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Biodegradation of expanded polystyrene (EPS) (Styrofoam) block as feedstock to *Tribolium castaneum* (Red Flour Beetle) imago: A promising plastic-degrading process

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ABSTRACT

The study was conducted to determine the biodegradation of Expanded Polystyrene (EPS) (Styrofoam) Blocks as feedstock to *Tribolium castaneum* (Red Flour Beetle) Imago. Three-hundred-sixty (360) *Tribolium castaneum* (Red Flour Beetle) Imagos were collected and acclimatized for two days before being exposed to experimentation. Incubation boxes were made using plywood. Three set-ups were prepared, with 30 *Tribolium castaneum* (Red Flour Beetle) each. Seven (7) grams of Styrofoam Block were placed in Set-up 1, seven (7) grams of Styrofoam, and seven (7) grams of Rice grain Bran in Set-up 2, and seven (7) grams of Rice grain Bran in Set-up 3 with three replications each. Mean percent mass loss of Styrofoam and Rice grain Bran, mean percent mass loss and survival rate of *Tribolium castaneum* (Red Flour Beetle) were measured at 5th, 10th, 15th and 20th day incubation periods. Maximum mean percent mass loss in the different set-ups was observed at the 10th, 15th and 20th incubation period, with losses of 7.14%, 10.71%, and 10.71%, respectively. Meanwhile, maximum mean percent increased weight of *Tribolium castaneum* (Red Flour Beetle) was observed at the 10th day incubation period, with 13.51%, 44.44%, and 37.93%, respectively. Microscopic observation, of a dark white smear on the gut of the specimen indicates that *Tribolium castaneum* (Red Flour Beetle) Imago really masticate and biodegrade the Expanded Polystyrene (EPS) (Styrofoam) blocks. The survival rate of *Tribolium castaneum* (Red Flour Beetle) in the diverse set-ups showed no significant difference. This implies that ingestion of Styrofoam Block had no lethal effects on the natural weight variation and health of the *Tribolium castaneum* (Red Flour Beetle). Hence, results indicate that Expanded Polystyrene (EPS) (Styrofoam) Block can be a feedstock for *Tribolium castaneum* (Red Flour Beetle).

Keywords: Biodegradation, Expanded Polystyrene (EPS) (Styrofoam) Block, Feedstock, *Tribolium castaneum* (Red Flour Beetle), Ingestion

1. INTRODUCTION

Polystyrene (PS) with chemical formula of $(C_8H_8)_n$ is a stable, stiff and brilliantly transparent synthetic resin produced by the polymerization of styrene. It is commonly used in the food-service industry as disposable cups and boxes or cushioning materials in packaging. Polystyrene is a plastic that was hard and rigid to a number of important plastic and rubber products because it is copolymerized or blended with other polymers. It is often made for a short and easy service use and time and one-time use as a result of more economical cost of this material.

The contrast between the durability of PS and the short service time of PS products led to the increasing acquisition of PS waste in our environment. Its abundance formed litter in the outdoor environment, particularly in waterways. Due to its poor recycling rate, polystyrene has polluted the environment (WHO, 1987). As stated in the research of South East Asia, there are several million tons of plastic waste in the ocean every year. Philippines is one of the top five countries in the SouthEast Asia that contributes 60 percent trash in the ocean, causing serious threat to both, wildlife and human health. In the environment, PS foam debris are eaten and ingested by water wildlife for being mistaken as food that cause harms to them. Fish, seabirds and sea turtles can sicken or die if they eat too much of it, or if a sharp piece slices up their insides. Some plastics also leach toxins, which end up in the meat of fish and shellfish.

To take action on this problem, various efforts were exerted to eliminate and recycle Expanded Polystyrene (EPS) Block waste has been done, such as burial incineration and use of plastic degrading microbes. However, these procedures make the foam more stable and have a long degradation time and inquire high energy to combust properly. On the other hand, there is no practical application invented as biodegradation technique, thus there are developing technologies and it is recommended to screen and find efficient organisms capable of degrading plastics properly (Kale, Deshmukh, Dudhare and Patil, 2015).

This study aims to investigate and determine the possibility of using *Tribolium castaneum* (Red Flour Beetle) Imago to masticate the Expanded Polystyrene (EPS) Block as a Standard diet, a type of PS Foam. *Tribolium castaneum* (Red Flour Beetle) is an insect species of beetle in the family Tenebrionidae, the darkling beetles together with *Tenebrio molitor* (Mealworm Beetle) a proven plastic eating worm.

This study focuses on feeding the *Tribolium castaneum* (Red Flour Beetle) imago with seven grams of Expanded Polytyrene (EPS) Block, combination of seven grams of Expanded Polystyrene (EPS) Block and ricegrain bran and seven grams of ricegrain bran. Feeding activity was monitored based on the larval weight of *Tribolium castaneum* (Red Flour Beetle) imago, percent mass loss of ricegrain bran and Expanded Polystyrene (EPS) Block, the survival rate of *Tribolium castaneum* (Red Flour Beetle) imago and the microscopic observation of their guts in each set-ups.

It will be beneficial to the nature, especially to the sea wildlife and human, because it aims to masticate the Polystyrene using *Tribolium castaneum* (Red Flour Beetle) imago. Henceforward, this will help minimize the plastics and its effect to the environment.

2. MATERIALS AND METHODS

2. 1. Collecting the Test Insect



Collecting 30 *Tribolium castaneum* (Red Flour Beetle) for each Set-up



Acclimatizing All 360 *Tribolium castaneum* (Red Flour Beetle) Imago

Three hundred-sixty adult *Tribolium castaneum* (Red Flour Beetle) Imagos were collected on Brgy. Pambisan Munti, Pinamalayan using gloves. It was brought in the Zoology Division of University of the Philippines-Los Banos for authentication. This 360 *Tribolium castaneum* (Red Flour Beetle) were acclimatized for two days with their natural diet in the incubation box before starting the experimentation.

2. 2. Building the Experimental Incubation Box

Plywood was cut measuring 30 cm × 20 cm × 20 cm using a saw and made a 12 incubation box. This was the experimental habitat for the test set-ups and its replications. Thirty acclimatized *Tribolium castaneum* (Red Flour Beetle) were used in every set-up.

2. 3. Preparing the feeding set-ups and study designs



Set- up Designs

Three Set-ups were prepared, Set-up 1 had 30 *Tribolium castaneum* (Red Flour Beetle) Imagos and fed with seven grams of Expanded Polystyrene (EPS) Block. Set-up 2 had 30 *Tribolium castaneum* (Red Flour Beetle) Imagos that fed with the combination of seven grams of Styrofoam and seven grams of husk of ricegrain. Set-up 3 had seven grams of husk of ricegrain with *Tribolium castaneum* (Red Flour Beetle) Imago. Each Set-ups had three replications for each test and monitored in 20 days.

2. 4. Mean Percent Mass Loss



Measuring the Weight of the Beaker



Measuring the Weight of Styrofoam



Measuring the Weight of Ricegrain Husk

Computing percent mass loss of Expanded Polystyrene (EPS) Block caused by *Tribolium castaneum* (Red Flour Beetle) was performed following the method mentioned in the study of Bozek, Hanus-Lorenz and Rybak (2017), the mass of each set up was measured using digital weighing scale at different incubation period. This test was done in triplicates. Percent mass loss was computed using the formula below:

$$\text{Mass Loss} = \frac{\text{Initial mass} - \text{final mass}}{\text{Initial mass}} \times 100\%$$

2. 5. Mean Percent Increased Weight



Measuring the Weight of the Beaker



Measuring the Weight of Alive *Tribolium castaneum* (Red Flour Beetle) Imago

Calculating the percent weight of *Tribolium castaneum* (Red Flour Beetle) Imago in every set-up was done following the methods of Yang *et al.* (2017), the mass of *Tribolium castaneum* (Red Flour Beetle) was measured using a digital weighing scale at different incubation periods. This test was done in triple replications. Percent weight was calculated using the formula below.

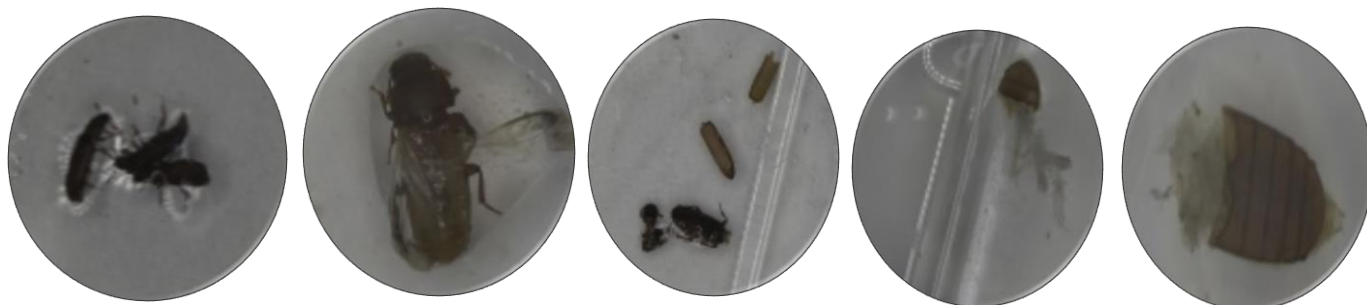
$$\text{Increased Weight} = \frac{\text{Initial mass} - \text{final mass}}{\text{Initial mass}} \times 100\%$$

2. 6. Survival Rate



The survival rate of *Tribolium castaneum* (Red Flour Beetle) Imago in every set-up was configured following the procedure that Kilic (2018) the survival rate of *Tribolium castaneum* (Red Flour Beetle) was calculated by counting the alive beetle at different incubation period. This test was done also in three replications.

2. 7. Dissecting the *Tribolium castaneum* (Red Flour Beetle) Imago and Analysis of the Gut



Five specimens from each Set-up was accumulated and emplaced on the glass plate with water. Under a digital microscope, clenched the beetle with tweezer while cut the head, pronotum and the elytra using the dissecting needle to easily got the abdomen. Separate the abdomen of the specimen in the other glass plate. In having the gut of the specimen, the abdomen was incised using tweezer and dissecting needle prior to obtained the gut of the specimen. The gut was analyzed using the digital microscope to examine the interior of the gut.

3. RESULTS AND DISCUSSION

According to Ho, Roberts and Lucas (2018) to evaluate the macroscopic changes on the surface structure of the biodegraded Styrofoam, the visual observation, like computing the percentage mean mass loss of Styrofoam can be used for an initial estimation of biodegradation property. A decrease in the average molecular weight, and the broadening of the molecular weight distribution, provide initial evidence of the degradation of a polymer. Change in molecular weight is an easy measurement of biodegradation, and when used with other methods, it can be a useful indicator of the degree of biodegradability. Measurement of weight loss for the estimation of biodegradation is a frequently used method when the polymer is exposed to a mixture of selected microbes and insect in the culture media with the polymer as sole source of their carbon. This method is standardized for in-field and simulation biodegradability tests.

Table 1. Mean Percent Mass Loss of Styrofoam Block and Ricegrain Bran as feeding diet of *Tribolium castaneum* (Red Flour Beetle) Imago in the Three Set-ups at Different Incubation Periods.

Incubation Time (Days)	Set-up 1 (%)	Set-up 2 (%)	Set- up 3 (%)
5	2.86	10.36	10.36
10	7.14	5.71	9.64
15	5.00	10.71	9.64
20	6.07	9.29	10.71

On the first five days, set-up 1 had 2.86 percent while Set-up 2 and Set-up 3 had the same mean percentage mass loss of 10.36%. These results were the same as on the study of Kaplan, Hartenstein and Sutter (1979) with 4-57% in the 5-11 days of incubation. There is an increase in percent weight loss in the set-up 1 as the incubation day increase from 5 to 10, however, an opposite result was observed in set-ups 2 and 3 in the same incubation days. The increase in percent weight loss in set-up 1 can be attributed to the very active on mastication of *Tribolium castaneum* on Expanded Polystyrene (EPS) Block while *Tribolium castaneum* in set-ups 2 and 3 were masticating rice grains as discussed by Bender, Revs, and Ward (2003).

While on the transition from 15 to 20 incubation days, Set-up 1 had 5.00% and 6.07%, respectively, set-up 2 had 10.71% and 9.29%, respectively, set-up 3 had 9.64%, and 10.71%, respectively. It shows that the mastication of *Tribolium castaneum* (Red Flour Beetle) Imago becomes slow. This also happened on the study of Yang *et al.* (2015a) with the carbon of the ingested Styrofoam egested as fecula was decreased from 73.6% to 49.2% from day 4 to day 15 suggesting that the activity for the digestion of ingested Styrofoam decrease progressively because the insect is adopting the food it had, and making it as its common food. They also reported that the larvae of *Tenebrio molitor* (Mealworm) eats 34-35 mg Styrofoam a day, supporting the result of the current study.

One-way analysis of variance (ANOVA) was used to further interpret the result above. A p-value of 0.008 was obtained, which is lower than the 5% level of significance. This implies that the null hypothesis is rejected. Therefore, there is a significant difference among the set-ups with different incubation time. Meanwhile, post hoc analyses using the Scheffé post hoc criterion supported that the set-ups 2 and 3 have no significant difference with a p-value of 0.664. This implies that Styrofoam can be a feedstock to *Tribolium castaneum*, thus providing an alternative solution to the problem involving disposal of Expanded Polystyrene (EPS) Block.

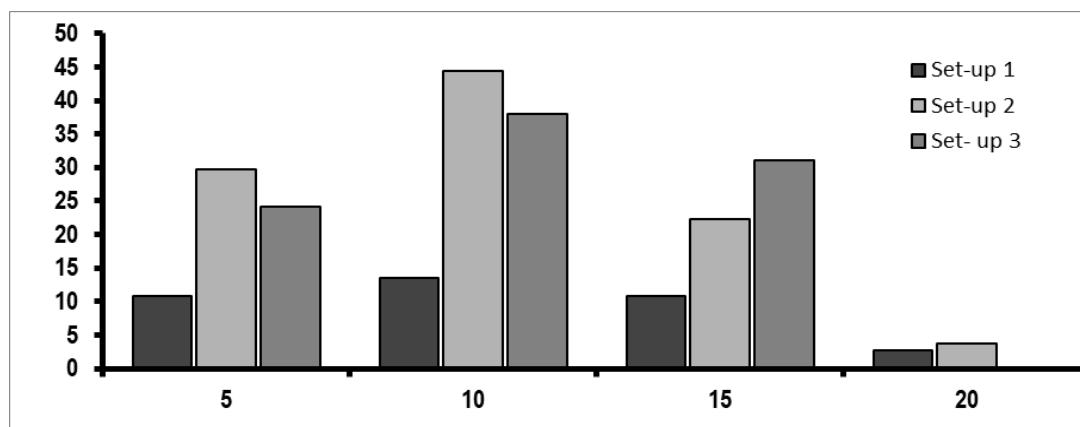


Figure 1. Percent Increased Mean Weight of *Tribolium castaneum* (Red Flour Beetle) Imago fed with Styrofoam Block Diet and Ricegrain Bran in Different Incubation Time

On the ten days of the three set-ups, Set-up 1 had percentage mean increased weight from 10.81% to 13.51%, and is lower than Set-up 2 from 29.63% to 44.44%, and Set-up 3 from 24.14% to 37.93%. It is because these two set-ups have a Rice bran and unlike Styrofoam, it has a proper water content and necessary growth nutrients, such as proteins, phosphorus, vitamins, and minerals. Additionally, Ricegrain bran also like yeast which is source of B vitamins, proteins, trace metals, and amino acids that could be easily assimilated through a simple digestion (Yang, 2015a; Gibson and Hunter, 2010).

Therefore, the lack of nutrients of the food with relatively poor biodegradability of *Tribolium castaneum* (Red Flour Beetle), provides a limited energy source for biomass synthesis or growth of the beetle. Similarly, the study of Butler and Buckerfield (1979) on the digestion of synthetic lignin hydrocarbons by termites in the absence of other nutrients indicated that 8.5-32.4% C form C- labeled lignin which was converted to carbon dioxide, while only a limited fraction (0.002-0.004%) of C was assimilated into termite bodies after 50 days' test

period (as cited in Yang *et al.*, 2015a). Meanwhile, after 20 days of incubation, all of the three set-ups had decreased the percent increased mean survival weight of *Tribolium castaneum* (Red Flour Beetle). Set-up 1 has decreased of 10.81% to 2.70%, set-up 2 has 22.22%-3.70%, and set-up 3 has 31.03% to 0% increased on their survival weight. It is because they easily adopt their diet and the nutrients they have. As reported in the study of Sreeramoju, Prasad and Lakshmipathi (2016), the weight of the *Tribolium castaneum* (Red Flour Beetle) apparently declines at pre-pupal stage to some extent (2.538 ± 0.07 mg per Beetle) and remains constant as it grows to an adult and throughout Imago. This results show that the Styrofoam didn't affect the natural weight variations and changes of the beetle.

One-way analysis of variance (ANOVA) was used to further interpret the result above. A p-value of 0.383 was obtained, which is higher than the 5% level of significance. This shows that the null hypothesis is accepted. Meanwhile, Post hoc analyses using the Scheffé post hoc criterion for significance indicated that Set-up 1 was not significantly different than were those in both Set-up 2 with a p-value of 0.447 and in Set-up 3 with p-value of 0.447. The pairwise comparison of Set-up Two and Set-up Three was nonsignificant ($p = 1.000$). Therefore, there is no significant difference among the set-ups with different incubation time. It also implies that Expanded Polystyrene (EPS) Block is adopted by the *Tribolium castaneum* as their feedstock that didn't show any lethal and bad effect on their weight variations.

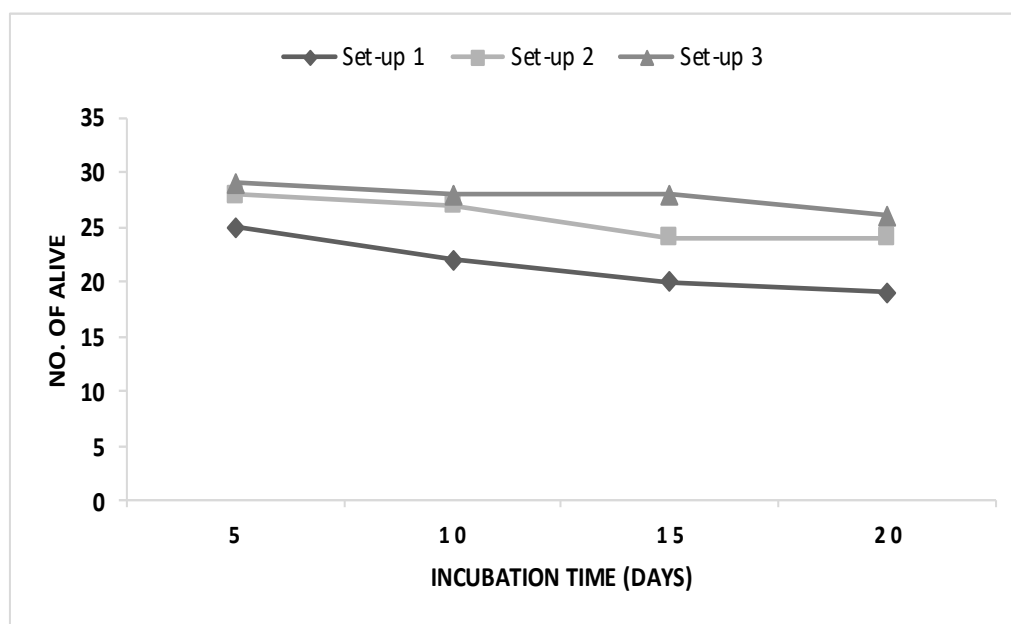


Figure 2. Mean Percent Survival Rate of *Tribolium castaneum* (Red Flour Beetle) Imago fed with Expanded Polystyrene (EPS) Block Diet and Ricegrain Bran in Different Incubation Time

At the end of the 20th day of incubation time, the survival rate of the *Tribolium castaneum* (Red Flour Beetle) in the Set-up 1 and set-up 2 fed with Expanded Polystyrene (EPS) block was 63% and 80%, respectively, which is not significantly less than the set-up 3 with ricegrain bran diet with a survival rate of 87%. This also implies that in the insect nutrition, it was

revealed that in the absence of yeast and ricegrain bran associates, the insect performance decreased (Vega and Dowd, 2005). These results are parallel with the study of Yang *et al.* (2017) with the results of $86.7 \pm 3.3\%$ of survival rate of mealworm fed with EPS alone, and on the controlled it had $90 \pm 0.8\%$ of survival rate.

Pearson Goodness-of-Fit Test (Chi-squared test) was used to further interpret the result above. A p-value of 0.449 was obtained, which is higher than the 5% level of significance. This shows that the null hypothesis is accepted. Therefore, there is no significant difference among the set-ups with different incubation time. It can be asserted that Expanded Polystyrene (EPS) (Styrofoam) Block is eatable and non-lethal to the *Tribolium castaneum* (Red Flour Beetle) Imago. Current findings seem to be confirmed what was reported that the mealworms (*Tenebrio molitor*) fed solely Styrofoam able to survive for a month. Such ability of mealworms in the biodegradation of Styrofoam was due to the role and activity of gut bacteria (Yang *et al.* 2015b). In addition, as reported by Genta, Dillion, Terra and Ferreira (2006) the non-lethal effect of the Styrofoam block in the mealworms is allegedly due to the detoxifying role of some gut microbiota of the insect

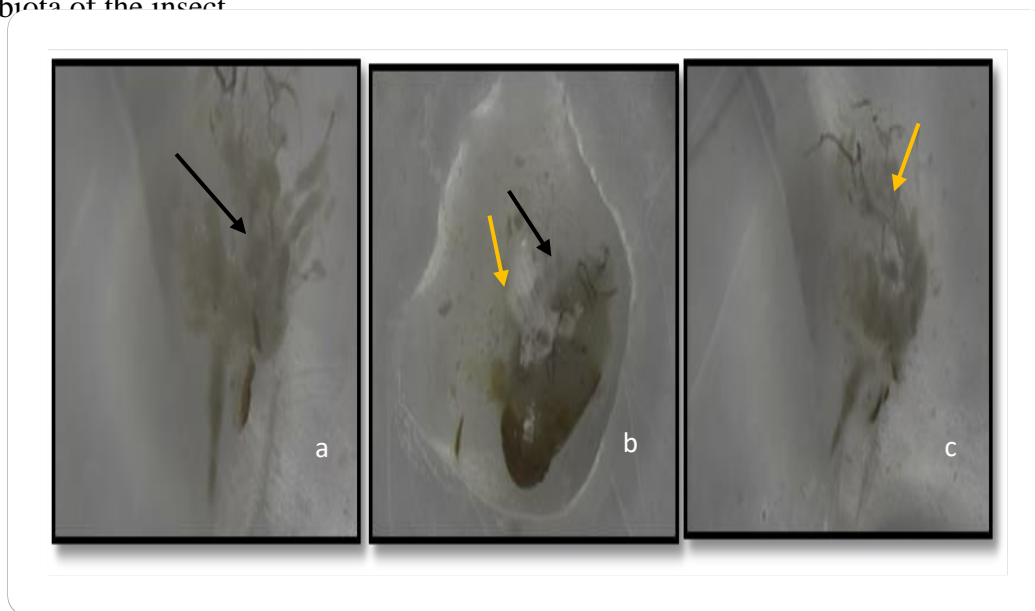


Figure 3. The microscopic observation of the gut of the *Tribolium castaneum* (Red Flour Beetle) Imago Specimen in Different Set-ups. Dark white (a) indicates the presence of Styrofoam, Dark white and yellowish portion (b) shows the presence of Styrofoam and ricegrain bran and yellowish part (c) proves the presence of rice grain bran.

According to Klimaszewski *et al.* (2013), microscopic examination of gut contents and molecular techniques to investigate dietary preferences of a beetle is highly important. For the beetles that are organisms with relatively strict feeding habitat requirements, such as fungal species that require lignocellulose, molecular gut analyses may lead to inferences on the importance of habitat elements, such as downed deadwood and other materials (Suh and Blackwell, 2005a;b). In the study by Klimaszewski *et al.* (2013), the use both, microscopic examination and molecular analysis of gut contents and digestive tract to more precisely characterize the feeding habits and what kind of food they masticate through their mandibles.

Based on the microscopic observation, there is a yellowish spot characteristic on the dissected gut on the Set-up 1 that shows that the specimens masticated the Ricegrain Bran as their common diet. Supported in the study of Yang *et al.* (2015b) they show yellowish spot on the gut of the Mealworms. When mealworms were fed with gentamicin-containing bran (30 mg/g of bran), gut bacteria were significantly suppressed on the basis of the estimation of gut bacterial numbers by the series dilution method of plate counting.

Both guts of the specimen dissected from Set-up 2 and 3 have a dark white spot on their guts that show the masticated and biodegraded Expanded Polystyrene (EPS) (Styrofoam) Block by the *Tribolium castaneum* (Red Flour Beetle) Imago. The study of Yang *et al.* (2014) show that the darker the gut of the beetle has more microbes and bacteria and the tendency is to have more biodegraded Expanded Polystyrene (EPS) (Styrofoam) Block.

Both, dissection and microscopic analysis of guts strongly suggests that *Tribolium castaneum* (Red Flour Beetle) can biodegrade and masticate the Expanded Polystyrene (EPS) (Styrofoam) Block by their mandibles. Utilization of an ingested food, in turn, is determined by the capacity of the insect to digest and absorb the various constituents present in the diet. The observations presented in this paper serve to elucidate how well adult *Tribolium castaneum* (Red Flour Beetle) are physiologically adapted to utilize the nutrients available in their given diet and feedstock.

4. CONCLUSIONS

Maximum mean percent mass loss in the different Set-up was observed by 10th, 15th, and 20th incubation period with 7.14%, 10.71%, and 10.71%, respectively. Meanwhile, maximum mean percent increased weight of *Tribolium castaneum* (Red Flour Beetle) was observed at 10th day of incubation period with 13.51%, 44.44%, and 37.93%, respectively. Furthermore, Survival Rate of *Tribolium castaneum* (Red Flour Beetle) in different Set-ups showed no significant difference. In addition, this study proved that setup 2 and 3 have no significant difference in terms of mean percent weight loss making it a good feedstock to the said test insect. In addition, the ingestion of Styrofoam block by *Tribolium castaneum* (Red Flour Beetle) did not affect the natural weight and survival rate. It implied that ingestion of Styrofoam Block had no lethal and bad effect on the natural weight variation and health of the *Tribolium castaneum* (Red Flour Beetle). These results proved that Expanded Polystyrene (EPS) (Styrofoam) Block can be a feedstock of *Tribolium castaneum* (Red Flour Beetle). Thus, on the microscopic observation, there is a dark white on the gut of the specimen that proved that the *Tribolium castaneum* (Red Flour Beetle) Imago really masticate and biodegrade the Expanded Polystyrene (EPS) (Styrofoam) Block. This study gave a reasonable data to conclude that Expanded Polystyrene (EPS) Block can be a feedstock of *Tribolium castaneum* (Red Flour Beetle).

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