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Keywords: organic waste; spiral economy; dynamic programming; risk management; post-decision state variable



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Article

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Hugo González-Lara ¹, Benito Parra-Pacheco ¹, Armando Gudiño-Calixto ¹, Ana Angélica Feregrino-Pérez ^{2*} and Juan Fernando García-Trejo ^{2,*}

¹ División de Investigación y Posgrado, Facultad de Ingeniería, Universidad Autónoma de Querétaro, Carretera a Chichimequillas Km. 1 s/n, Amazcala, El Marqués, Querétaro 76265, México

² Cuerpo académico de Bioingeniería Básica y Aplicada, Facultad de Ingeniería, Universidad Autónoma de Querétaro, Cerro de las Campanas s/n, Las Campanas, Querétaro 76010, México

* Correspondence: fernando.garcia@uaq.mx; feregrino.angge@hotmail.com

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

Keywords: organic waste; spiral economy; biofertilizer; phytostimulant

1. Introduction

The reduction of arable land and extreme weather conditions has led to a great proportion of crops, being cultivated in a soilless greenhouse production system to provide sufficient and high-quality food. Soilless production is a new kind of cultivation that uses organic or inorganic inert substrates, where the nutrients can be supplied from a nutrient solution in an optimal and innocuous way [1].

The most used growing media in soilless cultivation are peat, coir, soft-wood pine bark, wood fiber and composted organic wastes. These materials must offer the physical, chemical, and biological properties to support healthy root growth in the environment that contains it; and it must provide the practical requirements of the production system in which it is being used [2]. Peat moss is the most used substrate for plant germination due to its high moisture retention capacity and porosity. However, these properties generalize the proliferation of fungi that are harmful to the proper development of plants. It comes from moss ecosystems and its extraction destroys areas of ecological importance [3]. The search for new materials for plant germination has become a way to reduce environmental impact and at the same time improve seedling development. 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 the waste into the production chains [4].

Black soldier fly larva frass is a bioresource composed by excrement, not digested food and insect exoskeleton, which can be used in agriculture for many purposes, to improve plant growth, to increase production and soil improvement, this due to its high content of organic matter and nitrogen [5]. However, frass post-processing through anaerobic digestion or thermocomposting may be advised to avoid soil nitrogen deficiencies or impairing soil gas permeability, and thus have a better use of nutrients by plants [6].

Frass has been used as a germination substrate for vegetables, coming from Gainesville-type feeding, with any stabilization post-treatment (fresh frass), in combination with commercial peat, where 10% of frass showed the highest values in plant growth [7]. Frass stabilization by thermocomposting improves its physical and chemical properties, such as pH and significantly increases crop yield [8].

For this reason, the aim of this work is to evaluate black soldier fly larvae frass (BSFLF) generated from the treatment of fruit and vegetable waste, stabilized by thermocomposting, as a substrate for kale germination.

2. Materials and Methods

2.1. Obtention of Thermocomposted Black Soldier Fly Larva Frass

Black soldier fly larvae frass (BSFLF) was obtained from biotransformation unit pilot at Universidad Autonoma de Queretaro, Campus Amazcala – Mexico. The feedstock was organic residue integrated of a mixture of fruits and vegetables, from a municipal market as a biotransformation process. The residue was collocated in plastic boxes, filled with 20 kg residue and 9000 5-day-old larvae. 500 grams of sawdust was added to each box to reduce moisture. Fresh frass was collected by sieving after 14 days and placed in a circular tank made of geomembrane. The thermocomposting lasted 30 days using the static heap method until obtain a stabilized and ambient temperature frass.

2.2. Laboratory Analysis Methods of Thermocomposted BSFLF

After thermocomposting, frass was sieved to obtain 2 mm particle size and air dried. The Mexican norm NMX-FF-109-SCFI-2008 [35] was following to determine pH, electric 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. [36]; phosphorus was determined following the Mexican norm NMX-DGN-AA-32-1976 [37]; potassium, calcium and sodium concentration was determined by flamometry; magnesium concentration was determined according to Harris [38] and humic and fulvic acid was quantified following Kononova Belchikovas method [39].

2.3. Phytotoxicity Test of Thermocomposted Frass and Germination Index

To determine phytotoxicity the plant bioassay method was used, where seed germination index and plant growth were determined. The black soldier fly larvae frass was hand sieved with a 2 mm diameter sieved, then, extracts were obtained by diluting 5 g of frass, with 50 ml of distilled water (1:10 w/v) and shaken in Thermo Scientific maxQ 2506 reciprocating shaker for one hour. The samples were diluted in series of 20%, 40%, 60%, 80%, 100% and distilled water as a positive control. 5 ml of each dilution factor was taken and placed in the petri dish. Seeds of Blue ridge kale (SAKATA® Seed America, Inc.) (*Brassica oleracea*) were used as a test crop, where 10 seeds were placed in the petri dish in triplicate, laid on filter paper and moistened with 5 ml of black soldier fly larvae frass extract. The petri dishes were kept in an incubator Memmert IN30 under a controlled environment at 25 °C, measuring at 72 hours before. The germinated seed were counted, and their root lengths were measured. Germination index (GI) was calculated using the following equation [14]:

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

Where RSG is the relative seeds germination and RRG represents the relative root growth. RSG and RRG are calculated as follow equation.

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

Where SGCE is number of seeds germinated in thermocompost extracts and SGDW is number of seeds germinated in distilled water.

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

Where MRLCE is mean root length in thermocompost extract and MRLDW is mean root length in distilled water. To evaluate the phytotoxicity of frass, the GI value was calculated [14], where GI values below 50% were considered highly phytotoxic, values between 50% and 80% were moderately phytotoxic and values above 80% indicate no phytotoxic. When the value exceeds 100% can be considered a phytonutrient or phytostimulant.

2.4. Germination Substrate Preparation

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

2.5. Seedling Experiment

The experiment was carried out under a greenhouse with temperatures ranging from 8.1 to 38.2 °C, relative humidity ranging from 16.6 to 76.8 % and solar photoperiod (maximum intensity 2448 μM/m²s). Blue ridge kale (SAKATA[®] Seed America, Inc.) was sown manually 1 seed per pot. Styrofoam pots were used (diameter 8 cm and height 9 cm, 8 oz.) and filled with the different germination substrates. Each pot was arranged in a completely randomized design with 33 replicates and was manually irrigated every day. Thirty days after sowing, the following variables were measured on each replicate per treatment: Height of 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).

2.6. Antioxidants Content

The extraction was made according to Cardador et al. [40], 25 mg of dry sample and 200 mg of wet sample of kale were taken, and then 2.5 ml of methanol was added. The samples were kept in the dark and shaking, and after 24 h they were centrifugated at 5000 rpm for 10 min at 4 °C, the supernatant was taken only.

2.6.1. Total Content of Phenols, Flavonoids, and Tannins

The total phenols content of root and leaf were determined using the Folin-Ciocalteu method according to Singleton et al. [41], modified to 96-well microplate use. 4 μl equivalent to 0.01 g of the extraction was mixed with 250 μl of Folin-Ciocalteu reagent and 1250 μl of Na₂CO₃, incubated at room temperature for 2 hr. Absorbance was measured at 760 nm using spectrophotometer MULTISKAN GO, and the results were expressed as gallic acid equivalents per gram of sample.

The spectrophotometric method was used to determine the total flavonoids content in methanolic extracts according to Oomah et al. [42]. 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 spectrophotometer MULTISKAN GO, and the results were expressed as rutin equivalents per gram of sample.

Total tannins content was determined following Feregrino-Perez [43] modified method to 96-well microplate use. 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, and 50 µl of methanol and 200 µl of HCl. Absorbance was measured at 492 nm using spectrophotometer MULTISKAN GO, and the results were expressed as catechin equivalents per gram of sample.

2.6.2. Antioxidant Capacity Determination DPPH and ABTS

DPPH quantification was made according to Zenil et al. [44], 20 µl of methanolic extract and 200 µl of DPPH was deposited in in 96-well microplate. Absorbance was measured at 520 nm at 0, 10, 30, 60 and 90 minutes, using a spectrophotometer MULTISKAN GO, and the results were expressed as Trolox equivalents per gram of sample.

The spectrophotometric method for antioxidant capacity quantification by ABTS was made following Pellegrini et al. [45]. 230 µl of ABTS and 20 µl of sample was deposited in a 96-well microplate. Absorbance was measured at 734 nm using spectrophotometer MULTISKAN GO, and the results were expressed as Trolox equivalents per gram of sample.

2.7. Statistical Analysis

Data were analyzed using the software STATGRAPHICS Centurion, by one-way analysis of variance (ANOVA). The least significant difference was determined by Fisher’s test at $P<0.05$.

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.

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
Total N (%)	1.08 ± 0.018	1.98 ± 0.049
N-NO ₃ (mg/k)	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 ₂ O ₅ (%)	0.1 ± 0	3.57 ± 0.014
K ₂ O (%)	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 of Electrical conductivity (EC), Oxidizable organic matter (OOM), Oxidizable organic carbon (OOC), Cation exchange capacity (CEC), Apparent density (AD), Humic acid (HA), fulvic acid (FA).

3.2. Phytotoxicity of Thermocomposted BSFLF and Germination Index of Kale

All the treatments showed germination upper than 80 ± 26.46 % with no significant difference however, the 80% and 100% composted frass doses showed a moderately and highly phytotoxicity respectively, 20% and 40% treatments were greater than 100% which it means it has a phytostimulant properties, 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 ± 5.77 ^a	100 ± 0.00 ^a	No
20%	96.67 ± 5.77 ^a	108.54 ± 6.70 ^a	No
40%	90 ± 10.00 ^a	106.46 ± 13.60 ^a	No
60%	80 ± 26.46 ^a	69.70 ± 22.23 ^a	No
80%	90 ± 0.0 ^a	51.17 ± 6.13 ^b	Moderate
100%	96.67 ± 5.77 ^a	49.63 ± 4.48 ^b	High

Mean values ± standard deviation (n=30) with superscript as significantly difference at *p*<0.05 according to Dunnett test.

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

The percentage of 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, treatments GS5 and GS6 reached its highest emergence percentage on 7th day and then the plants die. The moderate and high phytotoxicity obtained for these treatments in the previous test was reaffirmed in this test. After 12th day, only treatments GS1, GS2, GS3 and GS4 survived, so the rest of the analyzes were for these treatments.

Table 3. Emergence percentage of kale in different germination substrate after sowing.

Germination substrate	Emergence (%)				
	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).

The growth of the kale seedlings was recorded with the variables of height, stem diameter, number of true leaves, length, and width of first true leaf and cotyledon. In Tables 4 and 5 are shown mean values of those variable, 12 day after the seeds were sown and day 40 when the seedlings were harvested. The greatest vegetative growth was performed by GS2 substrate, a mixture of composed 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 height and steam diameter by 32.7%, number of true leaves by 51.6%, length and width of first true leaf by 38.7% and 17.9% respectively, and cotyledon length and width by 25% and 33.7% respectively (Figure 1).

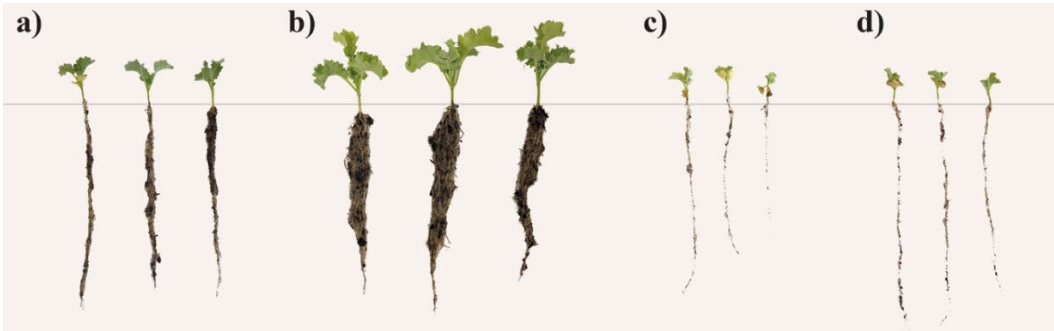


Figure 1. Representative kale seedlings harvested at day 40 grown in different germination substrates: (a) GS1= 100% Peatmoss (PM); (b) GS2= 20% composted Black Soldier Fly Larvae Frass (BSFLF) + 80% PM; (c) GS3= 40% composted BSFLF + 60% PM and (d) GS4= 60% composted BSFLF + 40% PM.

Table 4. Mean values of height, steam 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)	Steam 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 ^b	1.07 ^b	1.06 ^b	6.78 ^b	3.43 ^b	6.43 ^b	10.10 ^b
GS2	2.26 ^a	1.21 ^a	1.57 ^a	9.29 ^a	5.51 ^a	7.95 ^a	13.82 ^a
GS3	1.44 ^b	1.09 ^b	1.00 ^b	4.40 ^c	2.53 ^c	5.10 ^b	8.41 ^b
GS4	1.43 ^b	1.07 ^b	0.00 ^c	0.00 ^d	0.00 ^d	5.24 ^b	8.11 ^b

Mean values (n=33) with superscript as significantly difference at p<0.05 according to Fisher’s LSD test.

Table 5. Mean values of height, steam 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)	Steam 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 ^b	1.07 ^b	2.48 ^b	16.67 ^b	7.74 ^b	6.12 ^b	9.92 ^b
GS2	4.42 ^a	1.42 ^a	3.76 ^a	23.13 ^a	9.13 ^a	7.65 ^a	13.27 ^a
GS3	2.16 ^c	0.94 ^c	2.06 ^c	10.82 ^c	4.78 ^c	5.89 ^b	8.63 ^b
GS4	2.06 ^c	0.90 ^c	1.97 ^c	9.09 ^c	4.44 ^c	6.33 ^b	8.07 ^b

Mean values (n=33) with superscript as significantly difference at p<0.05 according to Fisher’s LSD test.

3.3. Antioxidants Content

The contents of phenols in kale leaf sample are expressed in milligrams equivalent of gallic acid, tannins in milligrams equivalent of catechin, and flavonoids in milligrams equivalent of rutin, in Table 6. The GS1 treatment (control treatment) shows the highest content of phenols and tannins and the GS2 treatment was the one with the lowest content of those phenolic compounds.

Table 6. This is a table. Tables should be placed in the main text near to the first time they are cited.

Treatment	Phenols (mg Eq. of gallic acid/g)	Tannins (mg Eq. of catechin/g)	Flavonoids (mg Eq. of rutin/g)
GS1	354.054 ^a	10.972 ^a	0.1045 ^b
GS2	170.954 ^c	7.985 ^c	0.0713 ^b
GS3	263.993 ^b	4.437 ^b	0.2177 ^a
GS4	244.744 ^b	5.115 ^b	0.2516 ^a

Mean values (n=3) with superscript as significantly difference at p<0.05 according to Dunnett test.

In flavonoids content, the GS4 treatment showed the highest amount and the GS1 treatment showed the lowest.

Antioxidant capacity is expressed in ABTS and DPPH percentage 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 percentage in leaf of kale seedlings different germination substrates.

Treatment	ABTS (%)	DPPH (%)
GS1	99.1718 ± 1.11 ^a	91.6772 ± 0.47 ^a
GS2	97.1877 ± 0.09 ^a	89.7525 ± 2.82 ^a
GS3	99.0338 ± 0.13 ^a	88.5828 ± 1.44 ^a
GS4	98.1481 ± 2.01 ^a	76.7695 ± 2.15 ^b

Mean values (n=3) ± standard deviation with superscript as significantly difference at $p<0.05$ according to Dunnett test.

4. Discussion

The Thermocomposted frass showed an alkaline pH (8.5), a higher value compared to other authors, 7.26 from brewery residue [9], 7.3 from brewery residue [8,10], 7.5 from a mixture of okara and wheat bran [11], 7.6 from brewery residue amended with sawdust [12], 7.7 from brewery residue [13] and 7.8 from brewery residue [14]. The rest of physical and chemical characteristics of thermocomposted frass used in this research are different from those reported in other works, this is because the origin of the waste with which the fly larvae were fed has a direct effect on the physical quality and quantity of frass nutrients [6].

Other experiment that used frass, from different waste compared to this work and even in a fresh state, showed a similar trend on seed of lettuce and radish, decreasing the percentage of frass in the aqueous medium, the GI value increased, however these values were higher in this work, which indicates that a composted frass has fewer effects due to phytotoxicity [15]. Another study carried out with fresh frass demonstrated GI values greater than 100%, which indicated zero phytotoxicity on seed of garden cress, however, it is important to consider the dilution values used in this work, it was at a ratio of 1:20 which means that it was a mostly diluted extract [7].

A study of thermocomposted frass derived from brewers spent grains biotransformation, used a dilution 1:10 made of 100% composted frass as a control, they obtained GI values of 62.4 ± 39.5 , upper average value compared to this work, but its standard deviation showed a lower GI [12].

Water extract (1:10) of a mixture of okara and wheat bran frass naturally composted was lower than 25%, which shows that the GI obtained at 100% in this work was higher. This comparison shows that even after a stabilization treatment by composting, frass has a high percentage of phytotoxicity, despite coming from the biotransformation of different types of waste. The high value of phytotoxicity reflected in the low percentage of GI may be due to the presence of phenols, chitin, and an excess of nutrients [11].

According to [16] substrates derived from thermocomposting contain some compounds that can cause phytotoxicity, such as ammonia, ethylene oxide, organic acids, phenols, salts, and heavy metals.

The substrate pH plays an important role in the determination of nutrient availability to the plant [17], while in seed germination, an acid pH can inhibit the action of enzymes necessary for germination and can have a direct effect by dissolving the seed coat [18]. The alkaline pH of the composted frass (8.5) and a high electrical conductivity (7.476 dS/m) could have reduced the germination of kale as the percentage of composted frass increased in the substrate, some authors report pH values between 5.5-6.9 and EC between 1.2-1.9 mS/cm (=dS/m) as suitable for kale germination [19–21].

At a higher percentage of inclusion as a germination substrate, the plants present an inhibition in their shoot and root growth. Macro and micronutrients are important for the development and growth of plants because they play important roles in plant physiology [22] however, plant hormones also play a very important role. Auxins and gibberellins are vegetative growth hormones that regulate plant height, cytokinins promote cell division and this is most clearly demonstrated in greater growth of cotyledons; regulates the growth of the stem and roots [23]. It has been reported that the greatest importance of frass is not due to its mineral nutrients, considering they are low compared to other alternative sources; The rhizobacterias and phytormones present in the frass, play a more important role as plant growth promoters [24].

[11] reported the highest number of leaves of pak choi using 10% composted frass with an increase of 41.67% in number of leaves compared to the control. In this study the highest kale growth was with 20% composted frass, with an increase of 51.61% in number of leaves compared to the control.

Therefore, thermocomposting, as a stabilization process for frass, increases the percentage of inclusion as a growth substrate, however, the type of crop produced must be considered because each plant has different demands for physical and chemical properties.

Some authors report that the application of organic fertilizers significantly increases the content of phenolic compounds in plants compared to inorganic fertilizers [25,26]. Considering that the production of phenolic compounds is the response of plants to biotic or abiotic stress [27], the increase in phenolic compounds in plants due to the application of organic fertilizers could be the response to abiotic stress due to the slow release of nutrients [28]. In that sense, a higher nitrogen application results in a decrease in the total phenolic and flavonoid contents [29,30].

Flavonoids has protective functions in plants including defense against phytopathogens and herbivores [31]. They influence the transport of auxin, a plant hormone that protects the plants from microbes and insects. Flavonoids play an important role inside the root during nodule meristem formation and as a defense against the attack of rhizobia soil bacteria [32]. In this way, the increase in flavonoids content as the percentage of composted frass in the substrate increased may have been in response to the defense of the microorganism contained in the frass, some of them are useful as agents that promote plant growth, nitrogen-fixing bacteria and phosphate solubilizing bacteria such as *Azospirillum*, *Rhizobium*, *Azotobacter* and genera *Bacillus* [33].

The difference between the obtained DPPH values can be attributed to the nitrogen content in the composted frass, because it has been reported that a greater amount of nitrogen applied to the plant, the percentages of ABTS and DPPH decreases, considering that this assay measures the activity of water-soluble antioxidants [29]. The same effect was reported by [30]. [34] reported no significant difference in the total phenol nether antioxidant capacity using fresh frass.

5. Conclusions

The addition of thermocomposted BSFLF derived from the biotransformation of organic waste, mixed with peatmoss generates the appropriate physicochemical conditions for the generation of a substrate for the agronomic performance of kale. The results obtained in this research show an alternative material for the germination, allowing greater production of seedlings under an environmentally friendly concept for soilless agricultural producers. The best dose was 20% of frass, however at higher doses there was an inhibition of growth and even the death of seedlings, so it would be necessary to improve the thermal stabilization process or even add another.

The origin of the residue bio transformed with soldier fly larvae influences the nutrient content in the frass, these nutrients can benefit plant nutrition. However, more research is needed on the content of other components of equal importance such as phytohormones. It is important to know the appropriate doses of composted BSFL in the phenological stages of germination, development, and production in different species of vegetables and fruits. Likewise, it is necessary to evaluate this germination substrate under biotic or abiotic stress, to take advantage of the agronomic benefits offered by the substrate and increase the antioxidant content in the seedlings.

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