

Biodegradation of plastic wastes by confused flour beetle *Tribolium confusum* Jacquelin du Val larvae

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Abstract

As a consequence of increasing the production of plastics which accumulate substantial quantities of plastic wastes in the natural environment and in landfills that could persist for centuries. The ability of confused flour beetle *Tribolium confusum* larvae to consume and biodegrade different types of plastics were investigated. The experiment was performed by starving the larvae then exposed to three different types of plastic (polystyrene (PS), polyethylene foam (PE), and ethylene-vinyl acetate (EVA) as diet in comparison to larvae reared on the conventional diet of wheat flour. The larvae were monitored under controlled conditions then the survival rate, the mass losses for both larvae and plastics resulting from the diet on plastic as a function of time (1, 14, 21 and 30 days) were measured. The results showed that with the increase of time; the averages of larvae survival on all types of plastic were decreased compared with control. The highest larval survival rate was found in the PS diet (70%), while the least was with larvae fed on EVA (30%) after 30 days. Decreasing in the mass weight of the larvae was confirmed during the study, which indicates that plastic materials are not an efficient source of energy for larvae except their survival. The mass loss was 26.2, 31.4 and 45.8 % recorded for larvae fed on PS, PE, and EVA respectively. The study recorded that *T. confusum* has the ability to degradation plastic, which can reduce the pollution caused by different types of plastic wastes.

Keywords: *Tribolium confusum*, Biodegradation, Plastic waste, FTIR

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Introduction

Plastic waste is one of the most severe and common environmental problems in the world in this era. Increased use and slow biodegrading have led to accumulating plastic waste in the outdoor environment in large quantities, as parts of municipal solid waste also dispersed as “white pollutants”

(Cózar et al., 2014; Jambeck et al., 2015). The long term accumulations of non-biodegradable polymers lead to many ecological and health problems, especially the marine (Przybylinska and Wyszowski, 2016) and terrestrial environment (Zhao et al., 2016). Further, plastic and its chemical compounds might have considerable concern about the adverse effects of these chemicals on wildlife as well as human health



(Kobrosly et al., 2014). Today the environmental hazards and risks from these chemicals associated with the plastic products are diversified with significant efforts to minimize plastic pollution, by reducing the consumption of plastic and encourage plastic recycling.

Polystyrene (PS) commonly known as styrofoam, is made from the monomer styrene. It is a synthetic aromatic hydrocarbon polymer which can be solid or foamed (Scheirs and Priddy, 2003). PS is durable and inexpensive materials used in various products such as food preservation containers, lids, trays, bottles, and disposable cutlery and the making of models. Polyethylene foam (PE) is an ordinary plastic polymer, which has a high load-bearing capacity. It is used to protect sensitive electronic products and material as shock absorbing, vibration dampening, insulation, barrier or buoyancy component, and as a material for cushioning products in packaging applications. Ethylene-vinyl acetate (EVA) is a thermoplastic copolymer derived from petroleum known as expanded rubber or foam rubber. EVA is materials like rubber in flexibility and softness used as a shock absorber material; packaging, textile, metal surfaces, coated paper, and cement render (Coto et al., 2012).

Degradation of plastic can be defined as any chemical or physical change in the polymer due to the effect of environmental factors, such as heat, light, moisture, chemical conditions or biological activity (Kale et al., 2015). Recent studies have focused on the ability of microorganisms including certain bacteria and fungi in biodegradation of polymers through different exoenzymes responsible under stress conditions (Ahmed, 2018). While studies on the ability of insects to degrade plastics are very limited and constricted. Insect pests, including red flour beetles *Tribolium castaneum*, cigarette beetles *Lasioderma serricorne*, cadelle beetles *Tnebrioide mauretanicus*, *Zophobas morio*, etc. and several moths and their larvae, also may degrade different plastic packing materials (Newton, 1988; Bombelli et al., 2017). Thus, the present study designed to investigate the efficiency of confused flour beetle, *Tribolium confusum* to degrade three types of plastic (PS, PE, EVA).

Material and Methods

Insect collection

The larval populations of confused flour beetle were collected from infested wheat godowns into glass

containers for further rearing at a constant temperature of 30 °C and 70± 5% R.H in continuous darkness (Abdulhay, 2012). The adults were moved to a new rearing media every week for further experiments.

Plastic collection

Three types of plastics collected in the study:

-Polystyrene (PS): Collected from disposable food storage containers (PS no.6).

-Polyethylene foam (PE): Obtained from the remaining electrical equipment packaging, used for electrical appliances to protect during transportation.

-Ethylene-vinyl acetate: Collected from the plastic on the car doors to protect against shocks.

All the plastics were cut into small discs with a diameter of 1cm and a height of 1mm, washed with distilled water and left to dry for at least a single day.

Experimental procedure

To investigate the ability of flour beetle *T. confusum* larvae to live, feed and survival on plastics; larvae almost with similar mass were starved for 24h and then placed into small plastic tubes (3.5 cm diameter and 5 cm height) containing two plastic dices from the same type. The experiment was carried out in four groups with ten larvae specimens for each as follows:

-Larvae fed on polystyrene (PS).

-Larvae fed on polyethylene foam (PE).

-Larvae fed on ethylene-vinyl acetate (EVA).

-Larvae feed on wheat flour as a typical diet considered as control.

The larvae were fed with the three types of plastic as their sole diet for 30 days. Before every experiment, the larvae and plastic discs were weighted. All the larvae were kept under the same conditions of rearing and monitored for 30 days. During the experiments, dead larvae and molted skins were removed immediately, and the survival was counted and weighted each week to determine the mass losses resulting from larvae diet on plastic as a function of time, compared to control. All tests were carried out in triplicate for each test.

Chemical group analysis (FTIR)

Functional groups responsible for linking ions were investigated by using Fourier Transform Infrared spectrophotometers (FTIR-8400) Shimadzu (Japan). Peaks were identified after recording the spectra from the residual plastics before and after larvae feeding.



Statistical analysis

The results were analyzed according to the complete random design of the experiments. All data were performed using one-way analysis of variance (ANOVA) and the least significant differences (LSD).

Results and Discussion

Measurement the survival ratio of *T. confusum* larvae

Based on data shown in table (1); we can find that *T. confusum* larvae fed on the three types of plastics: polystyrene (PS), polyethylene foam (PE), and ethylene-vinyl acetate (EVA) during one month. In general, the number of larvae did not change in the first week. While increasing in time; the average of larvae numbers for all types of plastics decreased compared to control. After 30 days from the beginning of experiments, the number of survival *T. confusum* larvae fed on PS, PE, and EVA were 70 ±4.0, 50 ±2.45 and 30± 6.04, respectively. No significant differences (P < 0.05) were found between the different feeding and control after 7 days (100± 0.0); also no significant differences were found between the number of survival larvae fed on PS and PE after 14 and 21 days from the experiment (Table 1).

Table-1: Survival rates of *T. confusum* larvae after 7, 14, 21 and 30 days of feeding on the different type of plastic

Plastic type	No. of survival <i>T. confusum</i> larvae mean± S.D (%)			
	7 day	14 day	21 day	30 day
Control	100± 0.0 a	100± 0.0a	90± 6.01a	90± 6.01 a
PS	100± 0.0 a	90±6.01b	75±4.82 b	70±4.0 b
PE	100± 0.0 a	81±5.5b	65±3.76 b	50±2.45 c
EVA	100± 0.0 a	70±4.82c	40±7.30 c	30±6.04 d
LSD (p<0.05)	8.23	9.0	12.45	18.10

- Mean values followed by the same letter within a column are not significantly different.

Current findings agreed with previously mentioned that mealworms fed solely on polystyrene were able to survive for two months. This ability for polystyrene biodegradation was due to the activity and role of bacteria found in mealworms gut (Yang et al., 2015). Several genera of bacteria have been identified from mealworms gut such as *Lactococcus*, *Weissella*, *Spiroplasma*, *Rahnella*, *Cronobacter*, *Enterococcus*, *Lactobacillus*, *Bacillus*, *Enterobacter*, *Clostridium*

and *Pantoea* (Wang and Zhang, 2015). In the gut of *T. confusum*, not only bacteria might exist but also various other microbes such as fungi and yeast. Some of fungi taxa are *Saccharomycetales*, *Capnodiales*, *Botryosphaeriales*, *Hypocreales*, *Xylariales*, *Chaetothyriales*, and *Pleosporales* have been isolated from these beetles (Rojas-Jiménez and Hernández, 2015). Also, some *Spiroplasma* spp. known as insect pathogen effects on the larvae of *T. confusum* without causing any harm (Jung et al., 2014). Although it is difficult to know which microbial genera or families are responsible for the degradation of polystyrene because only a few degrading bacteria have been isolated.

Measurement the mass changes in *T. confusum* larvae and the plastic materials

Table (2) showed the differences in the weight of *T. confusum* larvae fed on the three types of plastic (polystyrene (PS), polyethylene foam and EVA). Mass losses in larvae weight increased throughout the study from the initial mass 0.42± 0.92, 0.35± 1.01 and 0.31±1.20 mg, the final mass at the end of the experiment was 0.31± 0.75, 0.24± 0.98 and 0.17± 1.05 mg for larvae fed on the types mentioned before, respectively.

Table-2: The Mass losses in the weight of *T. confusum* larvae and the plastic materials after 30 days

Plastic type	<i>T. confusum</i>			Mass loss in plastic materials after 30 days (%)
	Initial weight (mg)	Final Weight (mg)	Mass loss after 30 days (%)	
PS	0.42±0.92	0.31±0.75	26.2 a	51.92 a
PE	0.35±1.01	0.24±0.98	31.4 a	46.84 a
EVA	0.31±1.20	0.17±1.05	45.2 b	2.9 b
			LSD (P<0.05) 7.33	LSD (P<0.05) 5.50

The results showed that the weight of *T. confusum* larvae gradually decreased, where the highest mass loss of larvae for EVA consumption after 30 days was 45.2 %. Meanwhile, 26.2% was the lowest mass loss was of larvae feed on PS (Table 2). No significant differences were found between the mass loss in larvae fed in PS and PE (p > 0.05) after 30 days. The observation throughout the study revealed that *T. confusum* larvae started to eat the PS then make a hole inside the plastic disk (Figure 1).

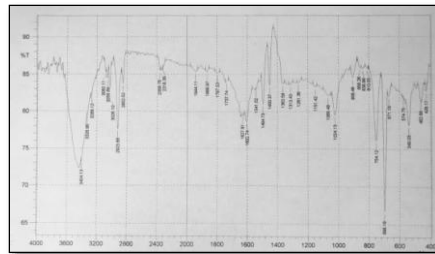




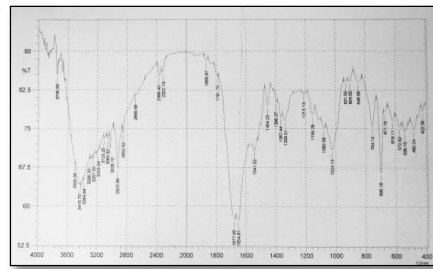
Figure-1: The holes of confused flour beetle, *Tribolium confusum* inside polystyrene (PS)

Furthermore, the results from table 2 conducted that polystyrene (PS) loss the highest mass (50.92%), suggesting that the larvae ingest this type of plastics as food. While the lowest mass loss concerning the mass of EVA, although a small but noticeable mass loss (2.9%) was recorded after 30 days of exposure to larvae, which may be probably due to the unavailability and difficulty of this material to *T. confusum* larvae. Among the three types of plastic, the significant differences ($p < 0.05$) were found only between mass loss of PS, PE and EVA, However, again no significant differences were found between PS and PE. Ethylene-vinyl acetate is one of the most challenging materials for biodegradation exhibit strong durability, high flexibility, low-temperature toughness, stress crack resistance and hot-melt adhesive waterproof properties (Stael et al., 2005). Also, it may be exciting and promising in the context of further studies.

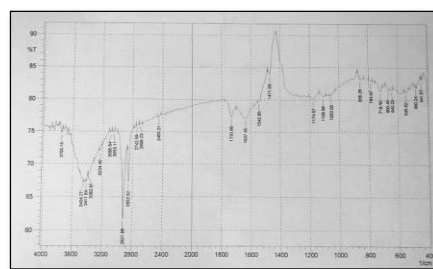
The degradation efficiency of PE's can be directly measured by the sample weight loss, as the depolymerization of materials can be observed by the formation of lower molecular weight compounds and molecular weight shift (Yang et al., 2014). Previous studies conducted the ability of insect larvae to chew and damage polyethylene, polyester and PVC films despite whether it was used to cover food, indicating that insect larvae may also eat and ingest plastics as food (Cline, 1978). Riudavets et al. (2007) mentioned the ability of some mandibulate insects to chew some plastic packages films, including polyethylene (PE), polyvinyl chloride (PVC), and polypropylene (PP).



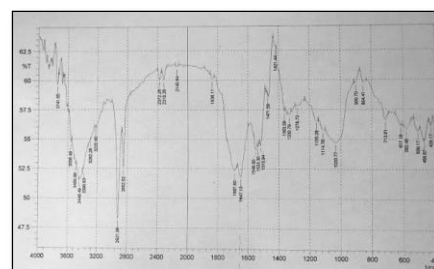
(A) Polystyrene sample before larvae consumption (Control)



(B) Polystyrene residual after 30 days from consumption



(C) Polyethylene sample before larvae consumption (Control)



(D) Polyethylene residual after 30 days from consumption

Figure-2: FTIR (A, B, C, D) spectra of polystyrene and polyethylene before and after larvae consumption

Elijah et al. (2015) used comparative biodeterioration of nylon by tested three species: *Tribolium*, *Sitophilus* and *Oryzaephilus* using three plastic types for 6 weeks. Results showed that the number of borings or holes (as an index of biodeterioration) increased and *Oryzaephilus* has the most active holes after 6 weeks. Other studies suggested the adaptability of the mealworm *Tenebrio molitor* L. gut microbiome enables degradation of styrofoam chemically dissimilar plastics effectively (Brandon et al., 2018). Different organisms need to mineralize large polymers into carbon dioxide and its constituent monomers as simple byproducts. Yang et al. (2014) mentioned that *Plodia interpunctella* and *Galleria mellonella* larvae could chew and degrade PE films. It is essential to mention that at the end of our experiments, some larvae completed their life cycle and transformed the pupae stage.

FTIR analysis

FTIR spectra of the samples are presented in (Fig. 2). The results of polystyrene after larvae consumption (Fig. 2.b) indicates the appearance of new absorption bands at $3500\text{-}2400\text{cm}^{-1}$ for hydroxyl groups (OH) and hydrogen bonding (Guilhaumou and Dumas, 2005). Moreover, a new absorption band at 1677cm^{-1} for carboxylic acid (CO) or amide compound. Aliphatic group (CH) at $2923\text{-}2852\text{ cm}^{-1}$ and aromatic (CC) at 1541 cm^{-1} were found in polystyrene after consumption. The polyethylene foam control sample showed the presence of NH group at $3100\text{-}3500\text{ cm}^{-1}$ and the same bond reflected at $1640\text{-}1560\text{ cm}^{-1}$. (CC) bond stretch was observed at 2100 cm^{-1} . Aliphatic group (CH) in CH_2 and CH_3 groups at $2900\text{-}2850\text{ cm}^{-1}$ and carbonyl group at $1650\text{-}1850\text{ cm}^{-1}$ were indicated (Ali et al., 2012). Regarding sample control, the variation in bond shape, width and peaks intensity were found in all bonds after larvae consumption. Amide bond (CONH) found in natural polymers can be hydrolyzed by proteolytic enzymes. The appearance of carbonyl groups is an essential sign of PE biodegradation which has demonstrated in a previous study on PE degradation (Arutchelvi et al., 2008).

Conclusion

We concluded that the confused flour beetle, *Tribolium confusum* has the ability for biodegradation different types of plastic (PS, PE, and EVA) so the

distribution of this insect in a diverse geographic region as a cosmopolitan can help to assist plastic biodegradation and minimize the massive amount of plastic wastes.

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Conflict of Interest: None.

Source of Funding: None.

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