BIODEGRADATION OF POLYSTYRENE BY PLASTIVORES GREATER WAXWORMS LARVAE (*Galleria mellonella*).

[1]Abbas, T . K. H. S. Abdulhay[2]
Researcher Prof.
[1]Coll. Environ. Sci. AL-Qasim Green University, Babylon.
[2]Dep. Biol . Coll .Sci. University of Baghdad ,Iraq.
altai.abbas@environ.uoqasim.edu.iq

ABSTRACT

This study was aimed to find and test biological methods for reducing the aggregation of plastics such as PS in the environment and study the ability of Greater Wax worms larvae (*Galleria mellonella*) to eat PS that similar in the its structure to beeswax .Weight loss, morphology changes ,FTIR spectroscopy and GC-mass analysis were performed which showed changes in chemical properties of the PS due to degradation. In this study the percentage of weight loss was 33% in the PS treated with *G. mellonella.* FTIR of PS frass showed the disappearance of aromatic cycle band that was found in the origin PS at region more than 3000 cm^{-1}. Also The PS frass samples from wax worms larvae revealed the creation of a new O-H stretching alcohols group or glycol substance at absorbance peak 3293cm^{-1} that no found in the origin PS that ensure the degradation of PS by wax worm larvae.

Key words: plastic pollution, bioremediation, insects, fourier transform infrared analysis.

التفكك الحياتي للبولي ستايرين بواسطة اكلات البلاستيك يرقات شمع العسل.

[1]عباس طالب خليف هند سهيل عبد الحي[2]
باحث أستاذ
[1]– كلية علوم البيئة , جامعة القاسم الخضراء, بابل.
[2]– كلية العلوم , قسم علوم الحياة , جامعة بغداد, العراق

المستخلص

تهدف الدراسة الحالية الى التحري عن طريقة بايلوجية للتقليل من المواد البلاستيكية مثل البولي ستايرين في البيئة ودراسة قدرة يرقة حشرة شمع العسل في التغذي على البولي ستايرين المشابه في تركيبه لشمع العسل. اجريت فحوصات فقدان وزن المادة البلاستيكية والتغيرات الشكلية للبوليمر وغيرها للتأكد من عملية التحلل. في هذه الدراسة كانت النسبة المئوية لفقدان وزن البولي ستايرين35% بعد معاملته مع يرقات شمع العسل. كذلك اوضحت نتائج الطيف للأشعة التحت الحمراء لبراز اليرقة بعد تغذيتها على البولي ستايرين اختفاء الحلقة الأروماتية التي كانت موجودة في البولي ستايرين الاصلي وكذلك تكون مجموعة هيدروكسيد مما يؤكد تحطم البولي ستايرين بواسطة اليرقة.

الكلمات الافتتاحية: التلوث البلاستيكي ، المعالجة الحيوية، حشرات، تحليل طيف الاشعة التحت الحمراء.

Received:3/1/2022, Accepted:2/3/2022

INTRODUCTION

Plastics are synthetic organic polymers that are utilized in a variety of applications, including water bottles, clothing, food processing, medical supplies, electronics, and construction materials (16). In our everyday lives , tens of thousands of plastic products are expected. PE (polyethylene), PP (polypropylene) PVC (polyvinylchloride), PET (polyethylene terephthalate), and PS (polystyrene) are some of the most widely utilized plastics (5). Plastics' beneficial features, such as simplicity of use, non-degradability, and low cost, have resulted in the replacement of normal materials and widespread apply to everyday things since 1960. Plastics production a rise in gradually over the last 50 years, from 1.5 million tons in 1950 to around 348 million tons in 2017 and is probable to double again over the next 20 years, with plastics serving a wide range of applications (8,12,15). Non-degradable plastic is manufactured in quantities ranging from 350 million to 400 million tons per year, with 5 to 13 million tons of excess plastic released into the ocean each year, posing a threat to the environment. When plastics are incorporated into natural habitats, they can be transferred from land to river and finally to the ocean (10). Plastics have been shown to leak several hazardous substances such as monomers, oligomers, and additives into the atmosphere(6). Polystyrene (PS) is a biodegradable material made up of several styrene molecules linked together. PS, a publicly traded petroleum-based plastic with Styrofoam (expanded PS foam) as its major product, produced almost 21 million tons globally in 2013 (20). Polystyrene (PS) is traditionally regarded non-biodegradable due to its large molecular weight and highly consistent structure, although researchers have had success isolating PS-degrading microbes such as *Rhodococcus ruber* (13). Many types of insects discovered that have the ability to degrade the plastic materials because the symbiotic relationship between the insects and its gut microbes or by enzymes of insects. The first indication of insect plastic-biodegrading possible derives from (17) when isolated an enzyme capable of degrading some bio plastics films made of polybutylene succinate (PBS).

After Two years (18) reported the first study concerning insects and their gut microbiome degrading polyethylene (PE) that represent non-biodegradable polymer. Tenebrio species Yellow mealworms (*Tenebrio molitor*) and (*Tenebrio obscurus*) larvae have the ability to degrade polyurethane, polystyrene, polyvinyl chloride and polylactic acid (4). Also-Abdulhay (1) showed the degradation of polystyrene ,polyethylene and ethylene –vinyl acetate by *Tribolium confusum* The present study used the larvae of wax worm *(Galleria mellonella)* to explain its ability in the plastic biodegradation. This ability of *G. mellonella* larvae may come from the similarity between the wax and plastic in the chemical composition.

MATERIALS AND METHODS

Farm hives in Babylon city were used to harvest greater wax worm larvae and honeycomb wax. Polystyrene(PS) materials were collected from local market . The collected wax worm larvae was morphologically recognized and confirmed by Iraq Natural History Research Centre and Museum /University of Baghdad .Greater wax worms were taken from honeybee comb and cultured in the lab under ambient conditions (temperature: 28±1ºC, humidity: 80±2%). Plastic actively feeding larva of greater wax worm (n=70) were placed in a 1000ml glass beaker with untreated PS films (3g) as their sole meal for biodegradation investigations. Polystyrene (PS) sliced into small sheets, cleaned with distilled water, and dried for at least one day. The experiment was carried out in two groups as follows:

- Larvae fed on Polystyrene(PS).
- Larvae fed on honeycomb wax as controls .

The larvae were fed with plastic (PS) as their sole diet for one month. The plastic sheets were weighted before each experiment. For 30 days, all of the larvae were housed in the same conditions of rearing and monitored. Dead larvae and molted skins were removed promptly during the studies, and survival was counted.

Test = for morphology

The morphology of the plastic before and after treatment with wax worm larvae was examined using an optical microscope (Electron Eyepiece, model Olympus, Japan)

mounted on a camera in the Engineering College / University of Babylon's laboratory.

Polystyrene weight loss study

After 30 days of feeding the larvae with the plastic material (PS), the weight loss of plastic sheets due by wax worm consumption was measured. Plastic residues were washed numerous times with a 2 percent (v/v) sodium dodecyl sulfate (SDS) solution followed by deionized water for precise weight measurement, then dried for 3 hours at 40 degrees Celsius and weight loss percentage computed using the procedure below (7):

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

Larvae excreta residue (ER) study

Collection of excreta residue (ER) samples

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

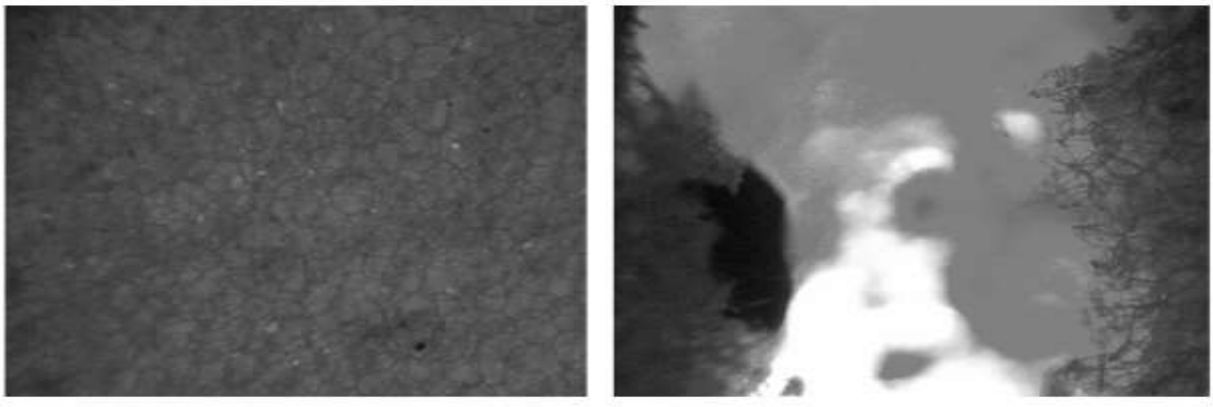
Gas Chromatography-Mass Spectrometry GC-MS analysis of E.R: For GC-MS analysis , sample about 1µl of ER was injected into the gas chromatograph: Agelint (7820A) USA GC Mass Spectrometer which is available in Ministry of Industry and Mineral/ Corporation of research and industrial Development / Ibn Baytar research Center/ Iraq . at an injector temperature of 250C Scan Range :m/z 40-400 .

Fourier transform infrared(FTIR) analysis

FTIR was used to check the mineralization of WC and plastic sheets (PS) by examining ER of the greater wax worm and plastic films. The ER samples were exposed to FTIR analysis instrument Type (Bruker) made in (Germany), which is available in laboratory of Pharmacy collage /University of Babylon.

RESULTS AND DISCUSSION

Characterization of plastic and rate of weight Loss: The creation of pits and holes on the external of (PS) sheets figure(1) under *Gaalleria mellonella* action shows the ability of *G. mellonella* larvae to degrade the plastic materials. This may depend on the type of natural food that eat by larvae of *G.mellonella* (beewax) that simillar in its structure to plastic materials. The *G. mellonella* were capable to crush and generate holes in the PS sheets after it was put in straight connection with the plastic film and consumption it as a source of energy. In this study the percentage of weight loss was 33% in the PS treated with *G. mellonella* because of the wide cutting that produce holes in the plastic, as a result of eating part of plastic reduced significantly the plastic weight during the experiment. The ability of *G. mellonella* to degrade the plastic and beeswax without relying on intestinal microbial proofed by Kong *et al* (10). Furthermore the ability of *G. mellonella* caterpillars to destroy PS and PE had been showed by Kundungal *et al* (11) when discovered the intermediaries of PE and PS degradation signifying metabolism of plastic by this caterpillars.



a- Control PS. **b- PS after eating by *G. mellonella* larvae**

Figure 1.Microscope picture explain PS before (a) and after (b) eating by *G. mellonella* larvae under 10x

Gas Chromatography-Mass Spectrometry (GC-MS analysis): Gas chromatography −mass spectrometry (GC−MS) was achieved to additional examine the intermediates and products of plastics biodegradation and to confirm the role or ability of *G. mellonella* in plastic damage. The qualitative study for the biodegradation of polystyrene (PS) and wax comb (WC) was detected by analyzing the Excreta residue (ER) of *G. mellonella* fed with WC, and PS films by GC-MS. In this study GC-MS technique has been applied to screen and estimate the generation of chemical compounds through the biodegradation of polystyrene (PS) with wax worm (*G.mellonella*) larvae (figure2,table1). Some fatty acids like tetradecanoic acid identified in this study. Also the frass of PS-fed larvae recognized long chain free fatty acids (FFAs), such as oleic acid ($C_{18}H_{34}O_2$), octadecanoic acid ($C_{18}H_{36}O_2$) and n-hexadecanoic acid ($C_{16}H_{32}O_2$). The existence of long chain FFAs and the reduction of the amounts of more complex long chain carboxylic acid esters structures showed digestion and biodegradation of PS, as reported by Liu and Chen (12). Beeswax is consist of esters that combine long-chain fatty acids with long-chain alcohols. Hence, the amount of long-chain fatty acids can be used as a measure of the degradation of beeswax by *G. mellonella*. Also the degradation of PS which are like to the chemical structure of beeswax and sources of environmental problems, was investigated through *G. mellonella* by Kong *et al* (10). Results were comparable with previous investigation, which gained methyl 9-octadecenoate and methyl hexadecanoate as intermediates of PS metabolism (10,11). In this study the frass of *G. mellonella* larvae examined by GC-MS to examine the creation of intermediates of wax metabolism including hydrocarbons and fatty acids in the beeswax and the result was the mineralized ER of wax revealed nearly similar compounds with PS difference in the intensity of spectrum as shown in (figur3,table2). From this result the biodegradation of polystyrene (PS) and wax comb (WC) by *G. mellonella* larvae take place may be by special enzyme present in the larvae or by the microorganisms (bacteria or fungi) that found in the gut of the larvae or its enzymes .

Fourier transform infrared spectroscopy (FT-IR)analysis: FTIR Analysis can be used for detecting chemical modifications in the building of many plastics as it will expose the variations like creation and the departure of chemical groups and chemical bonds. The FTIR spectra of polystyrene (PS) before treated with wax worms shows the peaks corresponding to the aromatic and aliphatic –CH- at region (2919-2849) cm^{-1} and aromatic =C-H at region 3081 cm^{-1} and 3059 cm^{-1}.Also aromatic C=C at (1583-1600) cm^{-1} and (1492-1451) cm^{-1} as shown in figures (4) .While after larvae feeding on the PS , the FTIR of PS frass figures (5) showed the disappearance of aromatic cycle band that was found in the origin PS at region more than 3000 cm^{-1} . Beside departure aromatic C=C band that was found in the origin PS at region(1583-1600) cm^{-1} and (1492-1451) cm^{-1} and convert of styrene cycle to H_2O ,CO_2 and energy and this ensure mineralization of PS in the larvae gut. Also The PS frass samples from wax worms larvae revealed the creation of a new O-H stretching alcohols group or glycol substance at absorbance peak 3293cm^{-1} that no found in the origin PS might be due to the oxidation in the gut of wax worms larvae, which was observed during PE degradation tests (2,14,19). FTIR for the wax comb (WC) frass, WC act as control and positive control for the degradation analysis, FTIR spectrum of control there was no high changes in the active group but only in the finger print region and mineralization of bee wax,when compare with PS frass there was no high difference and this confirm the ability of use PS as energy source by larvae. FTIR results ensured the ability of larvae to degrade PS and this capacity come from or because the similarity of polymer(PS) with the natural food (wax) in the chemical composition(3). Also in study by Kundungal,*et al* (11) showed PE degradation by wax worms is higher than the degradation rate of PE by other microbes.

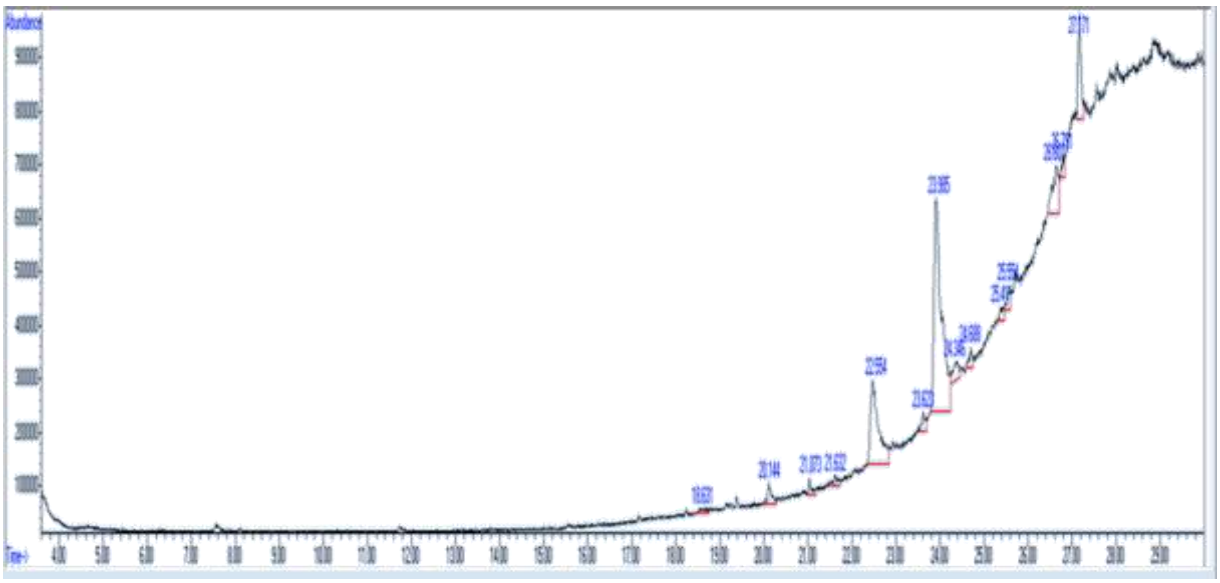


Fig 2. GC–MS chromatogram showing the Compounds detected PS frass of *Galleria mellonella* larvae

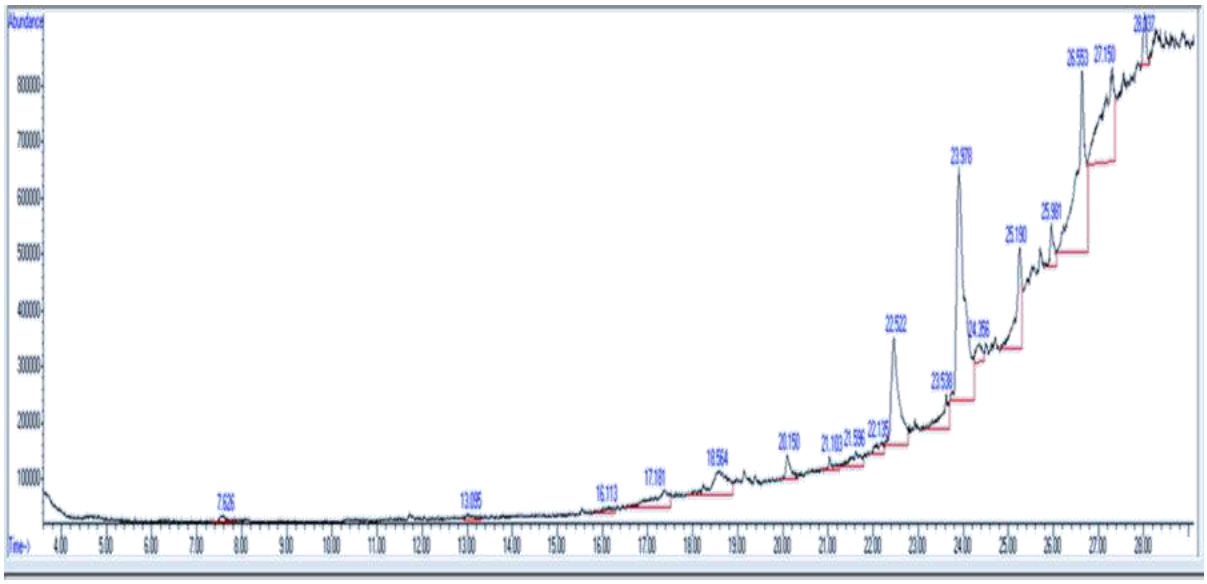


Fig 3. GC–MS chromatogram showing the Compounds detected wax frass of *Galleria mellonella* larvae

Table 1.Compounds detected PS frass of (*Galleria mellonella*) larvae

Peak	Relation time(min)	Compounds	Peak high%
1	18.632	Benzeneacetic acid, 4-(1,1-dimethy lethyl)-, methyl ester.	1.16
2	20.144	1-Propyne, 3-(ethenylthio)-	2.38
3	21.070	Oleic Acid	1.26
4	21.631	E-11-Tetradecenoic acid	1.30
5	22.557	Hexadecanoic acid, methyl ester	19.74
6	23.619	Fumaric acid, 2,4,6-trichlorophenyl tridecyl ester	1.93
7	23.984	cis-13-Octadecenoic acidmethy ester, 10-Octadecenoic acid, methyl ester	45.67
8	24.349	Oleic Acid , 13-Octadecenal	2.91
9	24.689	cis-11-Hexadecenal , cis-9-Hexadecenal	1.64

Table 2.Compounds detected wax frass of *Galleria mellonella* larvae

Peak	Relation time(min)	Compounds	Peak high%
1	7.662	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

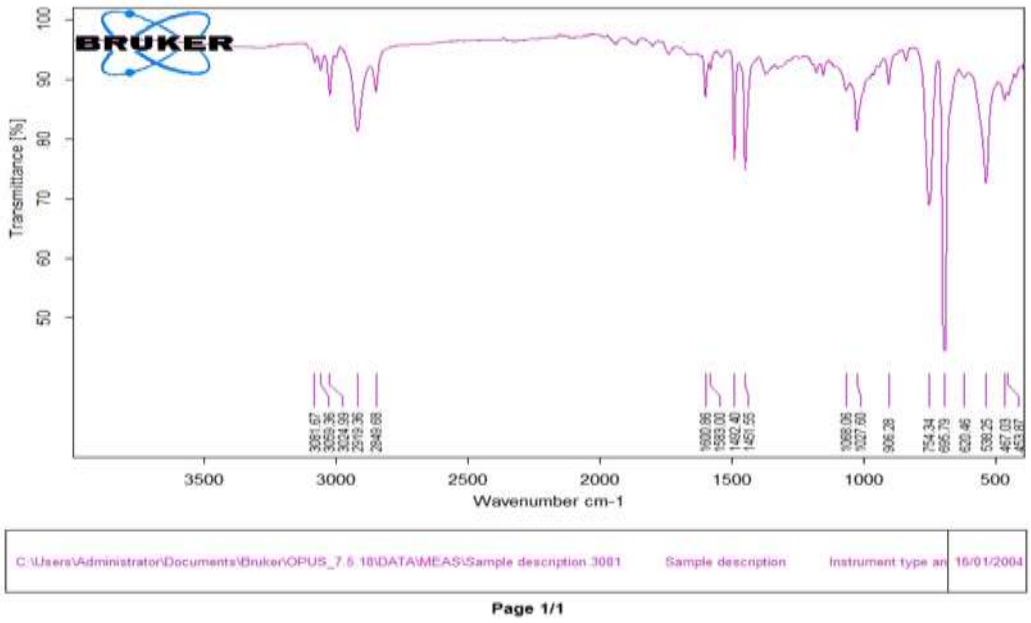


Fig 4. FTIR spectra of polystyrene (PS) before treated with *Galleria mellonella* larvae

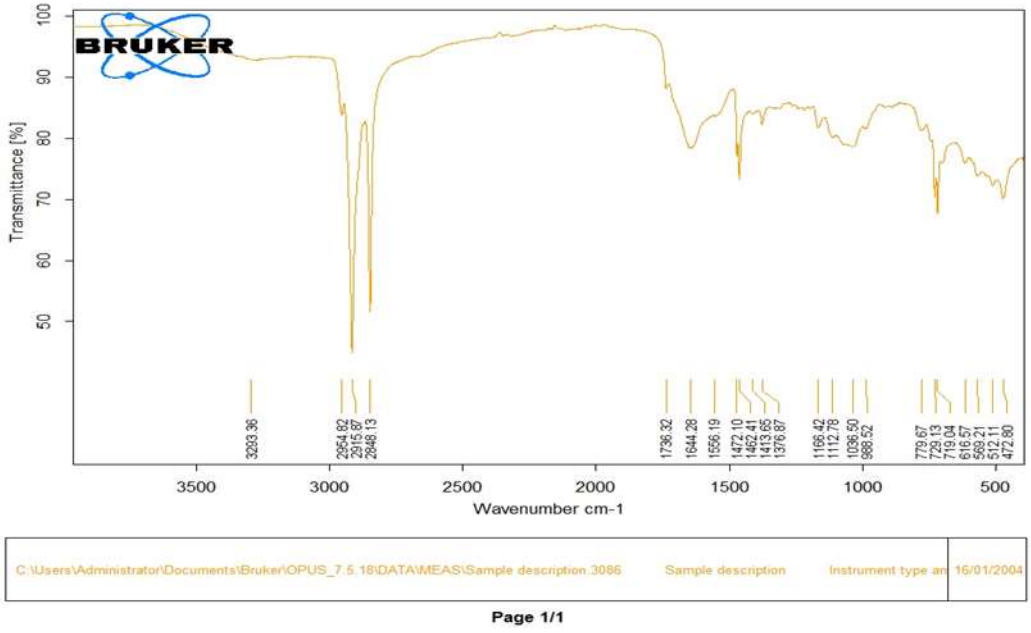


Fig 5. FTIR spectra of polystyrene(PS) frass sample of *Galleria mellonella* larvae

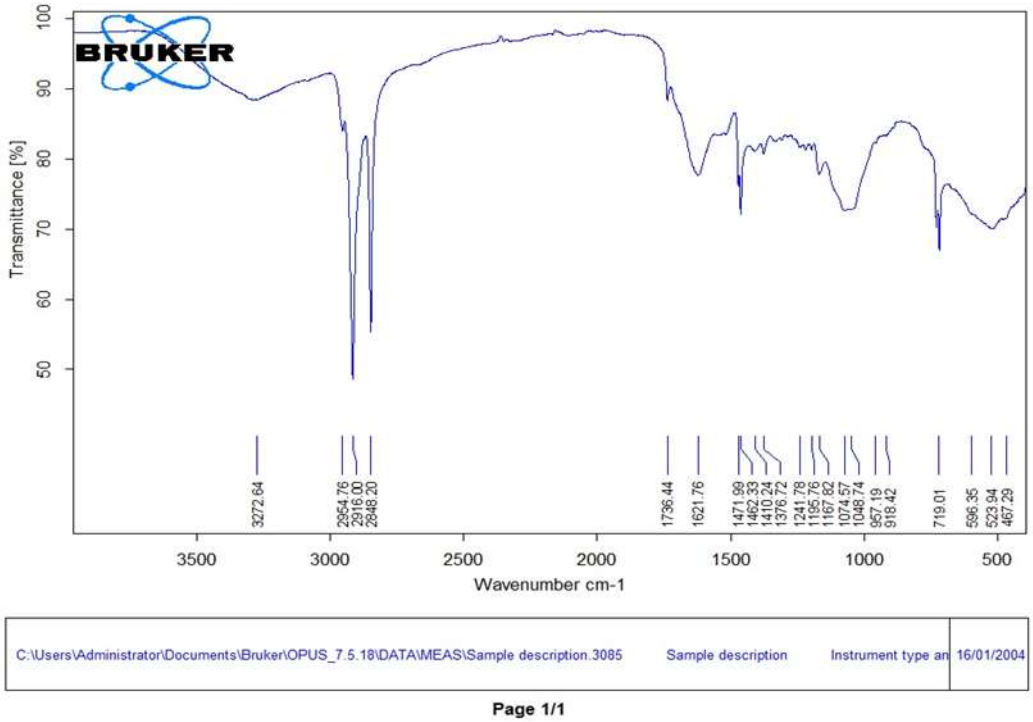


Fig 6. FTIR spectra of wax comb (WC) frass samples of *Galleria mellonella* larvae

REFRENCES

1- Abdulhay H.S. 2020. Biodegradation of plastic wastes by confused flour beetle Tribolium confusum Jacquelin du Val larvae .Asian J Agric & Biol.;8(2):201-206.

2- Asgari, P., O, Moradi., and B, Tajeddin,.2014.The effect of nanocomposite packaging carbon nanotube base on organoleptic and fungal growth of Mazafati brand dates. International Nano Letters, 4(1): 98

3- Bombelli, P., C. J, Howe., and F, Bertocchini. 2017. Polyethylene bio-degradation by caterpillars of the wax moth Galleria mellonella. Current biology, 27(8), R292-R293

4- Bożek, M., B, Hanus-Lorenz., and J, Rybak. 2017. The studies on waste biodegradation by *Tenebrio molitor*. In E3S web of conferences V. 17, p:\\00011). EDP Sciences

5- Bond, T., V, Ferrandiz-Mas., M,Felipe-Sotelo., and E, Van Sebille 2018. The occurrence and degradation of aquatic plastic litter based on polymer physicochemical properties: A review. Critical reviews in environmental science and technology, 48(7-9), 685-722

6- Chen, Q., A, Allgeier., D, Yin., and H, Hollert. 2019. Leaching of endocrine disrupting chemicals from marine microplastics and mesoplastics under common life stress conditions. Environment international, 130, 104938

7- Das, M. P., and S, Kumar. 2015. An approach to low-density polyethylene biodegradation by *Bacillus amyloliquefaciens*. 3 Biotech, 5(1), 81-86

8- Garside, M. 2019. Global plastic production from 1950 to 2018. Statista. Available online at: https://www. statista. com/statistics/282732/global-production-ofplastics-since-1950

9- Jambeck, J. R., R, Geyer., C, Wilcox., T. R, Siegler., M, Perryman., A, Andrady; and K. L, Law. 2015. Plastic waste inputs from land into the ocean. Science, 347(6223), 768-771

10- Kong, H. G., H. H, Kim., J. H, Chung., J, Jun., S, Lee., H. M, Kim., and C. M, Ryu. 2019. The *Galleria mellonella* hologenome supports microbiota-independent metabolism of long-chain hydrocarbon beeswax. Cell Reports, 26(9), 2451-2464

11- Kundungal, H., M, Gangarapu., S, Sarangapani., A, Patchaiyappan., and S. P , Devipriya. 2019. Efficient biodegradation of polyethylene (HDPE) waste by the plastic-eating lesser waxworm (*Achroia grisella*). Environmental Science and Pollution Research, 26(18), 18509-18519

12- Li, J., H, Liu., and J. P, Chen. 2018. Microplastics in freshwater systems: A review on occurrence, environmental effects, and

methods for microplastics detection. Water Research, 137,362-374

13- Lou, Y., P, Ekaterina., S. S ,Yang., B, Lu., B, Liu., N, Ren., ... and D, Xing. 2020. Biodegradation of polyethylene and polystyrene by greater wax moth larvae (*Galleria mellonella* L.) and the effect of co-diet supplementation on the core gut microbiome. Environmental Science and Technology, 54(5), 2821-2831

14- Mor, R., and A, Sivan. 2008. Biofilm formation and partial biodegradation of polystyrene by the Actinomycete *Rhodococcus ruber*. Biodegradation, 19(6), 851-858

15- Mukherjee, S., U, RoyChaudhuri., and P. P, Kundu. 2018. Biodegradation of polyethylene via complete solubilization by the action of *Pseudomonas fluorescens*, biosurfactant produced by Bacillus licheniformis and anionic surfactant. Journal of Chemical Technology and Biotechnology, 93(5), 1300-1311pp

16- Plastics Europe, 2019. Plastics-the facts 2019. https://www.plasticseurope.org/application/files/9715/7129/9584/FINAL_web_version_Plastics_the_facts2019_14102019.pdf. Accessed 20 March 2020).

17- Proshad, R., T, Kormoker., M. S, Islam., M. A, Haque., M. M, Rahman., and M. M. R, Mithu. 2018. Toxic effects of plastic on human health and environment: A consequences of health risk assessment in Bangladesh. International Journal of Health, 6(1),1-5.

18-Suzuki, K., H, Sakamoto.,Y, Shinozaki ., J, Tabata., T, Watanabe., A A, Mochizuki., ... and H. K, Kitamoto. 2013. Affinity Purification and characterization of a biodegradable plastic-degrading enzyme from a yeast isolated from the larval midgut of a stag beetle, Aegus laevicollis. Applied microbiology and biotechnology, 97(17), 7679-7688

19- Yang, J., Y, Yang., W. M, Wu., J, Zhao., and L, Jiang. 2014. Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. Environmental science & technology, 48(23), 13776-13784.

20- Yang, Y., J, Yang., W. M, Wu., J, Zhao., Y, Song., L, Gao., ... and L, Jiang.2015. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Part 1. Chemical and physical characterization and isotopic tests. Environmental science and technology, 49(20), 12080-12086

21- Yang, Y., J, Yang., W. M, Wu., J, Zhao., Y, Song., L, Gao., ... and L, Jiang. 2015. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: part 2. Role of gut microorganisms. Environmental science and technology, 49(20), 12087-12093.