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The Effect of *Zophobas morio* Superworms as the Agent towards Polystyrene Biodegradation to Reduce Plastic Waste Pollution

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Abstract

Plastic waste pollution, particularly polystyrene (PS), presents significant environmental challenges due to its durability and resistance to degradation. This study investigates the potential of *Zophobas morio* larvae, commonly known as superworms, in biodegrading polystyrene under various conditions. Utilizing their unique gut microbiota, these larvae were subjected to controlled laboratory experiments to determine the optimal conditions for plastic biodegradation. Key variables included temperature (21°C–23°C and 28°C–30°C), diet composition (PS, HDPE, LDPE, and mixed diets), and exposure duration (10 and 13 days). The results highlighted that superworms can effectively degrade polystyrene, with the highest efficiency achieved at lower laboratory temperatures (21°C–23°C) and a mixed diet at 13 days. The degradation was evaluated through weight loss measurements and larval health observations. Data analyses confirmed significant differences in degradation efficiency across the tested conditions. These findings underscore the potential of *Zophobas morio* larvae as a cost-effective, eco-friendly solution for managing plastic waste, offering a sustainable alternative to conventional methods such as landfilling and incineration. By optimizing conditions like diet and temperature, this research contributes to the growing field of biological waste management and offers promising insights into reducing plastic pollution's environmental footprint.

1. Introduction

Plastic waste pollution has emerged as a global environmental crisis, posing significant threats to ecosystems, human health, and socioeconomic well-being. Plastics are designed for durability, but this characteristic contributes to their persistence in the environment, often lasting for hundreds of years. Among various plastic types, polystyrene (PS) is widely used due to its lightweight, strength, and insulating properties. However, its

resistance to degradation exacerbates environmental challenges, as PS contributes significantly to the accumulation of non-biodegradable waste in landfills and marine environments (Ritchie, 2019).

In marine ecosystems, plastic debris is detrimental to wildlife through ingestion, entanglement, and habitat destruction. Animals often mistake plastic for food, leading to internal injuries, starvation, and even death. On land, improper disposal of plastic waste, including polystyrene, contaminates soil and water resources, posing risks to wildlife and human health. The fragmentation of plastics into microplastics further exacerbates these challenges, as they infiltrate food chains and pose potential health risks (De-la-Torre, 2020). Conventional waste management techniques, such as landfilling and incineration, have proven inadequate in addressing the growing problem of plastic waste. Landfills are rapidly reaching capacity, and incineration emits toxic pollutants, further harming the environment. The need for innovative, sustainable solutions to plastic waste pollution has spurred interest in biological degradation methods (Salisu and Maigari, 2019).

Zophobas morio larvae, commonly known as superworms, have shown significant potential in biodegrading polystyrene. These larvae host a diverse microbiome within their gut, enabling them to metabolize complex polymers such as PS. Studies have demonstrated that enzymes produced by the gut microbiota of superworms facilitate the cleavage of PS polymer chains, breaking them down into smaller, less harmful compounds. This biological process provides a promising alternative to conventional methods of plastic waste management (Hou and Majumder, 2021). The temperature and diet of *Zophobas morio* significantly influence their efficiency in degrading polystyrene. Optimal biodegradation occurs at temperatures between 21°C and 23°C, where metabolic activity is highest. Additionally, supplementing their diet with organic materials such as vegetables alongside polystyrene enhances their degradation efficiency. These findings underscore the importance of optimizing environmental conditions to maximize the biodegradation potential of superworms (Sun, 2022).

This study investigates the role of *Zophobas morio* larvae in mitigating plastic waste pollution, focusing on their ability to degrade polystyrene under varying conditions. By exploring factors such as temperature, diet, and duration, this research aims to establish the optimal parameters for biodegradation. The results have significant implications for sustainable waste management practices, offering a cost-effective and environmentally friendly solution to the persistent issue of plastic pollution (Kuan, 2022). Through rigorous experimentation and analysis, this study seeks to advance our understanding of biological waste management techniques. The findings can inform future strategies to combat plastic pollution while promoting ecosystem conservation and sustainability. By leveraging the natural abilities of *Zophobas morio* larvae, this research contributes to the global effort to reduce reliance on non-biodegradable materials and foster a cleaner, healthier environment (Turner, 2020).

2. Literature Review

The biodegradation of plastic waste has gained significant attention as an alternative to conventional waste management methods. Recent studies emphasize the potential of biological solutions, particularly involving organisms like *Zophobas morio* larvae, for addressing plastic waste challenges. These superworms have shown promising results in breaking down polystyrene (PS) and other plastic types through enzymatic activity facilitated by their gut microbiota (Hou and Majumder, 2021). Biodegradation offers several advantages over traditional methods, such as reduced environmental harm and cost efficiency. Unlike incineration or landfilling, biological approaches utilize natural processes to convert plastics into less harmful byproducts, making them a more sustainable option (Turner, 2020).

Polystyrene, a widely used plastic polymer, has unique physical and chemical properties that make it highly durable and resistant to natural degradation processes (Salisu and Maigari, 2019). Its lightweight structure and thermal insulation qualities have made it popular in packaging and consumer goods, but these same features contribute to its persistence in the environment (Luo, 2020). The environmental impacts of PS are particularly severe, as it often breaks down into microplastics that infiltrate ecosystems and food chains, posing risks to both human and wildlife health (De-la-Torre, 2020).

Zophobas morio larvae have garnered attention due to their ability to degrade plastics effectively. Their digestive systems host symbiotic microorganisms capable of enzymatically breaking down long polymer chains found in plastics (Peng, 2018). Beyond polystyrene, the larvae have demonstrated potential in degrading other plastics, such as high-density polyethylene (HDPE) and low-density polyethylene (LDPE), albeit at slower rates (Tay, 2023). The efficiency of degradation depends on various factors, including temperature, the larvae's diet, and the composition of the plastic being tested (George, 2022). Recent studies have highlighted that providing the larvae with a mixed diet of plastic and organic material can significantly enhance their degradation capacity, as the added nutrients improve their overall metabolic activity (Sun, 2022).

While superworms show great promise, challenges remain in scaling up this process for real-world applications. One key limitation is the time required for effective biodegradation, as the larvae typically need several days to show significant results (Hou and Majumder, 2021). Additionally, the health and lifespan of the larvae can impact the outcomes, with optimal degradation occurring during their larval stage (Kuan, 2022). Despite these challenges, the integration of biological agents like *Zophobas morio* into waste management strategies offers a viable path forward. This literature review highlights the importance of further research into optimizing conditions for superworm-based biodegradation, including temperature control, diet supplementation, and microbial activity analysis, to improve scalability and efficiency (Turner, 2020).

The life cycle of *Zophobas morio show* is a crucial consideration in their application for biodegradation. Starting as eggs laid by adult beetles, the larvae hatch and undergo a rapid growth phase, during which they consume large amounts of organic material. The larval stage, lasting three to four months, is the most metabolically active and effective for plastic degradation. After the larvae pupate and transition into adult beetles, their digestive processes change, making them less effective for biodegradation purposes. This highlights the importance of targeting larvae at their peak stage for use in waste management (Kuan, 2022). Furthermore, the specific impacts of superworms extend beyond just PS degradation. Studies have shown their ability to break down other plastic types, such as LDPE and HDPE, albeit at varying efficiency rates. Previous achievements in utilizing superworms for biodegradation have paved the way for scalable, eco-friendly waste management practices, demonstrating significant reductions in plastic waste under controlled conditions (Peng, 2018).

3. Methodology

This section describes the systematic approach undertaken to investigate the biodegradation potential of *Zophobas morio* larvae on PS, HDPE and LDPE. The methodology includes the preparation and handling of samples, the experimental setup at different locations, and the evaluation of key parameters such as temperature, diet, and exposure duration. By employing controlled conditions and robust analytical methods, the study aims to identify optimal biodegradation scenarios and provide reliable insights for sustainable waste management practices.

3.1 Location of Experiment

The experiments were conducted at two primary locations, each providing the necessary infrastructure and environmental conditions for the study. The Wastewater Laboratory at Universiti Tun Hussein Onn Malaysia (UTHM) was the primary site for conducting the experiments. This facility provided the necessary infrastructure for precise measurements, including temperature-controlled environments and specialized equipment for biodegradation analysis. The experiments in the lab ensured consistency in temperature, humidity, and other environmental factors critical to evaluating the degradation process. Additionally, the controlled setup helped isolate the influence of key parameters, such as temperature and diet composition, on the larvae's biodegradation capabilities. UTHM Pagoh Residential was used for initial preparations, including the storage of larvae, food waste, and plastic samples. This site ensured that all materials were maintained under proper conditions before transferring them to the lab for analysis. Larvae were housed in ventilated containers with adequate food and moisture to maintain their health and activity levels. The location also provided a clean and safe environment for handling samples, minimizing the risk of contamination or degradation before experimentation.

3.2 Sample Collection

The sample has been collected with three different types of methods that focusing on larvae collection from supplier, type of plastics which ar PS, HDPE and LDPE. *Zophobas morio* larvae were sourced from a local supplier specializing in insect cultivation, ensuring uniform size and health. Before starting the experiments, the larvae were acclimated to laboratory conditions for 24 hours to stabilize their physiological state. This acclimation period was critical to ensuring consistent behavior and activity during the biodegradation tests. The larvae were visually inspected for any signs of illness or lethargy, and only active and healthy specimens were selected for the study.

The plastics tested included polystyrene (PS), high-density polyethylene (HDPE), and low-density polyethylene (LDPE), each representing a common type of plastic waste. These plastics were collected from industrial and commercial sources to reflect real-world conditions. Prior to the experiments, the plastics were cleaned with distilled water to remove impurities, cut into uniform sizes, and air-dried. This standardization ensured accurate comparisons of degradation rates and minimized external variables influencing the results.

3.3 Sample Preparation

The sample has been collected for different types of location and store. Larvae were prepared by placing them in ventilated containers with adequate air circulation and food to maintain their vitality. They were not fed any plastic during the acclimation period to ensure that their digestive systems were not pre-conditioned to a specific diet. The larvae were divided into groups based on experimental conditions, such as diet composition and temperature settings. Each group was labeled appropriately to facilitate tracking and analysis throughout the study. Plastic samples were cleaned thoroughly with distilled water to eliminate surface contaminants and then dried in an oven at a low temperature to ensure consistency in weight measurements. Each piece of plastic was cut into 5x5 cm squares for uniformity across all test conditions. The prepared plastic samples were weighed using a precision scale, with weights recorded before being introduced to the larvae. This preparation ensured that any weight loss observed during the experiments was solely due to biodegradation by the larvae.

Fresh vegetable scraps, including green mustard and carrots, were chosen as the food waste component of the study due to their availability and high nutritional value. The vegetables were washed, chopped into small pieces, and stored in airtight containers at 4°C to maintain freshness. This ensured that the food waste provided adequate nutrition to the larvae while not introducing external contaminants into the experimental setup. A consistent amount of food waste was added to each test group to standardize the diet and enable accurate comparisons across different experimental conditions.

Blank samples were prepared using only plastic without the presence of larvae, serving as controls for the experiments. These samples were stored in identical conditions to those containing larvae to account for any non-biological degradation. By comparing the weight loss of blank samples to those exposed to larvae, the study could isolate the effects of biodegradation. This control setup was crucial for validating the results and ensuring that the observed degradation was directly attributable to the larvae. For a more realistic simulation, a mixture of plastic samples and food waste was prepared. This combination was used to observe how the presence of organic matter influenced the degradation process. The ratio of plastic to food waste was kept consistent across all groups to ensure uniformity. The mixture was placed in containers along with the larvae and monitored for any observable changes, such as weight loss or physical degradation of the plastics.

All prepared samples were stored in sealed containers under controlled environmental conditions to prevent contamination. Temperature and humidity levels were monitored and maintained to align with the parameters set for each experimental group. Samples were labeled with their respective conditions, such as diet type and temperature range, to facilitate accurate tracking. Proper storage ensured the reliability of the results and minimized external variables affecting the biodegradation process.

3.4 Sample Analysis

After finishing the experiment, there are a few parameters and data that has been collected and needed to analyze base on this study. The study began with a preliminary analysis to determine the optimal duration for biodegradation experiments. Tests were conducted over 10 and 13 days, with plastic weight loss recorded at each interval. This helped identify the time period where the highest degradation rates occurred. By focusing on these intervals, the study aimed to maximize the efficiency of subsequent experiments.

Pilot-scale experiments were performed to evaluate the effect of varying time periods on biodegradation efficiency. Groups of larvae were exposed to plastics at different time intervals under laboratory (21°C–23°C) and room temperature (28°C–30°C) conditions. Regular observations were made to assess changes in the plastics and the health of the larvae. This step was essential for validating the scalability of the process and identifying any challenges associated with prolonged exposure.

Temperature variation was explored to determine its influence on the degradation rate. Experiments were conducted at two distinct ranges: laboratory temperature (21°C–23°C) and room temperature (28°C–30°C). These ranges were chosen to reflect realistic environmental conditions and controlled settings. The results were compared to identify the temperature at which the larvae exhibited the highest biodegradation efficiency.

The weight of plastic samples was measured before and after each experiment using a precision scale. These measurements were used to calculate the percentage of weight loss, providing a quantitative assessment of degradation. By repeating the measurements across multiple trials, the study ensured statistical reliability. This data served as the primary metric for evaluating the effectiveness of the biodegradation process.

After completing the experiments, all waste materials, including residual plastics and organic matter, were sterilized using an autoclave machine. The autoclave effectively neutralized any biohazard risks by subjecting the materials to high pressure and temperature. The sterilized waste was sorted and disposed of following established waste management protocols. This ensured environmental safety and compliance with laboratory guidelines.

4. Results and Discussion

This section presents the findings of the study on the biodegradation of polystyrene (PS) shown in Figure 1 and other plastics by *Zophobas morio* larvae. The results are analyzed and discussed based on experimental parameters, including temperature, diet, and exposure duration. The subtopics include the determination of optimal conditions for biodegradation, the effectiveness of larvae diets, and comparative analysis across different scenarios.

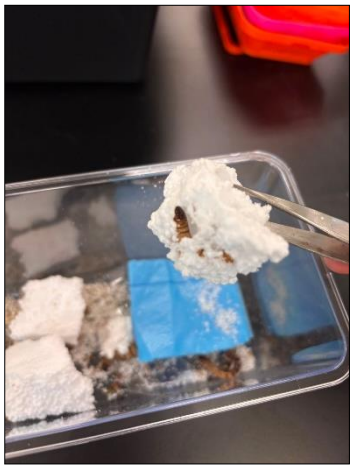

***Fig. 1*:** Biodegradation of Larvae towards Polystyrene

4.1 Optimization of Time Period

The study explored 7 days interval shown in Table l, to determine the optimal duration for biodegradation. Results showed that the degradation of polystyrene was more pronounced after 7days, with a lower reduction in the weight of plastic samples compared to the 10-day and 13-day period. This trend was consistent across all test groups, suggesting that longer exposure allowed the larvae to exhibit sustained enzymatic activity. Additionally, the health of the larvae remained stable during the extended period, confirming their adaptability to prolonged exposure to polystyrene. These findings highlight the importance of time as a critical factor in maximizing the efficiency of plastic biodegradation by superworms.

***Table 1*:** Optimization of Time Period

Food intake	Sample	Optimization Quantity / (ZM)	Quantity / g (food)	Period of monitoring/day	Initial weight / g	Final weight / g	Rate, %	mean %
Vegetables	1	10	5	7	5.0038	0.1673	96.66	97.5
	1		5	7	5.0003	0.0845	98.31	
Polystyrene (PS)	1	10	5	7	5.0003	4.0641	18	15.31
	2		5	7	5.0124	4.38	12.62	
High Density Polystyrene (HDPE)	1	10	5	7	5.0136	4.8912	2.18	1.43
	2		5	7	5.0116	4.968	0.68	
Low Density Polystyrene (LDPE)	1	10	5	7	5.0003	4.902	2.23	1.62
	2		5	7	5.0018	4.9616	1	
No Food (Control)	1	10	5	7	blank	blank	blank	blank
	2		5	7	blank	blank	blank	

4.2 Pilot Scale for Different Time Period

The pilot-scale experiments revealed that *Zophobas morio* larvae exhibited optimal performance in degrading plastics at laboratory temperatures of 21°C–23°C shown in Table 2 and 3. Over both the 10-day and 13-day intervals, significant weight reductions in polystyrene (PS) samples were observed, indicating efficient biodegradation. The cooler temperature range likely enhanced the metabolic activity of the larvae, facilitating greater enzymatic efficiency in breaking down the plastic polymers. Additionally, the larvae maintained stable

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health throughout the experiments, suggesting that the laboratory temperature provided an ideal environment for sustained biodegradation activity.

Further analysis demonstrated that extending the exposure period from 10 to 13 days amplified the degradation efficiency. This aligns with previous studies suggesting that longer durations allow for more prolonged enzymatic action, leading to higher plastic weight loss. The consistent results across all test groups underscore the significance of maintaining laboratory conditions for optimal biodegradation performance. These findings emphasize the potential of controlled environments in leveraging biological agents like Zophobas morio for plastic waste management.

Table 2: Pilot Scale for 10-Days in Laboratory Temperature

Pilot Scale for 10-Days								
Food intake	Sample	Quantity / (ZM)	Quantity / g (food)	Period of monitoring/day	Average Initial weight of food/ g	Final weight of food / g	Rate of food, %	Mean %
Vegetables	1	10	5	10	5.0031	0	100.00	100.0
	2		5	10	5.0113	0	100.00	0
Polystyrene (PS)	1	10	5	10	5.0012	3.5401	29.21	26.87
	2		5	10	5.004	3.7765	24.53	
High Density Polystyrene (HDPE)	1	10	5	10	5.0138	4.9716	0.84	1.63
	2		5	10	5.0004	4.88	2.41	
Low Density Polystyrene (LDPE)	1	10	5	10	5.0064	4.8913	2.30	2.40
	2		5	10	5.0075	4.887	2.41	
Mix	1	10	5	10	5.0116	2.967	40.80	40.40
	2	10	5	10	5.0048	3.0044	39.97	
No Food (Control)	1	10	5	10	blank	blank	blank	blank
	2		5	10	blank	blank	blank	

Table 3: Pilot Scale for 13-Days in Laboratory Temperature

Pilot scale for 13 days								
Food intake	Sample	Quantity / (ZM)	Quantity / g (food)	Period of monitoring/day	Initial weight of food / g	Final weight of food / g	Rate of food, %	mean, %
Vegetables	1	10	5	13	5.0231	0	100.00	100.0
	2		5	13	5.0125	0	100.00	0
Polystyrene (PS)	1	10	5	13	5.0006	2.967	40.67	40.40
	2		5	13	5.0036	3.0004	40.04	
High Density Polystyrene (HDPE)	1	10	5	13	5.0178	4.907	2.21	2.40
	2		5	13	5.009	4.88	2.58	
Low Density Polystyrene (LDPE)	1	10	5	13	5.0082	4.8617	2.93	2.50
	2		5	13	5.0591	4.9546	2.07	
Mix	1	10	5	13	5.0109	2.7316	45.49	43.43
	2	10	5	13	5.0013	2.921	41.60	
No Food (Control)	1	10	5	13	blank	blank	blank	blank
	2		5	13	blank	blank	blank	

In contrast, experiments conducted at room temperature (28°C–30°C) shown in Table 4 and 5 a comparatively slower degradation rate of polystyrene by the larvae. The higher temperature appeared to introduce stress on the larvae, as indicated by reduced activity levels and a slight decline in overall health during the 13-day period. While biodegradation still occurred, the efficiency was notably lower than that observed under laboratory conditions. This suggests that the larvae's enzymatic processes may be less effective at higher temperatures, potentially due to thermal stress on the gut microbiota responsible for polymer breakdown.

Interestingly, the difference in degradation efficiency between the 10-day and 13-day intervals at room temperature was less pronounced compared to laboratory conditions. This could indicate that extended exposure at higher temperatures does not provide significant additional benefits, likely due to the reduced metabolic performance of the larvae under these conditions. These results reaffirm the critical role of temperature in influencing the efficiency of plastic biodegradation and highlight the need for optimizing environmental conditions to maximize the larvae's performance.

***Table 4*:** Pilot Scale for 10-Days in Room Temperature

Food intake	Sample	Quantity / (ZM)	Pilot scale for 10 days Quantity / g (food)	Period of monitoring/day	Initial weight of food / g	Final weight of food / g	Rate of food, %	mean, %
Vegetables	1	10	5	10	5.0243	0	100.00	100.00
	2		5	10	5.0166	0	100.00	
Polystyrene (PS)	1	10	5	10	5.0013	3.965	20.72	21.47
	2		5	10	5.0046	3.893	22.21	
High Density Polystyrene (HDPE)	1	10	5	10	5.0178	4.9916	0.52	0.40
	2		5	10	5.001	4.9875	0.27	
Low Density Polystyrene (LDPE)	1	10	5	10	5.0077	4.8997	2.16	1.12
	2		5	10	5.0006	4.9964	0.08	
Mix	1	10	5	10	5.0014	3.1154	37.71	35.87
	2	10	5	10	5.0011	3.2987	34.04	
No Food (Control)	1	10	5	10	blank	blank	blank	blank
	2		5	10	blank	blank	blank	

***Table 5*:** Pilot Scale for 13-Days in Room Temperature

Food intake	Sample	Quantity / (ZM)	Pilot scale for 13 days Quantity / g (food)	Period of monitoring/day	Initial weight of food / g	Final weight of food / g	Rate of food, %	mean, %
Vegetables	1	10	5	13	5.0421	0	100.00	100.00
	2		5	13	5.0003	0	100.00	
Polystyrene (PS)	1	10	5	13	5.0003	3.3006	33.99	34.09
	2		5	13	5.004	3.2931	34.19	
High Density Polystyrene (HDPE)	1	10	5	13	5.0005	4.9504	1.00	1.15
	2		5	13	5.0096	4.9446	1.30	
Low Density Polystyrene (LDPE)	1	10	5	13	5.0004	4.9733	0.54	1.70
	2		5	13	5.0013	4.8587	2.85	
Mix	1	10	5	13	5.0109	2.954	41.05	40.85
	2	10	5	13	5.0013	2.9679	40.66	
No Food (Control)	1	10	5	13	blank	blank	blank	blank
	2		5	13	blank	blank	blank	

4.3 Effect of Different Temperature

Temperature emerged as a pivotal factor in the study. Tests conducted at 21°C–23°C demonstrated the highest biodegradation efficiency, with the larvae showing robust activity and minimal weight loss themselves. At 28°C–30°C, degradation was less effective, with signs of reduced activity among the larvae. The results align with existing literature, which suggests that *Zophobas morio* larvae thrive in cooler environments, where their enzymatic processes are optimized. These findings underline the importance of maintaining suitable temperature ranges to enhance the effectiveness of biological plastic degradation.

4.4 Weight of Plastic Degraded

The measurement of degraded plastic weight after the experiments of 13 days provided a direct quantification of degradation efficiency. Across all test groups, plastics exposed to larvae at 21°C–23°C and fed a mixed diet showed the greatest weight loss, indicating effective degradation. In contrast, plastics subjected to room temperature and a single diet type retained more weight, highlighting the combined impact of temperature and diet on biodegradation. These weight retention values serve as a robust metric for evaluating the efficiency of *Zophobas morio* larvae in breaking down different plastic types.

4.5 Effect of Diet on Degradation

The larvae's diet played a significant role in influencing the degradation process. Figure 2 and 3 show the result of mixed diets, consisting of polystyrene and vegetable scraps, yielded the highest degradation rates. The availability of additional nutrients in the vegetables appeared to enhance the larvae's overall metabolic activity, enabling them to process the plastics more effectively. Single-diet groups, fed exclusively on polystyrene, exhibited slower degradation rates, likely due to the lack of complementary nutrients. This highlights the importance of supplementing plastic diets with organic matter to optimize biodegradation *by Zophobas morio* larvae.

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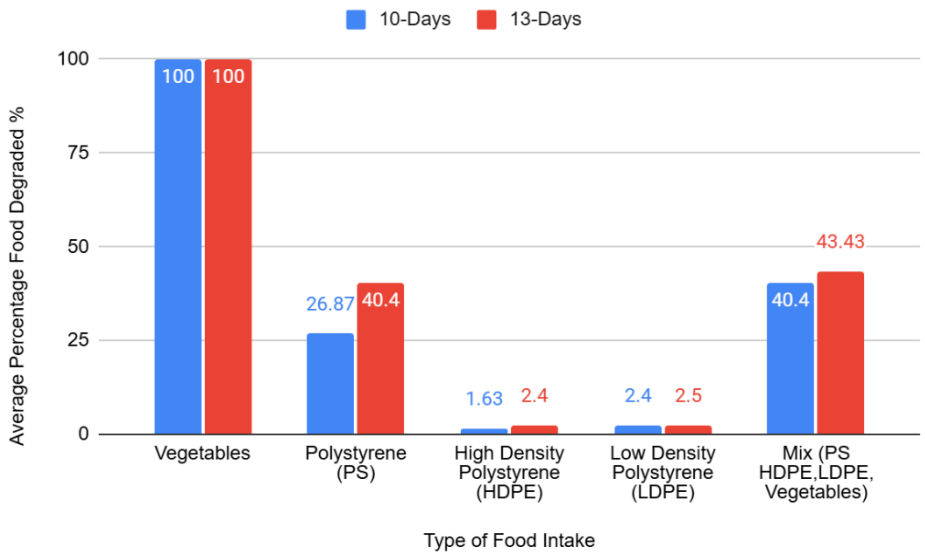

Fig. 3: Comparison between Food Degraded in Laboratory Temperature (21°C–23°C)

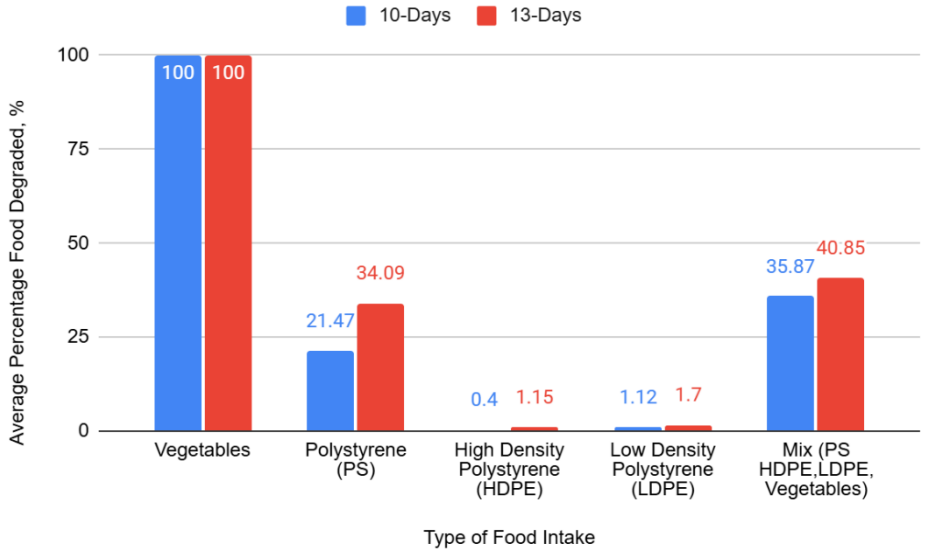

Fig. 3: Comparison between Food Degraded in Room Temperature (28°C–30°C)

4.6 Rate of Food Degraded Per Day

The rate of food degradation per day was calculated to assess the efficiency of Zophobas morio larvae in processing different substrates, including polystyrene (PS), high-density polyethylene (HDPE), low-density polyethylene (LDPE), vegetables, and a mixed diet. The data revealed that the larvae demonstrated higher degradation rates at laboratory temperatures (21°C–23°C) compared to room temperatures (28°C–30°C). This indicates that cooler, controlled conditions promote the larvae's metabolic activity and enzymatic efficiency, particularly when fed a mixed diet. The highest rates were observed with vegetables, followed by mixed diets, while plastics alone (PS, HDPE, and LDPE) exhibited relatively lower degradation rates.

***Table 6*:** Rate of Food Degraded Per Day (mg/day/worm)

Food intake	Sample	Rate of Food Degraded, mg/day/worm				
		Quantity of ZM	Lab Temperature		Room Temperature	
			10 Days	13 Days	10 Days	13 Days
Vegetables	1	10	0.0500	0.0502	0.0502	0.0504
	2	10	0.0501	0.0501	0.0502	0.0500
Polystyrene (PS)	1	10	0.0146	0.0203	0.0104	0.0170
	2	10	0.0123	0.0200	0.0111	0.0171
High Density Polystyrene (HDPE)	1	10	0.0004	0.0011	0.0003	0.0005
	2	10	0.0012	0.0013	0.0001	0.0006
Low Density Polystyrene (LDPE)	1	10	0.0012	0.0015	0.0011	0.0003
	2	10	0.0012	0.0010	0.0011	0.0014
Mix (PS, HDPE, LDPE, Vegetables)	1	10	0.0204	0.0228	0.0189	0.0206
	2	10	0.0200	0.0208	0.0170	0.0203

The table provided summarizes the degradation performance across different conditions. For laboratory temperatures, the degradation of vegetables reached a rate of approximately 0.0504 mg/day/worm, while PS degradation peaked at 0.0200 mg/day/worm when larvae were fed a mixed diet. Comparatively, under room temperature conditions, the degradation rates were slightly lower across all samples. For instance, vegetable degradation decreased to 0.0502 mg/day/worm, while PS showed a maximum rate of 0.0171 mg/day/worm with a mixed diet. These results highlight the importance of maintaining optimal environmental conditions to maximize biodegradation performance. The mixed diet proved particularly effective in enhancing the larvae's ability to process plastics, likely because the presence of food waste provided additional nutrients required for optimal metabolic function. This combination could improve the efficiency of biological waste management systems. The findings also reinforce the notion that integrating biodegradable food waste with plastics can serve as a viable approach to addressing plastic waste pollution while minimizing larval stress and maintaining productivity. Moreover, the lower degradation rates observed for plastics such as HDPE and LDPE compared to PS suggest that certain polymers may pose more challenges for microbial and enzymatic breakdown in the larvae's gut. This aligns with previous studies that indicate variations in the chemical structure and density of plastics significantly affect degradation rates. The need for further research into the mechanisms underlying these differences is crucial to refining and optimizing the use of *Zophobas morio* in practical waste management applications.

5. Conclusion

In conclusion, this study explored the potential of *Zophobas morio* superworms for polystyrene biodegradation by examining the optimum temperature, comparing the degradation efficiency of various plastics (PS, HDPE, and LDPE), and evaluating the degradation rate in relation to daily food waste intake at two temperatures. The results indicated that larvae demonstrated the highest polystyrene degradation efficiency at 25°C to 30°C, corresponding to optimal conditions for their gut microbiota's enzymatic activity. This temperature range enhances the larvae's metabolic processes, leading to effective polystyrene breakdown. Comparatively, *Zophobas morio* larvae degraded polystyrene more efficiently than HDPE and LDPE. Additionally, the degradation rate was influenced by the larvae's diet and environmental temperature, with a mixed diet improving retention, survival, and biodegradation efficiency. A balanced diet is crucial for sustaining larval colonies and preventing cannibalism, emphasizing the need for optimized rearing conditions. These findings demonstrate the potential of *Zophobas morio* larvae as a biological solution for mitigating plastic waste, especially polystyrene pollution.

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Conflict of Interest

Authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

This journal requires that all authors take public responsibility for the content of the work submitted for review.

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