








# Bioplastic Film from Black Soldier Fly Prepupae Proteins Used as Mulch: Preliminary Results

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Received: 18 May 2020; Accepted: 27 June 2020; Published: 29 June 2020



**Abstract:** A protein-based film (PBF), obtained from black soldier fly prepupae proteins, was assessed for its agronomic performance as mulch. PBF was investigated in a potting experiment and compared with Mater-Bi (MB), polyethylene (PE) and bare soil. During the experiment, degraded surface area, weight and thickness of the film, water evaporated from the pot, and the soil microbiological content (SMC) were recorded. In addition, films were buried to assess their biodegradability and impact on SMC. During the mulching process, the PBF showed a significant degradation. In terms of evaporated water, the PBF performed similarly to MB and worse than PE. Regarding SMC, film of any nature caused an increase in the *Clostridia* spp. and a decrease of total mesophilic aerobic bacteria and fungi contents. When buried, only PBF recorded a faster biodegradability, showing a reduction of surface area, thickness and weight. PBF and MB highlighted a significant increase in contents of *Clostridia* spp., mesophilic aerobic bacteria and fungi. Our results reported, for the first time, the valorization of black soldier fly (BSF) prepupae proteins as a biodegradable film for mulching purposes. However, further study is needed to reduce the PBF biodegradability and allow it to be used for the most important mulched crops.

**Keywords:** biodegradable materials; soil microbiota; sustainable agriculture; circular economy

## 1. Introduction

In the Mediterranean basin as well as in Northern Europe, the global temperature has steadily risen over the last years [1,2], negatively affecting agricultural irrigation water and energy consumptions [3].

Several studies demonstrated the usefulness of mulching in enhancing crop yield and quality [4], reducing the volume of irrigation water [4,5] and contributing to the avoidance of soil erosion phenomena [6]. In addition, mulching offers various benefits to crop production, such as weed control and early development due to the increase in the soil temperature and increased effectiveness of fertilizers [7].

Mulching materials are generally classified into three main groups: organic materials (agricultural, industrial and animal by-products), inorganic materials (plastic films) and special materials

(biodegradable plastic films) [3]. Concerning organic mulching, straw represents the most used material, showing numerous benefits after field application, such as increased soil moisture [8]. In addition, organic mulches are cheap, easily available and improve soil quality and crop yield [3]. Sometimes, natural mulches are not effective for weed control and can contain weed seeds [9]. In addition, when not properly managed, organic mulching can lead to excess moisture, creating new issues such as pest proliferation, anaerobic soil conditions and root rot. Finally, organic mulching is often characterized by a high carbon/nitrogen ratio (C/N), which might imbalance nutrient availability [10].

An estimated 2.5 million tons of plastics are utilized each year in agriculture to increase crop yield and quality [11]. Almost 50% of this amount is used as mulching films and to cover greenhouses [12].

The application of plastic mulching in agriculture has increased drastically worldwide since 2000 [13]. Several types of plastic mulching have been tested, and different compositions, thicknesses and colors have been assessed [14]. Currently, plastic mulching is used on all soil types, in all seasons and climatic zones; in particular, low-density polyethylene (PE), a plastic polymer derived from petroleum refining, is the most widely used material due to its low cost, good mechanical properties (durability) and positive effects on crop growth [10,15]. On the other hand, the durability of the PE film is much greater than that required for the crop production [16]. Although plastic mulching has many advantages, it has some negative consequences related to the management of waste disposal and the environmental impacts associated with this process [13]. In addition, considering its extended and repeated use in agricultural contexts, PE represents one of the most important sources of environmental pollution [17]. As a non-biodegradable material, PE can remain in the environment for hundreds of years before degrading [18]. Moreover, the incineration of PE can generate emissions of carbon dioxide and methane, which are greenhouse gases that contribute to climate change by increasing global temperatures [19].

In order to increase the agricultural sustainability, films based on biodegradable materials can be considered as favorable alternatives to conventional plastic films [20].

The development and optimization of specific bio-materials (i.e., those that can be decomposed or composted) could significantly reduce plastic waste [21]. Hence, investigators and farmers are called upon to find and use, respectively, alternative materials that can be used as mulching films, characterized by natural degradation and non-toxicity, allowing a drastic reduction of PE consumption and in turn increasing the agricultural sustainability [3,16,22].

Recently, a new group of materials, the “bioplastics”, have appeared on the market. Innovative and eco-friendly polymers are derived from renewable sources, such as oils, maize starch and vegetable fats, and from microorganism-mediated processes and agricultural by-products [18,23]. The degradation of bioplastic is carried out by bacteria, yeasts and/or fungi. They might also be composted and hence used as fertilizers and soil conditioners [24].

Most of the available biodegradable films are based on polysaccharides; currently, the ones of greater commercial interest are made of cellulose and starch. In addition, some more complex carbohydrates like chitosan, chitin and xanthan are also being considered [25]. The use of proteins to develop mulching sheets, a solution that has not yet been explored, could grant a greater benefit to the soil by naturally releasing nitrogen through their degradation. An added value would be if the proteins are derived from a process of full recovery and valorization of organic waste in a specific circular economy strategy.

In order to demonstrate the full circularity of the process, the aim of the present work was to assess the suitability of the innovative black soldier fly (BSF) protein bioplastic film for mulching purposes and to evaluate its biodegradability in the soil, comparing its performance with commercially available mulching films.

## 2. Materials and Methods

Recently, the exploitation of poultry manure by rearing larvae of a scavenging insect, the black soldier fly (BSF) *Hermetia illucens* (Diptera, Stratiomyidae), was proposed [26]. The larvae, after becoming prepupae, were fractionated to obtain proteins [27]. These proteins were investigated as base components for the rational design of biodegradable bioplastics, to be used for agricultural purposes [28], whereas the residual larval frass was used as a soil improver [26,29].

In the present study, for the preparation of the innovative protein-based film (PBF), BSF larvae were reared on an optimized mixture of chicken manure, water and zeolite [26], and the proteins were obtained after proper fractionation of the prepupae [27]. PBF was obtained by mixing 0.25 g of BSF proteins and glycerol (0.21 g) in distilled water (3.25 g), adjusted to pH 10 with NaOH (1 N) through a wet casting technique [30]. The obtained film had a thickness of 0.36 mm. For comparison, the following commercially available black mulching films were used: (a) a 0.02 mm thick biodegradable film made with the corn-starch-based Mater-Bi (MB) (Novamont S.p.A., Novara, Italy) and (b) a 0.05 mm thick non-biodegradable plastic film made with polyethylene (PE) (Lirsa, Ottaviano, Italy).

An assessment of the mulching properties of the films was performed in a short-term potting experiment, using a growth chamber (Binder KBW 720, Tuttlingen, Germany) located in the facilities of the University of Modena and Reggio Emilia, with programmed temperatures ranging from 25 to 19 °C (day/night), relative humidity ranging from 50 to 60% and a photoperiod 16:8 h L:D.

Plastic pots (diameter 10 cm and height 8 cm, 0.4 L) were filled with soil (sand 33.8%, silt 33.3%, clay 27.9%; pH 8.20, EC 0.16 dS m<sup>-1</sup>, N 1.9 g kg<sup>-1</sup>, P 44.0 mg kg<sup>-1</sup>, K 328.4 mg kg<sup>-1</sup>, organic carbon 15.0 g kg<sup>-1</sup>) collected from Rio Saliceto, Reggio Emilia, Italy (44°48′34.9″N 10°46′40.3″E).

The mulching properties were evaluated after covering the tops of the pots with disks (5.5 cm diameter) of the investigated films. An uncovered pot (bare soil) was used as control treatment (CTRL). Each pot was placed in a pot saucer and arranged in a completely randomized design with five replicates for each type of film.

Pots were manually irrigated (adding tap water into the pot saucer) every 2 days up to soil water capacity while recording evaporated water and film degradability. Evaporated water was controlled gravimetrically, weighing the pots every 2 days. After 10 days (T1) of mulching, PBF showed a faster degradability compared to the other films; therefore, at this time, the biodegraded disk area was measured for all the films using ImageJ software [31]. Disk thickness was measured using a caliper, and disc weight was also recorded.

Subsequently, each film was buried 5 cm from the surface, and film degradability was measured by weighing the films after 12 days (T2).

In addition, microbiological tests were carried out on soil contained in each pot at T1 and T2. The total fungi were assessed by counting the fungi on potato dextrose agar (PDA, Oxoid) at pH 6, supplemented with 150 mg L<sup>-1</sup> of nalidixic acid and 150 mg L<sup>-1</sup> of streptomycin [32]. The presence of *Clostridia* spp. was evaluated by mixing 10 g of each sample with 90 mL of peptone physiological solution in a sterile blender bag. The samples used for the determination of *Clostridia* spp. were thermally pretreated at 95 °C for 10 min to activate the spores. After an appropriate dilution, the plates were incubated at 30 °C for 48 h in an anaerobic environment. The medium used for the enumeration of spore-forming content was *Clostridium* reinforced agar (CM0149, Oxoid). The aerobic mesophilic bacteria were counted on selective medium (glucose, 1 g L<sup>-1</sup>; proteose peptone, 3 g L<sup>-1</sup>; yeast extract, 1 g L<sup>-1</sup>; potassium phosphate (K<sub>2</sub>PO<sub>4</sub>) buffer, 1 g L<sup>-1</sup>; agar, 15 g L<sup>-1</sup>) supplemented with actidione at 100 mg L<sup>-1</sup> [29]. Every sample was plated twice, and the test was repeated five times. Population densities were reported as log colony-forming unit (log CFU) g<sup>-1</sup> dry weight of soil.

The recorded data were analyzed using GenStat 17th edition software (VSN International, Hemel Hempstead, UK) for analysis of variance (ANOVA). Means were compared using Duncan's test at  $p < 0.05$ .

### 3. Results and Discussion

In the present study, a circular economic strategy was implemented to improve sustainability in agriculture. The mulching performance and biodegradability of an innovative bioplastic obtained from BSF prepupae reared on chicken manure [26] was assessed in a short potting experiment and compared with mulching films available on the market, based on polyethylene and corn starch (Figure 1).



**Figure 1.** Representative films used in the experiment (left to right): Mater-Bi, polyethylene, the innovative protein-based film.

The most important aspect of the mulching practice is the preservation of soil humidity. According the results on the amount of water evaporated from the soil (Table 1), PBF showed comparable values to MB and values that were 20% and 47% lower than those of PE and bare soil, respectively. Our data are in accordance with those available in the literature. In general, the most pronounced mulching effects reported in the literature concern water savings of up to 25% [33,34]. This characteristic is very important, especially in the cultivation of crops that require a great amount of irrigation, like tomato [35–38].

**Table 1.** The main characteristics of the assessed films during the pot experiment.

Parameter	CTRL	MB	PBF	PE
Area, T0 (cm <sup>2</sup> )	-	23.7 ± 0.02	23.7 ± 0.05	23.7 ± 0.03
Area, T1 (cm <sup>2</sup> )	-	23.7 ± 0.02 b	19.3 ± 0.04 a	23.7 ± 0.03 b
Evaporated water (g day <sup>-1</sup> pot <sup>-1</sup> )	29.1 ± 0.14 a	12.5 ± 0.11 cd	15.1 ± 0.09 c	18.9 ± 0.12 b
Fresh weight, T0 (g)	-	0.07 ± 0.01 c	0.84 ± 0.03 a	0.13 ± 0.02 b
Thickness, T0 (mm)	-	0.02 ± 0.003 c	0.36 ± 0.011 a	0.05 ± 0.006 b
Fresh weight, T1 (g)	-	0.07 ± 0.01 c	0.67 ± 0.04 a	0.13 ± 0.02 b
Thickness, T1 (mm)	-	0.02 ± 0.003 c	0.35 ± 0.009 a	0.05 ± 0.006 b
Fresh weight, T2 (g)	-	0.07 ± 0.01 c	0.30 ± 0.05 a	0.13 ± 0.02 b

CTRL = bare soil; MB = Mater-Bi; PBF = protein-based film from black soldier fly prepupae; PE = polyethylene. T0 = parameters recorded after 10 days of mulching. T1 = parameters recorded after burying. Data are presented as mean ± standard deviation. Means followed by the same letter do not significantly differ at  $p < 0.05$ . The statistical differences were tested only between the different treatments at each time point.

Considering the biodegradability, after ten days of mulching, the greatest degradation of the surface area was reported for PBF (~19%). The disk fresh weight and disc thickness of PBF were reduced by ~21% and ~4%, respectively, while MB and PE did not show any changes (Table 1 and Figure 2).

The same trend was also observed after the burying period. In fact, PBF was almost completely degraded, while MB and PE did not display any changes. Due to the high degradability of the PBF, only the fresh weight parameter of the remaining undegraded pieces was recorded (Table 1 and Figure 3). After being buried for 12 days, PBF showed a reduction of fresh weight of ~14%.



**Figure 2.** Representative films after ten days of mulching (left to right): Mater-Bi, polyethylene, the innovative protein-based film.



**Figure 3.** Representative films after being buried for twelve days (left to right): Mater-Bi, polyethylene, the innovative protein-based film.

The faster biodegradability of the PBF disks was probably due to its completely organic composition, as already reported by Barbi et al. [30], showing a high biodegradability of the material when completely submerged in tap water.

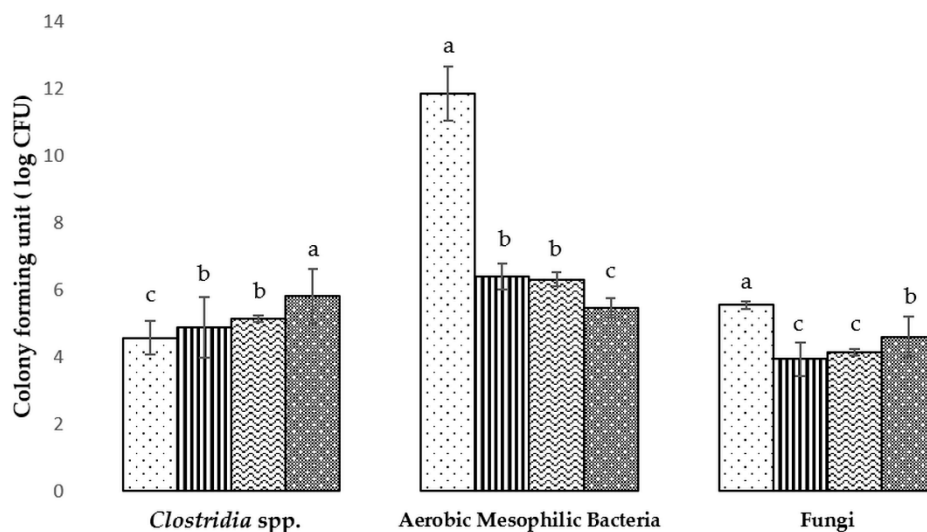
According to the literature, the main causes of mulching film degradation are photodegradation, oxidation and biodegradation. Biodegradation is performed by the action of natural microorganisms such as bacteria, fungi and algae that mineralize organic matter into carbon dioxide or methane and water [10]. These aspects should be verified on PBF in specific additional experiments.





The presence of water substantially influences the growth and development of the soil microbiota, and hence mulching, by altering this property, could also affect the quantity of the soil microbial population [3].

In the present study, the effect of the investigated films on soil microbiological contents was measured both at the end of mulching (T1) and after burying (T2).

As reported in Figure 4, all investigated films contributed to increase the *Clostridia* spp. content, with the highest value recorded using PE. Considering the mesophilic aerobic content, all assessed films led to a reduction of 5 orders of magnitude, and the lowest value was recorded by PE. Similarly to mesophilic aerobic content, a reduction in total fungi content was observed for all investigated films. PBF and MB behaved in the same way, decreasing the content by 1.5 orders of magnitude, while there was a smaller reduction in the case of PE. These data are in agreement with information available in the literature. In fact, mulching can alter soil–air gas exchange, leading to the formation of an anaerobic environment that can strongly alter the composition of the soil microbiota [39]. In addition, traditional plastic films reduce soil porosity and therefore air circulation, modifying microbial communities and reducing soil fertility [40].





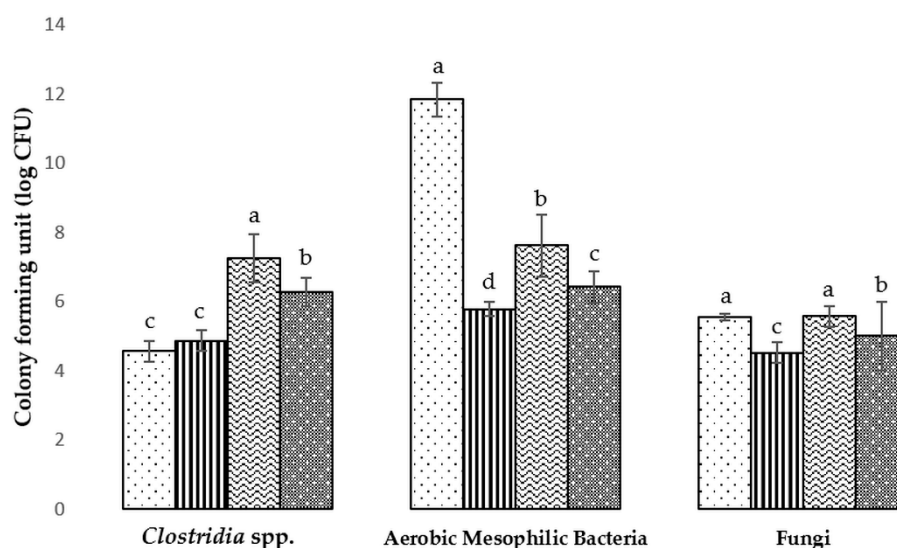
**Figure 4.** Main microbiological parameters assessed after 10 days of mulching. Bare soil = ; Mater-Bi = ; protein-based film from black soldier fly prepupae = ; polyethylene = . Whiskers indicate the standard deviation interval, while bars with the same letter do not significantly differ at  $p < 0.05$ . The statistical differences were tested only between the different treatments at each time point.





After the mulching period, the assessed films were buried to investigate their effect on the soil microbiological contents. As shown in Figure 5, different results for the *Clostridia* spp. content were obtained for the three investigated films when buried. PBF led to an increase of 2 orders of magnitude compared to bare soil, while MB and PE maintained the content recorded at the time of mulching. Analyzing the variation of the mesophilic aerobic content, the treatment with PBF led to an increase of 1 order of magnitude compared to bare soil, while MB and PE did not lead to any change in the aerobic microbial population compared to the content recorded at mulching time. Observing the variation in CFU of the total fungi, the highest values were recorded by bare soil and PBF. Compared to values recorded at mulching time, all films showed an increased fungi population. In particular, PBF led to an increase of 1.5 orders of magnitude, and an increase of 0.5 order of magnitude was recorded for MB.

In general, when biodegradable films are buried, an increase of the microbiological content is observed. Bacterial populations increase under organic mulch due to the different chemical composition and decomposition rates of these materials [41]. PBF caused a significant increase of *Clostridia* spp. and mesophilic aerobic bacteria contents and, in a less relevant way, also increased the content of fungi. These changes were probably due to the faster deterioration of the protein-based bioplastic in the soil, which caused the release of carbon and nitrogen compounds that ultimately fed and increased the soil microbial populations. This is in accordance with the findings of Kasirajan and Ngouajio [10], who reported that biodegradation performed by microorganisms such as bacteria and fungi is one of the main causes of degradation of mulching films. In addition, when organic mulch is decomposed by microbes, an increase of the soil carbon sequestration is recorded [42]. However, further studies are needed to verify this effect using the innovative biodegradable film investigated in the present study. Moreover, additional research is required to reduce the PBF biodegradability, allowing its use for the production of the most important mulched crops.

Insects like BSF already proved to be a useful tool for a circular economy in agro-food contexts by efficiently converting the biowaste into a biomass rich in proteins, lipids and chitin [43,44]. The residual frass after BSF larvae rearing can be used as a soil improver with compost-like properties [26,29] that is suitable for fertilizing horticultural crops and comparable with the traditional compost obtained by valorizing agro-industrial by-products [45,46]. Here, we report for the first time the valorization of BSF prepupae proteins for the development of innovative biodegradable protein-based films that can be

used for mulching purposes and can also increase the nitrogen content in the soil, adding value in the process to increase the agricultural sustainability.



**Figure 5.** Main microbiological parameters assessed after film was buried for 12 days. Bare soil = ; Mater-Bi = ; protein-based film from black soldier fly prepupae = ; polyethylene = . Whiskers indicate the standard deviation interval, while bars with the same letter do not significantly differ at  $p < 0.05$ . The statistical differences were tested only between the different treatments at each time point.

#### 4. Conclusions

Our study showed that it is possible to use the protein fraction of BSF prepupae as an organic-based material to produce a biodegradable film for mulching purposes. This innovative film can be a viable option, especially when the converted biomass (prepupae) cannot be used in livestock farming as a feed resource for regulatory reasons. The BSF-larvae-mediated bioconversion of agro-industrial by-products and organic waste to high-value-added material like proteins, fats and chitin is considered an interesting option. BSF can provide useful solutions to increase the agricultural sustainability, offering compost and bioplastic materials that can be used as fertilizer and mulch, respectively. This presents a valid solution that is especially useful for organic farming systems where synthetic products are not allowed. However, further studies are required to improve the properties of the PBF investigated in this study in order to reduce both its biodegradability and thickness when used for mulching purposes.

**Author Contributions:** Conceptualization, D.R.; methodology, L.S. and A.P.; software, D.R.; formal analysis, D.R.; investigation, L.S., A.P., R.D.L., S.M., E.F., L.M., L.I.M., M.M. and S.B.; resources, E.F. and L.M.; data curation, D.R.; writing—original draft preparation, D.R.; writing—review and editing, D.R., L.S., E.F., A.P. and L.M.; funding acquisition, L.M. and E.F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the framework of the project “ValoriBio” co-financed by 2014–2020 POR FESR, Emilia-Romagna Region, Italy, DGR 774/2015—CUP E42I15000110009.

**Acknowledgments:** The authors acknowledge all the staff and students of University of Modena and Reggio Emilia, who generously collaborated with the collection of the data.

**Conflicts of Interest:** The authors declare no conflict of interest.

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