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Algae for Fortification of Feeding Material of BSF, *Hermetia illucens* (L).

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Abstract

Attempt deals with rearing of Black Soldier Fly (BSF), *Hermetia illucens* (L) through the use of poultry feed fortified with the extractives of *Chlorella pyrinoidosa* (Chick) and *Scenedesmus dimorphus* (Turpin). The weight of larval stages of Black Soldier Fly (BSF), *Hermetia illucens* (L) of the control group was 321.32 (±42.786) units. Treating the poultry feed with the extractives (10 PPM, 25 PPM, 50 PPM, 75 PPM and 100 PPM) of *Chlorella pyrinoidosa* (Chick) or the extractives (10 PPM, 25 PPM, 50 PPM, 75 PPM and 100 PPM) of *Scenedesmus dimorphus* (Turpin) was resulted in significant increase in the body weight of larval stages of Black Soldier Fly (BSF), *Hermetia illucens* (L). The soluble protein and total protein contents (µg/mg body weight) of larval stages of Black Soldier Fly (BSF), *Hermetia illucens* (L) of the control group were 166.87 (±29.828) and 118.68 (±23.394) units, respectively. Treating the poultry feed with the extractives (10 PPM, 25 PPM, 50 PPM, 75 PPM and 100 PPM) of *Chlorella pyrinoidosa* (Chick) or the extractives (10 PPM, 25 PPM, 50 PPM, 75 PPM and 100 PPM) of *Scenedesmus dimorphus* (Turpin) was found resulted into significant increase soluble protein contents and total protein contents in the body of larval stages of Black Soldier Fly (BSF), *Hermetia illucens* (L). The BSF diet with supplementation of extractives of *Chlorella pyrinoidosa* (Chick) and *Scenedesmus dimorphus* (Turpin) at certain concentration (10 PPM to 100 PPM) exerts influence in weight and protein contents in Black Soldier Fly (BSF), *Hermetia illucens* (L).

Keywords: BSF, Chlorella pyrinoidosa, Scenedesmus dimorphus, Hermetia illucens

Introduction

Black Soldier Fly (BSF), *Hermetia illucens* (L.), is the insect of synanthropic category (animal living in close association with human being), polysaprophagous (feeding on different decomposed materials) fly. It is native to the Neotropic regions. But now, Black Soldier Fly (BSF), *Hermetia illucens* (L.) found in every zoogeographic region. The last two decades are witnessing about considerable utilization of the black Soldier Fly (BSF), *Hermetia illucens* (L.) for organic waste material. The black Soldier Fly (BSF), *Hermetia illucens* (L.) has been studied as efficient agents for the control of manure (Sheppard *et al*., 1994), for control of infestation of house flies in chicken production (Furman *et al*., 1959; Sheppard, 1983), as a fish meal (Bondari & Sheppard, 1981) and as a supplement in meal for pigs (Newton *et al*., 1977). Lalander *et al*. (2015) reported the highest conversion of waste material into the biomass and efficient reduction in Salmonella species through the use of the black Soldier Fly (BSF), *Hermetia illucens* (L.). Benelli *et al*. (2014) suggested that, the black Soldier Fly (BSF), *Hermetia illucens* (L.) might have been firstly brought to the Europe at around five hundred years ago. According to Lindnder (1936), the very first verifiable Palaearctic-record of the black Soldier Fly (BSF), *Hermetia illucens* (L.) is from southern-Europe (Malta) in the year: 1926. Subsequently, the spread of black Soldier Fly (BSF), *Hermetia illucens* (L.) in Europe has been significantly along the coast of Mediterranean-sea in Spain, in France, and in Italy in the year: 1950 and 1960 (Leclercq, 1969; Leclercq, 1997). Northwards spread of the black Soldier Fly (BSF), *Hermetia illucens* (L.) in the central Europe has been recorded by Ssymank and Doczkal (Ssymank & Doczkal, 2010). Further, Ssymank and Doczkal (2010) reported spread of the black Soldier Fly (BSF), *Hermetia illucens* (L.) from Germany. Roháček and Hora (2013) reported spread of the black Soldier Fly (BSF), *Hermetia illucens* (L.) from the Czech Republic. The apparent spread of the black Soldier Fly (BSF), *Hermetia illucens* (L.) along coastline and island suggested that, maritime-transport may have played a significant role in repeated and accidental introduction.

Due to high contents of proteins, the larval stages of *Hermetia illucens* (L), black-soldier-fly are edible. The rearing of the larval stages of *Hermetia illucens* (L), black-soldier-fly is possible through the use of organic materials. The range of organic materials for rearing of the larval stages of *Hermetia illucens* (L), black-soldier-fly is wide including waste materials from vegetable market, kitchen waste, canteen waste (Cammack & Tomberlin, 2017); cow-dung manure, fish-offal, by-products of brewery, sludge from sewage (St-Hilaire *et al*., 2007; Spranghers *et al*., 2017; Meneguz *et al*., 2018; Lalander *et al*., 2019). In comparison with the other insects, the larval stages of *Hermetia illucens* (L), black-soldier-fly are with the best capabilities of conversion of substrates (in the form of organic waste) into biomass and high-

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quality fertilizers (van Huis & Tomberlin, 2017). The nutritional composition of the larval stages of *Hermetia illucens* (L), black-soldier-fly is depending exclusively on the food substrates used for their rearing. With reference to chemical composition, on the basis of dry matter, the black soldier fly larval Stages (BSFLS) contain proteins (about forty percent) and fat (about thirty percent) (Barragan-Fonseca *et al*., 2017). Due to high contents of the proteins and fat, the larval stages of *Hermetia illucens* (L), black-soldier-fly have been utilized for the preparation of poultry feed, fish feed and feed for other animals (St-Hilaire *et al*., 2007; Schiavone *et al*., 2018) as one of the significant ingredients (Schiavone *et al*., 2018). Therefore, the European and American industries like aquaculture and poultry are exclusively depend on black soldier fly larval stages (BSFLS) (Cammack & Tomberlin, 2017; van Huis & Tomberlin, 2017). Lipid contents in the body of black soldier fly larval stages (BSFLS) and pupal stages are also appreciable. With reference to insect physiology, the higher lipid contents are for provision of energy to the non-feeding pre-pupal and pupal stages of *Hermetia illucens* (L), black-soldier-fly (Li *et al*., 2011; Kroeckel *et al*., 2012; Nguyen *et al*., 2013). The fat bodies are the most significant organs in the body of *Hermetia illucens* (L), black-soldier-fly with reference to collection, storage and utilization for metabolism (Pimentel *et al*., 2017). The contents of the nutrients in the body of *Hermetia illucens* (L), black-soldier-fly depends on the life stage (Liu *et al*., 2017) and the food quality. There are possibilities of relationship between the profiles of fatty acids and the modulations concerned with the genes of expression of metabolism of the lipids of larval stages and pre-pupal stages of *Hermetia illucens* (L), black-soldier-fly. There are four known genes of metabolism concerned with fat accumulation in the larval body of *Hermetia illucens* (L), black-soldier-fly (Zhu *et al*., 2019). In the larval stages of insects (like: *Hermetia illucens* L., black-soldier-fly), the energy (in the form of nutrients like fat) are stored for making available for each of the post feeding life stages. This system of storage of energy deserves significance and exert influence on reproduction. There is a positive correlation among the quality of clutch of eggs and fat stored during larval life (Georgescu *et al*., 2020). As the adult stage is non-feeding, *Hermetia illucens* L., black-soldier-fly is the best model for the attempts on study of the storage of nutrient-reserve during larval development. The utilization of nutrients by the adult stage of *Hermetia illucens* L., black-soldier-fly rely on the nutrient-reserves stored during the larval stage. The nutrient-reserves in the larval body of *Hermetia illucens* L., black-soldier-fly could be very much important for the attempt of reproduction by the adult.

The species of green algae, *Chlorella pyrenoidosa* (Chick) is recognized for richness in the nutrients. The contents (quality and quantity) of nutrients depends on the strain of *Chlorella pyrenoidosa* (Chick). *Chlorella pyrenoidosa* (Chick) is freshwater green algal species belongs to the Division: Chlorophyta. It has been used medicinally as a chelating agent, which bind with metal ions and help for the removal of metals that are toxic to human body (Nakano, 2007). Fibromyalgia, Hypertension and Ulcerative colitis are the fields of medicines for the possible utilization of *Chlorella pyrenoidosa* (Chick) (Merchant & Andre, 2001). The freshwater unicellular-green algal species, *Scenedesmus dimorphus* (Turpin) belong to the Class: Chlorophyceae. *Scenedesmus dimorphus* (Turpin) is able to synthesize products with high-energy-value (Grima *et al*., 2003; Walker *et al*., 2005). *Scenedesmus dimorphus* (Turpin) have also been recognized as a promising-feedstock for the biofuel industry. This is because of recognizable efficiency of photosynthesis and significant content of oil (Hu *et al*., 2008; Liang *et al*., 2009; Wijffels & Barbosa, 2010).

There are no reports on use of *Chlorella pyrenoidosa* (Chick) and *Scenedesmus dimorphus* (Turpin) as food substrates for the rearing the larval stages of black soldier fly (BSF), *Hermetia illucens* (L). This much is the background for planning the present attempt on the rearing of the larval stages of black soldier fly (BSF), *Hermetia illucens* (L) for biomass production and proteins through the use of *Chlorella pyrenoidosa* (Chick) and *Scenedesmus dimorphus* (Turpin) as food substrates.

Materials and Methods

The attempt concerned with the influence of food substrates on body weight and protein (soluble and Total) contents of black soldier fly (BSF), *Hermetia illucens* (L) was completed through the steps like: (A). Procurement of the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae); (B). Procurement of Extractives of *Chlorella pyrenoidosa* (Chick) and *Scenedesmus dimorphus* (Turpin); (C). Rearing of the larval-stages of black soldier fly (LBSF); (D). Preparation of the Silage of Selected Food Substrates; (E). Experimental Design; (F). Assay sample preparation; (G). Protein Bioassay (Lowry *et al*., 1951) and (H). Statistical Analysis.

Procurement of the Larval-Stages of Black Soldier Fly (LBSF), Hermetia Illucens (Linnaeus) (Order: Diptera, Family: Stratiomyidae)

For the present attempt, the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) of five days old were procured from the Black Soldier Fly (BSF) Unit of ICAR-National Institute of Abiotic Stress Management (Malegaon-Karhavagaj Road, Khurd, Baramati, Taluka: Baramati, District: Pune Maharashtra State-413115 India). Here, at ICAR-National Institute of Abiotic Stress Management, in the well-established insectary, the laboratory staff initiated the rearing of the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) through the use of commercial granular poultry feed. The weight of individual larval-stage of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) was recorded. The larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) were brought to Department of Zoology, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar (Malegaon Colony) Tal. Baramati, Pune, Maharashtra State- 413115 India (Khyade, 2021).

Procurement of Extractives of Chlorella Pyrenoidosa (Chick) and Scenedesmus Dimorphus (Turpin)

The extractives of *Chlorella pyrenoidosa* (Chick) and *Scenedesmus dimorphus* (Turpin) are selected for the fortification of food substrate (Poultry Feed) for rearing the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) in the present attempt. Both the extractives were procured from HERBSINO (20201, Dongxing, Building No. 64 Jiangong Rd. Xian, 710043 P.R. China) through the local dealer.

Rearing of the Larval-Stages of Black Soldier Fly (LBSF)

The larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) from the "Black Soldier Fly (BSF) Unit of ICAR-National Institute of Abiotic Stress Management" (Malegaon-Karhavagaj Road, Khurd, Baramati, Taluka: Baramati, District: Pune Maharashtra State-413115 India) were brought to the insectary (Green House) of Shardabai Pawar Mahila Mahavidyalaya, Shardanagar (Malegaon Colony) Tal. Baramati, Pune, India. The larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) were reared through standard method (Khyade, 2021). The culture was initiated through the use of commercial granular poultry feed. The content of the commercial granular poultry feed was taken in a plastic box (LBSF Rearing Bin) (with dimensions: 2x1.5x1.5). This LBSF rearing bin was with ventilation holes at the top lid. There was a rectangular plank placed at inclined position (with the angle of forty-five degree with the bottom of LBSF rearing bin. Purpose of keeping the plank inclined is to allow the self-harvesting for the mature larva as it turn into the pre-pupa. Little amount of water was used to spray on the feed contents (commercial poultry feed) in a LBSF rearing bin. This water spraying is for the initiation of the process of decomposition of the feed through bacteria intervention (Pedro *et al*., 2014).

Preparation of the Silage of Selected Food Substrates

Silage is the functional food substrate to be used for rearing the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) (Khyade, 2021). Silage was allowed for anaerobic fermentation for reduction in pH (potential of the hydrogen or power of hydrogen ion concentration (Jensen, 2004).

For preparation of silage of poultry feed, distilled water was used for addition (at the rate of 10 ml for one gram of commercial poultry feed in the form of pellet). The content was then processed in mixer (kitchen) for about five minutes. The content was collected in separate jar and kept for twenty-four hours at room temperature. Strengths of the extractives of *Chlorella pyrenoidosa* (Chick) and *Scenedesmus dimorphus* (Turpin) used for fortification of poultry feed were 10 PPM; 25 PPM; 50 PPM; 75 PPM and 100 PPM. 10 ml of each strength extractives were used for addition in the pellet-form poultry feed (at the rate of 10 ml extractives of each strength for one gram of commercial poultry feed in the form of pellet). This treatment was carried out separately. Treating the poultry feed with extractives, mixing well and keeping in a closed container for twenty-four hours at room temperature allows preservation in anaerobic condition through the method of fermentation. The anaerobic condition (no air inside container) of closed container is helping the production of lactic acid in the food substrate with the help of micro-organisms. The lactic acid in its turn, help to preserve the food substrate for longer duration.

Experimental Design

The individual weight of five days old the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) procured from ICAR-National Institute of Abiotic Stress Management (Malegaon-Karhavagaj Road, Khurd, Baramati, Taluka: Baramati, District: Pune Maharashtra State-413115 India) was recorded. The five days old the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) were divided into two control groups (untreated control group and solvent treated group) and ten (five + five) experimental groups, each in triplicate set (T.1; T.2 and T.3).

The first set of five experimental groups include: Experimental group fed with *C. pyrinoidosa* (10 PPM) Treated Poultry Feed; Experimental group fed with *C. pyrinoidosa* (25 PPM) Treated Poultry Feed; Experimental group fed with *C. pyrinoidosa* (50 PPM) Treated Poultry Feed; Experimental group fed with *C. pyrinoidosa* (75 PPM) Treated Poultry Feed and Experimental group fed with *C. pyrinoidosa* (100 PPM) Treated Poultry Feed. Another set of five experimental groups include: Experimental group feed with *S. dimorphus* (10 PPM) Treated Poultry Feed; Experimental group feed with *S. dimorphus* (25 PPM) Treated Poultry Feed; Experimental group feed with *S. dimorphus* (50 PPM) Treated Poultry Feed; Experimental group feed with *S. dimorphus* (75 PPM) Treated Poultry Feed and Experimental group feed with *S. dimorphus* (100 PPM) Treated Poultry Feed. Each group was with twenty-five the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae). All the sets of the attempt were kept at room temperature and allowed the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) to attain the age of twenty-one days. For the group of twenty group of larval stages, hundred grams of feed were used. For the aeration, with the help of kitchen fork, the culture of the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) in each group was turned the lower layer to upper surface. This was carried for four to five times for each group, daily at 7 – 8 a.m (Khyade, 2021).

Assay Sample Preparation

The soluble proteins and total proteins from the body of the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) were determined on twenty-first day. Counting of the age of the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) was from the day of their hatching. The selection of larval stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) from each group was made randomly. Number of the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) from each group was twenty. Weight of individual larva from each group was recorded. They were transferred to the deep freezer, at minus thirty-five degree Celsius. The duration of keeping the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) in deep freezer, at minus thirty-five degree Celsius was twenty-four hours. The process of keeping the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) in deep freezer, at minus thirty-five degree Celsius, for twenty-four hours was named here as, "thawing". After the process of thawing, through the use of chilled distilled water, the larvae were washed thoroughly. Purpose of using the chilled distilled water for thoroughly washing the larvae was to retain the chemical components of the larval tissue. The chilling allows no loss of the chemical components of living tissue. Thereafter, the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) were processed for homogenization. The prechilled mortar and pestle were used for the homogenization of the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae). Known volume of hilled distilled water was used for addition during the process of homogenization. The volumetric strength of the homogenized content was ten milligram per milliliter (10 mg/ml). This content was then subjected for centrifugation at 10000 rpm. The centrifugation was carried out for twenty minutes. Resulting supernatant was utilized as a sample for assay of soluble proteins. sample for estimation of soluble protein (Lowry *et al*., 1951).

For the purpose to estimate the total proteins, another set of the thawed larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) (with known weight) were selected. They were washed thoroughly using the chilled distilled water. Thereafter, the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) were processed for homogenization. The homogenization was carried in 1N sodium-hydroxide (NaOH). The content was subjected for precipitation in equal volume of ten percentage of trichloacetic acid (TCA) solution. The content was then subjected for centrifugation at 3000 rpm for ten minutes. The precipitate dissolved in 1N sodium-hydroxide (NaOH) and used as assay sample for estimation of total protein (strength of the content was 10 mg/ml) (Lowry *et al*., 1951).

Protein (Soluble and Total) Bioassay

The method of Lowry (1951) was followed for the bioassay of soluble proteins and total proteins from the assay sample of whole body of larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) (age: 21days). Triplicate set (for each assay sample three test tubes were taken) was followed for the bioassay of proteins. In each test tube, one ml assay sample was taken. Lowery's ―C solution (5.0 ml) was used for addition in each test tube. The content was mixed well. The set was kept for about fifteen minutes. This is to allow the copper protein complex formation. A blank test tube was also used to prepare simultaneously. 0.5 ml of Folin's phenol reagent was used for addition in each test tube and mixed well. The contents in each test tube were allowed for development of colour through waiting for about half an hour at room temperature. The optical density (OD) of contents from each test tube was recorded at 660 nm through the use of spectrophotometer (spectronic-20). The attempts were repeated for three times. The protein concentration of assay sample was calculated by referring the optical density (OD) obtained for sample and by using standard graph. The results were expressed in the unit as microgram (µg) proteins per mg body weight of the larval-stages of black soldier fly (LBSF), *Hermetia illucens* (Linnaeus) (Order: Diptera, Family: Stratiomyidae) (Khyade, 2021).

Statistical Analysis

For the purpose to get consistency in the results, each and every attempt in the experimentation was repeated for three times. The data on the attempt of contents of proteins (soluble and total) of black soldier fly (BSF), *Hermetia illucens* (L) (reared on different food substrates) was collected and subjected for statistical analysis. The student "t" - test (Bailey, 1955; Khyade & Eigen, 2018) was used for comparison of the significance of the data on the control group with the experimental groups.

Results and Discussion

The results on the on the attempt of the influence of different food substrates on weight of body, contents of total proteins and contents of soluble Proteins in Black Soldier Fly (BSF), *Hermetia illucens* (L) are given in summery form in **Table 1** and presented in **Figures 1-3**. The biomass (body-weight) (mg) of larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the untreated control group (reared on the silage of untreated poultry feed) and solvent treated control group (reared on the silage of solvent treated poultry feed) was recorded 321.32 (±42.786) units and 321.32 (±49.137) units **(Table 1, Figure 1)** respectively.

Table 1. The Weight (mg) and Protein Contents of Larval Stages of Black Soldier Fly (BSF), *Hermetia illucens* (L) reared on Poultry Feed Fortified with Extractives of *Chlorella pyrinoidosa* (Chick)

Serial No.	Group	Weight (mg) of BSF Larva (on 21 Day)	Soluble Proteins (µg/mg body Weight)	Total Proteins (µg/mg body Weight)
1.	Control group Fed with Untreated Poultry Feed.	321.32 (±42.786) 00.000	166.87 (±29.828) 00.000	118.68 (±23.394) 00.000
2.	Control group Fed with Solvent Treated Poultry Feed	321.32 (±49.137) 00.000	166.87 (±33.716) 00.000	118.68 (±27.168) 00.000
3.	Experimental group fed with *C. pyrinoidosa* (10 PPM) Treated P. F.	376.56 (±51.781) 17.191	180.21 (±39.107) 07.994	137.66 (±34.451) 15.992
4.	Experimental group fed with *C. pyrinoidosa* (25 PPM) Treated P. F.	384.23 (±73.663) 19.578	193.10 (±67.844) 15.718	143.51 (±49.678) 28.921
5.	Experimental group fed with *C. pyrinoidosa* (50 PPM) Treated P. F.	495.84 (±89.786) 54.313	218.45 (±83.428) 30.910	186.19 (±68.551) 56.884

6.	Experimental group fed with *C. pyrinoidosa* (75 PPM) Treated P. F.	543.03 (±96.408) 68.999	229.85 (±82.584) 37.741	213.42 (±76.681) 79.828
7.	Experimental group fed with *C. pyrinoidosa* (100 PPM) Treated P. F.	711.42 (±103.78) 121.40	273.78 (±87.768) 63.867	279.68 (±84.544) 135.65
8.	Experimental group feed with *S. dimorphus* (10 PPM) Treated P. F.	374.33 (±59.686) 16.497	226.49 (±89.113) 35.728	142.29 (±39.712) 19.893
9.	Experimental group feed with *S. dimorphus* (25 PPM) Treated P. F.	381.79 (±63.875) 18.819	285.33 (±83.643) 70.989	179.61 (±43.213) 51.339
10.	Experimental group feed with *S. dimorphus* (50 PPM) Treated P. F.	506.83 (±69.786) 57.733	363.41 (±79.903) 117.78	236.44 (±38.692) 99.224
11.	Experimental group feed with *S. dimorphus* (75 PPM) Treated P. F.	627.21 (±103.95) 95.197	369.93 (±91.786) 121.68	279..65 (±59.343) 135.63
12.	Experimental group feed with *S. dimorphus* (100 PPM) Treated P. F.	689.95 (±133.78) 114.72	373.88 (±106.43) 124.05	284..39 (±91.786) 139.62

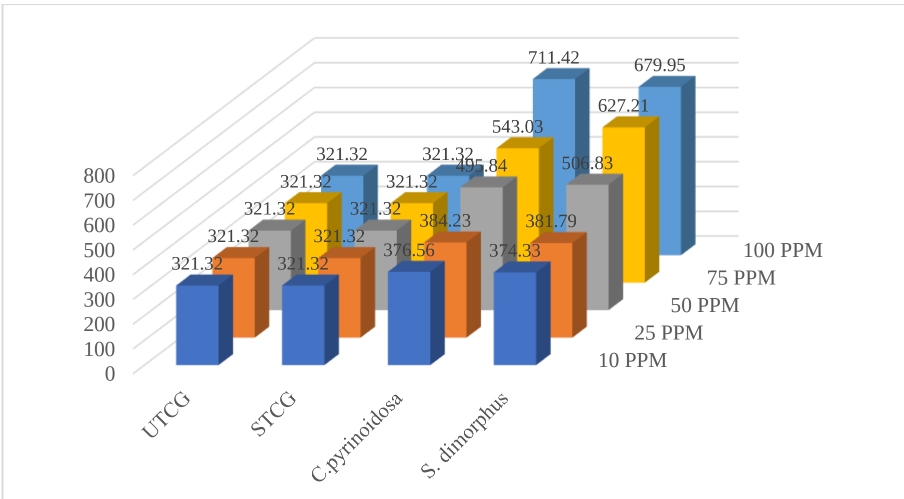

Figure 1. The Weight (mg) of Larval Stages of Black Soldier Fly (BSF), Hermetia illucens (L) reared on Poultry Feed Fortified with Extractives of Chlorella pyrinoidosa (Chick)

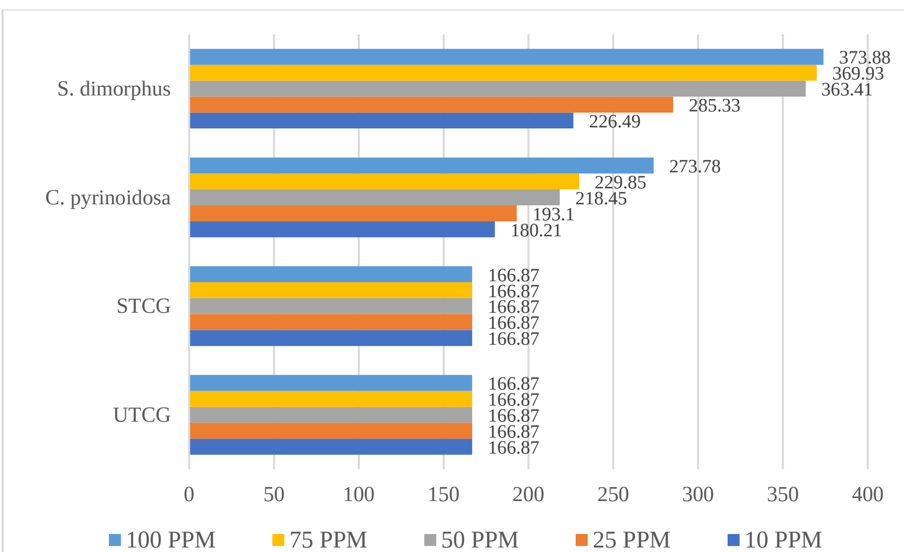

Figure 2. The Contents of Soluble Protein (Microgram Per mg body weight) in the Larval Stages of Black Soldier Fly (BSF), Hermetia illucens (L) reared on Poultry Feed Fortified with Extractives of Chlorella pyrinoidosa (Chick) and Scenedesmus dimorphus (Turpin)

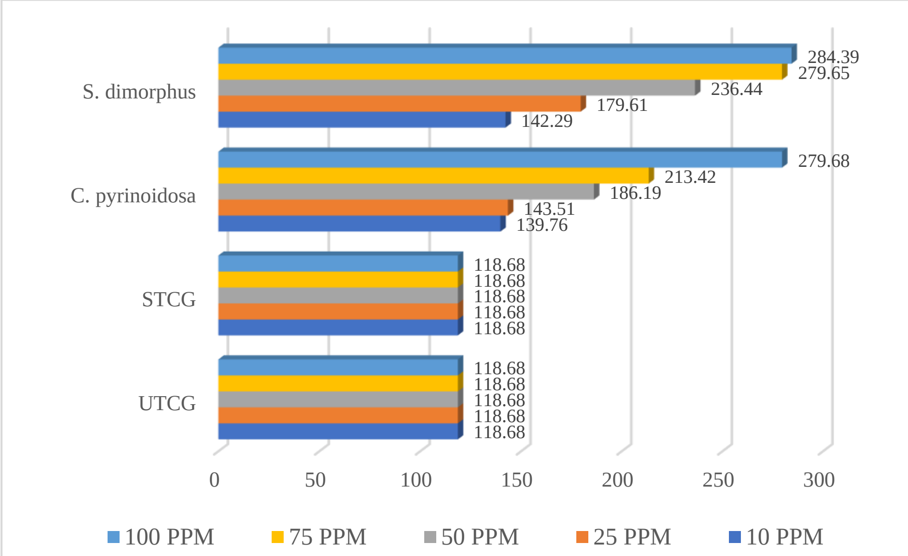

Figure 3. The Contents of Total Protein (Microgram Per mg body weight) in the Larval Stages of Black Soldier Fly (BSF), Hermetia illucens (L) reared on Poultry Feed Fortified with Extractives of Chlorella pyrinoidosa (Chick) and Scenedesmus dimorphus (Turpin)

The biomass (body-weight) (mg) of larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (10 PPM) was recorded 376.56 (±51.781) units **(Table 1, Figure 1)**. The biomass (body-weight) (mg) of larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (25 PPM) was recorded 384.23 (±73.663) units **(Table 1, Figure 1)**. The biomass (body-weight) (mg) of larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (50 PPM) was recorded 495.84 (±89.786) units **(Table 1, Figure 1)**. The biomass (body-weight) (mg) of larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (75 PPM) was recorded 543.46 (±96.408) units **(Table 1, Figure 1)**. The biomass (body-weight) (mg) of larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (100 PPM) was recorded 711.42 (±103.78) units **(Table 1, Figure 1)**. The range of improvement (percent increase) in the biomass (body-weight) (mg) of larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (strength: 10 PPM, 25 PPM, 50 PPM, 75 PPM and 100 PPM) in present attempt was 17.191 to 121.40 percentage.

The larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (10 PPM) weighed 374.33 (±59.686) units (mg) **(Table 1, Figure 1)**. The larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (25 PPM) weighed 381.79 (±63.875) units (mg) **(Table 1, Figure 1)**. The larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (50 PPM) weighed 506.83 (±69.786) units (mg) **(Table 1, Figure 1)**. The larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (75 PPM) weighed 627.21 (±103.95) units (mg) **(Table 1, Figure 1)**. The larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (100 PPM) weighed 689.95 (±133.78) units (mg) **(Table 1, Figure 1)**. The range of improvement (percent increase) in the biomass (body-weight) (mg) of larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (strength: 10 PPM, 25 PPM, 50 PPM, 75 PPM and 100 PPM) in present attempt was 16.497 to 111.68 percentage.

The soluble proteins (SP) deserve significancy with reference to their secretion, traversing the Golgi, packing and specificity of their working. The soluble proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the untreated control group (reared on the silage of untreated poultry feed) and solvent treated control group (reared on the silage of solvent treated poultry feed) was recorded 166.87 (±29.828) units and 166.87 (±33.716) units **(Table 1, Figure 2)** respectively.

The soluble proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (10 PPM) was recorded 180.21 (±39.107) units **(Table 1, Figure 2)**. The soluble proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (25 PPM) was recorded 193.10 (±67.844) units **(Table 1, Figure 2)**. The soluble proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (50 PPM) was recorded 218.45 (±83.428) units **(Table 1, Figure 2)**. The soluble proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (75 PPM) was recorded 229.85 (±82.584) units **(Table 1, Figure 2)**. The soluble proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (100 PPM) was recorded 273.78 (±87.768) units **(Table 1, Figure 2)**. The range of improvement (percent increase) in the soluble proteins (unit: (µg/mg body weight) of larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (strength: 10 PPM, 25 PPM, 50 PPM, 75 PPM and 100 PPM) in present attempt was 07.994 to 63.867 percentage.

The soluble proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (10 PPM) was recorded 226.49 (±89.113) units **(Table 1, Figure 2)**. The soluble proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (25 PPM) was recorded 285.33 (±83.643) units **(Table 1, Figure 2)**. The soluble proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (50 PPM) was recorded 363.41 (±79.903) units **(Table 1, Figure 2)**. The soluble proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (75 PPM) was recorded 369.93 (±91.786) units **(Table 1, Figure 2)**. The soluble proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (100 PPM) was recorded 373.88 (±106.43) units **(Table 1, Figure 2)**. The range of improvement (percent increase) in the soluble proteins (unit: (µg/mg body weight) of larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (strength: 10 PPM, 25 PPM, 50 PPM, 75 PPM and 100 PPM) in present attempt was 35.728 to 124.05 percentage.

The levels of total proteins are influenced by physiological conditions. The total proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the untreated control group (reared on the silage of untreated poultry feed) and solvent treated control group (reared on the silage of solvent treated poultry feed) was recorded 118.68 (±23.394) units and 118.68 (±27.168) units **(Table 1, Figure 3)** respectively. The total proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (10 PPM) was recorded 139.76 (±34.451) units **(Table 1, Figure 3)**. The total proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (25 PPM) was recorded 143.51 (±49.678) units **(Table 1, Figure 3)**. The total proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (50 PPM) was recorded 186.19 (±68.551) units **(Table 1, Figure 3)**. The total proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (75 PPM) was recorded 213.42 (±76.681) units **(Table 1, Figure 3)**. The total proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (100 PPM) was recorded 279.68 (±84.544) units **(Table 1, Figure 3)**. The range of improvement (percent increase) in the total proteins (unit: (µg/mg body weight) of larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *C. pyrinoidosa* extractives (strength: 10 PPM, 25 PPM, 50 PPM, 75 PPM and 100 PPM) in present attempt was 17.762 to 135.65 percentage **(Table 1, Figure 3)**.

The total proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (10 PPM) was recorded 142.29 (±39.712) units **(Table 1, Figure 3)**. The total proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (25 PPM) was recorded 179.62 (±43.213) units **(Table 1, Figure 3)**. The total proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (50 PPM) was recorded 236.44 (±38.692) units **(Table 1, Figure 3)**. The total proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (75 PPM) was recorded 279.65 (±59.343) units **(Table 1, Figure 3)**. The total proteins (unit: (µg/mg body weight) of whole-body homogenate in the larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (100 PPM) was recorded 284.39 (±91.786) units **(Table 1, Figure 3)**. The range of improvement (percent increase) in the total proteins (unit: (µg/mg body weight) of larval-instars of black-soldier-fly (BSF), *Hermetia illucens* (L) (Age: twenty-one days) of the experimental group, reared on the silage of poultry feed treated with *S. dimorphus* extractives (strength: 10 PPM, 25 PPM, 50 PPM, 75 PPM and 100 PPM) in present attempt was 19.893 to 139.62 percentage **(Table 1, Figure 3)**.

Proteins are the most significant nutrients for maintenance of the structure of the animal body. Generally, it is supposed that, the flesh (protein content) serves to make the flesh (protein content). Protein nature of the enzymes was proved by James Batcheller Sumner (American Chemist and Nobel Laureate) (Sumner, 1926). Higher degree of decomposition of silage (biowaste) (used for feeding the larval instars of the black soldier fly, *Hermetia illucens* L) is also responsible for qualitative results (Gold *et al*., 2018) with reference to biomass and prptein contents. The composition and strength of extractives of *C. pyrinoidosa* and *S. dimorphus* used for treatment of poultry feed (food substrate) is exerting the influence on the growth and efficiencies of conversions of ingested food (organic food substrate) into the biomass, total protein content and soluble protein content in the larval instars of black soldier fly (BSF), *Hermetia illucens* (L). The quality of food substrates and growing performance of the prepupal stages of black soldier fly (BSF), *Hermetia illucens* (L) goes hand in hand. Same is true for the qualities and quantities of soluble proteins and total proteins in the larval body tissues in the black soldier fly (BSF), *Hermetia illucens* (L). The high protein contents of *C. pyrinoidosa* and *S. dimorphus* (used for treating the poultry feed, the food substrate for larval instars of the black soldier fly, *Hermetia illucens* L) are contributed for the qualitative biomass and the protein contents (soluble and total). The cultivation of microalgae (*C. pyrinoidosa*) is possible through the use of waste water (Ji *et al*., 2013). The larval instars of black soldier fly (BSF), *Hermetia illucens* (L) are also expert in eating away the waste organic material. Both (algal species and BSF) may be utilized for waste management.

Conclusion

The composition and strength of extractives of *C. pyrinoidosa* and *S. dimorphus* used for treatment of poultry feed (food substrate) is exerting the influence on the growth and efficiencies of conversions of ingested food (organic food substrate) into the biomass, total protein content and soluble protein content in the larval instars of black soldier fly (BSF), *Hermetia illucens* (L). The quality of food substrates and growing performance of the prepupal stages of black soldier fly (BSF), *Hermetia illucens* (L) goes hand in hand. Same is true for the qualities and quantities of soluble proteins and total proteins in the larval body tissues in the black soldier fly (BSF), *Hermetia illucens* (L). The high protein contents of *C. pyrinoidosa* and *S. dimorphus* (used for treating the poultry feed, the food substrate for larval instars of the black soldier fly, *Hermetia illucens* L) are contributed for the qualitative biomass and the proteins (soluble and total).

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