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Research Article

Black Soldier Fly Larvae, a Viable Opportunity for Entrepreneurship

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Abstract

The larvae of *Hermetia illucens*, colloquially known as Black Soldier Fly (BSF) pose an enormous potential for small-scale entrepreneurship, especially for economically backward nations. Engaging these avid eaters in frugal application towards the societal benefit can open a new dimension to explore feasible business opportunities and mitigate unemployment issues. The present study tried to emphasize the various possible end applications of the BSF larvae in a holistic and wholesome way. The prime advantage of dealing with these larvae incorporates the minimization of the threat associated with the mortality rates, due to the ancillary applications of the dead larvae towards the nutriculture industry and production of alternative fuel. Furthermore, the nutrient-rich compost formation due to the larval digestion activity on the municipal organic waste (MOW) fraction creates a new entrepreneurial niche for lower and middle-income nations. The research yields synthesis of primarily three major end products in terms of compost, bio-diesel, and fish meal cake. Initially, the larvae were employed for the degradation and stabilization of MOW and the product of stabilization was further analyzed and identified as compost as per the Fertilizer Control Order (FCO) 1985. The departed counts have further compressed by means of compaction machine with capacity 3000 kN and body fluid was segregated. The crude liquid was purified using centrifugation and successively followed by Soxhlet extraction. Ultimately, the separated bottom sludge has been blended with the crushed body skeleton of the larvae and turned into protein-rich fish meal cake. Thus, it's evident to state that besides substantial societal benefits BSF poses magnificent potential to be explored by the entrepreneurial venture.

Keywords: Bio-Diesel; Black Soldier Fly; Compost; Fish Meal Cake; Struvite

Introduction

In a middle-income nation like India presently a wholesome solution for the overall good in terms of mitigation of unemployment issue is of utmost concern. In this scenario an approach associated with BSFL composting attributed with other ancillary facts could be the answer to the existing giant issue pertaining in various lower and middle income countries [1]. Rather than the waste treatment and minimization, the above process is also capable of addressing multidimensional issues such as rising demand of implementing renewable fuel, increment of protein content in farm and aquatic diet, yielding compost with high nutrient content, etc. The larvae of Black Soldier Fly consume the organic fraction of the waste ravenously and build a body composition with the higher amount of protein and fat contents. The protein content of the larvae generally used as a crude source of protein which replaces the expensive conventional protein source in pet food, poultry feedstock, fish meal, etc. and the body fat has been successfully converted into value-added co-products such as biodiesel. Other major advantages comprise higher consumption rates and faster rate of degradation and bio-conversion [2,3]. Hang., *et al.* [4] stated it is utmost feasible in capturing the nutrients from the waste stream which can be further utilized as agro-based plant manure and the bioconversion technique stabilizes the primary pollutants and minimizes the production of obnoxious gases and odour formation by 94.5%. The larval composting has also proven to be effective against the removal of *Escherichia coli* with an index range of 92.0% and successive reductions were observed by the same researcher in total weight, total Kjeldahl nitrogen and moisture content in stabilized waste around 67.2, 76.0 and 80.0%, respectively. The economic proficiency of the above technique even makes it more feasible with the yearly profit rages from US$33.4 - 46.1 per m^3, observed during the operational period. In order to interpret the above scenario an experimental study conducted by Tschirner and Simon [5] may be considered as a benchmark. They have investigated the influence of different growing substrates on the crude nutrient and they observed the impact of different fodder consumption on the larval body composition. They reported the yield of different nutrients for the experimental period of 15 day, where protein and fibre value ranges around 0.93 and 0.43 kg of wet mass, respectively. They also reported the changes in protein content due to the different substrate consumption, which showed the crude protein content values of 37.2, 44.6 and 52.3% of dry matter, respectively. In this context a baseline by Li., *et al.* [6] reported that synthesis of value-added co-products such as grease; biodiesel etc. is technically feasible, utilizing dairy manure as substrate. The extraction of grease from BSFL was performed with the help of petroleum ether, and thereafter biodiesel could be extracted in a similar manner with a two-step method. At last the remaining fraction of the manure could be anaerobically hydrolyzed to produce sugar. In their study, roughly 1248g raw dairy manure was converted by 1200 larvae into 273.4g compost material in 21 days. Once the stabilization got over approximately 15.8g of biodiesel was obtained from 70.8g dry BSFL, while 96.2g sugar was produced from the anaerobic digestion of treated dairy manure. After completion of grease extraction, the residual dry fraction of the larvae was used as a potential source of protein-rich food staff for the animals. In support of the above hypothesis Surendra., *et al.* [7] carried out the chemical analysis of the bio-oil secreted from BSF larvae and reported that body fluid comprises higher concentration (i.e. approximately 67%) of medium chain saturated fatty acids whereas, the concentration of polyunsaturated fatty acids found to be significantly lower, ranging from 13% - 15% of total fatty acids [8-13]. The above phenomenon confirms the bio-oil to be potentially acceptable as superior quality biodiesel. A study conducted by Nguyen., *et al.* [14] reported optimum recovery of bio-diesel from the live count by means of performing transesterification with zero requirement of any pretreatment facility. The study was executed using a 1:2 blend of n-Hexane and methanol which further enhanced the bio-fuel yield up to 94%. Ultimately, the optimal recovery of the bio-fuel was reported by Zheng., *et al.* [15] in which the larvae of *Hermetia illucens* were cultured on restaurant waste and production of bio-diesel was doubled performing raring activity prior to the segregation. The larvae also showed potential against pathogen removal, a study conducted by Qiaolin., *et al.* [16], reported the ability of BSFL for *E. coli* reduction from dairy manure and they introduced the larvae into 50, 75, 100, or 125g sterilized dairy manure homogenized and inoculated with *E. coli* and stored for 72 h at 270C. They concluded that the larvae composting potentially decreased the *E. coli* count in all the respective conditions. Not only this, pronto the black soldier fly larvae have been acclaimed as the most pertinent alternative to the costlier commercial animal protein sources. Nyakeri., *et al.* [17] has reported a complete nutrition profile; the researchers have performed experiments such as proximate analysis for vitamins and minerals and concluded that wild *Hermetia illucens* larvae consist approximately 40% protein, 33% crude fat, 12% crude fibre, 15% ash and remaining all sort of trace elements such as manganese, sodium, iron, potassium etc. It also includes the different variety of proteins namely, thiamine, riboflavin and vitamin E etc. The similar body composition was reported in another successive study conducted by Sara., *et al.* [18], who worked on the protein synthesis aspect and concluded that the protein extractability of larval flour fraction segregated was around 36% crude protein and 60% crude fat respectively. The further improvement in the protein quantity recovered was done by defatting operation and it yields increment in crude protein content by 47%, while depleting the crude fat content by 8.8%. The above nutrition profile of the black soldier fly larvae designated its ability, hence it should be considered as a potential and cheap alternative protein source. Consistently, Thomas., *et al.* [19] also stated that due to the greater amount of protein content in the body mass composition in prepupae stage BSFL can be considered as high-quality protein source for animal diet, though the substrate composition plays a vital role from the point of view of the variation in EE and ash content level.

Eventually, the entire research community has been agreed to validate black soldier fly larvae as the most unique and profitable conversion and stabilization agent of the new era with minimal hindrance. The technique is rampant and further research in this domain may help the lower middle-income nations to get read of the unsightly and unscientific dumping yards. Hence, composting utilizing BSFL should be recommended in India for sustainable waste management [20-24].

The primary objective of this research work was to emphasize the superiority of BSFL composting over the conventional, time consuming and primitive methods and also to substantiate the miscellaneous utilization of the larvae towards the frugal end application for societal good.

Methodology

The entire operation comprises three major operations such as compost recovery, bio-diesel preparation, and synthesis of by-products for the Nutri-culture industry. The details have been delineated in followings.

Compost Preparation

The trail was substantiated with the help of stabilizing and composting agents namely, BSFL. A maturation period of 14 days was allocated for individual trails and after 11th days addition of excessive moisture was restricted in order to drop down the moisture content. Once the treatment period was over the residue has been recovered from the bio-reactor and sent to the tipping floor for 48h to reduce the moisture level to 15 - 20%. Thereafter, the semi-fermented material was screened with the help of 20 mm trammel and plastics, inters, wooden materials etc. were separated as trammel reject. The permeate was further cured with the help of sunbath to reduce the stickiness for a period of 4 days and finally, fed to the 4 mm trammel. The final permeate passed through the 4 mm trammel was considered as compost.

Struvite Extraction

Nutrients from the cattle farm waste streams were recovered by aerobic treatment mechanism, utilizing fluidized bed reactor with dairy and goat manures as feed influents. In order to optimize the recovery, an effective pH value was determined and upheld by using a buffer solution of $MgCl_2.6H_2O$ and NaOH.

Larval Preparation

The larvae utilized for bioconversion and stabilization purpose was further separated into two fractions. One part of it was reverted back to the raring system for successive culture generation. Whereas, the other fraction was utilized for the derivation of value-added coproducts. In order to minimize the chances of possible contamination, the larval count was kept under the open sunlight in a closed container for 12h and then washed with alcohol. The live counts were further made operation ready by heating them at 105^0C for 2.5h and the volatile water content in the body has been reduced by 20% of the entire body mass. Ultimately the oven-dried mass was used for bio-diesel extraction.

Segregation of Body Fluid

The body fluid of the larvae was extracted using compaction testing machine with a capacity of 3000 KN. The sample was kept between the pressure plate of the apparatus and the observations were as follows (Table 1). The body fluid found to be separated easily and was collected at the extreme end by means of sample collection bottle.

Sl. No.	Parameters	Unit	Value
1	Modulus of Elasticity	N/mm^2	121.47
2	Strain at Max Stress	%	35.44
3	Displacement at Peak Load	mm	8.86
4	Max Load	kN	2429.9

Table 1: Compaction details.

Centrifuge Separation

The crude body fluid of BSF larvae comprises colloidal particles and other impurities and thus, the same has been centrifuged. A rotation speed of 4000 RPM was maintained over a period of 20 minutes and the supernatant liquid was separated.

Organic Extraction

The supernatant fraction was further purified by organic solvent extraction using n-Hexane as a solvent. The sample was placed inside the thimble the apparatus was operated for 1h to ensure optimal recovery. Once the extraction was over both the liquids formed an immiscible layer and further separated by funnel separator. The Bio-oil found to have higher viscosity and density when compared to the solvent and came to the bottom of the funnel once shaken properly.

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Homogenisation

The bottom sludge of the centrifuge tube was separated from the supernatant fluid using Whatman Filter Paper 40 micron pore size. The reject of the filter paper was further fused with the crushed body skeleton of the larvae and homogenized in the mixer grinder with the addition of a suitable quantity of water.

Cake Preparation

Post-homogenisation the successful conversion of the semi slurry into the nutrient-rich cake was the other challenging issue. The consortium was transferred into the grinder was operated for a period of 5 minutes at different RPM and finally, the paste was transferred to the mould. The mould was further placed in the oven and dried at a uniform temperature of 105^{0}C for 5h. The final yield of the process was utilized as fish meal.

Nutri-feeding

Utilization of the dead counts in an eco-friendly was undoubtedly one of the most delicate issues it has been further valorized by adopting them as poultry meal. Primarily, the hens were fed on the house fly larvae and the same resulted as a nutritious substrate for accelerated body growth and hence the BSF larvae were fed to hens kept in captivity over a week period of time and the factor of body growth was keenly observed.

Results and Discussion

The present study aimed to emphasize the economic feasibility of BSF larvae in terms to be used as a potential agent for small-scale entrepreneurship. An elaborative study has been conducted to explore the possible end use of BSF larvae towards the proclamation of waste derived value-added co-products and the results have been summarized as follows.

Compost Recovery

The mature larvae were allowed to feed on the organic fraction of the municipal solid waste and once the readily biodegradable matter got consumed the remaining fraction has been recovered from the reactor. Furthermore, the leftover fraction was cured over a period of 48h and the dried matter has been further sieved by using BIS standard 4 mm sieve. The recovery fraction recorded as 62.10% by weight [2,4,20,24]. But, due to the minimal maturation period, the quality of the compost found to be slightly inferior in some of the trails [25,26]. The different parameters values instructed by FCO [27] has been analyzed and the report has been tabulated in table 2.

Sl. No.	Parameters	Units	Compost Test Values	FCO standards
1	Particle size	%	96.28	Minimum 90% material should pass through 4.00 mm sieve
2	C:N Ratio	-	11.81	20.0 Max
3	Bulk Density	gm/cm^3	0.81	< 1.0
4	Moisture	%	24.19	15.0 - 25.0
5	Total Organic Carbon	%	11.24	12.0 min
6	Total Nitrogen as N	%	0.72	0.8 min
7	Total Phosphate as P_2O_5	%	0.29	0.4 min
8	Total Potash as K_2O	%	0.32	0.4 min
9	Pathogen	-	Not detected	Absent

Table 2: Grub compost analysis report.

As the recovered fraction was claimed from Indian municipal organic waste thus, a possibility of heavy metal contamination always sustains due to the absence of source segregation. Therefore, it was mandatory to undertake a complete analysis of the compost to ensure the nullification of possible contamination from persistent pollutants and the report has been tabulated in table 3.

Sl. No.	Parameters	Units	Compost Test Values	FCO standards
1	Arsenic as AS_2O_3	mg/kg	1.86	10.0 maximum
2	Cadmium as Cd	mg/kg	1.03	5.00 maximum
3	Chromium as Cr	mg/kg	23.59	50.00 maximum
4	Copper as Cu	mg/kg	85.44	300.00 maximum
5	Mercury as Hg	mg/kg	0.0059	0.15 maximum
6	Nickel Ni	mg/kg	3.65	50.0 maximum
7	Lead as Pb	mg/kg	3.19	100.0 maximum
8	Zinc as Zn	mg/kg	354	1000.0 maximum

Table 3: Analysis report of persistent pollutants.

Quality Enhancement

Starch-Iodine test has been performed on the final end products in the form of compost which yielded an appearance of brownish-green colour, signified semi-fermented nature of the material [28,29]. The interpretation of table 2 also explicitly reveals that the quality of the compost is moderately lower when compared to the prescribed limits mentioned in FCO [27] and therefore three different quality improvement drives have been carried out and delineated as follows [30].

Phosphate Rich Organic Manure (PROM) Preparation

The deficiency in phosphate content of the matured compost has been nominalized by the addition of rock phosphate powder. The powder was mixed with the grub compost in 30:70 ratio and a bottled culture of phosphate solubilising bacteria namely, *Enterobacter cancerogenus* was introduced with the consortium for fixation of phosphate into the ordinary compost [31,32]. Post mixing resulted in a tremendous improvement in the compost quality [33-35] and the same has been tabulated in table 4.

Sl. No.	Parameters	Units	Compost Test Values	FCO standards
1	Particle size	%	98.35	Minimum 90% material should pass through 4.00 mm sieve
2	C:N Ratio	-	12.24:1.26	< 20.0:1.0
3	pH @25⁰C	-	6.69	6.70 max
4	Bulk Density	gm/cm^3	1.09	< 1.60
5	Moisture	%	26.58	25.0 min
6	Total Organic Carbon	%	11.61	7.90 min
7	Total Nitrogen as N	%	0.98	0.4 min
8	Total Phosphate as P_2O_5	%	11.25	10.4 min
9	Pathogen	-	Not detected	Absent

Table 4: Detailed analysis report of PROM.

Bio Enriched Organic Manure Preparation

A liquid solution of Bio N, P, K has been added to normal grub compost in order to prepare bio enriched manure. An optimum dosing of 2% by the weight of compost has been practiced, attributed with the addition of a suitable quantity of water [36-38]. The process yield manure was further tested to ensure the quality component and the values are portrayed below in table 5.

Sl. No.	Parameters	Units	Compost Test Values	FCO standards
1	Particle size	%	96.74	Minimum 90% material should pass through 4.00 mm sieve
2	C:N Ratio	-	12.50	18.0 max
3	pH @25⁰C	-	7.71	6.50 - 8.00
4	Bulk Density	gm/cm^3	0.81	< 1.00
5	Moisture	%	35.15	30.0 - 40.0
6	Total Organic Carbon	%	16.38	14.0 min
7	Total Nitrogen as N	%	1.31	0.8 min
8	Total Phosphate as P_2O_5	%	0.79	0.5 min
9	NPK nutrients- Total of $N_2P_2O_5$ and K_2O	%	3.60	Not less than 3%
10	Total Viable Count (N, P, K, and Zn bacteria) or (N and K bacteria)	cfu/g	35x10^{-4}	5.0 x 10^{-6} (Within the date of Manufacture)

Table 5: Detailed analysis report of bio enriched organic manure.

Struvite Rich Organic Manure Preparation

Magnesium Ammonium Phosphate recovered from the waste stream of cattle farm is proven to be a vital source of nutrient for grub compost [39-41]. The aerobic method of struvite crystallization by means of fluidized bed reactor proven to be an economically feasible source of nutrient for ordinary compost. At the same time, the deficiency in organic carbon has been compensated by the addition of coir pith powder and tobacco crush [42]. The factor of quality enhancement has been emphasized in table 6.

Pathogen Reduction

Multiple studies reported the larvae of *Hermetia illucens* to be an efficient pathogen reducer from different substrate consortium. Numerous researchers concluded that BSF larvae efficiently reduce Escherichia coli and Salmonella spp. from pig, swine and diary manure respectively [16,43-45]. Thus, this study undertaken an approach to cross-examine the quality of manure yielded from BSFL composting in terms of pathogen removal. The compost specimen was analysed as per the standard procedure mentioned in FCO, 1985. The experiment resulted negative in terms of gas production and established the potential of BSFL against effective pathogen removal.

Sl. No.	Parameters	Units	Compost Test Values	FCO standards
1	Particle size	%	93.23	Minimum 90% material should pass through 4.00 mm sieve
2	C:N Ratio	-	12.38	20.0 max
3	pH @25^{0}C	-	7.09	6.50 - 7.50
4	Bulk Density	gm/cm^3	0.82	< 1.00
5	Moisture	%	26.97	25 min
6	Total Organic Carbon	%	17.59	14.0 min
7	Total Nitrogen as N	%	1.42	0.5 min
8	Total Phosphate as P_2O_5	%	0.88	0.5 min
9	Pathogens	cfu/g	Not Detected	Absent

Table 6: Detailed analysis report of struvite riched organic manure.

Characteristics of the Bio-fuel

The crude body fluid of *Hermetia illucens* larvae has enormous potential to yield supreme quality bio-fuel [6,7,10,12-15]. The solvent extraction [46] yielded the purest form of bio-fuel and that has been further analysed as per the standard procedure mentioned in IS 1448 [P: 2]: 2007 [47]. Table 7 portrays the analysis report and the ASTM standards prescribed for each parameter.

Sl. No.	Parameters	Bio-diesel	Diesel	ASTM Standard	Unit
1	Density	0.864	0.838	0.84 - 0.9	Kg/L
2	Viscosity	5.2	1.9 - 4.1	3.5 - 5.0	mm^2/s
3	Flash Point	155	60	Min 100	^{0}C
4	Solidifying point	-12	-50 - 10	-15	^{0}C
5	Total Base value	3.41	10 - 15	2-5	mg KOH/gm
6	Heating value	41	40 - 45	-	Mj/Kg

Table 7: Detailed analysis report of biodiesel extracted from BSFL.

Characteristics of the Fish Meal Cake

The homogenised body paste of BSFL was further heated at the required temperature and successfully converted into nutri-rich fodder for aquatic life [5,48-50]. The cakes were further analysed and the report has been enclosed in table 8. A research conducted by Katya., *et al*. [48] has reported the utmost acceptability of BSF meal for juvenile barramundi (*Lates calcarifer*) reared in freshwater. Whereas, the present study has undertaken substrate compatibility check with Oscar carp which found to be readily consumable attributed to major body growth (Figure 1).

Figure 1: Oscar carp (aquarium grade) feeding on BSFL cake.

Sl. No.	Parameters	Unit	Parameter Values
1	Physical State	-	Solid
2	Loss on Drying (LOD @ 105^0)	%	13.89
3	Crude Protein	%	38.6
4	Crude Fat	%	21.9

Acceleration of Growth Rate

The experiment explicitly revealed that the chicks fed on normal diet gained limited body growth. Whereas, the same chicks raised under captivity and supplied maggot meal showed higher growth rate factor [5,51,52]. Schiavone., *et al.* [53] have reported that partially defatted BSF larvae resulted in better digestibility when compared to highly defatted larvae. Though, the present study does not claim conduction of any defeating operation but, still the consumption of raw pre-pupae resulted in better body mass index. 14 chicks were raised under captivity from the day of emergence. The initial average weight of the chicks recorded as 40g. The anticipated body growth with ordinary meal observed to be varying with the ambient temperature. The optimal weight gain associated with a pertinent temperature of 23^0-28^0C found to be approximately 160g over a period of 1 week in the case of ordinary fodder practice [54,55]. The influence of temperature varied inversely proportional to the body growth over 28^0C and impacts were severe over the range of 350C which drastically reduced the factor of mass gain up to 100g per week [56]. Although, a certain drop in temperature up to 15^0C observed as ineffective over the feeding rate and body mass index of the chicks [57,58]. Furthermore, the minimization of the initiation period of 40 days (i.e. associated with minimum body weight of 1.8 kg to 2 kg) as per the commercial practice of dispatching the full grown hens provoked the idea of utilizing BSF larvae as alternative food resource. Three trails were conducted using different replacement ratios between ordinary diet and maggot diet such as, 75: 25, 50:50, and 25:75. Amid 75% replacement yielded optimal achievement of body growth with minimal initiation period of 32 days and a magical growth rate of gain of 185g/ week was recorded. The variation in weight gain for the chicks fed on normal diet and special diet portrayed in figure 2 whereas, figure 3 tried to represent the correlation of the temperature with anticipated body growth.

Figure 2: Graphical Representation of the body growth of chick fed over different ration.

Figure 3: Graphical Representation of the variation in body growth with temperature.

Conclusion

The prime aspect of this study was to explore the techno-economic feasibility of BSF larvae towards societal welfare. The research work yielded three major end products in the form of compost, biodiesel, and animal fodder. Synthesized products were individually analysed to ensure the bio-safety and minimal environmental interference. The Analysis yielded positive output and each item found to be satisfactory as per the prescribed limits. Since the maturation period was minimized the compost quality in terms of the presence of vital soil conditioning nutrient recorded as slightly sub-standard. Furthermore, the same has been compensated by means of introducing inexpensive and readily available nutrients, either extracted from waste stream or natural resources. Whereas, other two by-products readily met the quality bench mark and showed significant potential to be utilized as promising alternatives. Numerous frugal end applications and applicability with minimal environmental intervention interpolate a thought of using this holistic creature as a proficient agent for bioconversion and stabilization which can be further remoulded into a profitable entrepreneurial element.

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