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Polystyrene Consumption by Yellow Mealworm Tenebrio Molitor Larvae with and without Natural Food

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ABSTRACT: The yellow mealworm *Tenebrio molitor* larva has the aptitude to consume and degrade certain types of plastic waste. This study the biodegradation potential of *Tenebrio molitor* larvae toward polystyrene (PS) was evaluated under three feeding treatments. Larvae were provided with PS alone, PS supplemented with oats, and oats alone as the larval control. An additional plastic-only control containing PS without larvae was maintained under the same experimental conditions to determine weight loss caused by abiotic factors, over 40 days of experiment time Efficiency was assessed by measuring the plastic mass loss every 10 days, performing Fourier transform infrared (FTIR) spectroscopy on the polystyrene in the larval frass, and monitoring larval survival, growth, and weight changes. The results showed that the larvae fed polystyrene with added oats achieved the highest biodegradability rate of 78%, while the biodegradability rate of the larvae fed polystyrene alone did not exceed 30.5 %. FTIR analysis shown chemical changes occurred in the polystyrene structure following exposure to *T. molitor* larvae, as indicated by the appearance or increase of carbonyl (C=O) absorption bands and changes in the hydroxyl (O–H) region, suggesting oxidation and modification of the polymer chains. The biodegradation of polystyrene within the digestive tract of larvae *T. molitor* cusses Changes in the polymer's chemical structure and the survival rate of larvae that fed on plastic was high, and the mortality rate was almost nonexistent, approaching the control coefficient of approximately 98%. . These results highlight the role of supplementing mealworm larvae's diet in increasing their consumption of polystyrene, and contribute to identifying methods to enhance the larvae's efficiency in disposing of plastics as biodegradable and environmentally friendly agents.

INTRODUCTION

The increased interest in the development of industries and production has led to an increase in the production and consumption of plastic materials used in packaging. Plastic waste increased correspondingly. Plastics are inherently resistant to degradation and poor waste management has resulted in accumulation and persistence of plastics in terrestrial and aquatic ecosystems (Geyer et al., 2017). Environmental weathering has also been effect to further degrade plastic debris into smaller particles like (microplastics 1 µm–5 mm) and (nanoplastics <1 µm) that are widely distributed in all terrestrial, freshwater and marine environments (Dube and Okuthe, 2023). They are persistent and they interact with contaminants in the environment, increasing their ecological potential impacts (Singh and Tiwari, 2025). Such danger underscore the need for effective strategies to reduce plastic accumulation and to promote the biological degradation of synthetic polymers.

Recent research studies have shown that some species of insects They consume and contribute to the process degradation of, synthetic polymers. Yang et al. (2015) demonstrated that larvae of the yellow mealworm, *Tenebrio molitor*, could feed on polystyrene (PS). Later studies revealed the possibility of gut microorganisms' involvement in the transformation of plastics (Yang et al., 2018; Brandon et al., 2018; Przemenecki et al., 2020). Moreover, Galleria mellonella studies revealed weight loss and chemical modifications in PS and polyethylene as identified using FTIR following larval exposure (Khlaif and Abdulhay, 2023; Abbas and Abdulhay, 2024). These findings support the potential of insect plastivores in plastic biotransformation. However, only few studies have directly compared PS consumption of T. molitor larvae in presence and absence of a natural food supplement under the same experimental conditions.

Thus, the present study evaluated PS ingestion by *T. molitor* larvae in three different feeding conditions, PS alone, PS + wheat bran, and wheat bran alone. A plastic-only control was maintained under identical environmental conditions without larvae to account for weight changes due to abiotic factors. The experiment lasted for 40 days and the measurements were performed every 10 days. Larval survival and growth, PS weight loss, and frass production were evaluated under the different feeding conditions. The chemical structure of PS after exposure to larvae was characterized by the Fourier-transform infrared spectroscopy (FTIR). The present study compares the consumption of PS by *T. molitor* under different feeding situations and assesses the possible contribution of larval activity to PS transformation..

2. MATERIALS AND METHODS

2.1 Insect Rearing

The *T. molitor* larvae were obtained from commercial insect breeders. The larvae were morphologically identified and confirmed as yellow mealworms *T. molitor* by the Department of Entomology and Invertebrates, Iraqi Natural History Research Center and Museum, University of Baghdad. The larvae were reared under laboratory conditions to obtain a generation with uniform age and size. experment Larvae were fed oats and carrot slices (in small amount) supplied as moisture sources (Rovai *et al.,* 2021) and kept in plastic containers under controlled laboratory conditions at 25–28 °C, 60–75% relative humidity, and a 16:8 h light/dark cycle (Ribeiro et al., 2018; Eberle *et al.,* 2022; Mamtimin *et al.,* 2023).

2.2 Preparation of the Plastic Substrate

In this investigation, varieties of polystyrene (PS) plastics were employed from plastics collected in the study from disposable food plates, available in local supermarkets. The plates purchased were instantly transferred to the laboratory and maintained under sterile conditions. The plastic was cut into small sheets with a length of 6 cm and width of 3 cm and weighted according to the treatment. The plastic pieces were disinfected by washed with alcohol70% then and dried UV radiation in a laminar flow for 30 min (Nicolaou *et al.*, 2021). The aim of using this procedure was to prevent destruction of the polystyrene structure by standard sterilizing methods like washing or steam sterilization.

2.3 Experimental Design

This study aimed to evaluate the capacity of yellow mealworm T. molitor, larvae to eat and biodegrade polystyrene (PS) and to assess their survival during exposure to this plastic in laboratory conditions. The larvae were weighed equally and starved for 24 h before being transferred into rectangular plastic containers of 15 cm length x 10 cm width x 5 cm height. Each container was considered an experimental unit in the study.

In the experiment, a total of 150 *T. molitor* larvae were used, with 50 larvae per replicate, in three replicates. Larvae with similar development stages, body weight and length were selected to minimize variation between the experimental groups. Larvae were randomly assigned to three feeding treatments.

In the treatment T1, larvae were provided with 0.5 g of polystyrene (PS) as the sole substrate. In treatment T2, larvae were fed 0.25 g of PS combined with 0.25 g of oats, which is an equal mass mixture of PS and natural food source. Larval feeding control (T3): 0.5 g of oats were given to the larvae. All treatments were kept under the same experimental conditions and contained 50 larvae per replicate.

Beside the larval treatments, a plastic-only control (PC) containing 0.5 g of PS but no larvae was also maintained under the same environmental conditions. This control was used to measure any changes in PS mass caused by abiotic factors during the experimental period.

It was a 40 day trial. The larvae and plastic pieces were weighted before the experiment started. The larvae were reared under same rearing conditions during the trial period. The plastic fragments were cleaned and weighed every 10 days and 0.5 g of polystyrene was added after each sampling.

The dead larvae and shed skins were immediately removed from the experiment during the course of the experiment. Larval survival and body weight were measured every 10 days to assess changes over time with the plastic diet and compared to the control treatment. All tests were performed in duplicate.

2.4 Weight Loss Measurements

The weight loss mass was recorded on day 10, 20, 30 and 40 from the start of the experiment. The total weight and percentage of each variable were also measured. Additionally, larvae were weighed every 10 days and the cumulative weight of larvae, plastic and excrement was recorded. Larval activity was observed at intervals throughout the experiment.

2.5 Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

Polystyrene samples collected before and after larval exposure, together with frass samples produced during larval feeding, were analyzed using Fourier-transform infrared spectroscopy (FTIR) with a spectrometer equipped with an attenuated total reflectance (ATR) module. Spectra were recorded over the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1} using 32 scans per sample. The analysis focused on characteristic absorption bands associated with C–H stretching vibrations (2950–2840 cm^{-1}), C=O stretching vibrations (1700–1750 cm^{-1}), and C–H bending vibrations (1460–1370 cm^{-1}) (Przemenecki et al., 2018). FTIR analysis was performed to identify changes in the chemical structure of PS following larval exposure and to assess evidence of polymer modification associated with larval feeding and gut microbial activity.

2.6 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0. Data were expressed as mean ± standard deviation (SD) based on three independent replicates. The assumptions of normality and homogeneity of variance were assessed before analysis. Differences among the experimental treatments were evaluated using one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's honestly significant difference (HSD) test was used for multiple pairwise comparisons. Statistical significance was set at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect of dietary supplementation and polystyrene ingestion

The results showed significant differences in the mass loss of polystyrene between the dietary treatments. The greatest percentage loss of polystyrene was observed in larvae fed polystyrene with oats (78.19 ± 9.55% after 40 days), while the loss was lower (30.47%) in larvae that were fed polystyrene alone. Analysis of variance revealed significant differences among treatments indicating the composition of the diets affected consumption of polystyrene by Tenebrio molitor larvae. The higher mass loss recorded in the polystyrene–oat treatment suggests that nutritional supplement can improve larval feeding activity and thus increase the exhaustion of the polymer.

The effect of oat supplementation may be related to the nutritional requirements of the larvae. Larval growth, metabolism and feeding behaviour are influenced by dietary nutrient composition (Onincx et al., 2015). Polystyrene is not a very nutritious food and if it is the sole source of food, it cannot provide the nutrients that are necessary for normal growth and metabolic activity. The oats may have supplied the necessary carbohydrate, protein and other nutrients for maintenance and activity, which enabled the larvae to continue feeding activity throughout the experimental period. This is in agreement with previous results showing that mealworm larvae fed a nutritionally limited diet exhibited reduced development and survival compared to larvae fed a nutritionally balanced diet (Matyja et al., 2020).

The gravimetric results were supported by visible changes in the polystyrene samples. Larval feeding produced pits, cracks and cavities on the polymer surface, and these changes increased during the experimental period. The surface alterations were more pronounced in the polystyrene–oat treatment than in the polystyrene-only treatment, which is consistent with the greater mass loss recorded in the supplemented treatment. These observations indicate that the increased polymer loss was accompanied by physical alteration of the polystyrene surface. However, surface damage and mass loss alone cannot distinguish mechanical fragmentation from true polymer biodegradation. Additional evidence from chemical and structural analyses is therefore required to confirm polymer transformation.

Moreover, the observed mass loss may also be related to a higher susceptibility of the polystyrene to fragmentation by mechanical stress. Low density and the physical structure of expanded polystyrene provide a large surface area and may promote fragmentation during larval feeding. Additionally, the microorganism associated with the larval gut or polymer surface may be involved in the transformation of fragmented polymer particles. Microbial colonization can enhance surface modification and may contribute to the following degradation processes. Therefore, the greater mass loss observed under oat supplementation may reflect the combined effects of increased larval feeding, mechanical fragmentation and microbial activity.

The use of an abiotic polystyrene control improved the interpretation of the experimental results. No detectable change in polymer mass was observed in the control during the 40 day experiment. This result indicates that the experimental environmental conditions were not responsible for measurable non-biological mass loss of polystyrene. Thus, the more significant reduction in polymer mass in the larval treatments was attributed to larval exposure and feeding rather than environmental conditions.

Overall, the results suggest an association between dietary composition and mass loss of polystyrene by *T. molitor* larvae. Oats addition lead to a greater depletion of polystyrene than polystyrene alone. The mixed diet resulted in the greatest mass loss after 40 days. The results confirm the potential of *T. molitor* larvae in the laboratory-scale processing of polystyrene waste. However, further analyzes of the polymer chemistry, degradation products and microbial activity are required to determine the extent to which the observed mass loss is true biodegradation, rather than mechanical fragmentation and ingestion.

Table 1. Effect of oat supplementation on polystyrene consumption and biodegradation by Tenebrio molitor larvae

Tretment	Description	Initial plastic weight (g)	Final plastic weight (g) Mean ± SD	Weight loss (%) after 40 day	Degradation Rate (mg/day)[c]
T1	PS + Larvae	0.50	0.3477 ± 0.0078	30.47%	3.81 ± 0.10[a]
T2	PS + Oat+ Larvae	0.25	0.0545 ± 0.0239	78.19%	4.60 ± 0.18[b]
T3	Larvae +Oat	(Control)			—
T4	PS Only (Abiotic Control)	0.50	0.4978 ± 0.0031[a]	0.44[a]	0.06 ± 0.01[a]

Values are presented as mean ± SD (n = 3). Different superscript letters indicate a significant difference between treatments according to Welch's independent-samples t-test ($P < 0.05$)

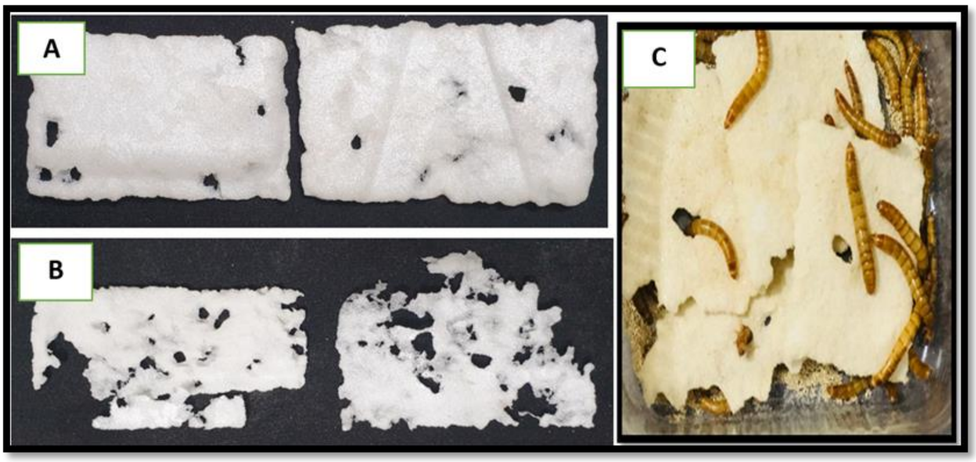

Figure (1). Yellow mealworm *T. molitor* larval consumption polystyrene

A-Polystyrene after consumption, in, 40 day T 1: larvae + polystyrene

B- Polystyrene pieces after consumption, , in 40 day T 2: larvae + polystyrene + oats

C - Larvae with polystyrene

3.2 Larval Survival and Biomass Changes

Survival of larvae was 98% during the 40-day test period for all treatments, which suggests that polystyrene did not have acute toxic effects under the experimental conditions. Both regimens of polystyrene feeding resulted in a decrease in larval weight. The highest weight loss was found in larvae fed on polystyrene alone (27.82%), while larvae fed on polystyrene supplemented with oats showed a lower weight loss (23.02%), suggesting that the supplementation with oats partially compensated the nutritional limitations of a diet based on plastic only (Table 2).

Table (2) Initial larval weight (ILW), final larval weight (FLW), weight change (Δ), and percentage weight change (%) of *T.molitor* larvae under different feeding treatments.

T	Description	Initial larval weight (g)	Final larval weight (g)	change (g) Larval weight	Larval weight change (%)
T1	PS + N	2.6593 ± 0.0863	1.9194 ± 0.0620	0.7399 a	27.82
T2	PS + Oat	2.3868 ± 0.1689	1.8381 ± 0.1450	0.5487 a	23.02
T3	Oat(Control)	2.4232 ± 0.3413	2.5329 ± 0.2204	−0.1097 a	−4.53

A reduction in larval body weight was observed throughout the experimental time ; however, the difference between the two dietary treatments was limited. This result indicates T. molitor larvae were able to maintain their body condition during long exposure to polystyrene in the experimental condition. Moreover, other studies have also reported that exposure or ingestion of polystyrene does not always result in significant larval mortality, although larvae fed plastic as a sole food source may have reduced growth due to the poor nutritional value of the polymer (Bozek et al., 2017; Brandon et al., 2018; Yang et al., 2015, 2021).

Larvae receiving oat supplementation retained more weight than those receiving polystyrene alone. This difference may be associated with the extra nutrients provided by oats, which could support larval maintenance and feeding activity while ingesting polystyrene. However, the present data do not allow a direct assessment of the changes in metabolism and therefore the observed weight response should be interpreted in relation to dietary supplementation and not be attributed to an enhanced metabolism.

The higher loss of polystyrene mass in the oat-supplemented treatment also indicates that the presence of a nutrient source can affect larval consumption of polystyrene. This effect may be due to the ability of larvae to continue feeding when nutritional requirements are partially met by a standard food source. The relatively low amount of oat supplementation in the present experimental design also limits the extent to which the nutritional effect can be assessed. Further experiments with different quantities and types of dietary supplementation could establish whether increased nutrient availability produces a dose dependent response in polystyrene consumption.

Future studies should further investigate the interaction between dietary nutrients and gut microbiota of T. molitor. In particular, the identification of specific nutritional conditions that support the growth and activity of polymer-associated or gut microorganisms may help to clarify the contribution of microbial processes to polystyrene transformation. Such experiments could help lay the ground for optimizing nutritional conditions for the improvement of insect-mediated plastic processing.

3.3 FTIR Spectroscopy

Comparative Analysis of Biodegradation Products of Polystyrene, and the Natural Diet (Oats) in the Feces of Yellow mealworm Larvae *T. molitor*

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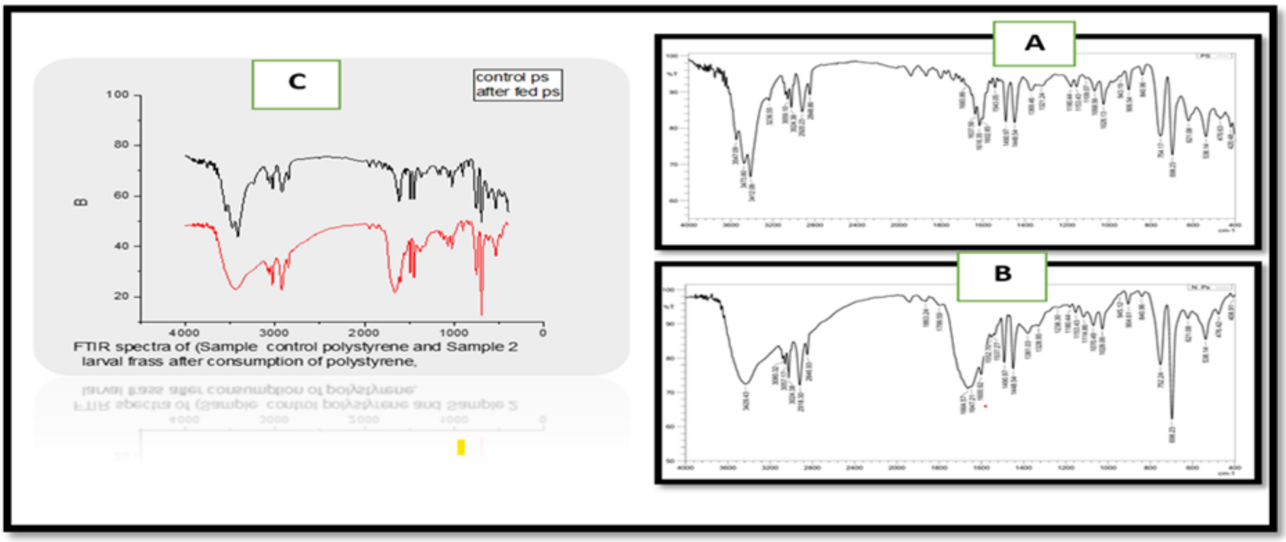


Figure (2). Fourier transform infrared (FTIR) spectra of polystyrene before biodegradation (A) and larval excrement after polystyrene consumption by yellow mealworm larva *T.molitior* (B). (C) shows the superimposed spectra for comparative analysis.

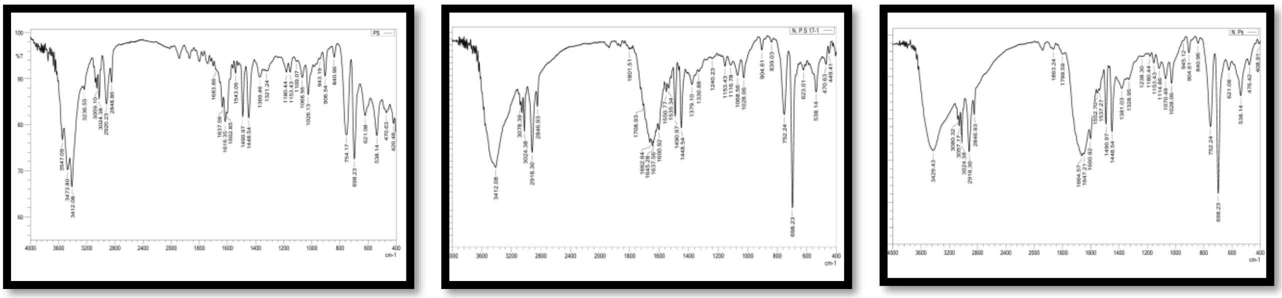
In this study, we offer a complete comparative spectroscopic examination by Fourier transform infrared (FTIR) spectroscopy of the feces of yellow mealworm larvae *T. molitor* comparing a control model of larvae fed naturally on oats and larvae fed on polystyrene. The spectroscopic data showed significant chemical changes in the frass of larvae fed on both polystyrene vs. baseline sample. Such spectrum alterations are indicative of oxidative modification and partial disintegration of the polymers during their passage through the larval digestive tract. Fourier transform infrared spectroscopy (FTIR) was used to assess chemical changes in the feces of oat-fed yellow mealworm larvae *T. molitor*, naturally fed oat larvae (NF) and polystyrene (PS). Spectroscopic measurements were made at two time intervals during the course of the experiment, i.e. 10 days after the start of the experiment and 40 days after the end of the trial to evaluate temporal changes in the composition of functional groups associated with polymer intake. Feces of larvae fed oat (NF) served as a baseline representing biological digestive products in the absence of synthetic polymers. Its spectra showed characteristic bands of biological materials, such as a large stretch band for O-H and N-H bonds in the 3400-3450 cm-1 region, C-H bond stretch vibrations at 2900-2960 cm-1, and amide I and amide II bands associated with proteins about 1650 and 1540 cm-1, respectively. The spectrum characteristics were quite stable in time, suggesting that no polymer-induced chemical changes occurred.

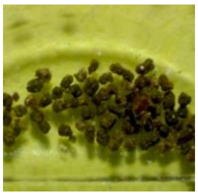
In contrast, the spectrum changes in the feces of larvae given PS were more gradual and were most notable in the regions linked with oxygen functional groups. The band of the O-H bond stretch at 3400-3450 cm -1 was increased and grew more apparent between day 10 and day 40 indicating the development of hydroxyl containing oxidation products during polymer processing in the larvae's stomach. Similar increases in the hydroxyl group absorption bands were seen in insect-mediated plastic degradation systems, where oxidation processes are a key initial step in polymer transformation (Yang et al., 2021; Brandon et al., 2022).

The most significant alterations were observed in the carbonyl region (1700-1720 cm−1) with broader carbonyl absorptions for the polystyrene residues (1664-1647 cm−1). These bands increased in strength with time showing that oxidized intermediates such as ketones, aldehydes and carboxylic acid derivatives are formed gradually. The presence of functional groups containing carbonyl is a well-known indication for the degradation of polymers by oxidation and partial chain scission (Gewert et al., 2015; Yuan et al., 2020). Weak spectral characteristics were identified in the high wavelength carbonyl region (1799–1863 cm^{-1}) of polystyrene feces. These features were faint at first but became more prominent in the spectra taken towards the end of the experiment. Due to their low intensity, they were identified as minor secondary oxidation products and not as dominating functional groups (Fenton et al., 2018) as seen in figure (5)

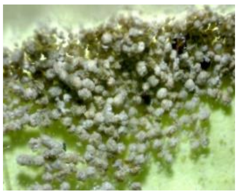
The spectral complexity of the C–O bond expansion region (1000–1250 cm-1) was increased in both polystyrene feces in comparison with the control sample. Multiple overlapping bands suggested the presence of oxidizing agents including alcohols, ethers and esters. The increase in complexity of the bands over time is consistent with oxidative alteration and partial breaking of the polymer chain in the gut of the larva. Weak spectral patterns were detected in the high wavelength carbonyl region (1799–1863 cm-1).

Overall, the Fourier transform infrared (FTIR) spectroscopy data suggested that polystyrene underwent oxidative alteration in a time-dependent manner during digestion in yellow mealworm *T. molitor* larvae. The increasing rise of the related hydroxyl and carbonyl bands from day 10 to day 40 suggests that biodegradation happened via progressive oxidation and partial cleavage of the polymer chain rather than complete mineralization.





Sample 1



Sample2

Sample 3

Figure (3). Fourier transform infrared (FTIR) spectra of frass of larvae normally fed on oats (Sample 1) Excrement; (Sample 2) Excrement of larvae after 10 days of polystyrene consumption; (Sample 3) Excrement of larvae after 40 days of polystyrene consumption.

CONCLUSION

In this study, biological breakdown of polystyrene in yellow mealworm Tenebrio molitor larvae was studied in a 40-day period by mass loss measurements and Fourier transform infrared (FTIR) spectroscopy. The study looked at the difference between those fed just on polystyrene larvae and groups given on a diet of polystyrene and oats in equal percentages. The results indicated the significant degradation of polystyrene by yellow mealworm *T. molitor* larvae. When the polymer was mixed with oats in the usual feed the polystyrene mass loss was enhanced. Hence the plastic consumption and breakdown efficiency was substantially improved. Thus, the nutritional supplement has a good effect on the metabolic activity of the larvae and the overall biodegradability performance.

The FTIR analysis evidenced a clear chemical modification of the polystyrene residues, with a progressive formation of functional groups containing oxygen. The increase with time of the hydroxyl (O-H) and carbonyl (C=O) bands suggests that the degradation occurs by the oxidation shift and partial rupture of the polymer chains. The large change in the spectrum from day 10 to day 40 shows that the process is time dependent and not instantaneous.

The data shown above suggest that *T. molitor* larvae could be used as a biological tool for the reduction of plastic waste, especially if paired with natural nutritional supplements. This work underlines the necessity of the integration of biological and nutritional aspects for improving the efficiency of insect based polymer breakdown systems.

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