Growth trials on vegetables, herbs and flowers using mealworm frass, chicken manure and municipal compost

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ABSTRACT

With the growth of the insect farming industry, increasing quantities of insect manure (called frass) must be upcycled. This research is providing one of the first source of information regarding the potential plant growth enhancement of *Tenebrio Molitor's* frass on garden plants. It aims at demonstrating that frass is a promising fertilizer for plant production. Nine vegetables, one herb, and three flowers were planted on the roof of "La Centrale Agricole" in Montreal. Plants were grown in 5% compost enriched substrate (v/v) (control) and fertilized with 0.5% (v/v) frass (treatment 2) or an isonitrogen concentration of hen manure (treatment 3). Plant growth (germination, height, N flowers) and productivity (biomass) were assessed regularly throughout the growing season. Although beets and carrots' seedling emergence was inhibited by both manures, this did not lead to reduced edible biomass compared to the control (germination was unaffected for corn, radish, and arugula). Similar to hen manure, frass resulted in a 16-fold increase of the edible biomass as compared to the control. Frass fertilized plants had larger and more numerous flowers than control plants. Our results confirm that insect manure should be recognized as a suitable fertilizer for multiple crops and regulated like other manures.

KEYWORDS: Frass, Agriculture, *Tenebrio molitor*, insect farm, plant growth, organic fertilizer, upcycling

SYNOPSIS: Upcycling food waste using edible insects yields manure which promotes plant growth similarly - and should be regulated similarly - to conventional animal manures.

1. INTRODUCTION

1.1. Novel organic sidestreams as circular economy substitute to conventional fertilizers

With rising concerns about the detrimental impacts of climate change on our environment, every potential solution to reduce waste and environmental pollution is to be considered. Using organic fertilizers, as a replacement or supplement for chemical or mineral fertilizers [1], is one of the ways to reduce the climate footprint of agriculture[2]. The estimated insect manure production from edible insect farms in Québec (Canada) was between 184 and 375 tons in 2020 and is expected to double in the coming years[3], and should reach 1,5M tons in Europe by mid-2020 [4]. In comparison, in 2017, the 168M broiler chickens [5] plus the 5,2M egg laying hens[6] of Québec produced an estimated 0,1 Kg of manure per head per day[7], for a total of 6,9M tonnes of manure per year, a by-product commonly used to enhance soil fertility and productivity[8]. Insect manure could also be a viable alternative to chemical fertilizers, while closing the nutrient loop between food production and waste management in an urban circular economy fashion. The increasing amount of insect farms (23 in 2020[9], grew to 30 in Québec in 2022[10]) could eventually ensure a reliable and local source of fertilizer for farmers[11]. With provincial agriculture plans aiming to reduce synthetic N-fertilizers by 10% and enrich soil carbon content to 4% by 2030[12], the nutrient and carbon content of this novel circular economy sourced fertilizer is appealing.

However, frass is still a new product with regulation challenges in different parts of the world (e.g. it cannot be considered as manure in Canada according to recent changes in the Fertilizer Act[13]). More information on its classification, nutrient content, application rate, and potential health hazards may alleviate regulatory hurdles and allow safer and more widespread use of this product. Depending on the way frass is used, it can be considered both as an amendment and as a fertilizer. In this article, it is the fertilization potential of frass that is under scrutiny.

1.2. Frass Composition

Insect manure, also known as frass, is the main by-product of insect farming. It is mostly composed of insects dejection, liter and exuviae. The nutrient and moisture content of frass is related to the type of feed, the species of insect farmed, and the growing conditions[14-18]. Frass contains plant macronutrients (nitrogen, potassium, and phosphorus) and micronutrients[19, 20]. Insect frass can also improve the growth, flowering, and rooting of plants by providing micronutrients such as calcium, magnesium, boron, copper, iron, manganese, and zinc[15-17]. Nitrogen is primordial for plant growth and biomass production[21] but excess nitrogen from insect frass (i.e. black soldier fly) can be phytotoxic[19, 22] and excessive nitrogen fertilization is associated to eutrophication[23] making it a critical nutrient to balance in fertilizers.

Analyzes of the nutrients contained in mealworm frass reveal a similarity with hen manure[24]. Similarly, Chavez et al. [25] suggests that the carbon, nitrogen and phosphorus concentrations of frass and manures are comparable. A difference observed in several studies is the decrease in total nitrogen and an increase in assimilable nitrogen between fresh manure and manure composted by insects [25]. By its rich content in organic matter (72%), mealworm frass improves the substrate's structure and properties. Poveda et al.[26] demonstrated that mealworm frass helps prevent damage from both water scarcity and water logging[26], which would aid crop development in extreme conditions. The nutrient and bacterial content of mealworm frass was also reported to stimulate beneficial soil microorganisms[20]. Insect-generated macromolecules, such as chitin or specific peptides, can also directly reduce the occurrence of insect pests and bacterial or fungi plant pathogens by enhancing the plants' defense mechanisms[15, 27-29].

1.3. Insect manure as an organic fertilizer

Information on the effectiveness of frass in agriculture is increasingly documented. A review of all the existing literature on this subject revealed that frass represents an important contribution of nitrogen and other nutrients to the soil, and promotes the growth and resistance of plants to diseases due to the presence of biomolecules and microorganisms in frass . A recent study on the quality of frass generated by nine species of edible insects concludes that frass may be suitable as an alternative source of fertilization for plants, given their high nutrient levels . Frass' macronutrient concentration is similar to other organic fertilizers[30] like chicken manure[31]. However, in a particular context of rooftop production, it is know that chicken manure may increase soil pH[8], limiting P bioavailability[32], while mealworm frass has been shown to decreases soil pH potentially enhancing plant nutrition- [33].

A study evaluating the potential of mealworm frass in a short-term potted experiment demonstrated that this type of frass can quickly supply essential plant nutrients like NPK, however, the rate of application can influence the nitrogen dynamic [24]. The availability of frass nitrogen also depends on the quality of the food given to the insect, for example, the nitrogen content of frass has been shown to increase when insects were fed plants that were fertilized [14]. Recent publications suggest that an application of mealworm frass leads to increases in the biomass of field crops such as barley and wheat as well as in the uptake of nitrogen, phosphorus, and potassium[34]. Fertilization trials show promising results on the growth and resistance of tomatoes fertilized with mealworm frass[35]. Fertilizing potential may vary depending on the diet of the insect, and despite the available information on the fertilizing potential of mealworm frass, questions about the optimal rate and timing of frass application for different crop types remain unresolved.

This study is one of the first to assess the effects of mealworm frass on the growth and yield of a variety of crops with the goal of characterizing which plants respond best to frass fertilization. In addition, the novelty of this study lies on the one hand, in the fact of having tested many crops at the same time under harsh climatic conditions in a context of rooftop farming. On the other hand, the effect of the reproducibility of the tests makes it possible to reduce the uncertainty, to seek maximum precision of the data. Hence, this study aims at widening what is known about this novel upcycled material with the intention of making it more accessible in our agricultural practices and inform policy makers of its potential. Finally, this study compares commonly used fertilizers, all from circular economy sources, thus promoting more ethical and ecological fertilizer sources.

1.4. Hen manure as an organic fertilizer

Hen manure has long been widely recognized as an organic fertilizer that promotes soil fertility and moisture retention. A recent meta-analysis from 90 studies investigated the influence of hen manure on crop productivity under different field conditions. This study showed that the effect of hen manure is comparable to that of inorganic fertilizers. Different factors such as soil type and properties, tillage, application practices and crop type influence the effectiveness of chicken manure [36]. An 11-year study demonstrated that organic matter and phosphorus content was higher in soils fertilized with hen manure, which resulted in increased corn and soybean yield [37]. However, the excessive use of this animal manure can have negative effects on the water quality of agricultural land, favoring the increase in the nitrate and orthophosphate content of water [38]. This manure must be properly treated before application to avoid risks to the soil and living organisms associated with the presence of various contaminants such as antibiotics or pesticides [39].

1.5. Urban and rooftop fertilization particularities

It is desirable in urban agriculture context to source fertilizers and soil amendments locally, notably materials upcycled from agrifood circular economy initiatives [40]. In 2018, compost production in the province of Québec was 0,215M tonnes, 35% of which were incorporated into potting substrates[41]. Plants grown in rooftop gardens must tolerate extreme environmental stressors like high wind, heat and drought [42]. Compost is a locally produced common organic soil and potting substrate amendment in urban agriculture[43]. Compost can also alleviate the stress caused by harsh environmental conditions[44]. Furthermore, organic fertilizers in urban agriculture context pose a special problem, notably with respect to odours, so it is desirable to find alternatives with a less offensive odour for this context (i.e. hen manure smells more strongly than mealworm manure)[40].

1.6. Objective

Growth trials were performed in summer 2020 in the context of urban rooftop agriculture to assess the effect of the addition of frass, as a fertilizer, on common horticultural crops and to compare it with another commonly used manure (hen). Our hypothesis is that the fertilizing effects of frass on the tested plants will be analogous to that of plants subjected to the hen manure treatment at an equivalent total nitrogen dosage, and that both will lead to increased productivity when compared to the manure-free control.

2. MATERIALS AND METHODS

2.1. Insect Farming

Yellow mealworm (*Tenebrio molitor)* frass was obtained from insects reared at TriCycle, an urban insect farm operating in short circuit circular economy to upcycle locally available agri-food by-products in Montreal (Canada). The growing units are plastic boxes with bottom dimensions of 30 x 43 cm and filled with 10,000 larvae, resulting in a density of 7.75 larvae/cm^2.

In each unit, 4.5 kg of wheat bran is provided as the feeding substrate. Larvae and adults were fed with organic wheat bran from *Boulangerie Jarry*, and fruit and vegetable pulp from *Les jus LOOP* twice a week. The units were kept in a controlled environment room where the temperature was kept at 27 ± 1 °C and the relative moisture was maintained at 57,5 ± 2,5%.

Frass was harvested at the same time as the larvae, after 12 weeks of growth, using mechanical sieving. Insects' molts and pieces were removed using a vacuum. The substrate and the larvae of different sizes were separated from the smaller frass particles using a square 500 micrometer screen. This frass is approved for organic agriculture by ECOCERT.

2.2. **Treatments**

Three treatments were carried out adopting a randomized-block experimental design with repeated measures and three replicates. The base substrate (control, referred to as treatment 1) in all three treatments was potting soil with mycorrhizae (AGRO MIX G2, Fafard, Saint-Bonaventure), supplemented with 5% (v/v) of municipal compost (food and yard residues compost obtained from the City of Montreal). Compost was homogenized manually in the substrate.

Treatment 2 was supplemented with 175g of frass (equivalent to 0.5% (v/v)) (Ferti-Frass™, TriCycle, Montréal), which represents 5.425g N_{tot} per plantation unit. Treatment 3 was supplemented with 108.5 g of hen manure (0.3% (v/v)) (Acti-Sol, Notre-Dame-du-Bon-Conseil) which represents 5.425g N_{tot} per unit. The frass fertilization rate was based on the results of a preliminary test on basil, mustard, radish and watercress growth[45]. We selected a homogeneous fertilization rate for all plants which was an acceptable compromise for different garden plants based on local fertilization guidelines [46] (Table 2), but added a second fertilizer dose for the most nutrient intensive plants. This concentration provided a comparable amount of total nitrogen between both manure treatments (isonitrogen). Hen manure granules were milled using a pestle and mortar and sifted (≤2 mm). Frass and hen manure were incorporated manually by mixing in the top 5 cm of the substrate. The chemical analysis of the potting soil, organic amendment, and fertilizers is presented in Table 1. For all plants, fertilization was carried prior to sowing or transplantation, though 3 plants required a second fertilizer dose at mid-season (see section 2.3).

Table 1. Physico-chemical properties of the potting soil, organic amendment, and fertilizers.

Properties	Individual substrates[a]

	Potting media	Compost	Frass	Hen manure
Density (kg/m^3)	287.5	300	350	680
Organic matter (%)	27	32	72.2	60.1
Total N per growing unit (g/kg)	0.40	9.00	33	50
NO_3 (g/kg)	0.075	0.088	0.034	na
P_2O_5 (g/kg)	0.01	4.69	32.80	32.28
K_2O (g/kg)	0.3	5.05	24.20	28.99
EC (ms/cm)	1.1	na	6.34	na
pH	5.80	7.55	5.61	7.10

Note: [a] Physico-chemical properties of the individual substrates based on manufacturer's technical specifications.

Substrates were sampled before plantation (June 2020, 12 samples pooled for analysis) and at mid-season (August 2020) for tomatoes in each treatment (3 samples per treatment pooled) and for radish in the frass treatment (3 samples pooled). Substrate's physico-chemical characteristics were analyzed by AgroEnviroLab (LaPocatière, Canada). They included density, total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and organic matter content, as well as pH. Additionally, the electric conductivity (EC) and pH of the substrates were measured (HI-9813-6, Hanna Instruments, Inc.) after diluting 5 grams of sample in 50 ml DI water (Data not shown).

2.3. Selected Plant Species and Disposition

Thirteen crops or horticulture flowers were used to assess the effects of organic amendments on a diversity of commonly grown plants: 3 leafy greens, 3 plants cultivated for their flowers, 3 root vegetables, 1 herb, and 3 fruit-vegetables. Cultivars of shorter height were selected to prevent strong wind damage on rooftop.

Dwarf sunflower (*Helianthus gracilentus*), nasturtium Dwarf Jewel mixed and Dwarf Double Cherry Rose (*Tropaeolum majus*), zinnia Dwarf Pumila Mixture (*Zinnia elegans*), arugula Esmee organic (*Eruca vesicaria*), carrot (*Daucus carota* subsp. sativus), radish Cherry Belle (*Raphanus raphanistrum* subsp. sativus), beetroot Detroit Dark Red (*Beta vulgaris*), large leaves sweet basil (*Ocimum basilicum)* and corn Canadian Early Supersweet Hybride (SH2) (*Zea mays* subsp. mays) were grown from seeds. Seeds were sowed on June 1st, 2020. Seeds were added as replacement of unsuccessful germination within two weeks. Nutrient requirements of plants, seeding depths and spacing are displayed in Table 3. The *Centre de référence en agriculture et agroalimentaire du Québec* (CRAAQ) recommends higher level of fertilization for nutrient-intensive plants such as tomatoes, corn, and cucumbers[46]. Hence these plants were re-fertilised with a second dose of the same fertilizers for each treatment at the mid-growing season (i.e. after 53 days).

Cucumber (*Cucumis sativus*), kale (*Brassica oleracea* L. var. *acephala*) and chard (*Beta vulgaris* subsp. *vulgaris*) plants were purchased from Pépiniaire Jasmin (Montréal), while yellow cherry tomato plants (*Solanum lycopersicum*) were provided by the Centrale Agricole. For every species, healthy individuals of the same height were planted after a short natural light acclimatation. At plantation, cucumber plants were at the stage of two real leaves, kale and chard were seedling of less than 8 centimeters and tomato plants had less than 30 cm of height. Tomato plants were supported by stakes for all the growing season.

The following planting duos were used: swiss chard and kale, beetroot and carrot, nasturtium and zinnia, radish and sunflower, and tomato and basil.

Table 2. Characteristics and needs for each plant used for the experiment.

Plant[1]	Plants disposition						Substrate requirements[2]			
	Days to sprout	Seeds spacing (cm)	Rows per unit	Plants per rows	Plants per pots	Number of plants	Kg-N /ha	Kg-P_2O_5/ ha	Kg-K_2O /ha	pH
Radish[a]	7-21	2,5	2	12	24	216	110	30-155	30-240	6,3
Dwarf sunflower [a]		30	2	12	24	216	0 - 230	0 - 310	0 - 340	6 - 7.2
Carrot[b]		2,5	1	24	24	216	80	30-170	30-225	6,3
Beetroot[b]	14	5	2	12	24	216	110	30-155	30-240	6,3
Cherry tomato[c]		40	1	2	2	18	35+ 100[3]	20-240	20-240	6,5

Basil[c]		40	1	2	2	18	0 – 230	0 - 310	0 - 340	6 - 7.2
Sweet Corn	7-14	10	2	3	6	54	20-50+ 75-90[3]	0-80	0-80	5,8-7,0
Cucumbers		30	1	2	2	18	80 + 35[3]	40-180	20-180	6,3
Arugula		1	2	24	48	432	0 – 230	0 - 310	0 - 340	6 - 7.2
Kale[d]		20	1	1	1	9	0 - 230	0 - 310	0 - 340	6 - 7.2
Swiss chard[d]		15	2	3	6	54	0 - 230	0 - 310	0 - 340	6 - 7.2
Zinnia[e]	5-7	1.3	1	8	8	72	0 - 230	0 - 310	0 - 340	6 - 7.2
Nasturtium[e]	7-21	2.5	2	6	12	108	0 - 230	0 - 310	0 - 340	6 - 7.2

Notes: [1]Plants identified by the same letter were sown in the same pots. [2] All values for substrate fertilization and pH requirements were retrieved from the CRAAQ's "Guide de Référence en Fertilization"[46]. [3]Nitrogen supplementation is required when tomatoes reach 2.5 cm, when corn plants reach the 6-7 leaf stage, or when flowering begins for cucumbers[46].

2.4. Plant Growing Conditions

Growth experiments were carried out in Summer 2020. Edible plant species were planted in long beds on the uncovered rooftop of the Montreal urban agriculture cooperative La Centrale Agricole, situated in the Marché Central area (Canada).

The fabric raised beds were made of air permeable geotextile (Smart Pot, Oklahoma City). They were 1.83 m long, separated in three growing units with cross sections of 40.6 × 40.6 cm and 61 cm length. Each growing unit had a volume of approximatively 0.10 cubic meter.

A drop-by-drop irrigation system provided municipal water (1.4 L/h) to plants four times per day for a total of 3 hours: one hour at 6am and 6pm, and half an hour at 11am and 2pm. The tubes were placed on the substrate surface and passed through the middle of each growing unit.

The average climatic conditions throughout the growing season (June 1st to September 11th) are provided by a local weather station (Pierre Elliott Trudeau International Airport, Montréal, Environment Canada)[47]. The total precipitation over the duration of the experiment was 303.8 mm. Daily average temperature varied between 9.2°C and 29.9°C and the total heating degree days was 873 DD5. For these four months, the monthly averages were 20.0°C, 24.2°C, 20.7°C, and 14.8°C. However, the roof on which the experiment was conducted is located in an urban heat island with temperatures between 10 to 20 degrees higher than the ambient temperature (unpublished data)[48]. The average speed of maximal gust registered at the weather station was 42.5 km/h with a maximal reported speed of 80 km/h[47]. Higher than ground average wind speeds were also observed on the experimental roof, located in an industrial area. The implications of such conditions will be further discussed in section 3.6.

2.5. Germination, growth rate, flowering, and yield Analysis

Germination success was evaluated by the average number of plants at the three stages of growth (seedling, cotyledon, and leaf). The plant's growth rate was evaluated once a week by total plant height (cm) and stem diameter (mm). Stem diameter was measured 5 cm above the ground with a vernier Caliper. A plateau in the growth rate of some plants coincided with a second fertilization (section 2.3). Flowering was assessed by the total number of flowers for each horticultural crop. Flowers were considered in bloom from buds opening to senescence, characterized by the loss of most petals. For sunflowers, the diameter (cm) was measured as the largest part of the disk flower prior to seed harvest.

Yield was assessed by the total edible biomass (g) of leaves, roots, or fruits depending on the plant. The fruits were harvested when mature and counted. The ratio of total edible (fresh weight) biomass yield (g) was used to compare frass or hen manure to compost treatments. Morphometric data were collected from harvested radishes and beetroots. Measurements included major diameter (mm), length of longest leaf (cm), root length including the longest secondary root and the taproot (mm), root biomass (g), above ground biomass (g), and water content (%). The water content was assessed by gravimetry using 105°C for 24h[49].

2.6. Statistical Analysis

In order to assess the efficacy of the treatments, the germination, growth rate, and flowering data were analyzed by analysis of variance (ANOVA). A repeated measures Anova was performed to quantify the influence of sampling date, treatment, and interactions between these two factors. JMP Software version 10 from SAS (Cary, United States of America) was used for statistical analysis. Data were assessed for normality and, where appropriate, nonparametric analysis was performed. Data were considered significant if the p value was less than 0.05.

3. RESULTS AND DISCUSSION

3.1. Soil's Physical Chemistry

Measurements were taken on pooled samples before the beginning of the growth experiment (1st of June 2020) while at mid-season (6th of July 2020) measurements were taken separately for each treatment and each plant. The pH increased significantly over time, from 6.63 ± 0.28 (± SD, n = 6) to 7.32 ± 0.18 (n = 72) (ANOVA, $p_{tot} = 0.0400$), and this was significantly impacted by crop type ($p_{crops} = 0.0023$) but not by treatment ($p_{trt} = 0.1293$) and there was no interaction between both factors ($p_{cropxtrt} = 0.6834$). The EC before plant growth (220 ± 28.6 µS/m, n=6) did not vary significantly at mid-growth (193.5 ± 108.1 µS/m, n=72) and was uninfluenced by crops or treatments ($p_{total} = 0.2202$). Table 3 shows the results of the physicochemical analyzes of the substrates before planting and at mid-season.

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Table 3. Substrate's physico-chemical characteristics before planting and at mid-season

Properties	Initial (June 2020)	Mid-season (August 2020)			
	Control (T1) Before planting	Control (T1) Tomato	Frass treatment (T2) Tomato	Frass Treatment (T2) Radish	Hen manure treatment (T3) Tomato
Density (kg/m^3)	200	210	220	190	230
N (g/kg)	14	14.7	15.3	15.6	15
P (g/kg)	0.145	0.144	0.236	0.291	0.210
K (g/kg)	0.481	0.526	0.388	0.534	0.543
Ca (g/kg)	4.09	4.14	4.78	4.60	4.97
Mg (g/kg)	0.361	0.402	0.512	0.541	0.474
OM (%)	68.0	67.4	66.2	67.8	64.1
pH	6.70	6.90	6.90	6.90	7.00
Substrate mass per growing unit (kg)	20.0	21.0	22.0	19.0	23.0
Total N per growing unit (g)	280	309	337	296	345

3.2. Germination

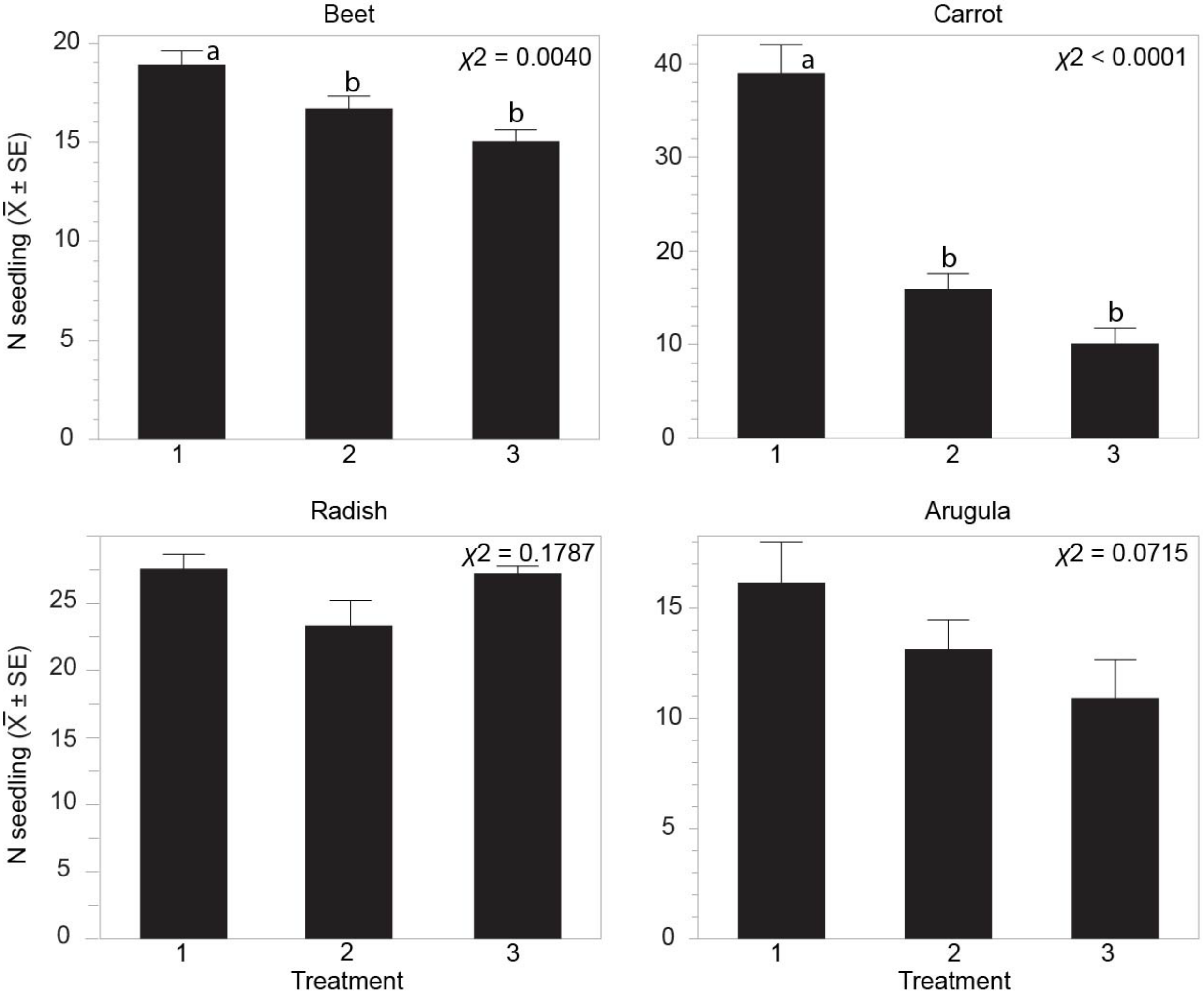


Figure 1: Number of plants at the seedling stage for the beet, carrot, radish, and arugula for the control (1), frass (2) and hen manure (3) treatments, respectively.

Though seedling, cotyledon, and first leave emergences were monitored individually, all plants displayed similar trends at those stages. Hence, germination performance is inferred from seedling emergence for radish, arugula, beets, and carrots (figure 1). The germination for radish (X^2 = 0.1787) and arugula (X^2 = 0.0715) was similar across the three treatments. However, germination of both beets (X^2 = 0.0040) and carrots ($X^2 \leq 0.0001$) was significantly reduced by both frass and hen manure compared to the control. Both manures had the same effect.

The decrease in the germination rate in certain vegetables could be due to the presence of phytotoxic compounds in manure. A similar effect has been also reported by Naiji and Souri[1], 2018 on sweet basil and Serri et al.[50] on coriander. In laboratory settings (10 seeds per petri dish on absorbent paper) exposure to increasingly concentrated water extracts of mealworm frass delayed germination and inhibited root and shoot development in radish and watercress [45]. A follow-up experiment showed that seedling inhibition was less severe when seeds were placed on potting media than when they were exposed on absorbent paper, suggesting that the phytotoxic compounds could be adsorbed on substrate particles or metabolized by substrate microbiota [45]. Bok choy seed germination reduction upon exposure to fresh black soldier fly frass was attributed to higher phenol concentrations in a test by Song et al.[51]. Furthermore, Beesigamukama et al.[52] demonstrated that composting black soldier fly frass can significantly increase the percentage of seed germination (greater than 80%) as well as the germination index of cabbage seeds. These authors suggest that *T. molitor* frass high salt concentration and electrical conductivity could explain seedling phytotoxicity[53]. Kebrom et al.[54] discussed the strong phytotoxic effects of hen manure on the germination of collard greens. Hence, our results confirm that germination decrease is no different between insects and hen manure.

3.3. Growth Rate

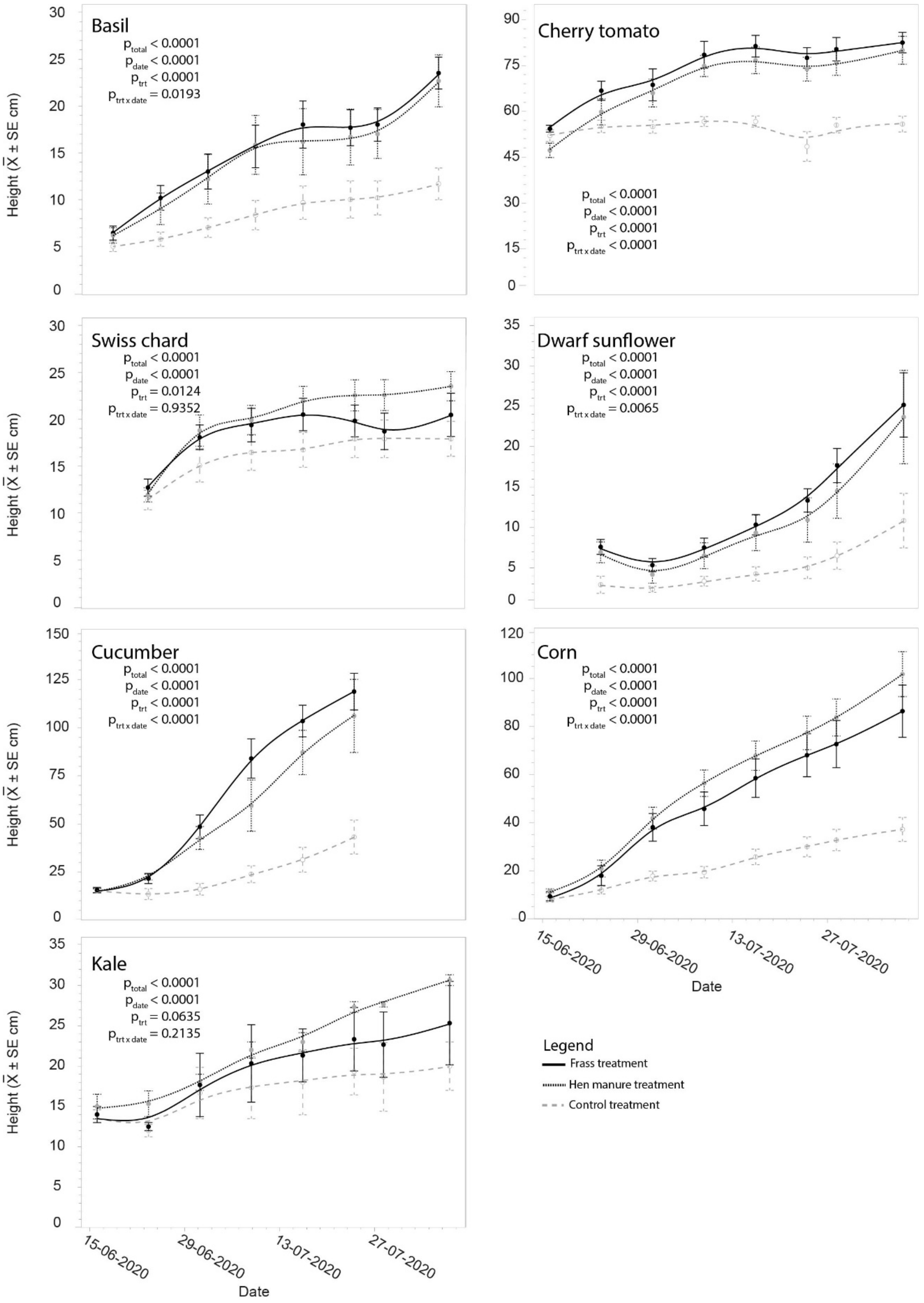


Figure 2: Height as a function of time for different crops in the control, frass or hen manure treatments.

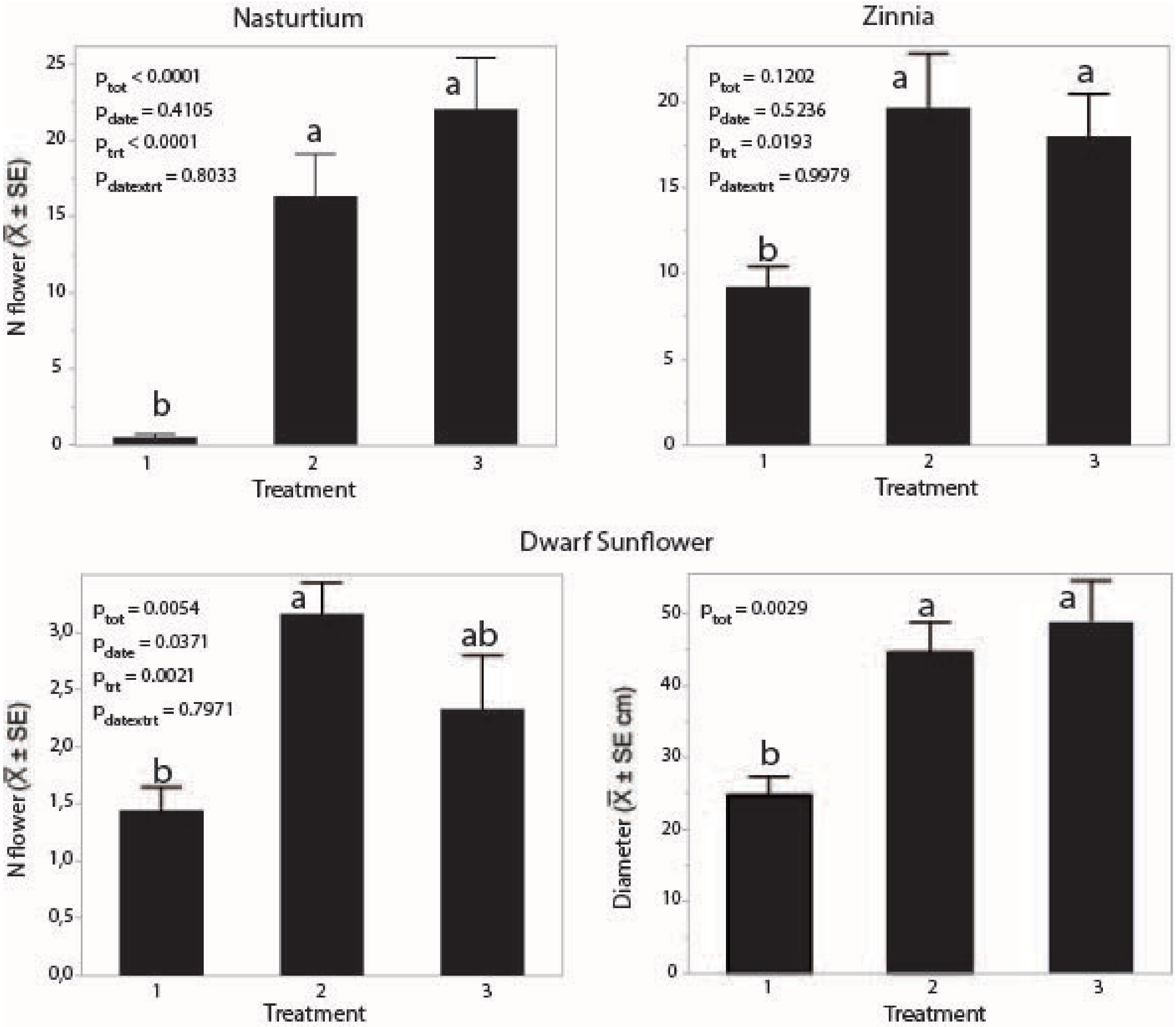
The treatment used for the basil ($p_{trt} \leq 0.0001$), cherry tomato ($p_{trt} \leq 0.0001$), swiss chard ($p = 0.0124$), dwarf sunflower ($p_{trt} \leq 0.0001$), cucumber ($p_{trt} \leq 0.0001$), and corn ($p_{trt} \leq 0.0001$) induced a significantly different growth rate, whereas it did not for kale ($p_{trt} = 0.0635$). The addition of frass resulted in a significantly increased growth rate for every plant, with the exception of kale, when compared to the control treatment. For every plant observed, the results obtained for the frass and the hen manure treatments were not significantly different.

Growth rate measured on plant height (Figure 2) displayed similar trends to that of stem diameter (Figure S1). For basil ($p_{trt}<0{,}0001$), corn ($p_{trt}<0{,}0001$), tomato ($p_{trt}<0{,}0001$) and cucumber ($p_{trt} = 0{,}0041$ (but not for sunflower : $p_{trt} = 0{,}2895$) stem diameter were smaller in compost treatments, and larger in frass and hen manure (with no significant difference between both).

Similar results were also observed for the edible length of carrot (figure S3), where the frass treatment showed an insignificantly bigger length than the 2 other treatments. The edible width of beetroot displayed a similar pattern to what is observed for the growth rate, whereas the edible width of carrot did not show any difference between the 3 treatments (figure S4). Finally, the leaf length of beetroot and kale also displayed a pattern similar to that of the growth rate, while the leaf length of carrot did not show any difference between treatments (figure S5).

Frass could have a beneficial effect on plant growth because it is rich in fertilizing elements, microbial biomass, chitin, growth hormones, and enzymes[55]. According to the United States Environmental Protection Agency, the chitin contained in frass and the chitosan derived from chitin can act as plant growth regulators through an increase in the plant's defense against diseases and fungal infections, respectively[56]. The use of black soldier fly frass improves the growth of sunflower (*Helianthus annuus*) by helping to expand its stem and increase the lipid content of the seeds[57]. In the current experiments, sunflower height (but not stem diameter) increased with the use of mealworm frass, though this effect was no different than with hen manure. In a study on the growth of cucumbers in a greenhouse, the use of black soldier fly frass led to a faster growth rate, taller plants with more leaves and greater production when compared to worm compost treatment[58]. These results concur with the current experiment using mealworm frass and municipal compost. The addition of frass equaled or increased the biomass of ryegrass, wheat, and alfalfa plants as compared to compost, digestate, conventional potassium fertilizer, and struvite[59]. On the other hand, Kagata and Ohgushi[14] have underlined the importance of using quality frass: they observed that nitrogen-deficient frass can have detrimental impacts on plant growth. Similarly, McTavish et al.[60] noted lower survival and growth rates of plants with frass from insects fed on polluted biomass from degraded environments. As with other mineral or organic fertilizers, the rate of application of frass should be controlled according to its fertilizing value. Too low, it can lead to nutritional deficiency. Too high, it can have a deleterious impact on plant growth[61].

3.4. Flowering



<u>**Figure 3:** Average number of flowers per unit for Nasturtiums, Zinnias and Dwarf Sunflowers subjected to the three treatments. Average flower diameter for Dwarf Sunflowers per</u>

treatments. Treatments 1, 2, and 3 correspond to control, frass, and hen manure treatment, respectively.

For the three crops, there was no significant effect of sampling date (p_{date}) nor interactions between the date and the treatment used ($p_{date\ x\ trt}$). On the other hand, the treatment used significantly affected nasturtium ($p_{trt} < 0.0001$), dwarf sunflower ($p_{trt} = 0.0021$), and zinnia ($p_{trt} = 0.0193$) (figure 3). Frass and hen manure always yielded an equivalent number of flowers, whereas in dwarf sunflowers only frass yielded a significantly higher number of flowers than compost.

As compared to the control treatment, the frass treatment resulted in a 32-fold increase in flowering for nasturtium and has caused the flowering of dwarf sunflower and zinnia to double (table S1). The frass treatment also resulted in a significant increase in the number of flower buds for the nasturtium (10-fold) and the dwarf sunflower (3-fold) as compared to the control treatment (table S1). For the three plants, the difference in number of flowers and buds between the frass and the hen manure treatment is not significant.

The diameter of sunflowers subjected to the frass treatment was 1.5 times larger than plants subjected to the control treatment. Similar results were obtained for the frass and hen manure treatments. Furthermore, similar results were obtained for the steam diameter of cherry tomato, sweet corn, basilic, and cucumber (figure S1). Although non-significant, a similar trend was observed for the stem diameter of dwarf sunflower (figure S1).

Though the chitin content of frass could not be measured in the current experiment (due to complex matrix effects), it is unlikely that it would have played a role in promoting the flowering because number and diameter of flowers were similar between frass and hen manure which is unlikely to contain significant concentrations of chitin. On the other hand, Ohta et al. (2004)[62] demonstrated that the flowering of torenia, exacum, begonia, gloxinia, lobelia, and monkey flower is accelerated in soil treated with chitosan. Indeed, the addition of chitin and chitosan can affect flowering phenology[63]. Chitosan used as a biostimulant in potted freesia cultivation promotes leaf and shoot development, flowering rate, and flower and bulb formation[64].

3.5. Yield

The edible biomass obtained in the frass treatment was on average 16,5 times greater in the frass treatment compared to the control treatment, ranging from no difference in radish to 86,5 times greater for sweet corn (table 4). The list of edible biomass yield increase response in decreasing order of magnitude (compared to compost) is corn, beets, cucumber, cherry tomatoes, nasturtium, kale, basil, arugula, swiss chard, carrots and radish. Beets were excluded from the average calculations because unfertilized beets never reached sufficient maturity to be edible. Corn, cucumber, and tomatoes showed the greatest yield increase but are also the most nutritionally intensive plants. In accordance with the CRAAQ's recommendation discussed in section 2.3, those 3 plants received a second fertilization at mid growing season (see table 3 for the fertilization requirements of every plant). For every plant, the yield results obtained when subjected to the frass and hen manure treatments are very similar (0.9-fold to 1.7-fold).

Similar trends were observed for the edible mass of beetroot, arugula, sweet corn, cherry tomato, cucumber, and radish (figure S2) and for the leaf mass of beetroot (figure S6), where the frass and hen manure treatments tend to yield higher results than the compost treatment. On the other hand, the results obtained for the edible mass of swiss chard and carrot (figure S2) and for the leaf mass of carrot (figure S6) did not show any difference between the 3 treatments.

The frass promotes an increase in plant yield. Houben et al.[20] suggest that applying mealworm frass at a rate of 10 tons per hectare results in increased barley (*Hordeum vulgare L.*) biomass as well as nitrogen, phosphorus and potassium uptake. The addition of frass equaled or increased the biomass of ryegrass, wheat and alfalfa plants when compared to compost, digestate, conventional potassium fertilizer, and struvite[59]. Rapid growth rate and high yield, resulting from an increase in plant height and leaves numbers and length, have been observed on cucumber plants fertilized with heat-treated black soldier fly frass[58].

Germination success may not have affected the final yield as carrots and beets were the seeds most hampered by frass upon germination and radish (with no biomass increase) was not hampered by frass upon germination. Zahn and Quilliam[22] proposed that the considerable proportion of nitrogen in the form of ammonium in black soldier fly frass may stunt plant growth. Accordingly, Selim and Zayed[65] have observed that phytotoxic compounds concentrations decrease with time. This may explain early seedling inhibition but absence of long-term effect during plant growth. Contrary to Beesigamukama[53] who suggested that prior composting of *T. molitor* frass was necessary to circumvent phytotoxic effects, our results suggest that soil processes after application are sufficient to promote plant growth and yield with *T. molitor* frass, but a recommendation to delay fertilization of a few sensitive seedlings species after germination could avoid early development phytotoxic effects.

Table 4. Edible biomass of eleven plants amended with compost only (control), frass, and hen manure.

Edible part	Plant	Number of plants	Total Edible Biomass (g) per treatment				Variation in yield of different treatments	
			Control (T1)	Frass (T2)	Hen manure (T3)	*p*	Frass/Compost X	Frass/Hen manure X
Leaf	Basil	18	129,0	1030,0	897,0	0,0529	8,0	1,1
	Swiss chard	54	45,5	174,5	202,7	0,0926	3,8	0,9
	Nasturtium	108	122,4^{b}	1196,4^{a}	1249,7^{a}	0,0216	9,8	1,0
	Kale	9	24,7^{b}	228,0^{a}	196,7^{ab}	0,0337	9,2	1,2
	Arugula	432	359,0^{c}	1507,5^{a}	1030,5^{b}	0,0003	4,2	1,5
Root	Beetroot	216	30,4^{b}	805,5^{a}	569,7^{a}	0,0042	26,5	1,4
	Carrot	216	244,6	288,3	166,6	0,6726	1,2	1,7
	Radish	216	576,4	590,4	1189	0,0766	1,0	0,5
Fruit/ Grain	Cucumber	18	509,0^{b}	8434,2^{a}	8743,8^{a}	0,0022	16,6	1,0
	Sweet corn	54	14,0^{b}	1211,0^{a}	1204,0^{a}	<0,0001	86,5	1,0
	Cherry tomato	18	217,9^{b}	3255,2^{a}	3255,2^{a}	<0,0001	14,9	0,9
Avg.[1]							16,5	1,1

Note: [1] The average difference between treatments excludes beets because these root vegetables harvested with compost alone were not large enough to be consumed. Same exponent letters represent numbers significantly similar.

3.6. Limitations and future implications

This study provided a first source of experimental data regarding the use of frass as a fertilizer on a wide variety of garden plants in comparison with conventional gardening practices (compost amendment only) and with common organic fertilizer (hen manure). A limitation to the extrapolation of our results to mass production food system stems from the fact that this experiment was conducted under optimal irrigation, fertilization, and plant spacing conditions and that plants were grown in pots and not soil. Furthermore, this experiment was conducted on a rooftop in an urban heat island, where temperature and wind speeds were observed to be higher than ground level average. According to Retuerto and Woodward[66], high wind speeds may reduce the number of leaves, stem length, leaf area, and dry weight of the total biomass of exposed plants compared to plants exposed to average wind speeds. On the other hand, Hatfield and Prueger[67] observed that high temperatures generally do not directly affect the growth rate and biomass production rate of plants, but can lead to water deficiency in the substrate which can negatively affect the growth of plants. Because higher-than-average temperatures and wind speeds can lead to detrimental impacts on the growth of plants the results obtained here may differ from those obtained under less extreme conditions. Our results are nevertheless pertinent in the context of climate change which may lead to more frequent and intense extreme heat and droughts episodes[68]. This had been emphasized by Poveda et al.[26] who demonstrated that mealworm frass could help plants tolerate abiotic stressors such as drought, waterlogging and salinity. A field experiment could provide more information on overall yield in a conventional gardening context. However, the results from this study remain relevant as they highlight urban agriculture challenges, which are not yet largely documented [40] [40].

Frass supplements can stimulate plant growth directly (nutrient effect) but can also promote plant growth via indirect effects such as the alteration of the substrate's pH, antagonism to potential pathogens, or modification in the chemical composition of the substrate[18]. In addition, it has been documented that frass can interact with compost in a manner that can affect plant health. For instance, the combination of both black soldier fly frass and humic acids can provide synergistic protective effects on bean plants against Pythium pathogens[69]. Further studies could attempt to extricate the specific mechanisms induced by frass and compost by adding separate and combination treatments.

Moreover, the fertilization potential of frass has been shown to vary according to the type of feed of insects[18]. Further experiments with different types of frass, other types of crops or ligneous plants, directly in soil and under varying environmental conditions including highly stressful conditions such as water deficiency, or in the presence of biotic stressors such as insect pests and diseases will help to refine our understanding of frass fertilization and tailor fertilization recommendations in different contexts. Nevertheless, it is evident from the current study that insect manure can be used as a plant fertilizer like hen manure.

At the moment, regulations regarding the use of frass are poorly adapted in different regions. Recently, modifications to the legal status of frass in the European Union (EU) (Regulation 142\2011) now enable insect excrements to be legally recognized as an organic fertilizer[70]. However, the EU-wide requirements regarding the safe use of frass for fertilization currently require a heat treatment (70C, 2h) which could alter the chemistry and microbiology of frass. Unfortunately, the most recent modification to the Fertilizer’s Act in Canada now precludes its categorization as manure, requiring burdensome registration of product before it reaches the market (contrary to other types of animal manures). Our paper suggests that frass should be classified with other manures as it promotes plant growth in a similar fashion to chicken manure.

SUPPORTING INFORMATION

Figure S1: Stem diameter of cherry tomato, sweet corn, basilic, dwarf sunflower, and cucumber
Figure S2: Edible mass of beetroot, arugula, sweet corn, carrot, cherry tomato, swiss chard, cucumber, and radish
Figure S3: Edible length of carrot
Figure S4: Edible width of beetroot and carrot
Figure S5: Leaf length of carrot, beetroot, and kale
Figure S6: Leaf mass of carrot and beetroot
Table S1: Quantity of flowers and flower buds of nasturtium, dwarf sunflower, and zinnia

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