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Effects of Using Thermocomposted Frass from Black Soldier Fly Larvae as a Germination Substrate on the Phytotoxicity, Germination Index, Growth and Antioxidant Contents in Kale (*Brassica oleracea*)

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Abstract: Frass generated during the production of black soldier fly larvae is attracting the interest of scientists and horticultural producers because it is a material made from the biotransformation of organic waste, it contains several nutrients that can be used by plants, and it has a biostimulant capacity that has become a recent focus. Thermal composting is a stabilization process that improves the physical and chemical properties of treated wastes, allowing better performance in plants compared to the waste in its fresh state. In this research, thermocomposted frass was evaluated as a germination substrate for kale seeds (*Brassica oleracea*). To achieve this, the phytotoxicity of increasing concentrations of frass was evaluated by examining the germination of kale seeds, and seedlings were grown for 30 days in germination substrates mixed with 20, 40, 60, 80, and 100% frass under greenhouse conditions. The treatment with 20% frass showed the highest values of seedling height, stem diameter, number of leaves, length and width of the first true leaf and length and width of cotyledons, and reduced the contents of phenols, tannins and antioxidants. However, the content of flavonoids increased compared to the control and the rest of the mixtures.

Keywords: biofertilizer; organic waste; phytostimulant; spiral economy

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1. Introduction

The most frequently used growing media in soil-less cultivation are peat, coir, softwood pine bark, wood fiber and composted organic wastes. These materials must provide physical, chemical and biological properties to support healthy root growth in the environment and must provide the practical requirements of the production system in which they are being used [1]. Peat moss is the substrate most frequently used for plant germination due to its high moisture retention capacity and porosity. However, these properties encourage the proliferation of fungi that are harmful to the proper development of plants [2]. Furthermore, this substrate is derived from moss ecosystems and its extraction destroys areas of ecological importance [3]. The search for new materials for plant germination is considered a means to reduce environmental impact and improve seedling development at the same time. The use of composted waste is a good option, since it offers physical and chemical properties suitable for the development of seedlings in addition to reincorporating waste (for example, livestock waste [4], municipal solid waste [5] and vegetable residues [6]) into the production chain.

Black soldier fly larva frass is a bioresource composed of excrement, undigested food and insect exoskeletons, and it can be used in agriculture for many purposes, including to

improve plant growth, increase production and improve soil. This is due to its high organic matter and nitrogen contents [7]. The food or residue used to feed the black soldier fly larvae has an effect on the chemical quality of the frass [8]. However, it has been reported that it is necessary to stabilize the frass through practices such as thermocomposting to improve its chemical properties and provide better uses for plants [9].

Frass post-processing through thermocomposting may be advised to avoid soil nitrogen deficiencies or impaired soil–gas permeability, and thus to improve the use of nutrients by plants [10]. This process reduces the C:N ratio, the pH (to neutral values) and the concentration of phytotoxins, and increases the germination percentage [11]. It also significantly increases the chlorophyll concentration, growth, yield and agronomic nitrogen use efficiency of maize [12].

Fresh frass has been used as a germination substrate for baby leaf lettuce (*Lactuca sativa* L., cv. Chiara), basil (*Ocimum basilicum* L., cv. ISI 602) and tomato (*Solanum lycopersicum* L., cv. Roma V.F.), in a Gainesville-type feeding process, in combination with commercial peat, where 10% frass showed the highest values in plant growth [13]. However, it has been reported that the use of immature composts can cause phytotoxicity in seed germination [9].

The phytotoxicity test is performed to qualitatively determine the delay in seed germination due to the presence of phytotoxins [14]. It has been shown that the application of the thermocomposting process to fresh frass reduces the potential presence of concentrations of phytotoxins such as phenols in the soil [11]. One way to quantify the impact of these compounds is to determine the antioxidant content of plants [15].

Antioxidants are secondary metabolites that plants produce under different types of biotic or abiotic stress, and their quantification is an indirect way of finding out if plants are subjected to any type of stress [16]. It has been reported that a deficiency of nitrogen, phosphorus and potassium generates an increase in flavonoid [17] and phenol [18] contents. The presence of beneficial bacteria from the Rhizobia family favors the production of flavonoids, and these also play an important role in the formation of the nodular meristem in the roots [19]; moreover, the main function of tannins is to provide protection against attack by insects and pathogenic microorganisms [20].

For this reason, the aim of this work was to evaluate the thermocomposted black soldier fly larva frass generated from the treatment of fruit and vegetable waste as a substrate and its effect on phytotoxicity, the germination index, growth and antioxidant content in kale.

2. Materials and Methods

2.1. Obtention of Thermocomposted Black Soldier Fly Larva Frass

Black soldier fly larva frass (BSFLF) was obtained from the biotransformation unit pilot at Universidad Autonoma de Queretaro, Campus Amazcala, Mexico. The feedstock was the organic residue of a mixture of fruits and vegetables from a municipal market which had undergone a biotransformation process. The residue was placed in plastic boxes filled with 20 kg organic residue, and 9000 5-day-old larvae and 500 g of sawdust were added to each box to retain water and reduce the moisture of the residue. Fresh frass was collected using a no. 10 mesh sieve after 14 days. After collection, the fresh frass was placed in a circular tank made of a black geomembrane for thermocomposting using the static heap method, until it reached its maximum temperature, oscillating around 65 °C, and then the ambient temperature, for a total time of 30 days.

2.2. Laboratory Analysis Methods of Thermocomposted BSFLF (TBSFLF)

After thermocomposting, frass was sieved to obtain 2 mm particles and then air dried. The Mexican guideline NMX-FF-109-SCFI-2008 [21] was followed to determine the pH, electrical conductivity, moisture content, organic matter, organic carbon, total nitrogen, carbon-to-nitrogen ratio, cation exchange capacity, apparent density and carbon-to-phosphorus ratio. Nitrate nitrogen was calculated according to Cataldo et al. [22]; phosphorus was determined following the Mexican guideline NMX-DGN-AA-32-1976 [23];

potassium, calcium and sodium concentrations were determined by flamometry; magnesium concentration was determined according to Harris [24]; and humic and fulvic acids were quantified following the Kononova–Belchikovas method [25].

2.3. Phytotoxicity Test of Thermocomposted Frass and Germination Index

The plant bioassay method was used [26] to determine phytotoxicity, and the seed germination index and plant growth were also examined. The black soldier fly larva frass was hand-sieved at a diameter of 2 mm, and then the extract was obtained by diluting 5 g of frass with 50 mL of distilled water (1:10 w/v) and shaking for one hour using a Thermo Scientific (Dubuque, IA, USA) maxQ 2506 reciprocating shaker. The extract was diluted in the same series as in the seedling experiment (described below), at 20%, 40%, 60%, 80% and 100%, with distilled water as a positive control, to determine the effect of the concentration of frass extract on germination, of which there are currently no reports. Next, 5 mL of each dilution factor was taken and placed in a Petri dish. Seeds of kale cv Blue ridge (SAKATA® Seed America, Inc., Morgan Hill, CA, USA) (*Brassica oleracea*) were used as a test crop, and 10 seeds were placed in the Petri dish in triplicate, laid on filter paper and then moistened with 5 mL of black soldier fly larva frass extract. The Petri dishes were kept in an incubator (Memmert model IN30 Eagle, WI, USA) under a controlled environment at 25 °C. The germinated seeds were counted after 72 h, and their root lengths were measured after 72 h. The germination index (GI) was calculated using the following equation [27]:

$$GI(\%) = \frac{(RSG\% \times RRG\%)}{100} \quad (1)$$

Here, RSG is the relative seed germination and RRG represents the relative root growth. RSG and RRG are calculated as follows:

$$RSG(\%) = \frac{SGCE}{SGDW} \times 100 \quad (2)$$

Here, SGCE is the number of seeds germinated in thermocompost extracts and SGDW is the number of seeds germinated in distilled water.

$$RRG(\%) = \frac{MRLCE}{MRLDW} \times 100 \quad (3)$$

Here, MRLCE is the mean root length in the thermocompost extract and MRLDW is the mean root length in distilled water. To evaluate the phytotoxicity of frass, the GI value is calculated; GI values below 50% are considered highly phytotoxic, values between 50% and 80% are moderately phytotoxic and values above 80% indicate no phytotoxicity. When the value exceeds 100%, the frass can be considered a phytonutrient or phytostimulant [27].

2.4. Germination Substrate Preparation

To assess the ability of TBSFLF as a growing media, six different germinations substrates were composed as follows (% volume): commercial peat moss (PM), PREMIER® Sphagnum Peat Moss Premier Horticulture, Inc., (Quakertown, PA, USA), PM 100% (GS1); PM 80% + TBSFLF 20% (GS2); PM 60% + TBSFLF 40% (GS3); PM 40% + TBSFLF 60% (GS4); PM 20% + TBSFLF 80% (GS5); and TBSFLF 100% (GS6).

2.5. Germination Test

A germination test with the prepared substrates was carried out under greenhouse conditions with temperatures ranging from 8.1 to 38.2 °C, relative humidity ranging from 16.6 to 76.8% and a solar photoperiod (maximum intensity 2448 $\mu M/m^2s$). Kale cv Blue ridge (SAKATA® Seed America, Inc., Morgan Hill, CA, USA) was sown manually, with 1 seed per pot. Styrofoam pots were used (diameter 8 cm and height 9 cm, 0.236 L) and filled with the different germination substrates. Each treatment contained 33 pots arranged in a completely randomized design with no repetition. Each pot was manually irrigated

every day. The number of emerged plants was recorded every day, and the accumulative percentage is reported.

2.6. Agronomic Performance of Seedlings

The experiment was carried out with the same conditions as the germination test. Each treatment (PM, GS1, GS2, GS3, GS4, GS5 and GS6) contained 11 pots with three repetitions arranged in a completely randomized design. Kale cv Blue ridge (SAKATA® Seed America, Inc.) was sown manually, with 1 seed per pot. Thirty days after sowing, the following variables were measured on each plant: height of the plant (mm), stem diameter (mm), total number of true leaves, true leave length (mm), true leave width (mm), cotyledon length (mm) and cotyledon width (mm).

2.7. Antioxidant Content

Extraction was carried out according to Cardador et al. [28]; a 25 mg dry sample and 200 mg wet sample of kale were taken, and then 2.5 mL of methanol was added. The samples were kept in the dark and shaken, and after 24 h they were centrifuged at 5000 rpm for 10 min at 4 °C; only the supernatant was taken.

2.7.1. Total Contents of Phenols, Flavonoids and Tannins

The total phenol contents of roots and leaves were determined using the Folin–Ciocalteu method according to Singleton et al. [29], modified to a 96-well microplate. Briefly, 4 µL equivalent to 0.01 g of the extract was mixed with 250 µL of Folin–Ciocalteu reagent and 1250 µL of Na_2CO_3 solution (20%), and incubated at room temperature for 2 h. Absorbance was measured at 760 nm using a spectrophotometer (Thermo Scientific ™ model MULTISKAN ™ GO, Dubuque, IA, USA). Gallic acid was used for the calibration curve (0 to 20 mg) and the results are expressed as gallic acid equivalents per gram of sample.

The spectrophotometric method was used to determine the total flavonoid contents in methanolic extracts according to Oomah et al. [30]. First, 50 µL of methanolic extract was mixed with 180 µL of distilled water and 20 µL of 2-aminoethyl diphenylborinate at 1% in a 96-well microplate. Absorbance was measured at 404 nm using a spectrophotometer, MULTISKAN GO. Extract absorption was compared with that of a rutin standard curve (up to 2 µg/mL). The results are expressed as rutin equivalents per gram of sample.

Total tannin content was determined following Feregrino-Perez [31]'s method, modified for a 96-well microplate: 50 µL of methanolic extract and 200 µL of solution 1:1 (v/v) of vanillin at 1% and HCl at 8% were deposited in 96-well microplate, along with 50 µL of methanol and 200 µL of HCl. Absorbance was measured at 492 nm using a spectrophotometer (Thermo Scientific ™ model MULTISKAN ™ GO), using (+) catequin (up to 0.1 mg/mL) as a reference standard. The results are expressed as catechin equivalents per gram of sample.

2.7.2. Antioxidant Capacity Determination: DPPH and ABTS

DPPH quantification was determined according to Zenil et al. [32]: 20 µL of methanolic extract and 200 µL of DPPH were deposited in a 96-well microplate. Absorbance was measured at 520 nm at different times (0, 10, 30, 60 and 90 min), using a spectrophotometer (Thermo Scientific ™ model MULTISKAN ™ GO), and the results were expressed as Trolox equivalents per gram of sample.

The spectrophotometric method for antioxidant capacity quantification by ABTS was used, following Pellegrini et al. [33]: 230 µL of ABTS and 20 µL of sample were deposited in a 96-well microplate. Absorbance was measured at 734 nm using a spectrophotometer (Thermo Scientific ™ model MULTISKAN ™ GO), and the results were expressed as Trolox equivalents per gram of sample.

2.8. Statistical Analysis

Data were analyzed using STATGRAPHICS Centurion software (version XVI). An ANOVA test was used for height, stem diameter, number of true leaves, first true leaf length, true leaf width, cotyledon length and cotyledon width of kale seedlings; for significant differences, a Fisher's LSD test was used to make a multiple comparison between treatments with parametric data. In addition, a significant difference was determined by Dunnett's pairwise comparison for germination, the germination index, antioxidant content, and antioxidant capacity to make a multiple comparison with the control because the data were nonparametric. The significance value of the data was $p < 0.05$ in all analyses. For percent emergence, the total number of plants is indicated.

3. Results

3.1. Chemical Composition of Thermocomposted BSFLF

The chemical composition of peatmoss and frass are reported in Table 1.

Table 1. Chemical composition of peatmoss and thermocomposted frass expressed in dry matter.

Parameter	Peatmoss	Thermocomposted Frass
Moisture (%)	64.70 ± 0.025	46.46 ± 0.615
pH	4.435 ± 0.017	8.506 ± 0.093
EC (dS/m)	0.6735 ± 0.019	7.476 ± 0.475
OOM (%)	91.735 ± 0.039	78.63 ± 0.750
OOC (%)	53.21 ± 0.021	45.61 ± 0.438
Total N (%)	1.08 ± 0.018	1.98 ± 0.049
N-NO_3 (mg/kg)	308.99 ± 4.458	96.50 ± 2.708
C/N	49.525 ± 0.725	23.13 ± 0.368
C/P	1263.135 ± 3.447	29.27 ± 0.453
CEC (Cmol/kg)	117.69 ± 0.647	55.57 ± 0.163
AD (g/mL)	0.1210 ± 0.007	0.2622 ± 0.017
P_2O_5 (%)	0.1 ± 0	3.57 ± 0.014
K_2O (%)	0.05 ± 0	1.71 ± 0.014
Ca (%)	0.62 ± 0.007	0.70 ± 0.035
Mg (%)	0.085 ± 0.004	0.24 ± 0.071
Na (%)	0.03 ± 0	0.11 ± 0
HA (%)	1.6455 ± 0.012	1.629 ± 0.144
FA (%)	2.613 ± 0.018	1.731 ± 0.144

Mean values ± standard deviation (n = 3) of electrical conductivity (EC), oxidizable organic matter (OOM), oxidizable organic carbon (OOC), cation exchange capacity (CEC), apparent density (AD), humic acid (HA) and fulvic acid (FA).

3.2. Phytotoxicity of Thermocomposted BSFLF and Germination Index of Kale

All the treatments showed a germination of 80 ± 26.46%, with no significant difference; however, the 80% and 100% composted frass doses showed moderate and high phytotoxicity, respectively, and the 20% and 40% treatments had values greater than 100%, which means that they had phytostimulant properties according to [27]. All values are shown in Table 2.

Table 2. Mean values of germination and germination index (GI) and phytotoxicity of different treatments in kale seed.

Treatment	Germination (%)	GI (%)	Phytotoxicity
Control	96.67 ± 7.07 [a]	100 ± 6.58 [a]	No
20%	96.67 ± 7.07 [a]	108.54 ± 6.58 [a]	No
40%	90 ± 7.07 [a]	106.46 ± 6.58 [a]	No

Table 2. *Cont.*

Treatment	Germination (%)	GI (%)	Phytotoxicity
60%	80 ± 7.07 [a]	69.70 ± 6.58 [b]	Moderate
80%	90 ± 7.07 [a]	51.17 ± 6.58 [b]	Moderate
100%	96.67 ± 7.07 [a]	49.63 ± 6.58 [b]	High

Mean values ± standard error (n = 3) with superscript letters indicating significant difference at $p < 0.05$ according to Dunnett's test.

3.3. Effect of Thermocomposted BSFLF as a Germination Substrate on Kale Seedlings' Growth

The percentage of the emergence of kale was affected by the percentage of inclusion of composted frass as a germination substrate; the results are shown in Table 3. Treatments GS2, GS4 and GS5 were the first to emerge, and treatments GS5 and GS6 reached their highest emergence percentage on the 7th day, and then the plants died. The moderate and high phytotoxicity obtained for these treatments in the previous test was reaffirmed in this test. After the 12th day, only treatments GS1, GS2, GS3 and GS4 survived, so the remaining analyses were for these treatments.

Table 3. Emergence (%) of kale seeds in different germination substrates after different days of sowing.

	Emergence (%)				
Germination Substrate	3rd Day	5th Day	7th Day	9th Day	12th Day
GS1	0	81.82	81.82	81.82	84.85
GS2	12.12	100	100	100	100
GS3	0.00	72.73	87.88	87.88	90.91
GS4	12.12	69.70	90.91	90.91	90.91
GS5	3.03	21.21	24.24	15.15	9.09
GS6	0	0	6.06	0	0

Mean values (n = 33) of accumulative percentage of total number of emerged plants are reported.

The growth of the kale seedlings was recorded with the variables of height, stem diameter, number of true leaves and length and width of first true leaf and cotyledons. In Tables 4 and 5, mean values of these variables are shown for 12 days after the seeds were sown and on day 30, when the seedlings were harvested. The greatest vegetative growth was seen with the GS2 substrate, a mixture of 80% peatmoss and 20% composted frass. At the end of the experiment, the GS2 treatment showed the best results compared to the GS1 control treatment, increasing the height and stem diameter by 32.7%; the number of true leaves by 51.6%; the length and width of the first true leaf by 38.7% and 17.9%, respectively; and the cotyledon length and width by 25% and 33.7%, respectively (Figure 1).

Table 4. Mean values of height, stem diameter, number of true leaves, length of first true leaf, width of true leaf, cotyledon length and cotyledon width of kale seedlings in different germination substrates at the initial measurement on day 12.

Germination Substrate	Height (cm)	Stem Diameter (mm)	Number of True Leaves	Length of First True Leaf (mm)	Width of First True Leaf (mm)	Cotyledon Length (mm)	Cotyledon Width (mm)
GS1	1.69 ± 0.07 [b]	1.07 ± 0.02 [b]	1.06 ± 0.05 [b]	6.78 ± 0.56 [b]	3.43 ± 0.31 [b]	6.43 ± 0.24 [b]	10.10 ± 0.39 [b]
GS2	2.26 ± 0.10 [a]	1.21 ± 0.02 [a]	1.57 ± 0.09 [a]	9.29 ± 0.64 [a]	5.51 ± 0.47 [a]	7.95 ± 0.29 [a]	13.82 ± 0.53 [a]
GS3	1.44 ± 0.04 [b]	1.09 ± 0.03 [b]	1.00 ± 0.00 [b]	4.40 ± 0.77 [c]	2.53 ± 0.58 [c]	5.10 ± 0.12 [b]	8.41 ± 0.26 [b]
GS4	1.43 ± 0.05 [b]	1.07 ± 0.02 [b]	0.00 [c]	0.00 [d]	0.00 [d]	5.24 ± 0.17 [b]	8.11 ± 0.27 [b]

Mean values (n = 33) ± standard error with superscript letters indicating significant difference at $p < 0.05$ according to Fisher's LSD test.

Table 5. Mean values of height, stem diameter, number of true leaves, length of first true leaf, width of true leaf, cotyledon length and cotyledon width of kale seedlings in different germination substrates at the initial measurement on day 30.

Germination Substrate	Height (cm)	Stem Diameter (mm)	Number of True Leaves	Length of First True Leaf (mm)	Width of First True Leaf (mm)	Cotyledon Length (mm)	Cotyledon Width (mm)
GS1	3.33 ± 0.11^{b}	1.07 ± 0.03^{b}	2.48 ± 0.09^{b}	16.67 ± 0.61^{b}	7.74 ± 0.40^{b}	6.12 ± 0.17^{b}	9.92 ± 0.39^{b}
GS2	4.42 ± 0.17^{a}	1.42 ± 0.04^{a}	$3.76^{a} \pm 0.11$	23.13 ± 1.17^{a}	9.13 ± 0.41^{a}	7.65 ± 0.25^{a}	13.27 ± 0.42^{a}
GS3	2.16 ± 0.09^{c}	0.94 ± 0.03^{c}	2.06 ± 0.06^{c}	10.82 ± 0.54^{c}	4.78 ± 0.21^{c}	5.89 ± 0.18^{b}	8.63 ± 0.30^{b}
GS4	$2.06^{c} \pm 0.05$	0.90 ± 0.02^{c}	1.97 ± 0.06^{c}	9.09 ± 0.32^{c}	4.44 ± 0.17^{c}	6.33 ± 0.21^{b}	8.07 ± 0.32^{b}

Mean values (n = 33) ± standard error with superscript letters indicating significant difference at $p < 0.05$ according to Fisher´s LSD test.

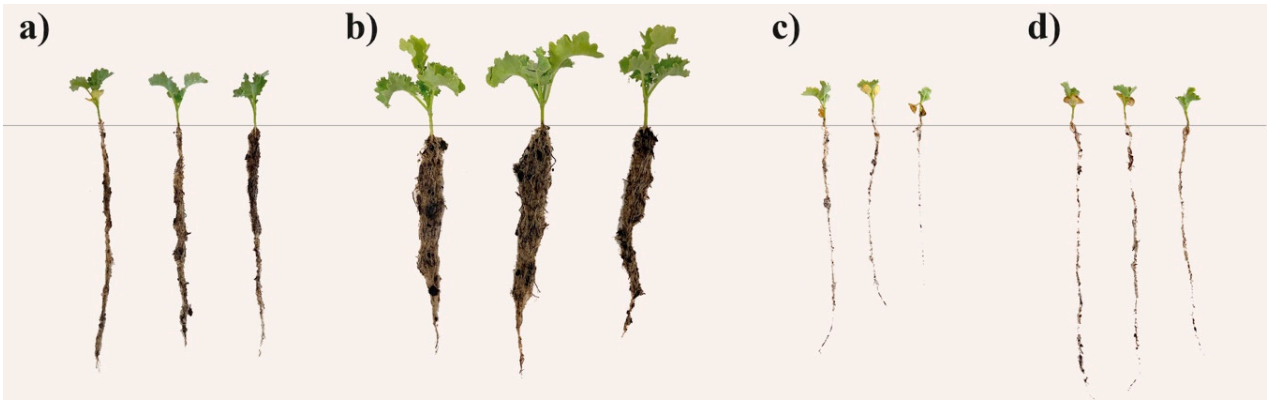

Figure 1. Representative kale seedlings harvested at day 40 and grown in different germination substrates: (**a**) GS1 = 100% peatmoss (PM); (**b**) GS2 = 20% TBSFLF + 80% PM; (**c**) GS3 = 40% TBSFLF + 60% PM; and (**d**) GS4 = 60% TBSFLF + 40% PM.

3.4. Antioxidant Content

In Table 6, the phenol content in kale leaf samples is expressed in milligram equivalents of gallic acid, tannins in milligrams equivalent of catechin and flavonoids in milligrams equivalent of rutin. The GS1 treatment (control treatment) shows the highest phenol and tannin contents, and the GS2 treatment was the one with the lowest amount of those phenolic compounds.

Table 6. Total phenol, tannin, and flavonoid contents in leaves of kale seedlings with different germination substrates.

Treatment	Phenols (mg Eq. of Gallic Acid/g)	Tannins (mg Eq. of Catechin/g)	Flavonoids (mg Eq. of Rutin/g)
GS1	354.054 ± 0.19^{a}	10.972 ± 0.45^{a}	0.1045 ± 0.28^{b}
GS2	170.954 ± 0.05^{c}	7.985 ± 0.21^{c}	0.0713 ± 0.11^{b}
GS3	263.993 ± 0.10^{b}	4.437 ± 0.09^{b}	0.2177 ± 0.18^{a}
GS4	244.744 ± 0.04^{b}	5.115 ± 0.17^{b}	0.2516 ± 0.3^{a}

Mean values (n = 3) with superscript letters indicating significant difference at $p < 0.05$ according to Dunnett's test.

For flavonoid content, the GS4 treatment showed the highest amount and the GS1 treatment showed the lowest.

Antioxidant capacity is expressed in ABTS and DPPH percentages in Table 7; only the GS1 treatment showed a significantly higher percentage of ABTS compared to the GS2 treatment. The GS1, GS2 and GS3 treatments showed a significantly higher percentage of DPPH compared to the GS4 treatment.

Table 7. ABTS and DPPH percentages in leaves of kale seedlings with different germination substrates.

Treatment	ABTS (%)	DPPH (%)
GS1	99.1718 ± 0.58 [a]	91.6772 ± 1.11 [a]
GS2	97.1877 ± 0.58 [a]	89.7525 ± 1.11 [a]
GS3	99.0338 ± 0.58 [a]	88.5828 ± 1.11 [a]
GS4	98.1481 ± 0.58 [a]	76.7695 ± 1.11 [b]

Mean values (n = 3) ± standard error with superscript letters indicating significant difference at $p < 0.05$ according to Dunnett's test.

4. Discussion

The thermocomposted frass showed an alkaline pH (8.5), and a higher value compared to previous works: 7.26 from brewery residue [34], 7.3 from brewery residue [12,35], 7.5 from a mixture of okara and wheat bran [11], 7.6 from brewery residue amended with sawdust [36], 7.7 from brewery residue [37] and 7.8 from brewery residue [27]. The other physical and chemical characteristics of the thermocomposted frass used in this research differed from those reported in other works because the origin of the waste with which the fly larvae were fed directly affects the physical quality and quantity of frass nutrients [10].

Other experiments with radish and lettuce seeds showed a similar trend with fresh frass, where decreasing the percentage of frass in the aqueous medium increased the GI value due to high phytotoxicity [38]. However, the GI was higher in this work, indicating that a thermocomposted frass has a low phytotoxicity and inhibition effect. Another study with fresh frass showed GI values greater than 100%, indicating zero phytotoxicity on garden cress seeds, but considering a 1:20 dilution [13], a highly diluted extract.

A study of thermocomposted frass from brewers' spent grain biotransformation used a 1:10 dilution, obtaining GI values from 22.9 to 101.9 [36], a broad range compared to the present work. Another study used a 1:10 dilution with a thermocomposted okara and wheat bran frass mixture, obtaining GI results lower than 25% and showing that even after a thermocomposting stabilization treatment, the frass had a high percentage of phytotoxicity that may be due to the presence of phenols, chitin, and an excess of nutrients [11]. According to Teresa Barral [14], substrates derived from thermocomposting contain some compounds that can cause phytotoxicity, such as ammonia, ethylene oxide, organic acids, phenols, salts and heavy metals.

The plants demonstrated inhibition in their shoot and root growth at a higher percentage of inclusion as a germination substrate. Macro- and micronutrients are essential for plant development and growth because they play important roles in plant physiology [39]. Additionally, the most significant importance of frass is not due to its mineral nutrients but rather the rhizobacteria and phytohormones present in the frass [40]. Song et al. [11] reported the highest number of leaves in pak choi (*Brassica rapa*) using 10% composted frass, an increase of 41.67% compared to the control. In this study, the highest kale growth was with 20% composted frass, an increase of 51.61% in the number of leaves compared to the control. The substrate pH is essential in determining nutrient availability to the plant [41]. In contrast, in seed germination, an acidic pH can inhibit the action of enzymes necessary for germination and can have a direct effect by dissolving the seed coat [42]. Some authors have reported pH values between 5.5 and 6.9 and EC between 1.2 and 1.9 mS/cm (=dS/m) as suitable for kale germination [43–45]. However, in this study, the alkaline pH of the thermocomposted frass (8.5) and high electrical conductivity (7.476 dS/m) could have reduced the germination of kale as the percentage of composted frass increased in the substrate.

Flavonoids have protective functions in plants, including defenses against phytopathogens and herbivores [46]. They influence the transport of auxins, plant hormones that protect plants from microbes and insects. Flavonoids play an essential role in the roots during nodule meristem formation and as a defense against attack by rhizobia soil bacteria [47]. In this way, the increase in flavonoid content as the percentage of composted

frass in the substrate increased may have been a defense response to the microorganisms in the frass, such as Azospirillum, Rhizobium, Azotobacter, and the genera Bacillus [48].

The difference between the obtained DPPH values can be attributed to the nitrogen content in the composted frass. A high amount of nitrogen applied to the plant decreases the percentages of ABTS and DPPH, which measure the activity of water-soluble antioxidants [49]. In this experiment, no differences in these variables were detected in accordance with Biesiada et al. [50] and Romano et al. [51], who reported no significant difference in the total phenol content or antioxidant capacity using fresh frass.

5. Conclusions

The addition of thermocomposted frass at 20% generates the appropriate physicochemical conditions for the generation of a substrate for improving the agronomic performance of kale, showing an alternative material for germination.

More research is needed on the content of other components in frass, such as phytohormones. It is important to know the appropriate doses of thermocomposted BSFL in the phenological stages of germination, development and production in different species of vegetables and fruits. Likewise, it is necessary to improve the thermal stabilization process or even add another, like vermicomposting.

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