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Effect of BSF (Black soldier fly) frass obtained from bioconversion of different waste materials on growth of red Amaranthus (*Amaranthus* sp.)

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Abstract
Amaranthus is a widespread leafy vegetable grown throughout India. This leafy vegetable is important in the diet as it provides essential nutrients and dietary fibre. It grows luxuriantly in soils with high organic matter content. Red amaranthus variety Arun is popular among farmers of Kerala which is commercially grown round the year in many areas. This crop responds well to organic farming practices and is presently grown with organic manures like vermicompost and farm yard manure. As insect farming is gaining popularity, the frass obtained from such facilities could be used for crop production. Black soldier fly (BSF) is an insect extensively used for converting various biodegradable waste materials of diverse physical and chemical properties. Evaluation of the frass obtained from BSF units for its chemical properties and evaluating its suitability for growing agricultural crops is very much required. Hence frass obtained from conversion of five types of wastes by BSF *viz*. food waste, fruit and vegetable waste, fish waste, slaughter house waste and coconut cake were analyzed for chemical properties. In addition to this, an assessment of BSF frass on the growth of amaranthus was also conducted. It was found that frass obtained from these wastes were odourless, dark brown coloured, neutral to alkaline, electrical conductivity, total organic carbon, NPK content and C:N ratio were as per FCO standards, they had microbial activity too as indicated by the dehydrogenase activity. The pot culture experiment revealed that all the frass materials significantly increased the growth of amaranthus compared to farm yard manure as per the recommended organic package of practice of Kerala Agricultural University. Hence the frass from BSF units could be used as organic manure for growing the leafy vegetable amaranthus.

Keywords: Amaranthus, black soldier fly, waste, frass, FCO, growth, organic

Introduction

Leafy vegetables are an important part of the human diet as they contain low calories and rich sources of vitamins, dietary fibre, minerals and antioxidants. Among the various leafy vegetables grown in India, Amaranthus has a prominent position both in cultivation and consumption. Leaf amaranth is considered as a native of India and major cultivars of amaranthus grown for leaf belong to *Amaranthus tricolor*, five major species of leafy amaranthus is found in India.It grows fast in organic manure rich soils. The leaves and tender stems are a very good source of minerals like iron, calcium, vitamins, dietary fibre and antioxidants (Sarkar *et al*., 2020) [14]. Red amaranth is popular and it is grown throughout Kerala except in heavy rains. According to Choudhary (2006) [7], 100 g of edible portion of amaranthus leaves contain 9200 IU vitamin A, 0.1mg riboflavin,0.01mg thiamine,99mg vitamin C, 4 g protein, 25.5 mg iron and 397mg calcium. It responds well to organic manures and the commonly used organic manures in Kerala include farm yard manure and vermicompost.

As insect farming, especially black soldier fly (BSF) farming is gaining momentum, thef frass-main byproduct from various waste streams needs evaluation for crop growth. Black soldier fly is a versatile insect used for managing diverse biodegradable waste materials since their larvae are voracious feeders which devour substrates and convert them into insect biomass and frass within a very short period thus helping in managing huge piles of waste which otherwise would have ended up in landfills and contributing to global warming and environmental pollution. Though native of American continent, the black soldier fly (*Hermetia illucens* L.) belonging to the family Stratiomyidae under order Diptera, is present across different countries across Europe, Africa, Asia and Oceanea which lies between latitude of 40° south and 45° north due to trade of goods and human migration It is not regarded as a pest and is not

included in the list of organisms that spread disease or act as a vector for infections. It typically occurs in moist, marshy areas with animal faeces, rotting fruit, or any other decomposing organic materials, which are ideal for larval growth. (Li *et al*., 2011a) [11]. Insect mass rearing generates massive amounts of frass, which is a mixture of uneaten substrate, excrement, and exuviae. This material has a significant potential to boost soil and crop productivity (Kagata and Ohgushi, 2012; Poveda *et al*., 2019; Barragan-Fonseca *et al*., 2022) [9, 13, 3]. As per the results of Garttling and Schulz (2021), BSF frass was nutrient rich compound fertiliser with a pH of 7.5 and a balanced N: P_2O_5: K_2O ratio of 1:0.9:1.1. Earlier reports also suggest the use of frass in crop production, especially vegetables. When applied as a soil ameliorant, BSF frass from an integrated waste disposal site improved the growth and yield of pakchoi (*Brassica rapa*) as well as the biochemical characteristics of the soil, suggesting that BSF frass might be employed as a biofertilizer (Agustiyani *et al*., 2021) [1].
As BSF farming is coming up across India, suitability of frass produced for growing crop needs assessment and hence the popular leafy vegetable is selected for evaluating the frass obtained after bioconversion of various waste materials through a pot trial.

Materials and Methods
The study was conducted at College of Agriculture Vellayani, Thiruvananthapuram, Kerala (N80 25'37.13952", E760 59'12.50736", 29 mamsl). Frass was collected from converting various waste materials *viz*. food waste, fruit and vegetable waste, fish waste, slaughter house waste and coconut cake with BSF larvae. Red amaranthus variety Arun was selected for a pot culture experiment. It is a photo insensitive variety developed by Kerala Agricultural University and popular among farmers of Kerala for its deep maroon coloured leaves and stems.
The frass was analysed for pH, electrical conductivity, total organic carbon, nitrogen, P_2O_5, K_2O, C:N ratio and dehydrogenase enzyme activity.
25 g of the sample were suspended in 50 mL of distilled water and agitated on a rotary shaker for two hours in order to determine pH., it was vacuum-filtered using Whatman No. 1 filter paper using a Buchner funnel and measured pH with the aid of a digital pH metre. A Conductivity meter was used for measuring the electrical conductivity of frass and total organic carbon (%) was found out by measuring total organic matter and dividing the value with 1.724. Total nitrogen (%) was estimated using Kjelplus Assembly by single acid digestion and distillation method. Di acid digestion was performed for determining phosphorus (P_2O_5%) and potassium (K_2O%). C:N ratio was calculated by dividing the organic carbon value with the total nitrogen value. Triphenyl tetrazolium chloride (2, 3,5salt) (TTC)-3% was the reagent used in determination of dehydrogenase activity. Intensity of purple colour formed by dehydrogenase activity in the methanolic extracts due to the formation of triphenyl formazan (TPF) was measured in a spectrophotometer at 485 n using methanol as blank. Dehydrogenase activity= microgram of TPF hydrolysed per gram of sample/24h; which was found out using a standard graph.
The pot culture experiment was conducted in completely randomised design with 6 treatments replicated thrice. Treatments are:

1. Frass from food waste
2. Frass from market waste
3. Frass from fish waste
4. Frass from slaughter house waste
5. Frass from coconut cake
6. Farm yard manure (Recommendation as per organic POP of KAU)

Instead of using farm yard manure, potting mixture consisting of soil, coir pith and appropriate treatments was placed inside plastic pots measuring 30 cm in height and 30 cm in upper diameter. A 1:1:1 mixture of soil, coir pith and farm yard manure (oragnic POP of KAU) was used as the control pot. Amaranthus variety Arun seedlings (15 days old) that were grown in a seedbed were meticulous uprooted, the uniform seedlings were transplanted in the pots @ one seedling per pot. Every week, *Pseudomonas fluorescens* was sprayed at a rate of 20 g L^{-1} of water to combat the leaf blight disease. The plants were uprooted carefully after one month and recorded the height, number of branches, fresh weight and shoot to root ratio.

Results and Discussion
Results of chemical analysis of frass is presented in table.1. Among various frass materials, the highest pH value recorded was from that of fruit and vegetable waste (8.8) which was followed by food waste (7.93), pH of all other materials were also in neutral to alkaline range and were as per the standards (6-8) of fertilizer control order for composts and organic fertilizers (KAU, 2017). When used as fertiliser, frass works best in a pH range of 6 to 8, where the predominate bacterial population facilitates the breakdown of organic materials and the cycling of nutrients for plants (Ahmad *et al*., 2023). Electrical conductivity was highest in the frass from food waste (7.4dSm^{-1}), but that of other materials were as per FCO standards (Maximum .5 dSm^{-1}). These were attributed to the larval activity in corresponding substrates.
Total organic carbon ranged from 34.72 to 58.53% and well within the FCO standards (Minimum.20%). The high amount of organic carbon might be due to the addition of insect exuvia added to the frass during moulting and also the presence of wood shavings added for absorbing moisture during BSF bioconversion process. Total nitrogen from frasses ranged from 3.13% to 6.3% and was significantly higher in frass from fish waste (6.3) and all these were as per FCO standards (Minimum.0.5%). According to Sarpong *et al*. (2019) [15], over time, the nitrogen concentrations may have increased as a result of the organic matter being broken down by the larvae and reducing its dry weight (re-concentration) in the substrate. There were two other possible reasons for this increase in nitrogen: the larvae's metabolic processes and the nitrifying bacteria secreted from its gut. While fruit and vegetable waste had the lowest recorded value (0.53%), both fish waste residue (2.93%) and coconut cake residue (2.83%) were on par and slightly superior in recording phosphorus as total P_2O_5. These values were likewise in line with the FCO criteria (Minimum.0.5%). The present findings are corroborated by research conducted by Sarpong *et al*. (2019) [15] which showed an increase in the concentration of total phosphorus in BSF composted organic waste. Significantly higher potassium as K_2O% was recorded by fruit and vegetable waste residue (5.03%) and coconut cake (4.74%) and all the materials were well above FCO standards (Minimum.0.5%). Rising potassium levels were also observed in the earlier investigations (Sarpong *et al*., 2019; Bernal *et*

al., 2009) [15, 5].

The CN ratio of all frass materials except from fish waste (6.43:1) were statistically on par and were as per FCO standards of CN ratio of less than 20:1. Manure with a C:N ratio less than 20:1 was good for plant growth due to the fact that it mineralised organic nitrogen into inorganic form. (Pan *et al*., 2012) [12]. All frass materials exhibited dehydrogenase activity which is an indicator of microbial activity in the frass. The residue, or frass, that was left over after conversion had a brownish-black colour and no unpleasant smell. Analogous results were seen in previous investigations that endorsed the presence of oxygen and compost maturity. (Sarpong *et al*., 2019; Diener *et al*., 2011) [15, 8].

Growth parameters of amaranthus are presented in table.2 and on perusal of data, it is evident that all the frass materials significantly improved growth and yield of amaranthus over control (FYM). Among various frass materials, except that from food waste, others were on par and superior with respect to plant height. Various frass materials recorded a height of 78.22 to 94.28 cm whereas farm yard manure (FYM) at recommended rate recorded a height of 61.5cm only. The fresh weight of the plant showed that coconut cake (247.33 cm) and frass from slaughterhouse waste (248.67 g) were both significantly superior than other options; this was followed by frass from food waste (163.89 g) and frass from market waste (156.56 g), both of which were considerably better than the control (53.89 g) and comparable to each other. The shoot to root ratio and branch count did not significantly change across the treatments. The increased level of nutrients reduced CN ratio and the presence of chitin in frass materials might have helped in increased yield of amaranthus over recommended dose of manure in the present study. In comparison to conventional compost, organic, and mineral fertilisers, Beesigamukama *et al*. (2020) [4] found that the enhanced yield of vegetables cultivated in BSF frass was caused by the increased supply and availability of nutrients from the frass. The BSF frass's quicker release of nutrients was further aided by the low C/N ratio. When used as fertiliser, black soldier fly frass improves the chemical composition, growth, and yield of cabbage in a manner similar to that of commercial fertiliser. BSF frass production had the additional benefits of managing various waste kinds and solving the issue of chemical fertiliser misuse (Choi *et al*., 2009) [6].

Table 1: Chemical parameters of BSF frass obtained from different waste materials

Treatments	pH	EC	OC%	N%	P_2O_5%	K_2O%	CN ratio	Dehydrogenase activity
Food waste	7.93^{b}	7.40^{a}	45.61^{b} (6.75)	4.01 ^{c} (1.99)	0.66 ^{c} (0.81)	0.95 ^{d} (0.97)	11.41:1^{a}	57.25^{c}
Market waste (fruit & vegetable)	8.80^{a}	3.15^{b}	34.72 ^{d} (5.89)	3.13 ^{d} (1.77)	0.53 ^{d} (0.73)	5.03 ^{a} (2.24)	11.10:1^{a}	146.16^{bc}
Fish waste	7.52^{bc}	2.73^{b}	40.45 ^{c} (6.36)	6.30 ^{a} (2.51)	2.93 ^{a} (1.71)	2.92 ^{b} (1.71)	6.43:1^{b}	146.64^{bc}
Slaughterhouse waste	7.18^{c}	0.11^{c}	46.35 ^{b} (6.81)	4.34 ^{c} (2.08)	1.86 ^{b} (1.36)	1.92 ^{c} (1.39)	10.73:1^{a}	268.86^{b}
Coconut cake	7.40^{bc}	0.19^{c}	58.53 ^{a} (7.65)	5.05 ^{b} (2.25)	2.83 ^{a} (1.68)	4.74 ^{a} (2.18)	11.62:1^{a}	466.61^{a}
CV	5.120	15.96	2.649	3.430	1.661	5.179	5.903	39.555
CD (0.05)	0.599	0.653	0.267	0.110	0.032	0.132	0.912	129.40

Table 2: Effect of BSF frass on growth and yield parameters of amaranthus

Treatments	Height of plant(cm)	Number of branches	Fresh weight of plant(g)	Shoot to root ratio
Frass from food waste	78.22 ^{b}	0.78	163.89 ^{b}	0.15
Frass from fruit& vegetable waste	89.44 ^{a}	0.78	156.56 ^{b}	0.16
Frass from fish waste	88.28 ^{a}	1.00	113.56 ^{b}	0.13
Frass from slaughter house waste	92.72 ^{a}	1.11	248.67 ^{a}	0.11
Frass from coconut cake	94.28 ^{a}	1.00	247.33 ^{a}	0.12
Farm yard manure as per POP	61.50 ^{c}	0.44	53.89 ^{c}	0.13
CV	5.663	NS	20.378	NS
CD	8.471		59.452	

Conclusion

It can be concluded that the frass obtained from different waste materials tested here increased growth of amaranthus. Though frass from food waste had a high EC and frass from fruit and vegetable waste had high pH, there was no detrimental effect in the plant growth. Hence, the farmers and entrepreneurs engaged in farming black soldier fly can utilize the frass obtained for growing crops. As the quality of frass depends on the quality of waste material used, it would be ideal to combine different wastes together in favourable proportion for better results.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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