

# Biodegradable material vs non-degradable materials: s consumption of compostable plastics less harmful than oil-derived plastics?

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January 27, 2025

## Abstract

Given the harmful impact of plastics on organisms' fitness, one question arises: is compostable material (derived from non-oil sources) any better? Here we assessed the fitness effects of consuming two oil-derived plastics (polyethylene and polystyrene) and one compostable product in insects, utilizing *Tenebrio molitor* beetles as the study system. Animals were fed during the larval stage either of four different treatments: a) polyethylene + apple/wheat; b) polystyrene + apple/wheat; c) compostable product + apple/wheat; and d) apple/wheat alone. Upon reaching the adult stage, insects were provided with wheat and apple for 7 days, allowed to mate, and lay eggs. We recorded developmental rate and mortality from larvae to pupa, weight and fecundity, and survival probability from one stage to the next. Mortality was higher when animals consumed any type of plastic. The probability of survival was also affected, particularly in the pupal and adult stages. Feeding with any type of plastic oil-derived or compostable plastic led to a reduction in body size and reproductive success (measured as surviving larvae). Notably, in some cases, the group fed with compostable plastic was the most affected. Delays in development at different stages could increase mortality, while the decrease in egg production in females and the reduction in adult size could imply carry-over effects on demography. Perhaps, the additional materials in compostable products imply toxic effects like those caused by plastics. Thus, the effects of compostable products are not any better than those of plastics.

Biodegradable material vs non-degradable materials: s consumption of compostable plastics less harmful than oil-derived plastics?

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Given the harmful impact of plastics on organisms' fitness, one question arises: is compostable material (derived from non-oil sources) any better? Here we assessed the fitness effects of consuming two oil-derived plastics (polyethylene and polystyrene) and one compostable product in insects, utilizing *Tenebrio molitor* beetles as the study system. Animals were fed during the larval stage either of four different treatments: a) polyethylene + apple/wheat; b) polystyrene + apple/wheat; c) compostable product + apple/wheat; and d) apple/wheat alone. Upon reaching the adult stage, insects were provided with wheat and apple for 7 days, allowed to mate, and lay eggs. We recorded developmental rate and mortality from larvae to pupa, weight and fecundity, and survival probability from one stage to the next. Mortality was higher when animals consumed any type of plastic. The probability of survival was also affected, particularly in the pupal and adult stages. Feeding with any type of plastic oil-derived or compostable plastic led to a reduction in body size and reproductive success (measured as surviving larvae). Notably, in some cases, the group fed with compostable plastic was the most affected. Delays in development at different stages could increase mortality, while the decrease in egg production in females and the reduction in adult size could imply carry-over effects on demography. Perhaps, the additional materials in compostable products imply toxic effects like those caused by plastics. Thus, the effects of compostable products are not any better than those of plastics.

Key words: plastic, compostable material, fitness, insect, *Tenebrio*, life history, survival, weight, fecundity

## Introduction

In a world full of threatening factors, plastics are part of the top list of stressors (MacLeod, et al. 2021; Santos et al. 2021; Martinho et al. 2022). Plastics are produced through the polymerization of monomers derived from oil and gas. Two well-known examples are polyethylene (PE) and polystyrene (PS) which are extensively used for plastic bags and containers (such as Styrofoam®) respectively (Andrady & Neal, 2009). Given the negative effects of these products, a proposed alternative is the use of bioplastics such as compostable plastics (CP), which are polymeric, non-oil derived materials from natural substances (Arikan & Ozsoy, 2015; Atiwesh et al. 2021). Unlike plastics, CP production presumably implies reduced greenhouse effects and energy-saving balance, faster degradation (Atiwesh, et al. 2021).

Although there were doubts about the effects of plastic ingestion on animal physiology, we now have a clearer understanding. Studies using insects have shown negative effects at molecular levels, such as a high production of reactive oxygen species, antioxidant enzymes, lipid peroxidation indicators, and oxidative damage in *Tenebrio molitor* larvae fed with PE (Peng et al. 2023). Similarly, also in *T. molitor* larvae, when PS was part of the diet, negative effects were observed on the lipidome, with high levels of ceramides and cardiolipins being produced, which are implicated in apoptosis and cellular stress processes (Tsochatzis et al. 2022). At the DNA level, PS consumption affected the expression of genes that encode heat shock and oxidative stress proteins during development, specifically at the pupal formation stage *Chironomus riparius* dipteran larvae (Carrasco-Navarro et al. 2021). These negative effects also take place systemically. In *C. riparius* larvae, an increase in basal phenoloxidase activity was found after PE ingestion (Silva et al. 2021). Also, *Bombyx mori* larvae fed with PS, showed an increased expression of antimicrobial peptide (AMP) genes, such as lysozymes and cecropins (Muhammad et al. 2021).

While the environmental advantages of using non-petroleum-derived plastics are known, there is still inconclusive evidence on their effects on insects. For example, consumption of polybutylene adipate terephthalate, a biodegradable and compostable copolymer, by *T. molitor* larvae over 4 weeks did not affect survival, mass, or molting rate (Kokalj et al. 2024). When black soldier fly was fed with the biodegradable polyester polylactic acid (PLA), their larval development, survival, as well as pupal development and rate, were not affected (Heussler et al. 2024). Conversely, *T. molitor* larvae fed with PBAT during the first and second generations, increased and decreased their molting rate in the first and second generation respectively (Kokalj et al. 2024). Finally, one other aspect that has lagged behind is that of the effects of both non-biodegradable plastics and bioplastics on insects when their ingestion is constant and long-term (i.e., during all or almost all the larval phase until adulthood). These elements are crucial since several future scenarios indicate that plastic waste

in the environment will be a constant for all organisms across ecosystems.

Here we show the effects of ingesting plastic versus compostable material on insect fitness (measured as development rate, mortality, weight, hatching rate, fecundity, and survival probability among different age stages) using the mealworm beetle *Tenebrio molitor* (Linnaeus, 1758) as our study subject. We chose this animal based on previous research indicating that larvae can consume polymers for short periods of time (Yang et al., 2015), and that microplastic, oil-derived consumption impairs survival, growth, and development (Matyja et al., 2020). The novelty of our work lies in its comparison of the fitness effects of ingesting plastics vs CP, as well as in examining how fitness surrogates covariate with each other. Our working hypothesis is that the ingestion of biodegradable plastics will have little or no effect on the variables analyzed, compared to PS or PE.

## Materials and Methods

### *Study species and experimental procedures*

*Tenebrio molitor* is a holometabolous insect with females capable of laying up to 500 eggs throughout their reproductive cycle (Frooninckx et al., 2022). The embryonic development takes 4 to 6 days, the larval development period spans 2 to 3 months, and the pupal stage lasts 6 days (Kim, 2015). After a few days, adults reach sexual maturity, and females begin ovipositing between days 4 and 17 after mating (Frooninckx et al., 2022). Individuals used in the study originated from several colonies in Mexico City and its metropolitan area. The animals were fed a standardized diet consisting of wheat bran and apple (Castro et al., 2017). Insects were housed in 15 plastic hinged containers (15 cm x 18 cm x 5.8 cm), each containing 200 individuals, to mitigate cannibalistic behavior. They were kept at a room temperature of 22.4 °C with a photoperiod of 12:12 h light:dark cycle. By the third generation, mealworms were transferred to a glass container (70 cm x 30 cm x 30 cm), and their resulting larvae were used in the experiments. All experiments commenced with stage 3 larvae, approximately 1.5 cm long. The treatments and feeding regime included: a) Polystyrene (PS): larvae were provided with 1 cm<sup>2</sup> sections cut from REYMA brand disposable plates provided ad libitum; b) Polyethylene (PE): fed with 5 cm x 5 cm sections of polyethylene bags from Poliexcel brand, commonly found in Mexican supermarkets; c) Compostable products (CP): fed with sections (5 cm x 5 cm) of Full Circle Fresh Air™ lemon-flavored bags (USA), made from cornstarch (Maizena) and other unspecified ingredients; and, d). Control: fed with 1 g of wheat bran and 2 cm<sup>2</sup> of apple provided weekly, with an additional 0.5 g of wheat bran and 2 cm<sup>2</sup> of apple every 15 days for the first three treatments.

Each treatment consisted of 50 larvae, and all individuals were sexed upon reaching the pupal stage (Pölkki et al., 2012). After reaching the adult stage, animals were paired, placed in separate containers, and fed ad libitum with wheat bran and apple cubes for seven days. After mating, the couples were separated, and individuals were monitored until death. Females ( $N = 10$  per group) were provided with filter paper for ovipositing until death.

### *Effects on developmental time, weight, hatching rate, and fecundity*

For all treatments, we recorded the number of days it took larvae to reach the pupal stage and adulthood, the number of dead larvae, and the time survived as adults. Each dead animal (as adult) was weighed (in g) after 48 hours using an electronic scale (VELAB electronic scale VE-1000). For females allowed to oviposit, we recorded the number of eggs and egg hatching success.

Due to non-normality and/or homocedasticity of the data (even after transformation), we used non-parametric tests. The Kruskal-Wallis test was employed to analyze the time to reach the next stage, with the Bonferroni post-hoc test applied when significant differences were observed ( $p < 0.05$ ). Analysis of variance (ANOVA) was used to analyze the number of dead individuals during each stage, with a Tukey's post-hoc test to identify significantly different groups. The Kruskal-Wallis non-parametric test was applied to compare the weight of the adults, and the Bonferroni post-hoc test was performed subsequently. To compare overall differences in the duration of each developmental stage for the four treatment groups, we calculated the ratio of the duration in days of the Pupa stage to the Larvae stage (ratioPL) and then applied an ANOVA

with a Tukey post-hoc test.

### Capture–Recapture Models

Το ζονστρυστ α σταβασε φορ προσεσσινγ ιν ζαπτυρε-ρεζαπτυρε φραμε μονελς, ωε οργανιζεδ τηε λιφε ταβλε οβταινεδ φορομ οβσερατιονς ζολλεςτεδ δυρινγ τηε εξπεριμεντ. Ωε ζονσιδερεδ ρεζαπτυρε εεντς εερψ 5 δαψς, ωιτη α ρεφερενζε το τηε δυπλασιαιον τιμε οφ 7 δαψς φορ α ποπυλαιον υνδερ οπτιμαλ ζονδιτιονς (Γοτελλι, 1995). Της ρεσυλτεδ ιν α λιφε ηιστοριψ σταβασε οφ 2197 σαμπλες ωιτη 35 ζαπτυρε-ρεζαπτυρε οςζασιονς οφ σαμε-αγε ινδιιδυαλς. Ας ουρ εξπεριμενταλ ποπυλαιον ις ζλοσεδ, ωε αππλιεδ α σινγλε στατε ορμασκ-Θολλψ-Σεβερ (“ΘΣ) μονελ, φολλοωεδ βψ α μυλτι-στατε μονελ ασζουντινγ φορ διφορεντ δεελοπμενταλ σταγες (Ααραε, Πυπα, ανδ Αδυλτ: Α, Β, ανδ Α ρεσπεςτιελψ). Δετεςτιον προβαβιλιτη ρεμαινεδ ζονσταντ τηρουγηουτ τηε εξπεριμεντ υνδερ ζοντρολλεδ ζονδιτιονς. Τηε “ΘΣ μονελς αππλιεδ ωερε: α) “ΘΣ μονελ ( , π) ωιτη ζονσταντ δετεςτιον ανδ συριαλ· β) Τιμε-αρψινγ συριαλ ανδ ζονσταντ δετεςτιον ( τ, π)· ανδ, ζ) ονσταντ δετεςτιον ανδ συριαλ δεπενδεντ ον τρεατμεντ ( τρεατ, π). Ιν αλλ ζασες, μονελ σελεςτιον ωας βασειδ ον λοωερ ΩΑΓ. Ιν μυλτι-στατε μονελς, ωε ζονσιδερεδ δεελοπμενταλ στατες (Α, Β, ανδ Α) πλυς δεαδ ασ στατες (φορ στατες ιν τοταλ), ωιτη τρανσιτιονς ονλψ ποσσιβλε φορομ Α το Β ανδ φορομ Β το Α, ανδ φορομ ανψ στατε το δεαδ. Δετεςτιον ωας ζονσιδερεδ ζονσταντ, ανδ ωε αππλιεδ τωο διφορεντ μονελς το εστιματε συριαλ: α) Α μυλτιστατε μονελ ωιτη ζονσταντ δετεςτιον ( Α, Β, Α, ψΑΒ, ψΒΑ, πΑ, πΒ, πΑ)· ανδ, β) Α μυλτιστατε μονελ ωιτη ζονσταντ δετεςτιον ανδ τρεατμεντ-δεπενδεντ συριαλ ( Α, Β, Α, ψΑΒ, ψΒΑ, πΑ, πΒ, πΑ, βτρεατ). Τηε λαττερ αιμεδ το ιδεντιψ α διφορεντιαλ εφφεζτ οφ τρεατμεντς ον τηε διφορεντ δεελοπμενταλ πηασες.

Results are indicated as mean ± STD unless indicated otherwise.

## Results

### Developmental rate from larvae to pupa

Time from larval to the pupal stage showed significant differences (Kruskal-Wallis  $H=403.94$ ,  $gl=3$ ,  $p<0.001$ , Figure 1). The control group ( $48.05 \pm 0.22$ ) had a faster development, compared to the other three groups (Bonferroni post-hoc tests  $p<0.001$ , in all comparisons). Development time was shorter in the group fed with PE ( $79.43 \pm 2.12$ ) compared to the groups fed with CP ( $83.82 \pm 1.38$ ;  $p<0.001$ ) or PS ( $101.78 \pm 0.89$ ;  $p<0.001$ ). There were no differences between CP or PS ( $p = 0.55$ ; Fig. 1), but there was a shorter pupal stage for the control group ( $7.67 \pm 0.05$ ,  $p<0.001$ ) compared to the other three groups (Fig. 2). No significant differences emerged for the other groups (CP,  $7.84 \pm 0.07$ ; PS,  $8.11 \pm 0.12$ ; PE,  $8.35 \pm 0.09$ ; Bonferroni post-hoc test  $p > 0.05$  for all cases).

### Mortality from larvae to pupa

The number of insects that died from pupal to adult stage was different among groups (ANOVA  $F_{3,16} = 10.61$ ,  $p < 0.001$ ). The control ( $2.2 \pm 1.16$ ) and PE ( $4.2 \pm 1.16$ ) groups showed a lower mortality compared to CP ( $9.8 \pm 0.73$ ; Tukey’s post-hoc test,  $p = 0.005$  in both cases). PS did not show significant differences in mortality with respect to the experimental groups ( $p = 0.05$  for PE, and  $p = 0.08$  for CP) or the control group ( $p = 0.05$ ).

### Final weight in adults

The four groups differed (Kruskal-Wallis  $H = 309.85$ ,  $gl = 3$ ,  $p < 0.001$ ): the Control group was heavier than the other three groups (Bonferroni’s post-hoc test  $p < 0.001$  for all comparisons). PE, PS and CP differed among them (Bonferroni’s post-hoc test  $p < 0.001$ , for all comparisons). While the PE group had the lowest weight ( $0.0314 \pm 0.00023$ ), the CP group had the highest weight ( $0.0385 \pm 0.00041$ ) and the PS group had an intermediate value ( $0.0340 \pm 0.00044$ ).

### Hatching rate and fecundity

The number of larvae hatched from surviving couples was different between the experimental groups and the control group (Kruskal-Wallis  $H_3 = 56.40$ ,  $p < 0.001$ , Fig. 3).

Females from the control group produced a higher number of larvae ( $27.46 \pm 0.80$ ), compared to females that were fed with PE or PS (PS,  $21.09 \pm 0.72$ ; PE,  $17.90 \pm 0.51$ ; Bonferroni's post hoc test  $p < 0.001$ , in both comparisons). Also, the number of larvae in the control group was higher compared to the adults fed with CP (CP  $22.41 \pm 0.67$ , Bonferroni's post-hoc test  $p = 0.028$ ). The number of larvae produced was higher in the group fed with CP, compared to the group fed with PE (Bonferroni's post-hoc test  $p = 0.009$ ). For the remaining comparisons of the experimental groups there were no significant differences (Fig. 3).

### *Survival rate among life stages*

Among CJS models, the one better representing our experimental set considers treatment as the main factor affecting survival, more than individuals' age (WAICs:  $\psi_{\text{trat,p}} = 14.343$ ,  $\psi_{\text{p}} = 15.809$ ,  $\psi_{\text{age,p}} = 16.148$ ). Also, being age less relevant than a constant condition we decided to consider stages more than age as factors affecting survival, so we applied multistate models. Multistate models considered different developmental stages as status: Larvae, Pupa and adult, plus dead stage as default. Age resulted as having an effect reducing survival probability of pupae and adult (Fig. 4A). In figure 4B we depict the three posterior distributions where pupae and adult show some variability. Considering the effect of treatment on survival in the multistate model, we obtained the mean and ranges of survival probability of the four different treatments for the three stages (Table 1). Treatment similarly affected the probability of survival compared to the control group, reporting major effects in pupal and adult stages (Figure 5).

## Discussion

Our results indicate that biodegradable plastics are not any better than common plastics. This is based on the adverse outcomes to fitness after consuming either plastic oil-derived or compostable plastics for almost all fitness variables. More specifically: compared to the control group, consuming these products delayed the developmental duration of both larval and pupal stages, hastened animal mortality, which resulted in lower weight (with the lightest in the PS and CP groups), and led to reduced survival. We will discuss each of these different effects below.

One negative effect of consuming plastics oil-derived and bioplastics is the delay in developmental time. Although, the presence of micro- and oil-derived nanoplastics did not influence larval growth in *Bombix mori* (Muhammad et al., 2021). A similar result was reported in *T. molitor* and *Hermetia illucens* larvae after eating bioplastics (Heussler, et al. 2024; Kokalj et al. 2024). Our results showed that both types of plastics delay the development time. The differences could be explained by time, since our animals fed on both types of plastic from very early larval stages compared to previous studies. One related question is that of the cost of delayed development. A consequence is that animals can face more threats, such as predators, parasites, and/or parasitoids (Nylin and Gotthard, 1998). Increasing development times is common in insects, largely explained by not reaching a certain body mass threshold necessary to complete metamorphosis (Nijhout, 2003). This aligns with our result of reduced weight in animals that consumed plastics or compostable products: reduced weight could prolong development. There are several non-mutually exclusive mechanistic explanations for this. First, stressed animals may not gather enough or do not have access to particular nutrients for their development (e.g. Welden and Cowie, 2016). Second, the presence of toxic elements in both petroleum-derived and compostable plastics may cause negative effects (Wang et al., 2020; 2021; Zimmermann et al., 2021). Third, either plastics or compostable products may obstruct the digestive system and impede nutrient acquisition (Sigler, 2014). And fourth, plastics may affect the animal's microbiota (Antonelli et al., 2022).

Our results also showed that mortality increased when larvae were fed with biodegradable and non-biodegradable plastics, where even mortality was higher in the CP group. Similar studies showed that *Drosophila melanogaster* males had a higher mortality compared to females after ingesting PS microplastics (El Kholly and Al Nagar, 2023). In contrast, in yellow mealworm beetles and black soldier fly larvae that were fed bioplastics did not show increased mortality (Heussler, et al. 2024; Kokalj et al. 2024). However, similar to other studies (Wang et al., 2021), we found a trade-off between investment in development and survival. Also, the number of offspring after reproduction in surviving pairs of treatment groups was signifi-

cantly lower than control. As explained above, the trade-offs may be understood by the different mechanisms underlying the ingestion of non-natural material. One challenge to clarify is how the different effects plastics non-biodegradable and bioplastics can cause on survival, reproduction, and growth may balance each other, resulting in some traits being less affected than others. For example, we are aware of the effects of oil derived microplastics on antioxidative stress response, sex hormone disruption, and disturbed transcription of steroidogenic genes as main drivers of impaired reproduction (Wang et al., 2021). How these effects may be balanced with the effects of the same material on, say, survival, is unclear. One other challenge is to explain whether the observed trade-offs are adaptive or are an artifact of an animal being unable, in general, to deal with, say, a toxic material.

Finally, the idea that plastic ingestion has detrimental effects on life history traits and that this can lead to apparent trade-offs is not new (Santos et al., 2021). What is new about our work is the trade-off and outcome of ingesting compostable, which plays as much a detrimental role as ingesting plastics. For example, animals may trade off fecundity for development time after eating compostable products. Why would compostable products produce similarly acute patterns as plastics? At the proximate level, compostable products may contain similarly toxic components to those of common plastics. It is known that added components to plastics produce inflammatory responses, endocrine disruption, neurotoxicity, oxidative stress, and metabolic alterations that all together affect immunity, reproduction, and digestion in insects (reviewed by Sánchez-Hernández, 2021). This may be the case for compostable products too. For example, a study analyzed 43 biobased and biodegradable products and found that 67% showed baseline toxicity, 42% led to oxidative stress, and 23% induced antiandrogenicity (Zimmermann et al., 2021).

## Conclusions

Considering the assertion that compostable products can serve as an alternative to conventional plastics, our work presents contradicting evidence: insects fed with compostable products may experience fitness effects (growth, fecundity, and survival) as detrimental as those observed in insects fed with plastics. The cause of this phenomenon is currently unknown, and one potential explanation is the presence of added toxic components in compostable products. Consequently, our work serves as a cautionary note against the argument advocating for the safety of using compostable materials.

## Acknowledgements

This project was financed by a UNAM-PAPIIT grant IN204921.

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## Tables

Table 1. Survival estimations obtained from the Multistate model with developmental stage as state and treatment as covariate.

age ->	Larvae	Pupa	Adult			
Treatment	Phi mean	range	Phi mean	range	Phi mean	range
Polyethylene (PE)	0.961	0.954 - 0.967	0.852	0.803 - 0.897	0.767	0.727 - 0.803
Polystyrene (PS)	0.963	0.958 - 0.968	0.876	0.844 - 0.908	0.788	0.760 - 0.813
Compostable products (CP)	0.965	0.959 - 0.970	0.897	0.857 - 0.924	0.807	0.782 - 0.827
Control	0.967	0.958 - 0.974	0.915	0.861 - 0.947	0.824	0.795 - 0.856

Table 2. Results of the Tuckey test as ANOVA post hoc of evidencing differences in ratioPL, ratio of the duration in days of Pupa stage on Larvae stage, between treatments

	difference	range	p
control Vs CP	0.060	0.056 - 0.066	< 0.001
PE Vs CP	-0.008	-0.013 - -0.003	< 0.001
PS Vs CP	0.000	-0.005 - 0.006	0.995
PE Vs control	-0.069	-0.073 - -0.064	< 0.001
PS Vs control	-0.060	-0.065 - -0.055	< 0.001
PS Vs PE	0.008	0.003 - 0.013	< 0.001

## Data accessibility statement

Data are available at [10.6084/m9.figshare.25135070](https://doi.org/10.6084/m9.figshare.25135070).

### Figure captions

Figure 1. Average time from the larval to pupal stage in *Tenebrio molitor* according to treatment (after eating two different plastic types, compostable products and the control group) ( $\pm$  SE). The data refers to all 5 repetitions and total sample sizes are shown for each group.

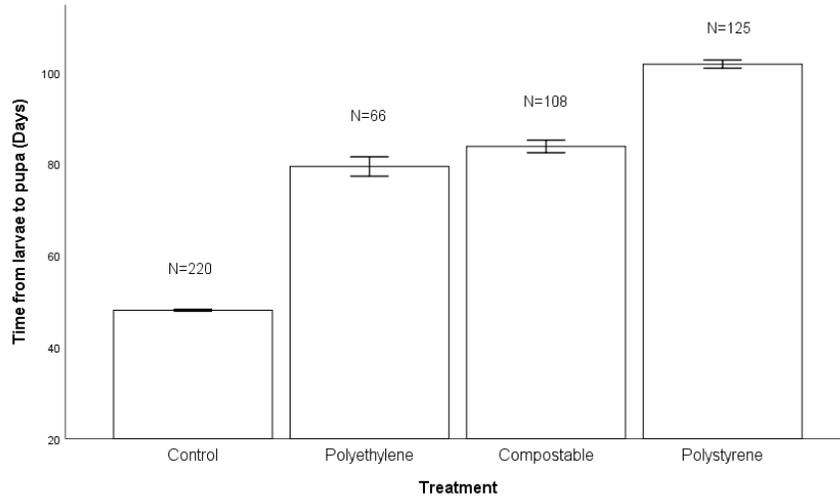


Figure 2. Average time from the pupal to adult stage in *Tenebrio molitor* according to treatment (after eating two different plastic types, compostable products and the control group) ( $\pm$  SE). The data refers to all 5 repetitions and total sample sizes are shown for each group.

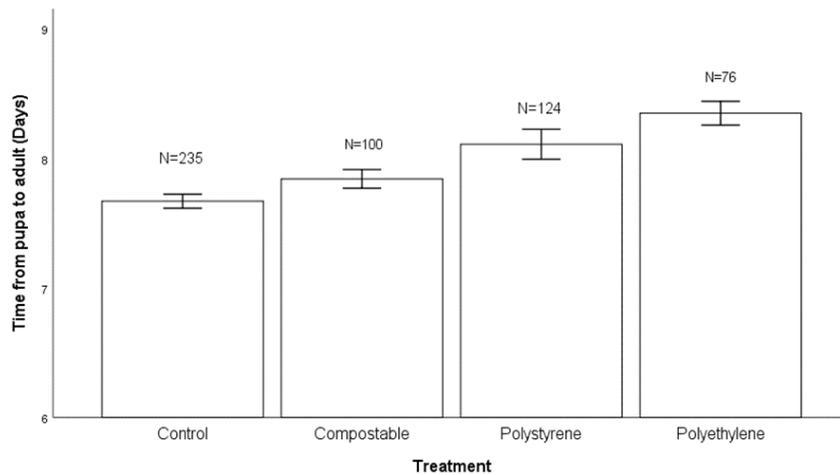


Figure 3. Average number of larvae produced in *Tenebrio molitor* females according to treatment (after eating two different plastic types, compostable products and the control group) ( $\pm$  SE). The data refers to all 5 repetitions and total sample sizes are shown for each group.

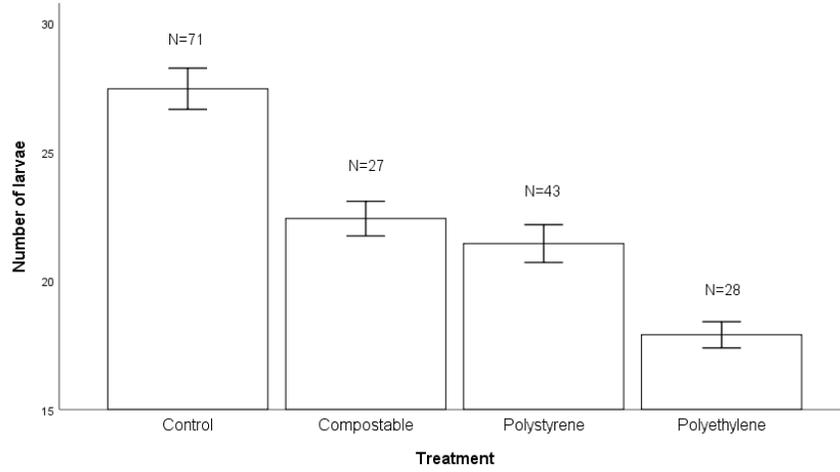


Figure 4 Boxplot of the ratio of the duration in days of the pupal stage on larval stage, ratioPL, for individuals according to treatment (after eating two different plastic types, compostable products and the control group).

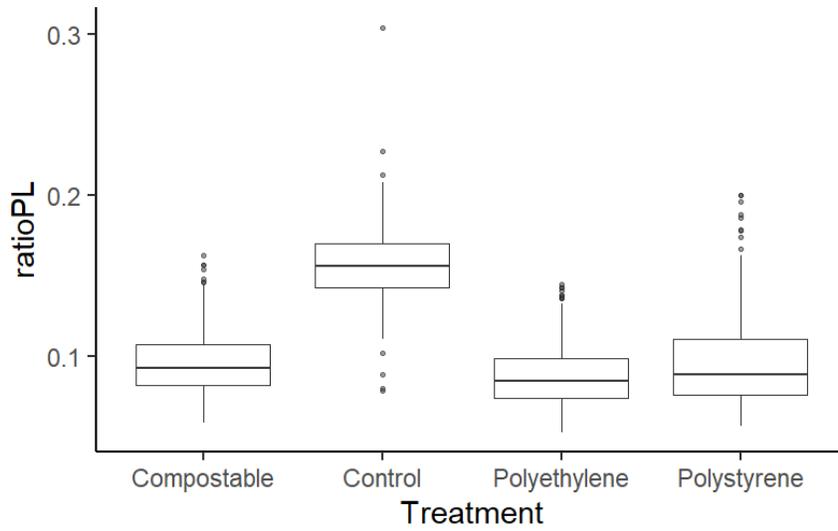


Figure 5A and B. Probability of encounter,  $p$  and of survival,  $\phi$  of individuals of different developmental stages (larvae [J], pupa [P] and adult [A]) in *Tenebrio molitor*.

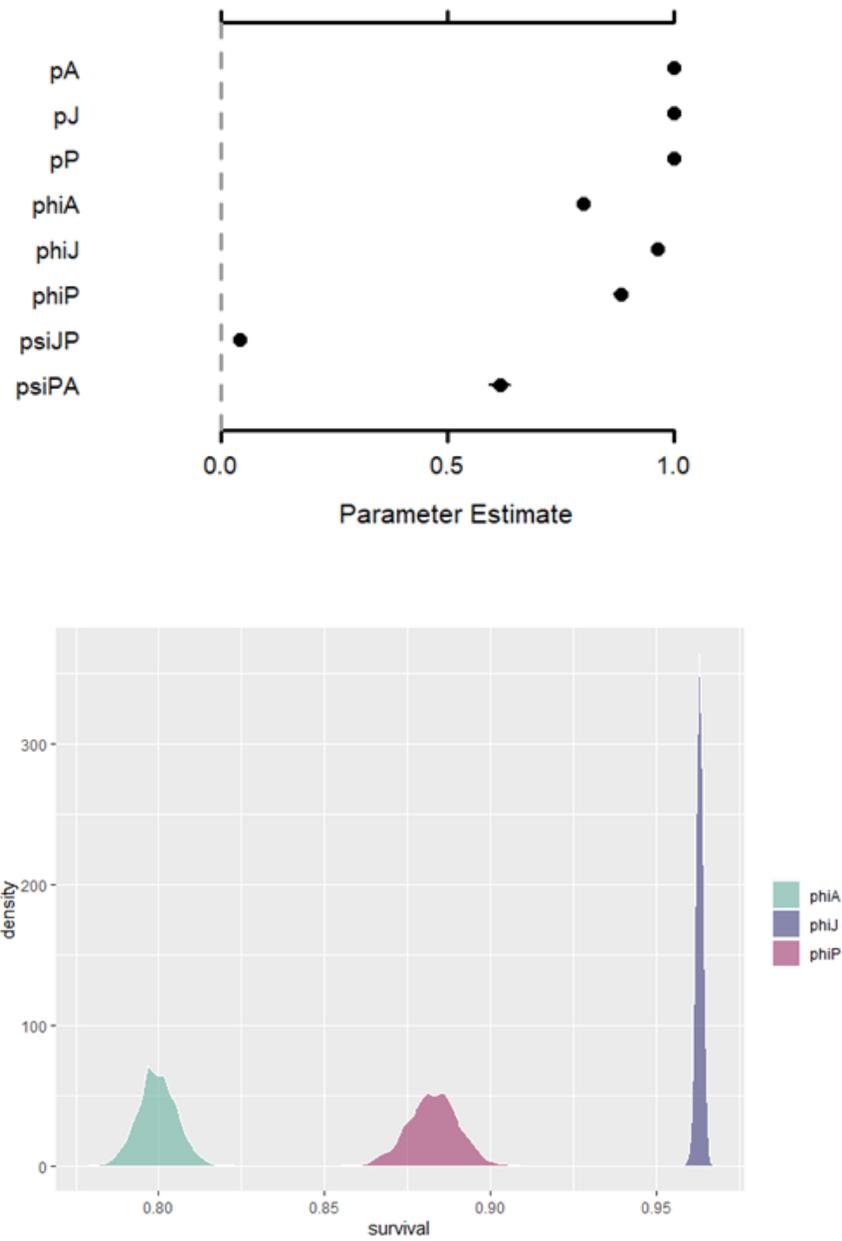


Figure 6. Survival probability of *Tenebrio molitor* individuals according to development stage according to treatment (after eating two different plastic types, compostable products and the control group).

