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Polyethylene Degradation by Plastivores Greater Wax Worms Larvae (*Galleria mellonella*)

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Abstract
In this study, wax worm larvae (*Galleria mellonella*) were used to examine their ability to degrade and assimilate polyethylene (PE) as an energy source. This idea came from the similarity of wax, that is used as the sole diet for larvae, with PE in composition. Morphology changes, weight loss, FTIR analysis and GC-Mass test were studied to prove the degradation of PE by *G. mellonella*. The maximum depth of holes on the plastic surface and 16% PE weight loss was due to extensive cutting. The creation of a novel O-H stretching alcohols/phenols group absorbance peak at 3293cm^{-1} observed in wax worm larvae PE frass samples may be due to the oxidation in their gut. Accordingly, the biodegradation of PE by wax worm larvae is ensured.

Keywords: Plastic, Contamination, Insects, Bioremedation, Environmental problems.

تحلل البولي اثيلين بواسطة يرقات ديدان الشمع (*Galleria mellonella*)

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الخلاصة :
تهدف الدراسة الحالية الى معرفة قابلية يرقات حشرة الشمع على التغذي على المادة البلاستيكية البولي اثيلين كمصدر للطاقة. درست التغيرات الشكلية وفقدان وزن المادة البلاستيكية وكذلك الطيف للأشعة تحت الحمراء لبراز اليرقة وغيرها من الفحوصات للتأكد من عملية التحلل. لوحظ تكون ثقوب (الفتحات) على سطح المادة البلاستيكية بعمق 3 سم وكذلك فقدان الوزن للبولي اثيلين (المادة البلاستيكية) بنسبة 16% نتيجة القطع او التغذي من قبل اليرقة، بالاضافة الى ذلك بينت نتائج الطيف للأشعة تحت الحمراء لبراز اليرقة بعد تغذيتها على البولي اثيلين تكون مجموعة هيدروكسيد مما يؤكد تحطم البولي اثيلين بواسطة اليرقة.

1. Introduction:

Plastics are synthetic polymers and their use in daily life has increased in the last few years. Polyethylene (PE) is an example of widely used plastics in which has various

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applications such as shopping bags, medical equipment, packing requirements, etc. [1]. Because of its properties and cost, low-density polyethylene (LDPE) is the most common type of polyethylene that is used in various industries that has resulted in its discharge to the environment after use thus creating problems due to its resistance and aggregation in large volumes [2]. Polyethylene (PE) resists degradation because of the long-chain structure, high molecular weight and hydrophobic nature of the action of microbial degradation [3]. Many methods have been used to reduce harmful effects of plastic on the environment like incineration. Another problem has also risen out because of the gases and by products of plastics that may be produced from their thermal treatment. Recently many studies have implemented bioremediation to reduce the accumulation of polymers in the environment [4]. Bacteria and fungi are isolated from soil, compost, sludge, and water and are used to control plastic accumulation via degradation and employing plastics as a carbon source [5][6]. Many factors such as plastic composition, the existence and absence of functional groups, the molecular weight of plastic and the ability of microbes to degrade the plastic as well as the type of microbes and the produced enzymes play an important role in the biodegradation process [7]. Extracellular enzymes that are produced by microbes degrading and cleaving the chain of plastics into low molecular weight remains that are used by microbes as energy to grow.

Several kinds of insects that can degrade plastic materials have been discovered because of the symbiotic relationship between the insects and their gut microbes or by enzymes of insects [8]. Yellow mealworms larvae of *Tenebrio molitor* and microbes isolated from the gut of insects have been used in research to check its ability to biodegrade plastics [9]. Furthermore, the degradation of polystyrene, polyethylene and ethylene-vinyl acetate by *Tribolium confusum* have also been explained [10]. The present study used the larvae of wax worms *Galleria mellonella* in their ability to biodegrade plastic. The basis of this idea came from the similarity between wax and plastic in their chemical composition.

2. Materials and Methods

Greater wax worm (*Galleria mellonella*) larvae and honeycomb wax were obtained from hives of a farm in Babylon city. Low Density Polyethylene (LDPE) was obtained from a local market. Plastics were cut into small sheets, diameters of 5 cm and 5 cm in height, and cleaned with distilled water before being dried for a day.

2.1. Greater Wax Worm (Galleria mellonella) Larvae Identification: The collected wax worms (*G. mellonella*) were morphologically identified and validated by the Iraqi Natural History Research Centre and Museum /University of Baghdad.

2.2. Biodegradation Studies of Plastic (PE)

The greater wax worms (*G. mellonella*) larvae were collected and reared in the laboratory under conditions (Temperature: 28±1ºC, Humidity: 80±2%). For the biodegradation studies of plastic, actively nurturing larvae of greater wax worm (the number of larvae (n)= 70 larvae) was done to study degradation and (n=100 larvae) their survival rate. Ther larvae were isolated in a 1000ml glass beaker with untreated plastic sheets (3g) as their sole source of energy. They were subjected to a 48 hour starvation period before initiating experimental diets. The larvae were divided into two groups [11]:

- Larvae fed on polyethylene (PE).
- Larvae fed on honeycomb wax as controls.

The larvae were fed plastic (PE) as their sole diet for one month. The plastic sheets were weighed before each experiment. The larvae were housed under same conditions and monitored for 30 days. Dead larvae and molted skins were removed promptly during the study and their survival rate was counted [10].

2.3. Determination of Weight Loss

The weight loss of plastic sheets caused by the wax worm was measured after 30 days of feeding the larvae with the plastic materials. For the accurate weight measurement, plastic residues were washed with 2% (v/v) sodium dodecyl sulfate (SDS) $NaC_{12}H_{25}$ So_4 solution, followed by washing with deionized water several times and dried for 3h at 40ºC. Weight loss percentage was calculated by using the following formula [11]:

Weight loss (%) = Initial weight – Final weight \ Initial weight × 100

2.4. Tests for Morphology

The morphology of the plastic before and after treatment with wax worm larvae was examined via an optical microscope (Electron Eyepiece, model Olympus, Japan10x) mounted on a camera in the Engineering College, University of Babylon's laboratory.

2.5. Larvae Excreta Residue (E.R) Study.

2.5.1. Collection of Excreta Residue (ER) Samples.

For 30 days, fifty active wax worm larvae were fed their natural food wax comb (WC) as control and plastic sheets (PE) as their sole diet. To avoid un-ingested feed mingling with the accumulated ER, the ER of WC and PE fed worms were collected every 12 hours. For further investigation, the ER samples were collected in an airtight container and refrigerated at 4°C [14].

2.5.2. Gas Chromatography-Mass Spectrometry (GC-MS analysis)

For GC-MS analysis a sample of about 1μl was injected into the gas chromatograph: Agilent (7820A) USA GC Mass Spectrometer which was available in the Ministry of Industry and Mineral, Corporation of Research and Industrial Development, Ibn Baytar Research Center, Iraq at an injector temperature of 250ºC scan range: m/z 40-400.

2.5.3. Fourier Transform Infrared Spectra (FTIR) Analysis.

FTIR was used to assess the mineralization of WC and plastic sheets (PE) by examining ER of the greater wax worms and plastic films [11]. The ER samples were exposed to FTIR analysis instrument Type (Bruker made in (Germany) which was available in the laboratories of College of Pharmacy,/University of Babylon.

3. Results and Discussion

3.1. Characterization of Plastic (PE) and Rate of Weight Loss

The surface morphology of plastic films under wax worm action (Figure1) exhibits the creation of pits and cavities in comparison with the control which might be due to the surface degradation of the plastic by larvae. When the wax worm is left in direct contact with the plastic film, it is able to gnaw and make holes in it and use it as a source of energy. The cracks on the surface of plastic had a maximum depth of about 2-3cm (Figure2). The wax worms after going through all stages of development (larvae, pupa and adult moth), reached the end of their life cycle. Greater wax worms were fed WC and PE and virtually completed their life cycle in 30 days. The larval interval of WC and PE-fed wax worms differed significantly. WC fed worms showed a longer larval stage length of 25 days than PE fed worms which was 15 days) Studies on the life phase of insects proved that the larval phase period lifetime relies on the ecological conditions as well as the obtainability and type of food [12]. Besides, PE unlike WC does not have the essential nutrients and suitable water content necessary for progress and development which may also be the cause of fewer biomass gain. The number of larvae did not alter in the first week. The number of larvae feeding on plastics reduced with time, in relation to the control. After 30 days of the beginning of the tests, the numbers of survival of great wax worms fed on WC and PE were 90 and 70 respectively. It revealed that greater wax worms fed only PE could continue by consuming and digesting PE. However that survival rate was poor when associated with WC fed wax worms. It was found that these larvae could decompose a type of plastic, polyethylene (PE), and degrade it into ethylene glycol which is degraded instantly [13]. It was observed that the capacity to degraded plastic was due to the existence of several bacteria inside the larvae gut. The bacteria that existed in the wax worm gut were isolated and two strains of bacteria namely *Bacillus* and *Enterobacter* seem to have the ability to digest polyethylene [4].

To determine weight loss following biodegradation in controls, samples of plastic films were collected. In this study, the percentage of weight loss was 16% due to extensive cuts, causing holes in the plastic that was a result of eaten part of plastic which significantly decreased the plastic weight during the experiment. The loss of plastic weight was proven by *Bombelli et al.*[14] who found that hundreds of wax worms can degrade 92 mg of PE within 12 hours and that spread of worm homogenate on plastic film results in 13% loss in the mass of PE. PE degradation by wax worms was higher than the degradation rate of PE by other microbes [15]. Also, the ability of *G. mellonella* to degrade the PE and beeswax without relying on intestinal microbial was proved by Kong *et al.*[16]. This was supported by GC-MS analysis that revealed less important changes in PE degradation by *G. mellonella* fed on PE compared with antibiotics treatment and *G. mellonella* fed on PE without antibiotics treatment.

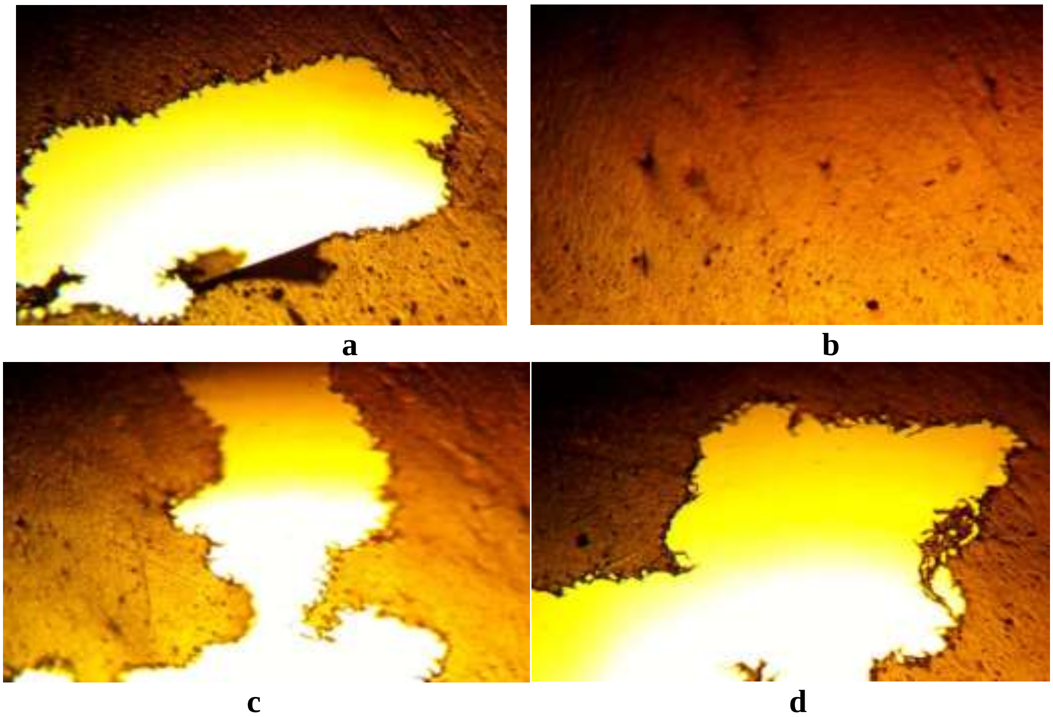

Figure 1: Plastic (PE) surface before (a) and after (b, c, d) treatment with wax worm larvae under microscope 10x .

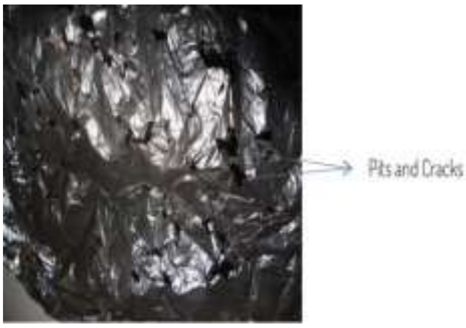

Figure 2: Picture of the naked eye before and after the decomposition by larvae of polyethylene

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3.2. GC-MS Analysis

The GC-MS examination of ER from the wax comb (WC) and plastic film (PE) revealed about 20 compounds (Table 1 and 2). The mineralized products from wax worms fed on WC and plastic, after feeding by wax worms, included terpenes, fatty acids and other functional group compounds. Gas chromatography−mass spectrometry (GC−MS) was performed to further investigate the intermediates and products of plastics biodegradation. The results were comparable with the previous investigation which found methyl 9- octadecenoate and methyl hexadecanoate as intermediates of PE metabolism [17][18]. Fragrance and flavor compounds such as limonene and 3-carene had the highest estimated concentration which is similar to previous studies [19]. It was reported that limonene and D-limonene are monomers [20]. In this study the frass of *G. mellonella* larvae were examined by GC-MS to study the creation of intermediates of wax metabolism including hydrocarbons and fatty acids in the beeswax. The results showed the mineralized ER of wax which revealed nearly comparable compounds of PE with changes in the intensity of spectrum as shown in (Table 2).

Table 1: Compounds detected after GC–MS analysis of polythene frass of greater wax worm (*Galleria mellonella*) larvae.

Peak	Relation time	Compounds	Peak area%
1	5.167	Alpha-pinene,3 Carene.	2.32
2	6.144	Beta-pinene.	12.61
3	7.427	D-Limonene, Limonene	71.49
4	8.098	3-Carene	12.40
5	22.455	Hexadecanoic acid, methyl ester	0.33
6	23.874	11-Octadecenoic acid, methyl ester, 9- Octadecenoic acid	0.35
7	26.635	Octadecane,1(ethenyloxy)-	0.10
8	27.161	2-methyldocosane, 4-Methyldocosane	0.40

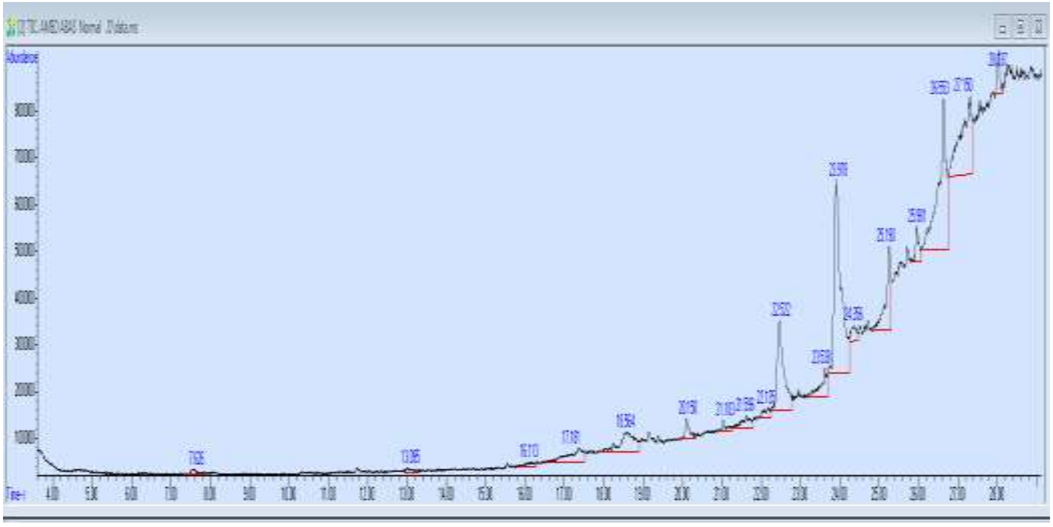

Figure 2: GCMS of ER of wax worms fed on PE.

Table 2: Compounds detected after GC–MS analysis of wax frass of greater wax worms (*Galleria mellonella*) larvae.

Peak	Relation time	Compounds	Peak area%
1	7.622	Benzaldehyde, 4-((4-(dimethylamino) phenyl) azo)-	0.78
2	13.093	4-Acetamidobutyric acid, Octanal	0.49
3	16.117	4-Acetamido-1-pentanol	0.78
4	17.179	Piperazine, 2-methyl-	3.78
5	18.564	Benzeneacetic acid, 4-(1,1-dimethyl)-, methyl ester	5.06
6	20.153	Heptadecanoic acid, heptadecyl ester	1.47
7	21.104	9-Octadecenoic acid, (E)-	0.79
8	21.597	E-11-Hexadecenoic acid, ethyl este	1.90
9	22.132	Dichloroacetic acid, 2-tridecyl ester	1.06
10	22.523	Hexadecanoic acid, methyl ester	10.26
11	23.534	Oleic Acid, 6-Octadecenoic acid	3.37
12	23.976	cis-13-Octadecenoic acid, methyl ester	22.90

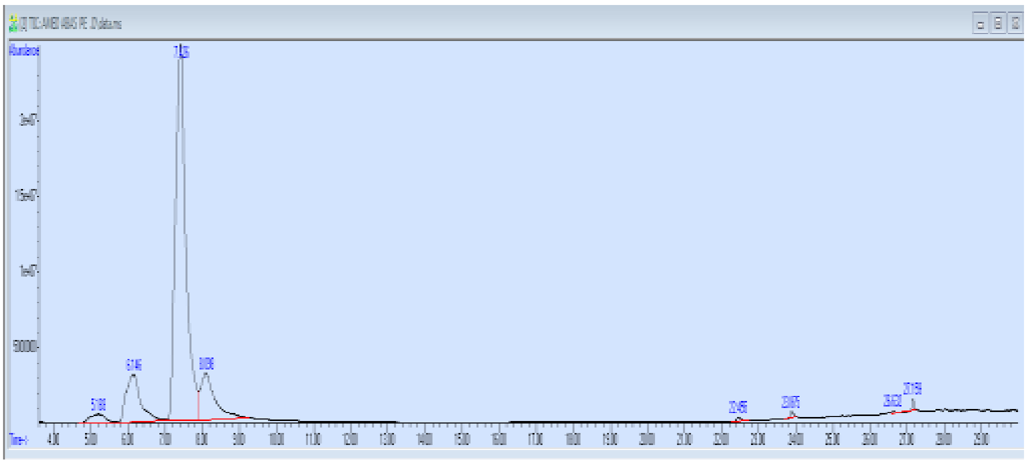

Figure 3: GCMS of ER of wax worms fed on WC.

3.3. FTIR Studies

The FTIR study supported examining the alterations in functional groups of ER of *G. mellonella* by chemical bond breakage, transformation, and creation of compounds and complete reduction of WC and plastic sheets. The FTIR spectra of polyethylene (PE) before being treated with wax worms (Figure 4) showed the peaks corresponding to the C-H, CH_2 at peak (2847, 2915cm^{-1}) respectively and the appearance of peaks at 3011, 3119 represented or returned to the pigment (black) that treated the PE. The FTIR of PE frass (Figure 5) samples from wax worms larvae exhibited the creation of a new O-H stretching alcohols/phenols group absorbance peak (3293cm^{-1}) which could be due to the oxidation in the gut of wax worms larvae. It was detected through PE degradation tests [21][22] and the conversion of active groups to energy. FTIR for the wax comb (WC) frass (Figure 6) actED as a control and positive control for the degradation analysis and FTIR spectrum of control was also studied. There were no high changes in the active group but only in the fingerprint region and mineralization of bee wax. When compared with PE frass there was no high difference which confirmed the ability of the wax worm to use PE as an energy source by larvae. From the results of weight loss of PE, GC-Mass analysis and FTIR test showed the capacity of wax worms larvae to degrade the PE and use it as a source of energy.

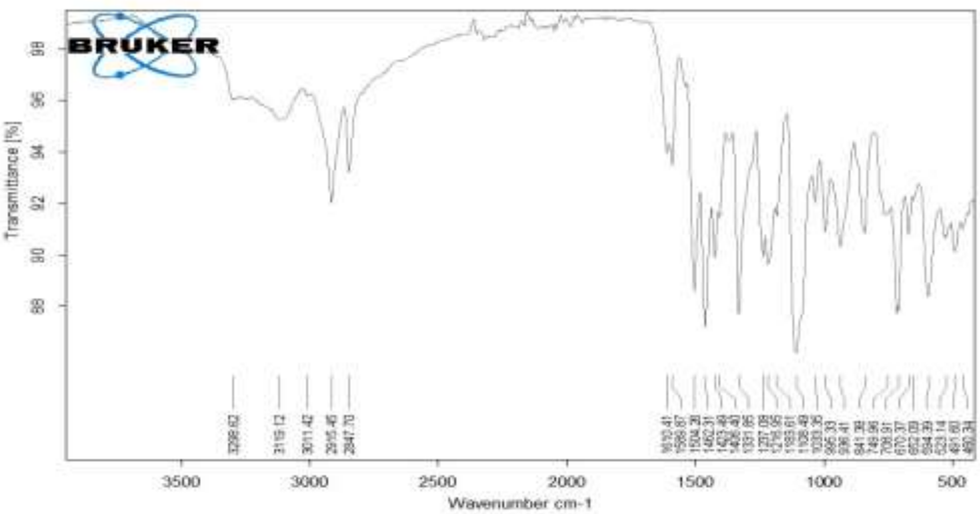

Figure 4: FTIR spectra of polyethylene (PE) before being treated with wax worms.

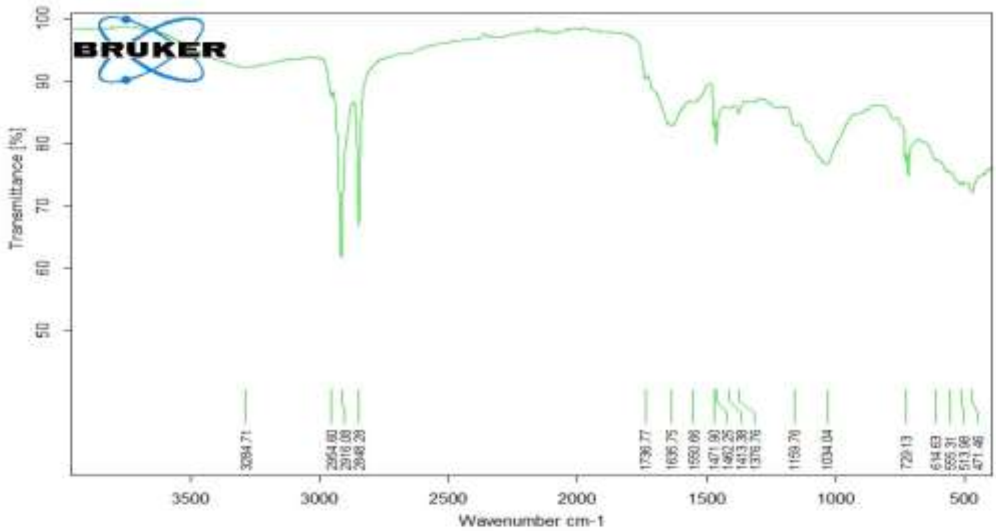

Figure 5: FTIR spectra of the PE frass samples from wax worms.

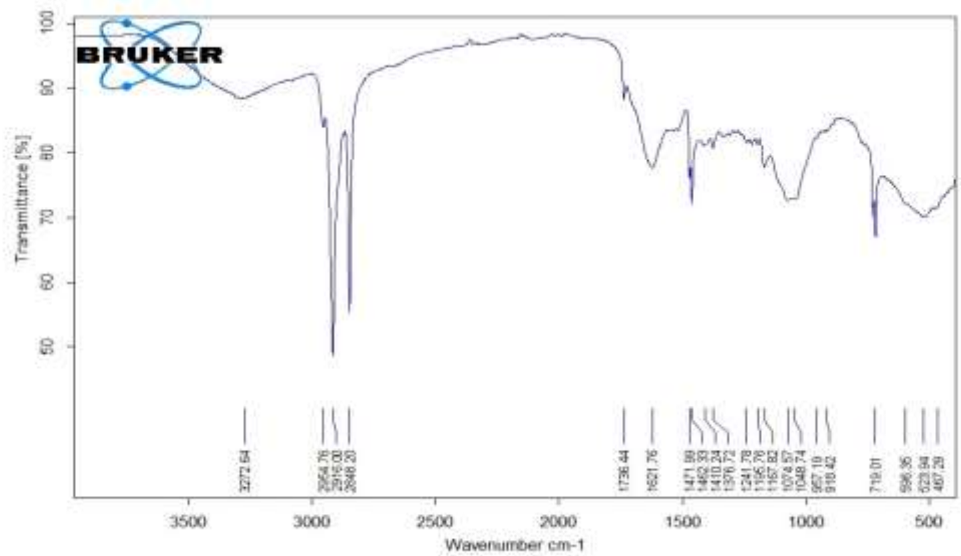

Figure 6: FTIR spectra of wax comb (WC) frass samples of wax worms.

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