

EVALUATION OF PHYTOTOXICITY OF BLACK SOLDIER FLY LARVAE (*Hermetia illucens* L.) MEDIATED COMPOST ON CHINESE CABBAGE (*Brassica rapa*) USING THE SEED GERMINATION BIOASSAY

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ABSTRACT

A lab experiment was conducted at Department of Entomology, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad during September to December 2021. The phytotoxicity of six different entocomposts obtained from rearing black soldier fly larvae on different feed substrate was tested on Chinese cabbage by seed germination bioassay. For each compost type, the effect of four concentrations of water extracts of compost on seed germination and root growth of Chinese cabbage were investigated. Composts were also analyzed for C/N ratio, pH and electrical conductivity. The C/N ratio, pH and EC ranged from 12 to 43.10, 6.87 to 8.81 and 5.70 to 11.30 dSm⁻¹ respectively. Highest relative seed germination (88.44 %) was recorded in frass from Gainesville diet (12.5 %), highest relative root growth (91.23 %) was recorded in frass from fruit waste (12.5 %), and sheep droppings (12.5 %), and highest germination index (73.41) was recorded in the frass from sheep droppings (12.5 %) aqueous extract. Since all the examined BSFL composts extracts were not mature as reflected by germination index of less than 80 resulting in different levels of phytotoxicity, hence suitable neutralizing additives may be added before application in crop.

KEYWORDS: BSFL, compost, Chinese cabbage, phytotoxicity, maturity, germination index

Composting is a natural process that transforms organic waste into nutrient-rich soil conditioner, promoting sustainability and reducing environmental impact. There are various types of composting, one innovative and increasingly popular method is black soldier fly larvae (BSFL) based composting. Black soldier fly larvae efficiently consume various streams of organic waste, converting it into high-quality compost while minimizing greenhouse gas emissions and offensive odors associated with traditional composting (Akumah *et al.*, 2021).

One challenge in BSFL mediated composting, is the determination of compost maturity. The rapid and voracious feeding habits of black soldier fly larvae can lead to incomplete decomposition of organic matter, resulting in a compost that may not be fully stabilized. Additionally, the presence of undigested residues or chitin-rich exoskeletons of the larvae in the final compost can affect its quality. Compost maturity is crucial as immature compost may contain phytotoxic substances, inhibiting plant growth and potentially causing harm to the environment. Moreover, mature compost promotes

nutrient stability and availability, fostering a balanced microbial community that contributes to disease suppression, improved soil structure, and enhanced plant resistance (Sayara *et al.*, 2020).

Some of the on-site parameters that help in testing the maturity and stability of a compost are phytotoxicity, temperature, colour, odour and moisture. The laboratory parameters that help in testing the maturity and stability of a compost are germination Index (GI), volatile solids (VS), C/N ratio, organic matter, microbial respiration, and biological tests (Oviedo-Ocana *et al.*, 2015).

Among the maturity parameters, GI is considered as the most sensitive parameter for identifying the phytotoxicity of compost and assessing its suitability for use as soil amendments or growing media. It is an integrated parameter which combines the relative radical elongation and relative germination. According to Zucconi *et al.* (1985) GI values below 50% indicate high phytotoxicity, values between 50% and 80% indicate moderate phytotoxicity; and values above 80% indicate absence of phytotoxicity. When

the index exceeds 100%, the compost can be considered as phytostimulant or phytonutrient.

Application of any compost product is only possible if the compost is mature enough and is free from potentially phytotoxic components. Studying the impact of mature compost on seed germination or plant growth is very much essential for its application.

MATERIAL AND METHODS

Six different feeding substrates *viz.* Vegetable waste (T_1), fruit waste (T_2), ground maize shank (T_3), sheep droppings (T_4), chicken droppings (T_5) and Gainesville diet (T_6) were used for production of BSFL compost. The nitrogen (%), phosphorus (%) and potassium (%) were ranged from 0.98 to 3.20, 0.62 to 2.74 and 0.27 to 0.51 respectively in all the composts. The C/N ratio, pH and EC of all the composts were analyzed as per the standard procedure (Walkely and Black, 1934; Subbaiah and Asija, 1956; Jackson, 1973).

Fresh aqueous extracts (1st extract) were prepared by shaking 10 g of compost with 100 ml of deionized water (1:10 w/v) on a rotary shaker for 1 h. The solutions were centrifuged at 5000 rpm for 15 min, and then filtered through filter paper. A range of dilutions (25%, 50% and 75%) of the aqueous extracts were prepared using deionized water as diluent (Zucconi *et al.*, 1981).

The phytotoxicity of compost extracts was evaluated by the seed germination technique. The test crop Chinese cabbage seeds were surface sterilized by immersion in 75% alcohol for three minutes followed by transferring in 0.001 HgCl₂ solution for two minutes with periodical agitation and finally thoroughly washed with sterilized distilled water to get rid of toxic chemicals. Ten seeds were set for a germination test on Whatman filter paper wetted with 5 ml aqueous extracts (25%, 50%, 75% and 100% dilutions) and deionized water (as control) in 100-mm-diameter and 25-mm-height Petri dishes and incubated at 25 °C. After 3 days, the number of germinated seeds and the radicle length of each germinated seed were recorded. A seed with a radicle length of at least 2 mm was considered as germinated. The whole experiment including preparation of fresh aqueous extracts and germination test was replicated three times, and the results were analyzed by determining the relative seed

germination (RSG), relative radicle growth (RRG), and germination index (GI) as per the Barral and Paradelo (2011)

RESULTS AND DISCUSSION

Relative seed germination (RSG) of Chinese cabbage

At 12.50 % aqueous extract of BSFL compost Gainesville diet showed highest RSG of 88.44 % followed by sheep droppings, chicken droppings, ground maize shank and fruit waste with an RSG of 80.40 %, 77.39 %, 74.37 % and 39.20 % respectively, whereas vegetable waste showed the lowest RSG of 36.18 %. At 25 % aqueous extract of BSFL compost sheep droppings showed highest RSG of 49.25 % followed by Gainesville diet, ground maize shank, chicken droppings and fruit waste with RSG of 48.24 %, 44.22 %, 40.20 % and 30.15 % respectively, whereas vegetable waste showed the lowest RSG of 19.10 %. At 50 % aqueous extract of BSFL compost chicken droppings showed highest RSG of 24.12 % followed by fruit waste, Gainesville diet, ground maize shank and sheep droppings with RSG of 20.10 %, 18.09 %, 17.09 % and 17.09 % respectively, whereas vegetable waste showed the lowest RSG of 13.07 %. At 100 % aqueous extract of BSFL compost fruit waste showed highest RSG of 19.10 % followed by ground maize shank and Gainesville diet with similar RSG of 15.08 %, vegetable waste and sheep droppings with similar RSG of 12.60 %, whereas chicken droppings showed the lowest RSG of 7.04 % (Table 2).

Relative root growth (RRG) of Chinese cabbage

At 12.50 % aqueous extract of BSFL compost fruit waste and sheep droppings showed highest RRG of 91.23 % followed by ground maize shank, vegetable waste and chicken droppings with RRG of 80.70 %, 77.19 %, 77.19 % respectively, whereas Gainesville diet showed the lowest RRG of 66.67 %. At 25 % aqueous extract of BSFL compost fruit waste showed highest RRG of 80.70 % followed by sheep droppings, with RRG of 77.19 %. Vegetable waste, ground maize shank and Gainesville diet showed similar RRG of 66.67 %, whereas chicken droppings showed the lowest RRG of 59.65 %. At 50 % aqueous extract of BSFL compost fruit waste showed highest RRG of 70.18 % followed by ground maize shank, with RRG

of 66.67 %. Sheep droppings and Gainesville diet showed similar RRG of 59.65 %. Whereas vegetable waste and chicken droppings showed the RRG of 56.14 % and 49.12 %, respectively. At 100 % aqueous extract of BSFL compost sheep droppings and Gainesville diet showed highest similar RRG of 49.12 %. Fruit waste, ground maize shank and chicken droppings showed similar RRG of 45.61 %, whereas vegetable waste showed lowest RRG of 38.60 % (Table 2).

Germination index (GI) of Chinese cabbage

When Chinese cabbage seeds were treated with 12.50 % aqueous extract of BSFL compost, sheep droppings, ground maize shank, chicken droppings and Gainesville diet and recorded moderate phytotoxicity with a GI of 73.41, 60.07, 59.79 and 59.01, respectively. Vegetable waste and fruit waste showed high phytotoxicity with GI of 27.95 and 35.79. Similarly when 25%, 50% and 100% aqueous extract were all the treatments showed high phytotoxicity, where chicken droppings recorded lowest GI of 3.21 (Fig.1).

The dilution of compost consistently led to an increase in RSG, RRG and GI (Table 2). Moldes *et al.* (2006) reported similar findings where GI increased when the extract was diluted or previously washed compost was used, potentially due to a reduction in salinity in the extract.

In the present investigation, the pH of the compost varied from 6.87 to 8.81 (Table 1). The typical pH range for conventional horticulture applications generally falls between 5.5 and 6.5, whereas for organic farming, it may extend from 5.5 to 8.0 (Liu *et al.*, 2021). Different crop seeds exhibit optimal germination at different pH levels, and variations in germination have been observed among different genotypes at various pH levels (Wijayanto *et al.*, 2021). While pH alone

may not always have a significant impact on germination, it can influence nutrient availability, ultimately affecting various germination properties (Follmer *et al.*, 2021).

A high salinity may also cause phytotoxicity. In fact it has been observed that GI is more affected by this property than by degree of stability, in organic amendments with a wide range of EC (Lasaridi and Stentiford, 1998). In present study compost EC ranged from 5.70 to 11.30 dSm⁻¹ (Table 1) which was much higher than threshold value of 3.5 dSm⁻¹ for organic growing media (Liu *et al.*, 2021). Campitelli and Ceppi (2008) observed a strong negative correlation between GI and EC. Similarly, Aslam *et al.* (2008) discovered a negative correlation between relative germination and the electrical conductivity (EC) of the compost.

The Germination Index (GI) of BSFL compost generated from golden needle mushroom was 65.7 for Chinese cabbage and 52.9 for rapeseed (Cai *et al.*, 2017). In Italy, Setti *et al.* (2019) used lettuce (*Lactuca sativa*) seeds and found germination indexes above 70%, indicating no signs of phytotoxicity. In another study, Liu *et al.* (2019) treated chicken, pig, and cow manure with BSF larvae and observed high levels of phytotoxicity in the chicken manure treatment through a seed germination test. In contrast, the compost from the other manures showed stabilized characteristics with reduced electrical conductivity and a higher germination index. The authors suggested that the lack of maturity in the compost derived from chicken manure might be attributed to the high electrical conductivity and concentration of N-NH₄⁺ in the feed substrates.

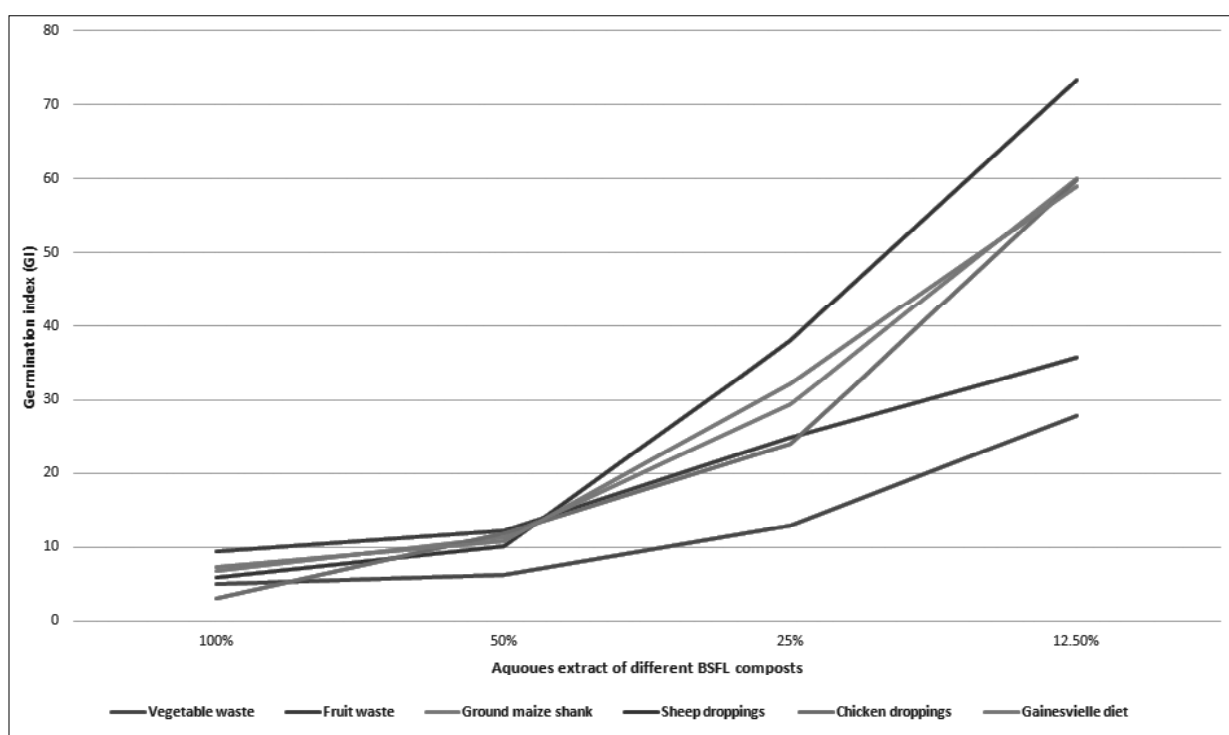
To reduce the pH and EC in compost, various methods can be employed, including the addition of biochar, pine needles, oak leaves, citrus peels, elemental sulfur or sulfur-containing compounds.

Table 1. Physiochemical properties of black soldier fly larvae compost obtained from different feeding substrates

Treatments	T ₁ (Vegetable waste)	T ₁ (Fruit waste)	T ₁ (Ground maize shank)	T ₁ (Sheep droppings)	T ₁ (Chicken droppings)	T ₁ (G.V. diet)	CD(0.05)	CV
C: N ratio	26.10	25.90	43.10	27.20	19.40	12.00	2.29	5.03
pH	8.81	8.37	6.89	7.76	8.16	6.87	0.71	5.11
EC (d Sm ⁻¹)	11.30	7.83	5.70	9.90	8.73	8.07	0.70	4.80

Table 2. Effect of black soldier fly larvae compost obtained from different feeding substrate on RSG (%), RRG (%) and GI of Chinese cabbage

	% aqueous extract	BSFL Compost					
		T ₁ (Veg. waste)	T ₂ (Fruit waste)	T ₃ (Ground maize shank)	T ₄ (Sheep droppings)	T ₅ (Chicken droppings)	T ₆ (G.V. diet)
RSG	12.50%	36.18	39.20	74.37	80.40	77.39	88.44
	25%	19.10	30.15	44.22	49.25	40.20	48.24
	50%	13.07	20.10	17.09	17.09	24.12	18.09
	100%	12.06	19.10	15.08	12.06	7.04	15.08
RRG	12.50%	77.19	91.23	80.70	91.23	77.19	66.67
	25%	66.67	80.70	66.67	77.19	59.65	66.67
	50%	56.14	70.18	66.67	59.65	49.12	59.65
	100%	38.60	45.61	45.61	49.12	45.61	49.12

**Fig.1: Germination index of Cabbage seeds treated with different aqueous extracts of BSFL composts**

Simultaneously, reducing electrical conductivity (EC) in compost can be achieved through dilution with neutral materials, leaching with water to wash out soluble salts, incorporating low-EC ingredients, promoting aeration during composting to enhance microbial activity, and avoiding the inclusion of salty materials. Biochar addition generally resulted in higher GI especially in BSFL treatments. The highest GI (147) was observed

for the feedstock amended with 15 % biochar (Akumah *et al.* 2021). Biochar with its alkaline pH must have neutralized the acidic effect of the organic acids produced culminating in higher GI. At maturity, compost with more than 10 % inclusion rate of biochar had the highest cabbage seed germination indices (> 100 %) (Beesigamukama *et al.*, 2020).

CONCLUSION

The results of the present study indicate that the BSFL compost derived from various waste streams have been observed to be immature at all the dilutions exhibiting phytotoxicity to Chinese cabbage. The reasons could be high pH, EC and C/N ratio which should be addressed before application to the crop. Hence there is scope for exploring additives to enhance the maturity of the compost, in view of the rich nutrient (N, P, K and micronutrient) status of ento-composts.

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