Plastic agriculture using worms: Augmenting polystyrene consumption and using frass for plant growth towards a zero-waste circular economy.

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ABSTRACT

Polystyrene or PS is one of the plastics contributing to environmental pollution with its durability and resistance to biodegradation. Recent research has found that mealworms (*Tenebrio molitor*) and superworms (*Zophobas morio*) are able to utilize polystyrene as a carbon food source and degrade them without toxic effects. In this study, we studied food additives to augment the plastic consumption and found that small additions of sucrose and bran were able to encourage PS consumption. To close the plastic carbon cycle, we also evaluated the use of the worm frass for dragon-fruit cacti (*Hylocereus undatus*) growth and found that superworm frass supported rooting and growth better than mealworm frass and control media over a fortnight. From the results, superworms are shown to be a suitable natural solution to the PS plastic problem that can also support plant growth towards a zero-waste sustainable bioremediation cycle.

Keywords: Biodegradation, Mealworm, Superworm, Frass, Polystyrene, rooting, agricultural support, waste management

Introduction

Polystyrene (PS) or commonly known as Styrofoam, was being invented and synthesized around the 1950s[1]. It is a very light polymer, have low heat conductivity[2], and can be synthesized to different shapes and sizes, making it a highly versatile material. From insulating material for buildings to packaging material widely used in food and beverages, Styrofoam can be found throughout the world. However, it does not have an innocuous place in the marine or terrestrial environments, resulting in accumulation and pollution due to its resistance to chemical degradation[3]. While one current large-scale management of PS waste is by incineration, this method can lead to toxic fumes in air pollution [4–6], causing harm to human health as well [6].

Worms such as superworms (*Zophobas morio*) and mealworms (*Tenebro molitor*) belongs to the darkling beetle (*Tenebrionidae*) family and are naturally voracious pests in agriculture, consuming dry grain stock even though they are food sources themselves in many societies [7]. Mealworms have been recently shown to be able to consume and metabolize plastics [8] safely, a capability attributed to commensal gut bacteria in these worms confirmed with ^{13}C-carbon isotope tracing experiments [9]. At the worm stage, they can be kept at high density, and excrete nitrogen-rich waste in the form of frass[10] that can be potential fertilizers for plant crops. The worms are also rich in chitin [11,12], a chemical shown to improve the growth and the yields of plants [13–15]. More recently, superworms are also been reported to consume PS, and at a higher rate than mealworms [16], showing promise in the use of superworms in the fight against plastics.

Since PS food containers make the bulk of PS waste, they are often contaminated with food waste, complicating recycling methods that require clean plastic waste. In this aspect, the use of worms, and the evaluation of possible food contaminants to speed up plastic degradation may be a natural solution that has yet to be fully exploited, especially if the worm waste can in

turn be used to support plant growth, especially food crop agriculture production, thus making worms the key to turn plastic waste into fertilizers for food production with zero waste.

To evaluate the possibility, this study aims to investigate the role of food additives to plastic degradation by the worms, and the use of worm frass for the dragonfruit (*Hylocereus undatus*) plant that could be grown easily even indoors. The dragon fruit cactus was chosen as it is an easy growing indoor fruit plant with potential urban farming applications.

Materials and methods

Insect rearing and frass collection

Superworms (*Zophobas morio*) and mealworms (*Tenebro molitor*) fed on bran were purchased as pet-food from various pet stores in the Clementi area, Singapore. For various experimental conditions, they were weighed and transferred to polypropylene (PP) containers (which the worms cannot eat, Figure 1A & B) with the respective test food condiments. The collection of worm frass were performed by sifting the contents of the containers with a mesh sieve to remove uneaten PS/food and worm parts. The worms were kept in cardboard boxes with a constant humidity of ~50% and a temperature of ~25ºC. The environmental parameters were monitored by assembled Arduino devices (not shown).

PS consumption rate experiments

The natural rate of PS consumption (mg PS / g of worm per day) by superworms and mealworms were determined by rearing each worm type separately. To control for different worm sizes, total worm weights between 6.22 -10.76 g per experimental setup with 0.3-0.39 mg of similarly sized PS balls of diameters ranging between 0.4 to 0.5 cm (Art friend, Singapore) were set up (Figure 1A & B). For experimental setup with food additives, the PS balls were premixed with 25 mg of either cinnamon (Masterfoods, Australia), bran (Bob's

Redmill, America), table sucrose (Lippo group) or no additive (control) in polypropylene containers. To allow better adherence of the food additives to the PS balls, 0.9 ml of water was added to the mix. The PS balls were collected after 4 days and weighed on an analytical balance to determine the unconsumed amount. Dead worms and final total live worm weights were also recorded. Only live worm weights were used for analysis. All experiments were repeated in sextuplicate.

Worm frass and Dragon fruit (*Hylocereus undatus*) experiment setups

Frass from superworms and mealworms reared solely on PS balls were used as 100 % soil media for *Hylocereus undatus* cacti. The stock cacti were grown from seeds in indoor office environments for more than four years. The grafting method was used to expand cacti successfully multiple times on spent Oolong tea leaves. For the experiment, the same grafting method was used to transplant cacti branches onto the test media and grown in recycled plastic wineglasses in individual setups (Figure 1C). Test conditions used were spent tea leaves, bran, superworm, and mealworm frass to cover the grafted cacti sufficiently to stand.

The grafted cacti were lined up against a window ledge and were watered every 2-3 days to wet the media. As much as possible, equal conditions were applied for all the setups in triplicates batches of 3-5 technical replicates. The heights of the grafted cacti were measured before during the grafting and after a period of two weeks. The cacti were straightened where necessary, with the heights being between the tip and the bottom stem (not including any roots) for each plant. Observed rooting of the grafted cacti were recorded qualitatively with photographs.

GC-MS analysis of superworm frass

To characterize the frass, ~20 mg of PS balls or frass from superworms reared on either polystyrene or bran were first filtered to remove uneaten PS, dissolved in gas chromatography grade dichloromethane solvent and incubated in 2 ml tubes on a shaker rack for 10 minutes. The tubes were centrifuged (14800 rpm for 5 minutes) to remove undissolved solids. The solvent soluble samples were passed through a Teflon Syringe 0.22 µm filter and analysed using the GC-MS system (HP 6890 gas chromatography HP-5MS column and HP 5973mass spectrometry). The GC oven temperature was held at 50°C for 1 minute, then heated up to 250°C by ram up rate at 10°C/minute, followed by held at 250°C for 5 minutes. PS balls dissolved in dichloromethane was used as positive control.

Results

Effects of food additives/condiments on polystyrene consumption.

Mealworms and superworms were reared on PS with/without common food additives such as cinnamon, sucrose, and bran. Bran was used as a control as it was the food source with which the worms came purchased. It was also previously reported to increase the rate of PS consumption in previous studies when supplemented as half the weight of PS[17]. From our results (Figure 2), the addition of all three food additives significantly increased the rate of polystyrene consumption in both types of worms ($p < 0.1$ - 0.05) except for mealworms fed with cinnamon additives (Figure 2) . The addition of small amounts of sugar or bran were enough to almost double the PS consumption rate from an average of 1.035 and 1.397 mg / g of worm per day to 1.787 and 2.142 when bran was used as an additive and 2.025 and 3.19 mg / g of worm per day when sugar was added to PS in superworms and mealworms, respectively.

No significant worm weight change (ranging from -0.7 to 2.7%) was observed in either the mealworms or superworms over the period of four days near the complete consumption of the PS (See Figure S2 in the supplementary data) with respects to worms fed on PS only without additives.

Effect of Superworm and Mealworm frass on Plant growth and rooting.

We sought to determine if frass from worms fed on PS only can be used as an alternative growth media for plants. From our results (Figure 3), the superworm frass supported both a significantly higher proportion of rooting for cacti when compared to those grown on spent Oolong tea leaves or bran. In superworm frass media, nine cacti rooted (90%) compared to the tea leaves with five cacti rooting (45.5 % rooted, $p = .03$) ; or to those grown on bran, four cacti rooted (36.4 % rooted, $p = .01$). With respect to cacti height, superworm frass media plants gained an average height of 0.5 cm that was not significantly different from plants grown on tea leaves (average gain of 0.14 cm). Mealworm frass media alone significantly impaired the growth of plants which lost an average height of 0.52 cm ($p < 0.05$). It was also observed that 5 cacti out of a total of 11 plants across triplicates died when grown on mealworm frass alone.

A loss of 0.43 cm was also experienced in plants grown on bran, but not statistically significantly different. There were no significant differences between the number of rooting cacti of mealworm frass to both tea leaves and bran. Superworm frass supported rooting better compared to the other media (Figure 3).

GC-MS analysis of superworm frass

To investigate if the frass of the worms contained toxic degradation products from PS, the frass were collected and analysed using Gas chromatography–mass spectrometry (GC-MS). 20 mg

of the frass of mealworms and superworms fed on bran or PS were collected and dissolved in dichloromethane. The GC-MS analysis results showed that frass of superworms fed on PS and frass of superworms fed on bran have similar results (Figure 5) with no major additional products observed in Sample A compared to Sample C (Figure 5). The GC-MS library search result did not find major toxic degradation product such as styrene in the filtered worms frass.

Identified peaks are summarized in the supplementary data Table S2. The identified major peaks in the frass of the superworms are identified as octadecenamide or oleamide. The oleamide (C18H35NO) is the amide derived from fatty acids, and is labelled as a fatty acid primary amide (FAPA)[18]. Given its presence in both PS and bran fed superworm frass, it is likely to occur naturally in the frass.

Discussion

We set out to investigate the effects of food additives on the rate of PS consumption by mealworms and superworms, and the possibility of their frass alone to support plant growth using the dragon fruit cacti, measuring height gain and rooting.

On the food additives, small additions of table sucrose (25 mg) were able to double the rate of PS consumption in both superworms and mealworms. Bran, which was previously reported to double the rate of PS consumption when supplemented as half the weight of PS substrate [17], increased the rate of PS consumption by ~1.7 and ~1.5 folds, respectively, in both superworms and mealworms in our study. This was better than cinnamon and in the absence of food additives. It should be noted that cinnamon did have a significantly higher rate than no additive control for superworms, but this was not shown to be the case for mealworms, which showed no statistical difference. While this may be due to many factors from taste receptors to

differences in metabolic/microbial processing of cinnamon by the different worms, it is shown that cinnamon did not have negative impact on mealworm consumption of PS. Given that most PS waste are food packages, this bodes good potential for the use of worms to deal with plastic waste given that both mealworms and superworms can also consume organic waste and be grown in high densities with very little weight loss (see Figure S2 of supplementary data on worm weight changes after PS consumption). Our breeding of these worms did not show notable abnormalities during the development of these worms to beetles, and we have a second generation of superworms grown entirely on plastics alone (data not shown).

One added advantage of mealworms and superworms over other worms, is that unlike black soldier flies that are commonly used for food waste [19], the mature darkling beetles have fused wings/elytra and do not fly, making their biocontainment easier for any plastic degradation setup.

Comparing mealworms and superworms by the PS consumed by weight of worms per day, our results showed no significant difference between the two worm types (by the control conditions in Figure 2), which was contrary to a recent report [16] reporting the superiority of superworms over mealworms in PS consumption. This difference in findings for superworms could be due to strain variations or unknown environmental/laboratory differences.

Given that both mealworms and superworms have also been evaluated to be nutritious as fish feed [20] and poultry [11], the mealworm and superworm are valuable plastic degraders that can also be used to provide cost-effective feed in food production in urban farming. While further research is required to ensure that plasticizers or other plastic degradation products do not bioaccumulate and get introduced into the food chain to humans (see review on plasticizer accumulation in the food chain [21,22]), the ability of our frass analysis shows promise for reasonably safe degradation of PS, a finding in agreement with many other reports [8,9,16,17,23].

In our frass analysis, we did not test the mealworm frass as they did not support our cacti growth, and the literature on plastic degrading mealworms was already quite extensive [14,23]. From our focus on superworm frass, we did not detect notable additional toxic degradation products in the filtered frass of superworms fed on PS as compared to those fed on bran. This suggests that the superworms were able to safely degrade the PS balls, although this should be further evaluated in a wider range of PS products, including coloured or other PS products with additives before implementation in real-life settings.

For ease of operation, the dragon fruit cacti (*Hylocereus undatus*) was chosen as it is an easy to grow indoor plant that is both an ornamental and food crop that could be used to evaluate frass for easy urban farming. It was found that superworm frass alone was better at supporting rooting (90% rooting in superworm frass as compared to 45.4% in used tea leaves) and was at least as effective as spent tea leaves in supporting growth measured by cacti height gain over a fortnight. Mealworm frass on the other hand, resulted in a lot of failed grafts, while bran media resulted in poor height and rooting support. It is possible that chitin in the superworm frass may have augmented rooting (chitin was previously reported to support rooting [24]) , or that there was some other auxin like compound present that would require further analysis. It should also be noted that the researchers found the superworm frass to be less pungent than the ammonia-tinted smell of mealworm frass, giving more support beyond obvious rooting and comparable cacti growth for the use of superworm frass.

The lack of support of mealworm frass on dragon fruit cacti growth is unexpected given a previous report [14]**.** This difference may be due to the usage of 100% frass for our evaluation or due to the different nutritional requirement of the dragon fruit cacti, or the difference in frass from mealworms that are fed purely on PS. It may be possible to tailor this to looking in other plants.

Given that there was no known benefit of mealworm frass for the cacti in our setups, and that the consumption of PS by both mealworms and superworms showed no difference, the use of superworms over mealworms is proposed in closing the loop from plastic to fish/poultry feed to frass-supported agriculture. Much remains to be studied on the possible accumulation of plasticizers or other plastic-derived chemicals as well as the frass on a variety of other food crops, but the current results are promising. In fact, given that some plants are able to clear up toxins from the environment [25] , it is possible that any potential toxic substances arising from other plastics, could be combined with phytoremediation [25,26].

There are exciting research based on enzymes isolated from bacteria present in plastic eating worms [27–30], but the implementation of these process towards complete degradation into harmless substances at industrial scale will still require further research and engineering in the face of an increasingly pressing problem of plastic waste. In the meantime, the natural solution of worms can be investigated for more immediate implementation, especially given that they can also simultaneously provide for urban farming in both fish/poultry feed and their frass for food crops. Worms are natural more resistant to environmental factors compared to pure enzymes and can overcome obstacles for enzymes in plastic crystallinity or accessibility of the polymer chains, While protein engineering of such enzymes [31] are promising, there is still much to optimize.

The setups of both PS consumption by worms and frass-supported cacti growth were all performed indoors, demonstrating the possibility of worms to be an environmentally friendly urban solution to plastic waste and food sustainability that can be implemented widely, even within homes. With evidence that food additives augment rather than antagonize PS degradation, the answer of nature in worms is a very fitting scalable solution to both the plastic pollution and food (aquaculture and agriculture) production problems.

AUTHOR CONTRIBUTIONS

DWSK, BYXA, JYY, SKEG performed the worm culturing and plant growth experiments. ZX performed the GCMS experiments. DWSK, JYY, SKEG, analysed the results and wrote the manuscript. SKEG designed and supervised all aspect of the study. All authors read and approved the manuscript.

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Conflicts of Interests: The authors declare no competing financial interests.

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Table 1. Effect of different medias on number and proportion of rooting cacti. n=44, df=1

Media	Control	Proportion of Cacti rooted	Observed Total Number of Cactus Rooted	Observed Total Number of Cactus Not Rooted	Total	Pearson's χ^2 *P*-value	Pearson's χ^2 totals
Tea leaves	Bran	45.5%	5	6	11	.66	0.2
Bran	Tea leaves	36.4%	4	7	11	.66	0.2
Mealworm frass ^	Tea leaves	15%	2	4	6	.49	0.5
	Bran					.63	0.2
Superworm frass ^	Tea leaves	90%	9	1	10	.03*	4.7
	Bran					.01*	6.4

Note. * denotes $P < .05$. ^ Five out of eleven cactus plants with mealworm frass died. One out of eleven cactus plants with superworm frass died

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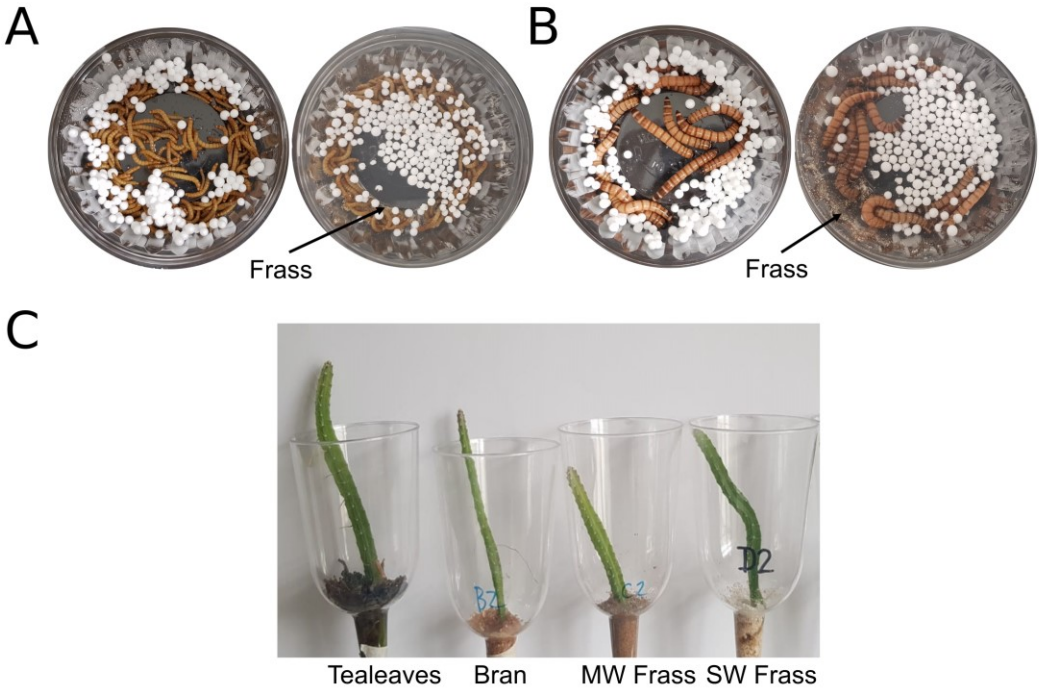

Figure 1: Representative images of the setups for testing PS consumption rates by A) mealworms; B) Superworms. For both A and B, the left are the initial setups, and the right showed the setup after four days where frass was produced from the PS consumption. (C) Setup of the dragon fruit cacti grafted onto the test media of tea leaves, bran , MW and SW frass.

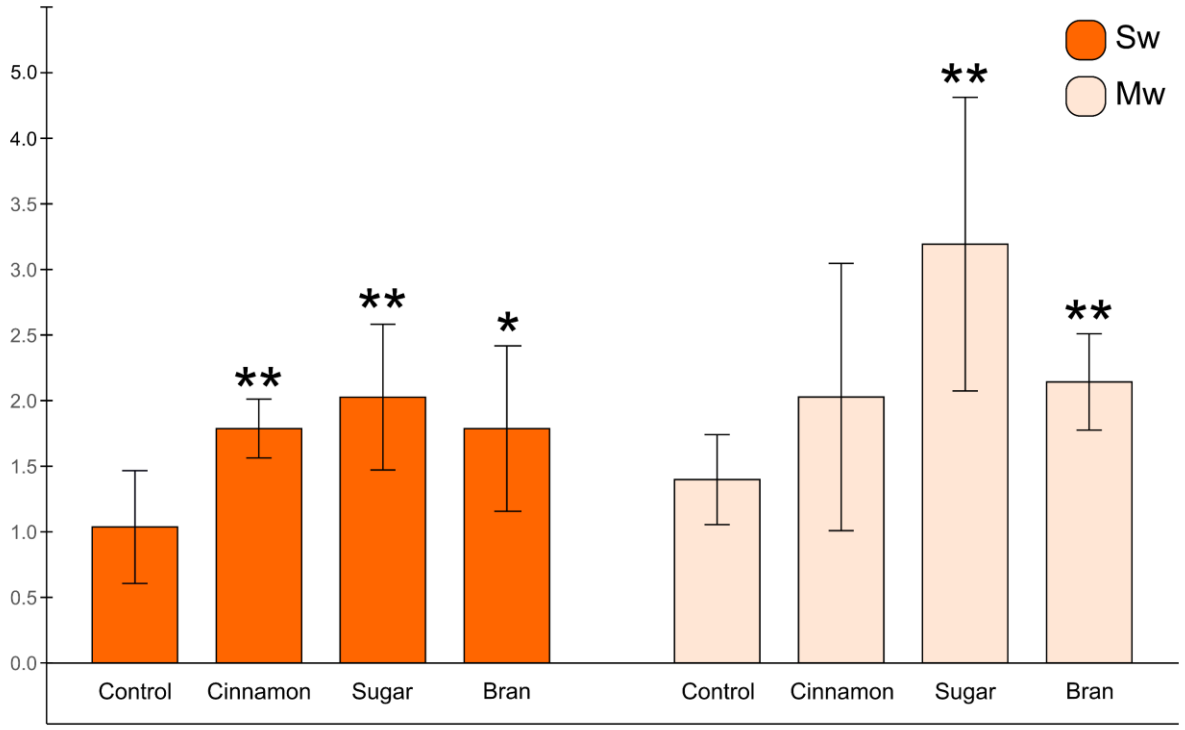

Figure 2 Average rate of PS consumption (mg / g of worm per day) by superworms (Sw) and mealworms (Mw) with and without food additives (cinnamon, sugar and bran). Additives were mixed with PS balls and sprayed with DI water to allow the additives to adhere to the styrofoam balls. The residual PS were weighed after four days. Results are reported as standard error of means from 6 replicates, statistical analysis were performed with one tail Student's T-test. * = $p < 0.1$, **= $p < 0.05$. Significant differences in PS consumption rate were observed only when additives were compared with controls.

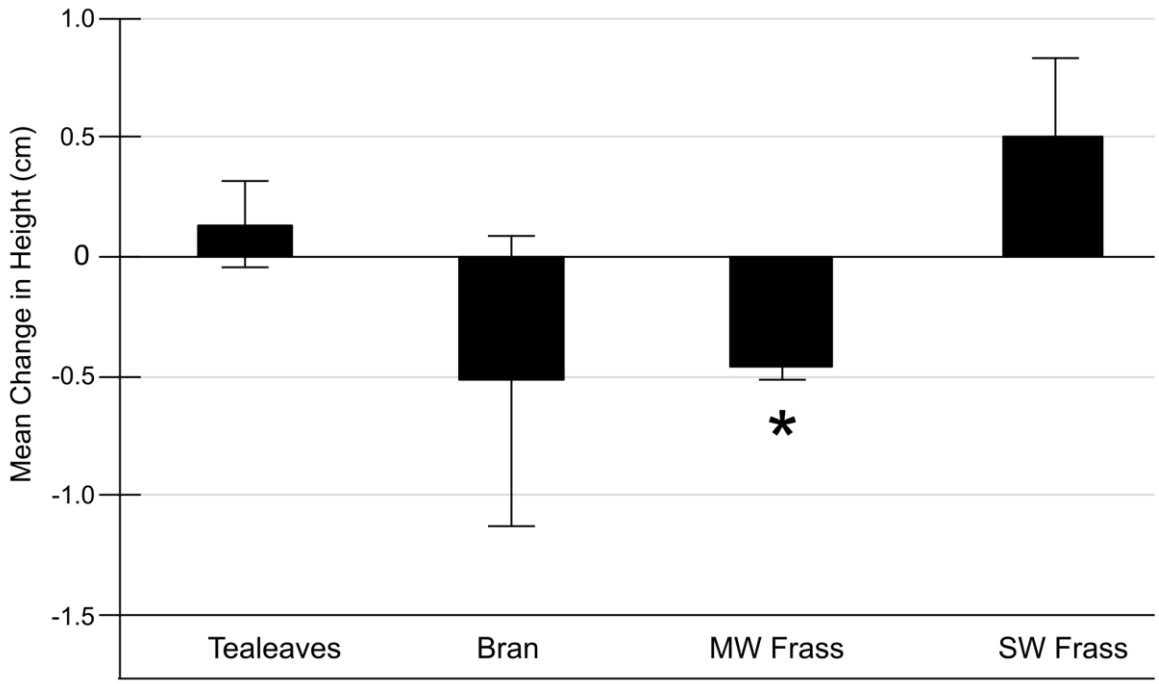

Figure 3: Mean cacti height differences grown on the respective media over a fortnight with standard error from three replicates with 3-5 technical replicates each. * = a significant change in cacti height compared to tea leaves control ($p < 0.05$, two-tailed student's T-test). MW = mealworm frass, SW = superworm frass.

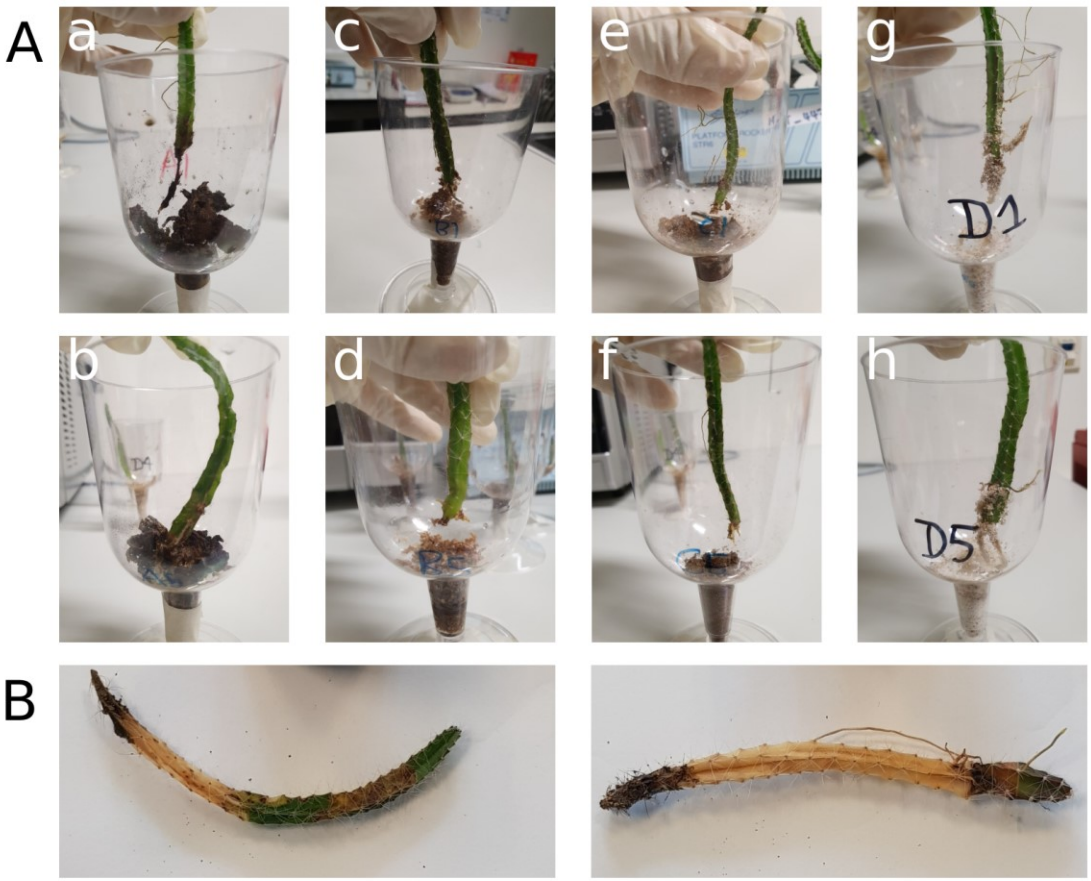

Figure 4: Dragon fruit cacti grown on frass, bran and tea leaves after a fortnight. (A) Two technical replicates of cacti grown on tea leaves (a,b), Bran (b,f), Mealworm frass (c,g) and Superworm frass(d,h). (B) Dead cacti from mealworm frass setups.

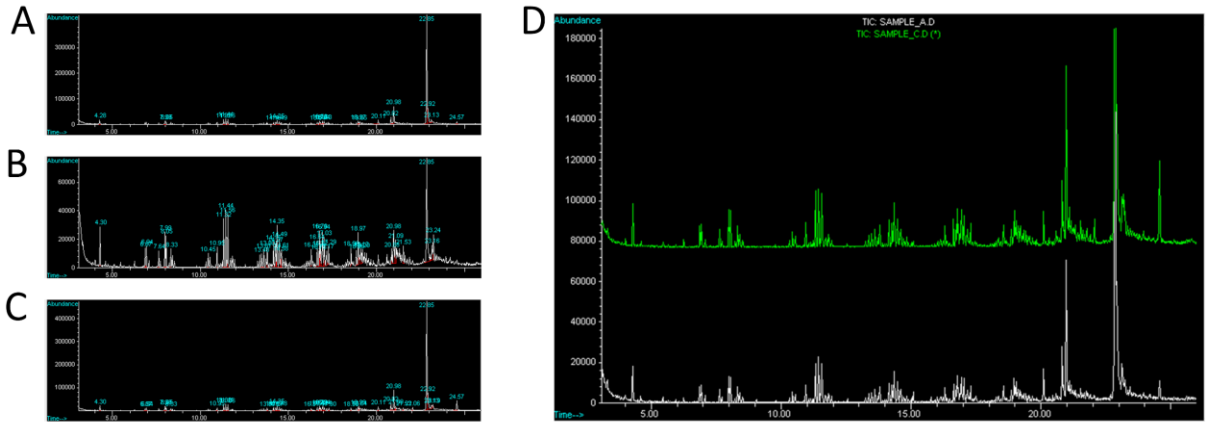

Figure 5: GC-MS graphs (A) frass from PS fed superworms, (B) PS balls, and (C) frass from superworms worms fed on bran , (D) Comparison of (top) frass from PS fed worms and (bottom) frass from bran-fed superworms.