minutes before being poured into plates for subsequent cellulase production testing.

2.5. Cellulase Activity Assays

Fungal samples stored at -20 degrees Celsius were thawed at room temperature. Agar pieces containing the fungi were placed on CMC agar and incubated in the dark for 3 days. After the incubation period, the diameter of each fungal colony was measured in triplicate to obtain an average size. Iodine solution (1%) was then applied to the plates, which were allowed to stand for 5 minutes. A color change from cloudy white to dark purple indicated cellulase activity, with clear zones indicating cellulase production. The diameter of these clear zones was measured in triplicate and averaged, and the ratio of the average apparent zone size to the average colony diameter was calculated, along with the standard deviation.

2.6. Temperature and pH Optimization

2.6.1. Temperature

To study the effect of temperature on cellulase activity, a minimal medium was prepared by dissolving glucose (1 g), monopotassium phosphate (9 g), sodium citrate (0.5 g), magnesium sulfate (0.1 g), and ammonium sulfate (1 g) in 1,000 mL of distilled water. The solution was sterilized at 121.5°C for 15 minutes, poured into flasks, and inoculated with fungal isolate EW41. The inoculated medium was incubated on a shaker for 3 days, after which the crude enzyme was extracted by filtration and stored at 4°C. To test the cellulase activity at varying temperatures (30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C, and 80°C), 0.5 mL of crude enzyme was mixed with 0.5 mL of 1% CMC solution (adjusted to pH 7) and incubated at the designated temperatures for 30 minutes. The enzyme reaction was halted by boiling the samples, and reducing sugars were measured using the DNS method. Absorbance at 540 nm was recorded, and cellulase activity was calculated using a standard glucose curve. The optimal temperature for cellulase activity was determined based on enzyme performance across the temperature range.

2.6.2. pH

To investigate the effect of pH on the cellulase activity of fungal isolate EW41, a minimal medium was prepared by dissolving glucose, monopotassium phosphate, sodium citrate, magnesium sulfate, and ammonium sulfate in distilled water, followed by sterilization at 121.5°C. Fungal isolate EW41 was inoculated into the medium, incubated on a shaker for 3 days, and crude enzyme was extracted. The cellulase activity was tested at pH levels ranging from 3.0 to 9.0 by mixing 0.5 mL of the crude enzyme with 0.5 mL of 1% CMC solution, adjusted to the respective pH values. The enzyme mixtures were incubated at 50°C for 30 minutes, and the reaction was stopped by boiling the samples. Reducing sugars were measured using the DNS method, with absorbance recorded at 540 nm.

2.7. Agricultural Waste Degradation Tests

Agricultural materials (Coconut Coir, Sugarcane Bagasse, Rice Straw) and 1 gram of CMC were weighed and placed into Erlenmeyer flasks, with one flask designated for each type of material. Seven flasks were prepared for each material, with one acting as a control. All flasks were sterilized in an autoclave at 121.5°C and 15 psi for 15 minutes. After cooling, four flasks per material were filled with 50 mL of distilled water, followed by a second round of sterilization. The remaining three flasks per material were filled with 50 mL of fungal culture broth from EMW41. All samples were shaken at 120 rpm and maintained at 50°C for 24 hours. After the incubation period, reducing sugar levels were measured, with the average value calculated from three replicates. Statistical differences were analyzed using Duncan's multiple range test at a confidence level of $p < 0.05$.

2.8. Statistical Analysis

Data were analyzed using ANOVA ($p < 0.05$), and means were compared using Duncan's multiple range test. All analyses were performed using SPSS version 30.

3. Results

3.1. Earthworm Classification

A survey conducted on agricultural plots of farmers in Nakhon Pathom province included two types of orchards: banana and guava. 166 earthworm samples were collected, with 57 samples from the banana orchard and

109 samples from the guava orchard. In the banana orchard, the samples were classified as follows: 7 juvenile specimens (12.28%), 10 sub-adult specimens (17.54%), and 40 adult specimens (70.16%). In the guava orchard, 6 juvenile specimens (5.50%), 9 sub-adult specimens (8.26%), and 94 adult specimens (86.24%) were identified, as summarized in **Table 1**.

Table 1. Stages of Earthworm Development.

Stages	Areas	
	Banana Orchard Samples	**Guava Orchard Samples**
Juvenile	7 (12.28%)	6 (5.50%)
Sub-adult	10 (17.54%)	9 (8.26%)
Adult	40 (70.16%)	94 (86.24%)
Total	57	109

Adult earthworms were classified morphologically in the banana orchard into two families and four species. The family Megascolecidae included the species *Amynthas alexandri* (7.50%), *Metaphire posthuma* (15.00%), and *Polypheretima elongata* (75.00%). The family Moniligastridae included only one species, *Drawida* sp. (2.50%). Adult earthworms were identified within two families and two species in the guava orchard. Megascolecidae comprised Metaphire posthuma (67.02%), while Moniligastridae contained *Drawida* sp. (2.50%). See **Table 2** and **Figure 2**.

Table 2. Diversity of Earthworm Species in the Survey Area.

Diversity of Earthworm		Survey Area	
Families	Species	Banana orchard (%)	Guava Orchard (%)
Megascolecidae	*Amynthas alexandri*	7.50	
	Metaphire posthuma	15.00	67.02
	Polypheretima elongate	75.00	
Moniligastridae	*Drawida* sp.	2.50	86.24

Figure 2. Earthworm Species. **A**,**B**: *Amynthas alexandri*; **C**,**D**: *Metaphire posthuman*; **E**,**F**: *Polypheretima elongata*; **G**,**H**: *Drawida* sp.

3.2. Soil Characteristics

This research involved the collection of soil samples from two agricultural areas: a banana plantation and a guava orchard. The analysis of the soil in the banana plantation revealed that it is a black-gray loamy soil with a pH of 5.92 and an electrical conductivity of 45.6 µs/cm. The organic matter content was found to be 1.63%. The carbon-to-nitrogen ratio measured 2:1, with nitrogen content at 0.60%, phosphorus at 0.37%, potassium at 0.65%, calcium at 1,399 ppm, and magnesium at 645 ppm. In contrast, the soil from the guava orchard was identified as sandy loam, with a pH of 7.32 and an electrical conductivity of 184.0 µs/cm. The organic matter content was measured at 0.28%, with a carbon-to-nitrogen ratio of 0.3:1. The nitrogen content was 0.61%, phosphorus was 0.58%, potassium was 0.75%, calcium was measured at 1,768 ppm, and magnesium at 519 ppm (**Table 3**).

Table 3. Soil Properties in the Earthworm Sampling Areas.

Soil Properties	Sampling Areas	
	Banana Orchard	**Guava Orchard**
pH	5.92	7.32
Electrical Conductivity (μs/cm)	45.6	184.0
Organic Matter (%)	1.63	0.28
C/N Ratio	18:1	22:1
Nitrogen (%)	0.09	0.07
Phosphorus (%)	0.37	0.58
Potassium (%)	0.65	0.75
Calcium (ppm)	1,399	1,768
Magnesium (ppm)	645	519
Soil Type	 Black-gray loamy	 Sandy loam

3.3. Cellulase Activity

The assessment of cellulase production capabilities among 48 fungal isolates found that 7 isolates exhibited an apparent zone-to-growth ratio exceeding 1.4. These isolates included EW16, EW25, EW28, EW40, EW41, and EW44 (**Table 4**), demonstrating precise zone formation as illustrated in **Figure 3**.

Upon testing the fungal isolate EW41, which exhibited the highest clear zone-to-growth ratio, the cellulase enzyme activity was found to be at a maximum of 32.9767 units/mg. The amount of reducing sugar measured was 0.5496 mg/ml (**Table 5**). Morphological studies revealed that fungal isolate EW41 belongs to the genus *Penicillium*, characterized by white colonies with green spores and smooth colony edges (**Figure 4**).

Table 4. Clear Zone-to-Growth Ratio of Fungal Isolates.

Fungal Isolates from Banana Orchard	The Ratio of the Clear Zone to the Colony (± SD)	Fungal Isolates from Guava Orchard	The Ratio of the Clear Zone to the Colony(± SD)
EW1	0.78 ± 0.45bc	EW2	1.06 ± 0.42b
EW3	1.33 ± 0.26ab	EW15	0.25 ± 1.37e
EW6	1.23 ± 0.18ab	EW18	0.89 ± 0.34b
EW8	0.95 ± 0.16b	EW23	1.10 ± 0.25b
EW10	0.79 ± 0.42bc	EW25	1.45 ± 0.31a
EW12	0.84 ± 0.19bc	EW26	1.10 ± 0.40b
EW13	1.27 ± 0.11ab	EW28	1.43 ± 0.37a
EW16	1.41 ± 0.25a	EW30	0.94 ± 0.34b
EW17	1.00 ± 0.22ab	EW31	0.85 ± 0.56b
EW19	0.94 ± 0.16b	EW37	1.00 ± 0.42b
EW22	0.69 ± 0.20c	EW38	1.01 ± 0.44b
EW24	1.00 ± 0.21b	EW39	1.32 ± 0.13ab
EW33	0.68 ± 2.13d	EW40	1.42 ± 0.27a
EW36	0.93 ± 0.24b	EW41	1.58 ± 0.22a
		EW42	0.89 ± 0.45b
		EW43	1.15 ± 0.45b
		EW44	1.52 ± 0.33a
		EW45	1.31 ± 0.25ab
		EW46	0.85 ± 0.26b
		EW47	0.95 ± 0.20b

Notes: The fungi isolated from the banana garden were derived from the intestinal samples of the earthworm *Metaphire peguana* (EW1, EW6, EW8, EW12, EW13, EW16, EW22, EW33); The fungi isolated from the banana garden were derived from the intestinal samples of the earthworm *Polypheretima elongata* (EW3, EW10, EW19, EW36); The fungi isolated from the banana garden were derived from the intestinal samples of the earthworm *Amynthas alexandri* (EW17, EW24); The fungi isolated from the guava garden were derived from the intestinal samples of the earthworm *M. posthuma* (EW2, EW15, EW18, EW23, EW25, EW26, EW28, EW30, EW31, EW37, EW38, EW39, EW40, EW41, EW42, EW43, EW44, EW45, EW46, EW47); The fungi isolated from the guava garden were derived from the intestinal samples of the earthworm *P. elongata* (EW25, EW43).

Table 5. Reducing sugar content and cellulase enzyme activity.

Fungal Isolate Code	Cellulase Enzyme Activity (Unit/ml)	Reducing Sugar Content (mg/ml)	pH of the Solution After Incubation	Average Dry Weight of Fungi (mg)
EW41	32.97	0.5496	5.33	145.56

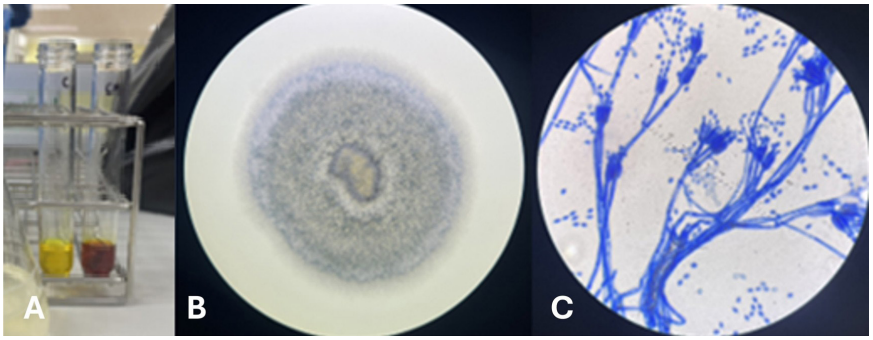

Figure 4. Color change of DNS reagent solution (**A**) with control on the left and test tube on the right. (**B**,**C**) show colonies and spores of fungal isolate EW41 captured under a stereo microscope and a compound microscope at 1,000× magnification, respectively.

3.3.1. Study of the Optimal Temperature for Cellulase Activity Produced by Fungal Isolate EW41

The study on the optimal temperature for cellulase enzyme activity of fungal isolate EW41 revealed that enzyme activity increased rapidly at 40 °C, reaching its peak at 60 °C. Beyond this temperature, enzyme activity decreased steadily (**Table 6** and **Figure 5**).

Table 6. Cellulase enzyme activity from fungi Isolate EW41 at different temperatures.

Temperature (°C)	Cellulase Enzyme Activity at Different Temperatures (Unit/mL)
30	34.99 ± 1.78c
35	45.71 ± 2.01b
40	45.93 ± 1.29b
45	48.19 ± 1.69ab
50	48.99 ± 0.39ab
55	48.97 ± 0.21ab
60	52.39 ± 1.09a
65	48.21 ± 1.98ab
70	45.41 ± 0.89b
75	44.99 ± 1.07b
80	42.53 ± 1.11bc

$p \leq 0.05$.

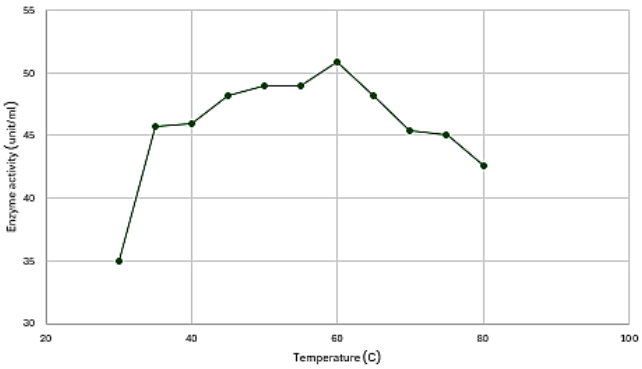

Figure 5. Cellulase enzyme activity from fungi isolate EW41 at different temperatures.

3.3.2. Study of the Optimal pH for Cellulase Activity Produced by Fungal Isolate EW41

The study investigated the cellulase enzyme activity of the EW41 fungal isolate at varying pH levels: 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. The results revealed that cellulase enzyme activity was highest at a pH of 5.0, reaching 88.59 units/ml (**Table 7** and **Figure 6**).

Table 7. Cellulase Activity of Fungal Isolate EW41 at Different pH Levels.

Fungal Isolate	Cellulase Activity at Different pH (Unit/mL) of EW41						
	3.0	4.0	5.0	6.0	7.0	8.0	9.0
EW41	75.89c	84.39ab	88.59a	85.61ab	82.61b	81.11bc	80.41bc

$p \leq 0.05$.

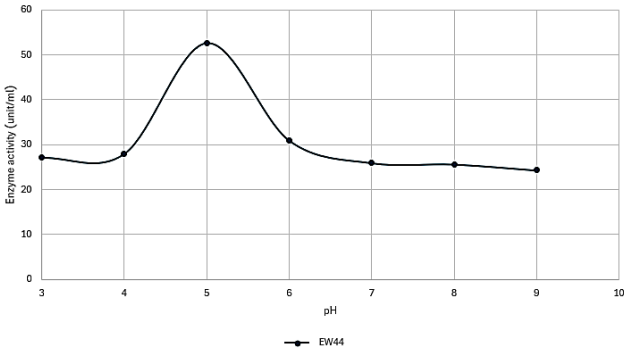

Figure 6. Cellulase enzyme activity from fungi isolate EW41 at different pH.

3.4. Agricultural Waste Degradation

The fungal filtrate of EW41 was used to degrade agricultural wastes for 24 hours. It was found that the cellulase enzyme was able to degrade dry coconut coir, producing an average of 0.83 mg/mL of reducing sugar. For dry sugarcane bagasse, the reducing sugar measured was 1.98 mg/mL, while dry rice straw yielded 0.73 mg/mL of reducing sugar (**Figure 7** and **Table 8**).

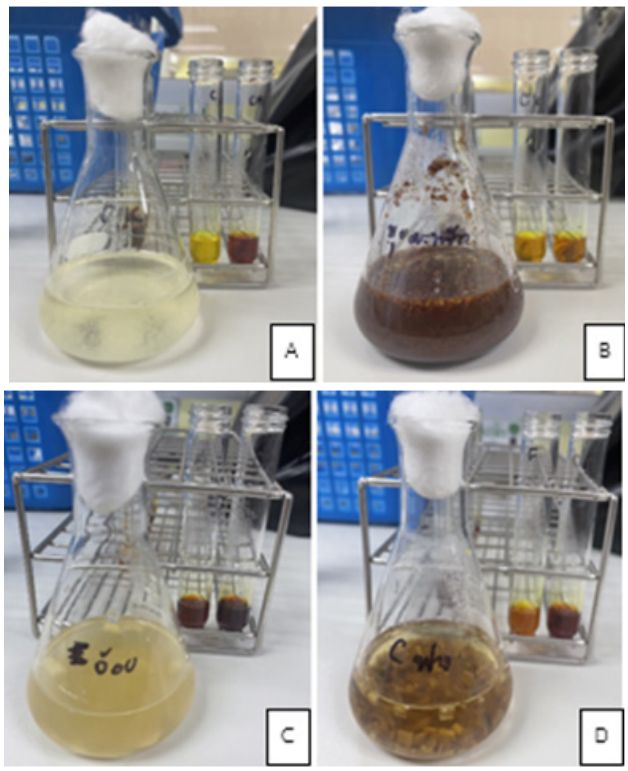

Figure 7. Efficiency of Agricultural Waste Degradation (Coconut Coir, Sugarcane Bagasse, Rice Straw) by Cellulase Enzyme Produced from Fungal Isolate MEW41 at 24 Hours. Co = Control Tube, T = Test Tube. A = Synthetic Cellulose, B = Dry Coconut Coir, C = Dry Sugarcane Bagasse, and D = Dry Rice Straw.

Table 8. Cellulase Activity of Fungi at Different pH Levels.

Agricultural Materials	A540 nm	Measured Reducing Sugar (mg/mL)
Synthetic Cellulose (CMC) (control)	1.37	1.39 ± 0.21b
Dry Coconut Coir	0.81	0.83 ± 0.11c
Dry Sugarcane Sheath	1.95	1.98 ± 0.18a
Dry Rice Straw	0.71	0.73 ± 0.09b

$p \leq 0.05$.

4. Discussion

The classification of earthworm species in the agricultural regions of Nakhon Pathom province revealed significant diversity in both banana and guava plantations. In the banana plantation, 70.16% of the earthworms were adults, while in the guava plantation, this percentage increased to 86.24%. Within the family Megascolecidae, Polypheretima elongata was the predominant species in the banana plantation, comprising 75.00% of the population. This suggests a strong adaptation to the local environmental conditions. In contrast, *Metaphire posthuma*, also from the Megascolecidae family, was the dominant species in guava plantations, making up 67.02% of the earthworm population. The high prevalence of Megascolecidae species in agricultural areas aligns with previous findings that reported similar distributions in Thai agricultural landscapes, particularly in fruit orchards where chemical fertilizers are used less frequently [10]. Farming practices, soil health, and the amount of organic matter present in the soil can influence the diversity of earthworm species. This finding emphasizes the essential role of earthworms in enhancing soil health and fertility [11].

Soil analysis in both plantations highlighted the strong influence of soil composition on earthworm populations. In the banana plantation, the soil had acidic properties (pH 5.92), which potentially affected plant nutrient absorption, particularly phosphorus, as phosphorus is less available in acidic soils. Guava plantation soil, with a pH of 7.32, falls within the optimal range for nutrient absorption and plant growth. Furthermore, the higher electrical conductivity of the guava plantation soil (184.0 µs/cm) indicates excellent nutrient retention. This is consistent with observations that soils with greater electrical conductivity typically contain more soluble nutrients [12]. Ahmed N, et

al. (2022) also demonstrated that higher nutrient content in soil is directly correlated with earthworm activity and diversity [13,14].

The carbon-to-nitrogen (C/N) ratio analysis further illustrated the differences between the two plantations. Guava plantation soil had a low C/N ratio (0.3:1), indicative of rapid organic matter decomposition and nutrient cycling [15]. In contrast, the banana plantation had a higher C/N ratio (2:1), likely experiencing slower decomposition and delayed nutrient availability, a reflection of the accumulation of decaying banana leaves. C/N ratios are critical for understanding the rate of organic matter breakdown and its subsequent influence on plant nutrient availability. Further analysis explored the fungal capabilities in earthworm intestines to produce cellulase enzymes. Seven fungal isolates, including EW16, EW25, EW28, EW40, EW41, and EW44, demonstrated high cellulase production capacity, with a clear zone-to-growth ratio exceeding 1.4. The ability of these fungi to facilitate cellulose decomposition is likely enhanced by organic materials within the earthworm's intestines to high cellulase production from fungi in earthworm intestines [16]. Additionally, K Song et al. (2020) observed that cellulase production in fungi is highly efficient when aided by earthworm digestive processes, particularly in organic-rich soils [17,18].

Regarding enzyme performance, isolate EW41 showed significantly higher activity ($p < 0.05$) compared to other isolates at 37 °C, with optimal activity at a pH of 5 (88.59 units/ml). The importance of pH in optimizing enzyme activity is highlighted, as deviations from the optimal pH can lead to significant reductions in enzyme efficiency. This underscores the importance of optimizing pH conditions to maximize cellulase activity for efficient breakdown of agricultural materials [19,20].

Temperature also played a critical role in enzyme activity, with rapid increases observed at 40 °C and a peak activity of 52.39 units/ml at 60 °C. Beyond this temperature, enzyme efficiency declined significantly, dropping activity to 42.53 units/ml at 80 °C. This highlights the need to maintain optimal temperatures for maximum enzyme performance which demonstrated similar patterns in enzyme activity under temperature fluctuations [21–23]. When evaluating the degradation efficiency of agricultural materials, dried coconut coir, dried sugarcane sheath, and dried rice straw, EW41 demonstrated the highest efficiency, yielding 1.98 mg/mL of reducing sugars from dried sugarcane sheath. In comparison, coconut coir and rice straw yielded 0.81 mg/mL and 0.71 mg/mL, respectively. Additionally, Civzele et al. (2023) noted that fungal cellulases are particularly effective in breaking down lignocellulosic materials, which aligns with the observed efficacy of EW41 in this study [24]. In conclusion, the cellulase enzyme produced by the fungal isolate EW41 shows considerable potential for degrading agricultural waste. Future studies should investigate the influence of other environmental factors, such as substrate variability and temperature modulation, to enhance cellulase production. Utilizing this enzyme in industrial composting could provide sustainable solutions for managing agricultural waste, thereby contributing to improved soil health and increased crop yields. Shi et al. (2024) emphasized the critical role of earthworms in composting processes, further supporting the potential application of cellulase-producing fungi in agricultural waste management [25]. This study demonstrates the role of earthworms in enhancing soil quality and organic matter decomposition. The results align with previous studies in other regions, such as research from Brazil and South Africa, which found that earthworms play a crucial role in improving soil health for sustainable agriculture [26]. These studies indicated that earthworms help increase cation exchange capacity (CEC) and nutrient cycling in soils with lower chemical fertilizer use, particularly in fruit orchards where organic fertilizers are more frequently applied. Additionally, the findings of this study are consistent with research from Africa, which found that Eisenia fetida plays a significant role in decomposing lignocellulosic materials in organic-rich soils [13,27,28]. This is linked to the observation that earthworms in banana plantations in Nakhon Pathom decompose banana leaves, which have high carbon content.

5. Conclusions

This study highlights the significant influence of soil properties on earthworm diversity in Nakhon Pathom's agricultural areas. The Megascolecidae family, particularly *Polypheretima elongata* and *Metaphire posthuma*, dominated banana and guava plantations, with soil pH and C/N ratios playing a key role in nutrient availability and organic

matter decomposition. Fungal isolate EW41, sourced from earthworm intestines, exhibited high cellulase activity, peaking at 37 °C and pH 5. Its effectiveness in degrading agricultural waste, particularly sugarcane sheath, demonstrates its potential for sustainable waste management. Further research into environmental factors could enhance cellulase production, offering promising applications in industrial composting to improve soil health and crop productivity. Future studies should test EW41 cellulase efficacy under field conditions and explore large-scale composting applications using diverse crop residues.

Author Contributions

Investigation, formal analysis R.K., A.T. and S.W.; validation, R.T., P.P., N.P., K.S. and W.N.: verification of research output, T.J., D.R., and T.M.; supervising responsibility for valuable ideas, T.M. and R.K.; research administrator, T.M. All authors have read and agreed to the published version of the manuscript.

Funding

This work received no external funding.

Institutional Review Board Statement

The staff approved the protocol for collecting earthworm samples at each site of each garden, and the research received animal research ethics approval from the committee and department head (BTU.MD.002/2024).

Informed Consent Statement

Not applicable.

Data Availability Statement

We encourage all authors of articles published in our journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required.

Acknowledgments

The researcher extends special thanks to Bangkokthonburi University and Maejo University for funding this research. Special thanks are extended to the Department of Biology and Microbiology at the Faculty of Medicine, Bangkokthonburi University, for providing facilities for the research experiments. Appreciation is also given to the microbiology lab scientists at Bangkokthonburi University for their assistance and support throughout the research study.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Liu, H., Li, J., Carvalhais, L.C., et al., 2021. Evidence for the plant recruitment of beneficial microbes to suppress soil-borne pathogens. New Phytologist. 229, 2873–2885. DOI: https://doi.org/10.1111/nph.17057

[2] Liu, Z., Liu, J., Yu, Z., et al., 2020. Long-term continuous cropping of soybean is comparable to crop rotation in mediating microbial abundance, diversity and community composition. Soil and Tillage Research. 197, 104503. DOI: https://doi.org/10.1016/j.still.2019.104503

[3] Li, X., Rui, J., Mao, Y., et al., 2014. Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. Soil Biology and Biochemistry. 68, 392–401. DOI: https://doi.org/10.1016/j.soilbio.2013.10.017

[4] Sun, R., Zhang, X.-X., Guo, X., et al., 2015. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biology and Biochemistry. 88, 9–18. DOI: https://doi.org/10.1016/j.soilbio.2015.05.007

[5] Adejuyigbe, C.O., Tian, G., Adeoye, G.O., 2006. Microcosmic study of soil microarthropod and earthworm interaction in litter decomposition and nutrient turnover. Nutrient Cycling in Agroecosystems. 75, 47–55. DOI: https://doi.org/10.1007/s10705-006-9010-5

[6] Ai, C., Zhang, S., Zhang, X., et al., 2018. Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation. Geoderma. 319, 156–166. DOI: https://doi.org/10.1016/j.geoderma.2018.01.010

[7] Eisenhauer, N., 2016. Plant diversity effects on soil

microorganisms: Spatial and temporal heterogeneity of plant inputs increase soil biodiversity. Pedobiologia. 59, 175–177. DOI: https://doi.org/10.1016/j.pedobi.2016.04.004

[8] Lauber, C.L., Strickland, M.S., Bradford, M.A., et al., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry. 40, 2407–2415. DOI: https://doi.org/10.1016/j.soilbio.2008.05.021

[9] Gates, G.E., 1939. Thai earthworms. Journal of the Thailand Research Society. 12, 65–114.

[10] Chanabun, R., Aoonkum, A., Seesamut, T., et al., 2023. Four new terrestrial earthworm species from the northeast Thailand (Oligochaeta, Megascolecidae). ZooKeys. 1176, 195–219.

[11] Tuanudom, R., Yurayart, N., Rodkhum, C., et al., 2021. Diversity of midgut microbiota in laboratory-colonized and field-collected Aedes albopictus (Diptera: Culicidae): A preliminary study. Heliyon. 7, e08259. DOI: https://doi.org/10.1016/j.heliyon.2021.e08259

[12] Ali, A., Das, B., Dhakar, M.K., et al., 2024. Enhancing soil health and fruit yield through Tephrosia biomass mulching in rainfed guava (Psidium guajava L.) orchards. Scientific Reports. 14, 13913. DOI: https://doi.org/10.1038/s41598-024-64814-x

[13] Ahmed, N., Al-Mutairi, K.A., 2022. Earthworms Effect on Microbial Population and Soil Fertility as Well as Their Interaction with Agriculture Practices. Sustainability. 14(13), 7803. DOI: https://doi.org/10.3390/su14137803

[14] Karimifard, S., Saberi-Pirooz, R., Ahmadzadeh, F., et al., 2024. Investigating the impacts of agricultural land use on soil earthworm communities: A case study of northern Zagros Mountains of Iran. Heliyon. 10, e23523. DOI: https://doi.org/10.1016/j.heliyon.2023.e23523

[15] Jílková, V., Straková, P., Frouz, J., 2020. Foliage C:N ratio, stage of organic matter decomposition and interaction with soil affect microbial respiration and its response to C and N addition more than C:N changes during decomposition. Applied Soil Ecology. 152, 103568. DOI: https://doi.org/10.1016/j.apsoil.2020.103568

[16] Yang, Y., Callaham, M.A., Wu, X., et al., 2023. Gut microbial communities and their potential roles in cellulose digestion and thermal adaptation of earthworms. Science of The Total Environment. 903, 166666. DOI: https://doi.org/10.1016/j.scitotenv.2023.166666

[17] Havlin, J., Heiniger, R., 2020. Soil Fertility Management for Better Crop Production. Agronomy. 10(9), 1349. DOI: https://doi.org/10.3390/agronomy10091349

[18] Song, K., Sun, Y., Qin, Q., et al., 2020. The Effects of Earthworms on Fungal Diversity and Community Structure in Farmland Soil With Returned Straw. Frontiers in Microbiology. 11, 594265. DOI: https://doi.org/10.3389/fmicb.2020.594265

[19] Gentili, R., Ambrosini, R., Montagnani, C., et al., 2018. Effect of Soil pH on the Growth, Reproductive Investment and Pollen Allergenicity of *Ambrosia artemisiifolia* L. Frontiers in Plant Science. 9, 1335. DOI: https://doi.org/10.3389/fpls.2018.01335

[20] Neina, D., 2019. The Role of Soil pH in Plant Nutrition and Soil Remediation. Applied and Environmental Soil Science. 2019, 5794869. DOI: https://doi.org/10.1155/2019/5794869

[21] da Silva, G.J., Rivadavea, W.R., de Lima, J.D., et al., 2021. Plant Growth-Promoting Microbes for Sustainable Biotic and Abiotic Stress Management. In: Mohamed, H.I., El-Beltagi, H.E.-D.S., Abd-Elsalam, K.A. (eds). Springer International Publishing: Cham, Switzerland. pp. 133–155.

[22] Daunoras, J., Kačergius, A., Gudiukaitė, R., 2024. Role of Soil Microbiota Enzymes in Soil Health and Activity Changes Depending on Climate Change and the Type of Soil Ecosystem. Biology. 13(2), 85. DOI: https://doi.org/10.3390/biology13020085

[23] Chen, Y., Zhang, Y., Shi, X., et al., 2022. The succession of GH6 cellulase-producing microbial communities and temporal profile of GH6 gene abundance during vermicomposting of maize stover and cow dung. Bioresource Technology. 344, 126242. DOI: https://doi.org/10.1016/j.biortech.2021.126242

[24] Civzele, A., Stipniece-Jekimova, A.A., Mezule, L., 2023. Fungal Ligninolytic Enzymes and Their Application in Biomass Lignin Pretreatment. Journal of fungi. 9(7), 780. DOI: https://doi.org/10.3390/jof9070780

[25] Shi, J.-H., Hu, X.-J., Zeng, Z.-X., et al., 2024. Earthworms promote crop growth by enhancing the connections among soil microbial communities. Journal of Applied Ecology. 61, 1867–1880. DOI: https://doi.org/10.1111/1365-2664.14713

[26] Nxele, T.C., Lamani, S., Measey, G.J., et al., 2015. Studying Earthworms (Annelida: Oligochaeta) in South Africa. African Invertebrates. 56(3), 779–806. DOI: https://doi.org/10.5733/afin.056.0319

[27] Tao, J., Griffiths, B., Zhang, S., et al., 2009. Effects of earthworms on soil enzyme activity in an organic residue amended rice–wheat rotation agro-ecosystem. Applied Soil Ecology. 42, 221–226. DOI: https://doi.org/10.1016/j.apsoil.2009.04.003

[28] Scheu, S., 1993. Cellulose and lignin decomposition in soils from different ecosystems on limestone as affected by earthworm processing. Pedobiologia. 37, 167–177. DOI: https://doi.org/10.1016/S0031-4056(24)00096-9