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# *Hermetia illucens* (L.) larvae as chicken manure management tool for circular economy

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## ABSTRACT

The increased request for poultry meat and eggs of a rising human population requires more efficient and cleaner methods to manage increasing quantities of chicken manure. The black soldier fly *Hermetia illucens* is known as an efficient bio-converter of organic waste in proteins and fats, with the advantage that the larval frass is supposed to have compost-like properties. In the view to identify the operating conditions for the sustainable management and valorization of livestock waste at a pre-industrial scale, this study is aimed at: i) optimizing the growth of *H. illucens* on a mixture of chicken manure, chabazite and water; ii) assessing the soil amendment properties of the larval frass obtained from the optimized mixture. Preliminary trials allowed defining the basic rearing conditions in terms of temperature and substrate components. A mixture design based on a special cubic model allowed identifying the best mixture for *H. illucens* larvae growth, which consists in 34.5% chicken manure, 58.3% water and 7.2% coarse chabazite. This mix led to about 86% of alive prepupae weighting 90 mg on average, and to a reduction of the initial substrate amount by more than 75%. The larval frass obtained from this mixture showed soil improver properties, suggesting its use to supply the common peat based growing media for potted baby-leaf lettuce production. Overall, *H. illucens* larvae have proved to be a useful tool to favor a more sustainable management of chicken manure by strongly reducing its amount and closing its recovery cycle obtaining high value products for agricultural purposes.

## 1. Introduction

Chicken manure is rich in organic matter (OM), nutrients and pathogens. This substrate has a low C/N ratio, high humidity content, phytotoxic compounds, and an unpleasant odor that could become a problem during the long-period-composting process. The increase of poultry production due to an increasing demand for meat and eggs, results in increased problems with management of chicken manure (Xiao et al., 2018). Chicken manure has traditionally been used as soil improver. However, over-application of this material can lead to eutrophication of water bodies, spread of pathogens, phytotoxicity, air pollution and emission of greenhouse gases (Abouelenien et al., 2010). Developing efficient methods to manage chicken manure is therefore crucially important to prevent it from becoming a serious pollutant source (Nie et al., 2015).

Zeolites are aluminium-silicates, with alkali metals, occurring as secondary minerals in diagenized volcanic rocks; thanks to their high and selective cation exchange capacity (CEC), zeolitized tuff with a medium-high content of zeolites such as chabazite, phillipsite, and clinoptilolite are commonly used as molecular sieve.

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**Abbreviations**

| | |
|---|---|
| AER | adults emerging ratio |
| Anth | anthocyanins |
| BSF | Black Soldier Fly |
| BSFL | BSF larvae |
| BSFLF | BSF larval frass |
| CEC | cation exchange capacity |
| Chl | chlorophyll content |
| CP | commercial peat |
| D | diameter |
| DUD | days until larvae death |
| EC | electric conductivity |
| Flav | flavonols |
| FTR | fraction of biomass to root |
| FWC | field water capacity |
| G | soil improver from pruning shears of urban green |
| GI | germination index |
| GM | growing media |
| H | plant height |
| H/D | height-to-stem diameter ratio |
| HI | Harvest index |
| LA | leaf area |
| LAR | leaf area ratio |
| LD | larval density |
| LF | larval frass |
| M | chicken manure |
| Mortality | percentage of larvae mortality |
| MWD | days necessary to obtain the maximum average weight of prepupae |
| NBI | chlorophyll to flavonol ratios |
| OM | organic matter |
| PCA | Principal Component Analysis |
| PM | peat moss |
| PPD | days necessary to obtain the maximum number of prepupae |
| Pprep | percentage of insects individuals that have reached the prepupae stage |
| Pupae | percentage of pupae |
| Puparia | percentage of puparia |
| RCBD | randomised complete block design |
| RDW | root dry weights |
| RFW | roots fresh weights |
| RH | relative humidity |
| SF | solid fertilizer |
| SFW | shoots fresh weights |
| SLA | specific leaf area |
| SPAD | index of chlorophyll concentration |
| SWD | shoot dry weights |
| SWL | percentage weight loss of the substrate |
| TDW | total dry weights |
| TFW | total fresh weights |
| VE | vermiculite |
| W | water |
| Z | chabazite |

The addition of zeolites to chicken litter allows a considerable adsorption of ammonium, thereby decreasing its pollutant effect (Kithome et al., 1999) and nasty odor. Another common approach to manage chicken manure is methane fermentation via biogas recycle (Abouelenien et al., 2010).

Inadequate recycling process of organic waste often causes water pollution or loss of potential crop fertilizers (Lalander et al., 2015). Bioconversion through insects that use manure and other types of organic waste as food source might represent a valuable alternative. In particular, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), also known as Black Soldier Fly (BSF), is able to convert manure in a biomass rich in proteins and fat, in ranges between 32 and 43% and 33% on a dry base, respectively (Gold et al., 2018). Besides reducing considerably the initial waste volume, BSF larvae (BSFL) significantly decrease the levels of unpleasant odors in livestock manure (Beskin et al., 2018), lower the proliferation of pathogens by producing antimicrobial peptides (Erickson et al., 2004; Liu et al., 2008), and prevent house flies development (Bradley and Sheppard, 1984). BSF adults are non synanthropic flies and are not vectors of diseases (Wang and Shelomi, 2017).

Closed-loop agri-food supply chains have a high potential to reduce environmental and economic costs resulting from waste disposal (Borrello et al., 2016). Valorization of manure using insects represents a sustainable solution that harmonize with circular economy (Rumpold et al., 2017). Furthermore, especially in Europe, there are several challenges about soil management and nutrient supply (Tittarelli et al., 2016), thus the approach on crop production is changing, both in protected and in soilless production (Pignata et al., 2017). The use of BSFL maximizes the reduction of waste leading to the production of insect biomass, whose fractions (proteins, fat, and chitin) are useful for many industrial purposes. Moreover, the larval frass (LF) has useful soil improver's characteristics (Setti et al., 2019; Kebli and Sinaj, 2017). Being rich in macronutrients, micronutrients and OM, BSFL frass (BSFLF) could increase the OM content of intensively cultivated soils (Pan et al., 2010), partially replace chemical fertilizers and reduce the emissions of greenhouse gases (Favoino and Hogg, 2008).

The potential of BSFL for bioconversion of livestock manure is widely described (Diener et al., 2009; Lalander et al., 2015; Rehman et al., 2017; Zhou et al., 2013). This procedure is not applicable for food or feed production within the EU, where livestock can only be reared on certificated feed which does not include manure (Rumpold et al., 2017). Conversely, insect fractions can be considered for non-food applications. Due to BSFL high fat content, the potential and feasibility of rearing them on manure for biodiesel production was demonstrated (Li et al., 2011; Zheng et al., 2012b), whereas the use of the protein fraction was not considered (Rumpold et al., 2017).

Previous works investigated the feasibility of the use of BSFL to treat organic waste, focusing mainly on larval growth and survival rate, waste reduction index and feed conversion rate (Li et al., 2011; Newton et al., 2005; Oonincx et al., 2015; Xiao et al., 2018). This study is part of a project aimed at developing a circular economy strategy at a pre-industrial scale, based on the sustainable valorization of bio-waste using BSFL in a demonstrative plant to obtain high value products for agricultural uses. Specifically, the project focused on poultry manure and the BSF prepupae biomass was fractionated (Caligiani et al., 2018) to obtain proteins that were used for the production of biodegradable mulching sheets (Barbi et al., 2018), whereas LF was meant to be used as soil improver. The specific aim of this study was to acquire practical knowledge on the operative conditions to run a pilot plant with BSFL using as substrate the chicken manure from a zeolite-added chicken litter, and to demonstrate the full circularity of the process by showing the soil improver properties of the obtained LF on a horticultural crop, baby-leaf lettuce.

BSF are omnivorous, but growth and survival rate of the larvae are strictly dependent on substrate's composition and humidity (Cammack and Tomberlin, 2017; Cheng et al., 2017). Chicken manure proved to be a suitable substrate for BSFL (Erickson et al., 2004; Rehman et al., 2017; Xiao et al., 2018). In poultry farms, the addition of zeolite to chicken litter is a quite common practice to reduce the pollutant effects and nasty odors associated with chicken, however no data is available in literature on the biocompatibility of zeolite towards BSF larval growth *in vivo*. The specific aims of this work are to: i) achieve the operating conditions to rear BSFL in a pilot plant using as substrate chicken manure and chabazite (a type of zeolitic tuff), optimizing the insect biomass yield for further industrial applications, and ii) assess the agronomic use of the LF obtained from the optimal chabazite-added manure.

## 2. Materials and methods

### 2.1. Starting conditions and preliminary tests

Before planning the experimental design aimed at optimizing the production of BSF prepupae preliminary tests were conducted and the ingredients of the mixture to be used as substrate for larvae growth were characterized. Subsequently, an experimental set-up was planned according to Fig. 1, and described in detail in Section 2.1.3, 2.2, and 2.3.

### 2.1.1. Maintenance of BSF colony

Insects belonged to a colony reared in the laboratory of Applied Entomology of the University of Modena and Reggio Emilia (Italy) since 2016, established starting from larvae purchased from CIMI srl (Cuneo, Italy). The colony was maintained at 26 ± 0.5 °C, 60–70% relative humidity (RH), and a photoperiod 16:8 L:D h. Larvae were reared on Gainesville House Fly Diet consisting of 50% wheat bran, 30% alfalfa meal, and 20% corn (Hogsette, 1992), with the addition of equal volume of water, as suggested by Sheppard et al. (2002). Adults were kept in BugDorms® (BD4S3030, MegaView Science Co., Taiwan), and fed with sugar cubes and water.

### 2.1.2. Composition of substrate ingredients

Manure and chabazite-rich zeolitic tuff (hereafter named "chabazite") were characterized for the main chemical and physical features. Chemical analyses of manure were performed with a wavelength dispersive Philips PW 1480 X-ray fluorescence (XRF) spectrometer, using the methods from Franzini et al. (1975) and Leoni and Saitta (1976), on ashes obtained heating manure at 750 °C for 12 h. The measurements were carried out on pulverized ashes kindly ground in an agate mortar and after pressed at 12 tons to obtain tablets 30 mm in diameter. Manure weight loss at 105 and 750 °C was measured on the same samplings, as well. The results are reported in Table A1 (appendix); the comparison with literature data (Vassilev et al., 2010) for major elements concentration values did not highlight significant differences. Manure, after drying at 105 °C for 12 h was also characterized for N, C, and S content. The elemental analyses were performed using a FLASH 2000 Thermo-Fischer Scientific Elemental Analyser on finely ground powder obtained using a mechanical dry mill. The results are reported in Table A2 (appendix).

Chabazite is from the Piandirena quarry in Sorano (central Italy) and has already been extensively characterized in previous works (Faccini et al., 2015; Malferrari et al., 2013). However, in order to assess the concrete correspondence of the material, chemical composition (major elements) and CEC were once more measured through the methods reported in Malferrari et al. (2013). The results are shown in Table A3 (appendix) and highlight only minor

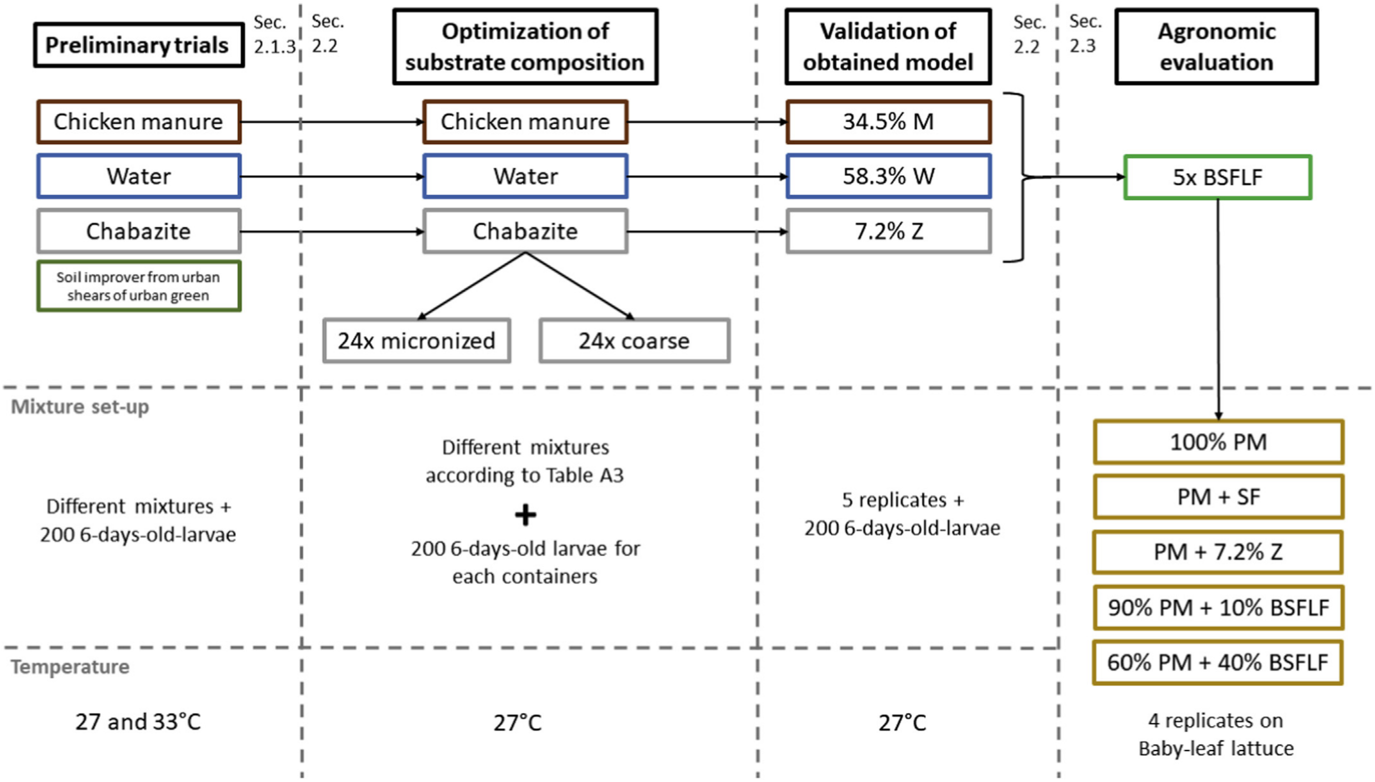

**Fig. 1.** Flow diagram of experimental set-up used in this research (Sec = detailed section of Materials and Methods; M = chicken manure; W = water; Z = coarse chabazite; BSFLF = black soldier fly larval frass; PM = peat moss; SF = solid fertilizer).

deviations with respect to data reported in the cited references that can be related to the variability between samplings taken in different times and locations of the quarry. In fact, these minor changes in chemical composition of chabazite were observed and discussed also in Malferrari et al. (2013).

#### 2.1.3. *Preliminary trials*

Several preliminary tests were conducted at 27 and 33 °C to determine a proper temperature for BSFL rearing. The substrate was composed of chicken manure (M), water (W), chabazite (Z) and soil improver from pruning shears of urban green (G).

Different parameters about rearing effectiveness were recorded: percentage of larvae mortality (Mortality), days until larvae death (DUD), percentage of insects become prepupae (Pprep), days to obtain the maximum number of prepupae (PPD), adults emerging ratio (AER) and percentage weight loss of the substrate (SWL).

Principal Component Analysis (PCA) was applied to the autoscaled data used as an exploratory data analysis tool, in a way to highlight informative trends for the experiments conducted in the different conditions and to recognize possible outlier experiments. The scree plot of the eigenvalues of the principal components (PCs) was used to choose the number of PCs to retain in the model. PCA analysis was performed using the PLS Toolbox (ver 8.1.1, Eigenvector Research Inc., USA) running in the MATLAB environment (ver. 7.12, The Mathworks Inc., USA).

### 2.2. *Optimization of substrate composition*

#### 2.2.1. *Design of experiments*

The composition of the substrate used for BSFL rearing was defined by means of an optimal mixture experimental design (Design Expert software Ver. 10, Stat-Ease Inc., USA) with three factors: chicken manure (M, range: 20–45% w/w), water (W, 50–70% w/w) and chabazite (Z, 5–25% w/w). The factors ranges were defined based on the results of the preliminary trials and on the information about the substrate ingredients composition. It has to be noted that the high amount of moisture in chicken manure forced us to consider a quantity of water greater than 50% in the mixtures (the quantity of water used to prepare the mixtures includes both the moisture of the manure and the added liquid water). Percentage of individuals become prepupae (Pprep, expressed in %) and maximum average weight of prepupae (Wprep, expressed in g) were considered for optimization.

A simplex-centroid mixture design with constraints, expanded with 5 replicate points and 3 additional center points, for a total of 24 experimental runs, was planned (Table A4, appendix). Since grain size can affect cation exchange reactions, the same design was repeated with two types of chabazite, i.e., coarse and micronized, with average particle size of 0–3 mm and less than 10 μm, respectively. The greater surface area of 10 μm chabazite makes it potentially more reactive to cation exchange reactions than coarse chabazite. Furthermore, the smaller size may favor interactions with larvae, including its ingestion.

The chosen design would allow to fit the following polynomial model:

$$y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12}\, x_1\, x_2 + \beta_{13}\, x_1\, x_3 + \beta_{23}\, x_2\, x_3 + \beta_{123} x_1 x_2 x_3 \qquad (1)$$

The statistical significance of model terms was verified by analysis of variance (ANOVA) and the variation sources were compared by using the Fisher distribution ($p < 0.05$).

The validity of the obtained model was evaluated performing a validation experiment with five replicates in correspondence of the predicted optimal point, determined by numerical optimization.

#### 2.2.2. *Experiment execution*

Tree-hundred-grams mixtures were placed in 21 × 13 × 6 cm glass containers according to the experimental design, for a total of 48 boxes (24 different mixtures containing coarse chabazite, and 24 containing micronized chabazite). Two hundred 6-days-old larvae were added to each container, which were arranged randomly on the different shelfs, into a climatic chamber at 27 ± 0.5 °C with 80–90% RH, in dark conditions. The first check was performed after 6 days to avoid larvae disturbance, and then with a frequency of three times per week. The prepupae observed at each check were manually collected, weighted, and then divided in two groups; one was frozen for further chemical analysis, and the other one was kept in a climatic chamber (27 ± 0.5 °C, 60–70% humidity, photoperiod 16:8 h L:D) to evaluate adults' emergence, survival rate and breeding performance. The experiment was completed when all the larvae became prepupae or died.

The validation test of obtained model was conducted as described above, and the best substrate was collected and preserved in fridge (4–5 °C) until agronomic analysis.

### 2.3. *BSFLF trial on lettuce*

#### 2.3.1. *Growing media characteristics*

Solid substrates used as growing media (GM) in this experiment had the following characteristics: peat moss (PM) technique®, Free Peat B.V. (The Netherlands) – organic C (23%), organic N (0.5%), OM (46%), pH 7.4, and EC 0.2 dS $m^{-1}$; BSFLF from the entomological experiment; chabazite, which characteristics were described by Malferrari et al. (2013); solid fertilizer (SF) NPK Origina Gold®, Compo, MB, Italy, at a concentration of 3.79 g $pot^{-1}$; a layer (2–3 mm) of vermiculite (VE) Saint-Gobain PPC S.p.a. (particle size 0.5–4.0 mm, pH 8.0, density 105 kg $m^{-3}$± 15%) was added on each pot. The field water capacity (FWC) was determined by the gravimetric method for each GM according to Young et al. (2014), with two replicates. pH and EC were determined on wet material (1:5 ratio) using a CRISON pH meter basic 20 and CRISON GLP 31 EC m, respectively, as suggested by Violante (2000).

#### 2.3.2. *Lettuce production and agronomic traits*

A baby-leaf lettuce (*Lactuca sativa* L.) Batavia blonde type cultivar 'Chiara' (ISI Sementi S.p.A., Fidenza, Italy), a fresh-cut lettuce with a medium-short growing cycle (20–25 days), was used to test the agronomic effectiveness of the BSFLF. The experiment was performed in a fitotron (University of Modena and Reggio Emilia, Reggio Emilia, Italy), under long-day conditions (16:8 h L:D; light intensity 180 μmol $m^{-2}s^{-1}$) at 65% of relative humidity. A randomized complete block design (RCBD) with four replicates (corresponding to four pots with 20 lettuce seeds each) *per* treatment was used for each experiment.

Five solid substrates were tested in total: GM1) 100% PM; GM2) PM + SF; GM3) PM + chabazite 7.2%; GM4) PM 90% + BSFLF 10%; GM5) PM 60% + BSFLF 40%. All proportions are expressed by v/v.

At the end of the crop cycle, the following agronomic traits were recorded: plant height (H), diameter (D), height-to-stem diameter ratio (H/D), shoots (SFW), roots (RFW), and total fresh weights (TFW). For the physiological traits, chlorophyll (Chl), flavonols (Flav), anthocyanins (Anth) and chlorophyll to flavonols ratios (NBI), were measured by Force-A Dualex Scientific+™ (FORCE-A, Orsay, France). Shoot (SDW), root (RDW) and total dry weights (TDW) were measured after 65 °C desiccation, up to the reaching of a stable mass weight. Harvest index (HI), fraction of biomass to root (FTR), as the TDW/RDW and SDW/H ratios were estimated. Specific leaf area (SLA), expressed as the ratio between the leaf area (LA) and SDW, and leaf area ratio (LAR), expressed as the ratio between LA and TDW, were also investigated.

### 2.3.3. Germination Index test

Germination index test was performed according to Zucconi et al. (1981), for each GM and the BSFLF to evaluate the lettuce's germination rate. The water extracts of each GM were obtained by stirring the GM for 3 h at the concentration of 50 g $L^{-1}$. Then, 4 ml of each water extract, plus a water control, were added to Petri dishes containing Whatman filter paper (Ø 90 mm). Three replicates (20 seeds *per* plates) were prepared, and the plates were incubated 36 h at 25 °C in a Binder ED53, Tuttlingen (Germany) stove. In order to calculate the Germination Index (GI) the following formula was used, according to Tiquia and Tam (1998):

$$GI\% = 100 \times (Gt/Gc) \times (Rt/Rc) \quad (2)$$

where.

$G_t$ = number of germinated seeds of the GM extract;
$G_c$ = number of germinated seeds of the control;
$R_t$ = average root length (mm) of the GM extract;
$R_c$ = average root length (mm) of the control.

The same experiment was repeated for garden cress (*Lepidium sativum* L.), a control crop for the BSFLF phytotoxicity test's.

### 2.4. Agronomic data analysis

GenStat 17.0th edition software was used to perform a one-way ANOVA on the agronomic data. Factors' means were compared using Duncan's multiple range test at $p$-value < 0.05 level.

## 3. Results

### 3.1. Results from preliminary trials

PCA performed on the dataset acquired during the preliminary trials stage, which included experiments conducted at different temperatures and with different substrate compositions, suggested to carry out the subsequent experiments at 27 °C (Figure A1, appendix).

PCA was then repeated on 27 °C dataset including the substrate composition variables (Figure A2, appendix). Among other outcomes, PCA evidenced that soil improver increases the larvae mortality and reduces the substrate weight loss. Thus, soil improver was definitively removed from the substrate composition in the subsequent experimental design.

### 3.2. Mixture design results

Initially, many parameters indicative of the rearing trend were recorded for thoroughness. In addition to Mortality, PPD, AER and SWL, also the number of days necessary to obtain the maximum average weight of prepupae (MWD), percentage of pupae (Pupae), and percentage of puparia (Puparia) were recorded.

PCA performed on all data obtained using both coarse and micronized chabazite suggested that the chabazite grain sizes influences insect development, although the effect of the substrate composition is predominant (Figure A3, appendix). Since micronized chabazite slows down the larvae development, we decided to consider the results of the experimental design conducted just with coarse chabazite.

### 3.2.1. Model for Pprep response variable

An inverse transformation was applied to the Pprep response, as suggested by the Box-Cox plot. In Table A5 (appendix) the ANOVA results of the fitted quadratic model are shown. Just the model terms retained as significant are reported ($p$-values < 0.05: significant terms; $p$-values > 0.10: insignificant terms). The model is highly significant, while lack of fit is not significant indicating that the fitted model is adequate for prediction purposes. The coefficient of determination (R-squared = 0.91), the adjusted coefficient of determination (Adj R-squared = 0.88, which considers the number of predictors in the model) and the coefficient of determination in prediction (Pred R-squared = 0.81) of the model are reported, as well. These values indicate that the model is good, and it should have predictive capability. The quadratic model equation, expressed in actual factors, resulted to be:

$$1/(Pprep) = +0.083002*W + 0.15274*M + 0.81958*Z - 0.38496*W*M - 1.13666*W*Z - 0.83979*M*Z \quad (3)$$

In Equation (3) all the coefficients of the pure components are positive and chabazite has the largest coefficient, followed by that of manure and water. Since the response is inverted, to maximize the percentage of prepupae, the mass fraction of chabazite relative to manure and water should be decreased. Concerning the interaction terms, they all have an antagonistic effect on 1/(Prep).

In Fig. 2, the response surface plot for Pprep is represented: the maximum percentage of prepupae is obtained in the domain region where the amount of chicken manure is high, the amount of water is quite high and the amount of chabazite is low.

### 3.2.2. Model for Wprep response variable

In this case, the preliminary inspection of the model results suggested to not transform the response Wprep. In the bottom part of Table A5 (appendix), the ANOVA results of the significant model

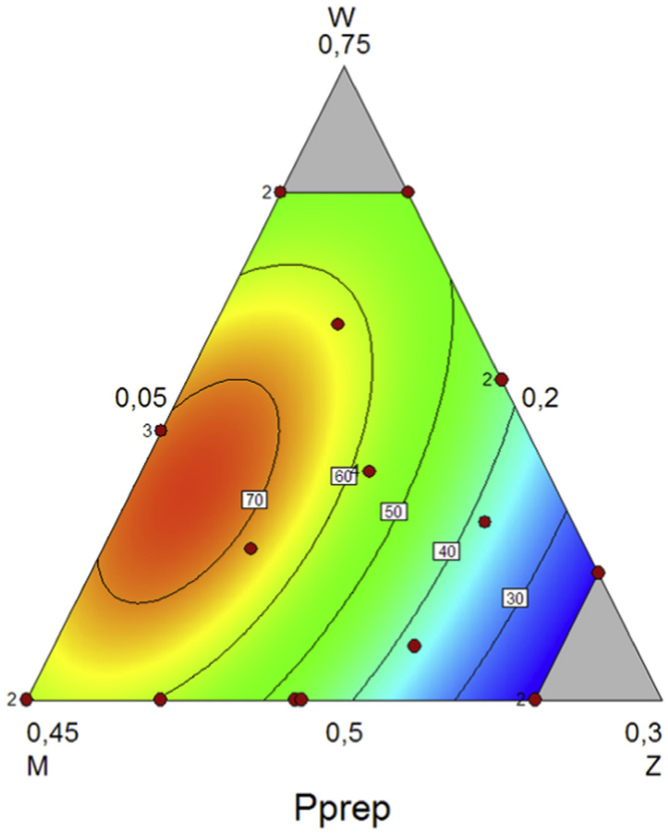

**Fig. 2.** Response surface plot for Pprep. Red points correspond to the design points. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

terms, together with the model performance parameters, are reported. Although the quadratic model is significant and the lack of fit is not significant, as would be desirable, the model performance is poorer with respect to the Pprep model (R-squared = 0.66, Adj R-squared = 0.60, Pred R-squared = 0.50).

The model equation in actual factor is the following:

$$\mathrm{Wprep} = +\,0.046148{*}W + 0.10400{*}M - 0.20494{*}Z + 0.48377{*}W{*}Z \tag{4}$$

Linear mixture and just W*Z two-factor interaction have effect on the weight of prepupae. The quadratic term is the most influential on prepupae weight with a synergistic effect; concerning linear mixture, the increasing of chabazite influences the production of low-weight prepupae, whereas both chicken manure and water have positive effect on the increase of prepupae weight.

Fig. 3 reports the response surface plot for Wprep: the domain region where the response is maximized correspond to high values of chicken manure and minimum values of chabazite.

### 3.2.3. Model validation with optimized conditions

Considering the results of the models from the two responses, we decided to optimize only the percentage of prepupae as the model for Pprep showed better performance, resulting in greater predictive power.

Numerical optimization has been used to find out the mixture in the domain having the maximum Pprep value. The following mixture gives the best solution: M = 34.5%, W = 58.3%, Z = 7.2%.

The replicates of the validation experiment were elaborated by T-test (prob 95%, n = 5). The comparison of the replicates' mean with the expected value based on the model confirmed the model validation. In fact, as shown in Table A6 (appendix), the obtained mean is within the confidence interval.

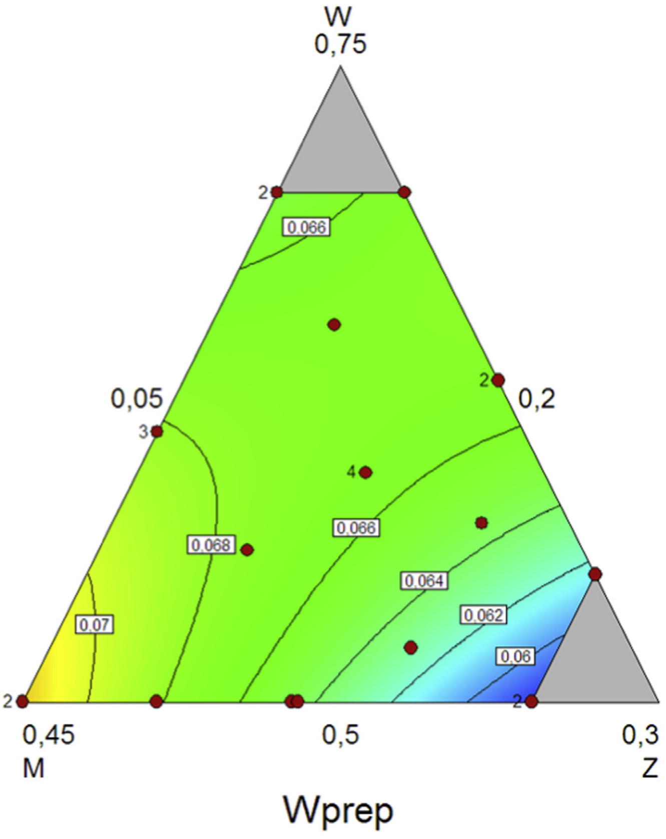

**Fig. 3.** Response surface plot for Wprep. Red points correspond to the design points. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

It must be reported that, for the same experiment, a value of Wprep = 0.090 ± 0.010 g was obtained, which is remarkably high.

### 3.3. Modification of substrates

The reduction of substrate after BSFL growth was 75.68 ± 2.82% (mean ± SD). To assess whether the substrate, after rearing BSFL, could be used as a fertilizer, LF was characterized for the parameters reported in Table A7 (appendix) to comply with the main regulations for compost. The results indicated that the material underwent a substantial depletion of carbon and nitrogen but maintained features that make it suitable as a soil improver. Moreover, the presence of chabazite undoubtedly represents a benefit both for plant growth and, more generally, for the overall quality of soil. The pH and EC showed a value of 8.51 and 8.26 dS $m^{-1}$, respectively. The results of the phytotoxicity test showed values of 140.12% and 83.52% for roots and shoots, respectively. The total N was 2.66%, while the C/N ratio was 11.7.

### 3.4. Agronomic evaluation of BSFLF

The characteristics of GM are described in Table A8 (appendix). The pH ranged between 7.5 (GM3) and 8.5 (GM5), while the EC ranged between 1.18 dS $m^{-1}$ (GM3) and 2.56 dS $m^{-1}$ (GM5). The values of FWC ranged between 84.33% (GM5) and 87% (GM1, GM2).

The biometrical and physiological results are reported in Table 1. Regarding the biometric traits, GM2 showed the highest D value (0.60 cm), 50% higher than the average. As a result, GM2 showed the lowest H/D value. GM1 and GM2 showed the highest value even for the emergence, with a value of 98% and 97%, respectively. Regarding the GI, the GM5 showed the highest value (184.70%). Considering the physiological traits, GM2 showed the highest values of both Chl (21.98) and NBI (20.67), 26.98% and 26.04% above the average, respectively.

The agronomical results are reported in Table 2. The highest values of SFW were recorded for GM1 (23.50 g $pot^{-1}$), GM2 (31.34 g $pot^{-1}$), GM3 (24.25 g $pot^{-1}$) and GM4 (24.63 g $pot^{-1}$). Regarding TFW, GM2 (34.46 g $pot^{-1}$) and GM4 (27.26 g $pot^{-1}$) showed the highest values.

## 4. Discussion

This work presents quantitative data useful for the successful exploitation of BSFL at a pre-industrial scale for a cleaner and more sustainable management of livestock waste, which ends up with the development of high value products for agricultural uses. Specifically, this investigation is part of a bigger project aimed at maximizing the BSF larval growth on chicken manure and chabazite for industrial-agronomic scopes, using the proteins from BSF prepupae for the development of biodegradable mulching sheets (Barbi et al., 2018) and the residual larval frass as soil improver. These objectives fit in a circular economy system, due to a full and profitable valorization of the chicken manure and to the absence of residual waste at the end of the cycle, because both the biodegradable mulching sheets and the larval frass finally feed back into the system as soil improvers to increase nutrients availability (especially nitrogen) for the crops.

Considering the optimization of the BSFL rearing, the larval density (LD) in these experiments was 0.7 larvae/$cm^2$, slightly lower than the LD of 1–2 larvae/$cm^2$ suggested by Parra Paz and collaborators (2015), and was selected to favor the best development of larvae avoiding competition for the food source. The

**Table 1**

**Main biometrical (a) and physiological (b) investigated traits.** The lowercase letters indicate significant difference at $p < 0.05$ according to Duncan's test. n.s. = not significant. GI = Germination index; H = Height; D = diameter; H/D = height/diameter ratio; Chl = Chlorophyll; Flav = Flavonols; Anth = Anthocyanins; NBI = Nitrogen Balance Index; SLA = Specific Leaf Area; LAR = Leaf Area Ratio.

A) Biometrical traits

| Treatment | GI (%) | | Rate of emergence (−) | | Emergence (%) | | H (cm) | | D (mm) | | H/D (−) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GM1 | 109.90 | c | 3.89 | n.s. | **98.00** | **a** | 11.94 | n.s. | 0.40 | b | **31.30** | **a** |
| GM2 | 139.1 | bc | 3.74 | n.s. | 74.50 | c | 11.55 | n.s. | **0.60** | **a** | 19.33 | b |
| GM3 | 139.1 | bc | 2.74 | n.s. | 89.00 | b | 1.75 | n.s. | 0.30 | b | **39.90** | **a** |
| GM4 | 168.60 | ab | 3.11 | n.s. | **97.00** | **a** | 12.00 | n.s. | 0.38 | b | **32.00** | **a** |
| GM5 | **184.70** | **a** | 3.19 | n.s. | 93.00 | ab | 11.19 | n.s. | 0.30 | b | **38.30** | **a** |
| Average | 148.28 | | 3.33 | | 90.30 | | 11.69 | | 0.40 | | 32.17 | |

B) Physiological traits

| Treatment | Chl | | Flav | | Anth | | NBI | | LA ($cm^{-2}$) | | SLA ($cm^2\ g^{-1}$) | | LAR ($cm^2\ g^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GM1 | 18.40 | ab | 1.09 | n.s. | 0.66 | n.s. | 16.86 | b | 1162.60 | n.s. | 1369.00 | n.s. | 1100.00 | n.s. |
| GM2 | **21.98** | **a** | 1.06 | n.s. | 0.65 | n.s. | **20.67** | **a** | 979.00 | n.s. | 841.00 | n.s. | 698.00 | n.s. |
| GM3 | 14.11 | b | 1.02 | n.s. | 0.65 | n.s. | 13.80 | b | 1192.10 | n.s. | 1587.00 | n.s. | 1236.00 | n.s. |
| GM4 | 16.13 | b | 1.05 | n.s. | 0.62 | n.s. | 15.44 | b | 1222.60 | n.s. | 1537.00 | n.s. | 1213.00 | n.s. |
| GM5 | 15.91 | b | 1.05 | n.s. | 0.62 | n.s. | 15.21 | b | 813.40 | n.s. | 1506.00 | n.s. | 1223.00 | n.s. |
| Average | 17.31 | | 1.05 | | 0.64 | | 16.40 | | 1073.94 | | 1368.00 | | 1094.00 | |

**Table 2**

**Main agronomic investigated traits.** The lowercase letters indicate significant difference at $p < 0.05$ according to Duncan's test. n.s. = not significant. SFW = Shoots Fresh Weight; RFW = Root Fresh Weight; TFW = Total Fresh Weight; SDW = Shoots Dry Weight; RDW = Root Dry Weight; TDW = Total Dry Weight; HI = Harvest Index.

| Treatment | SFW ($g\ pot^{-1}$) | | RFW ($g\ pot^{-1}$) | | TFW ($g\ pot^{-1}$) | | SDW ($g\ pot^{-1}$) | | RDW ($g\ pot^{-1}$) | | TDW ($g\ pot^{-1}$) | | HI | | SDW/H ($g\ cm^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GM1 | **23.50** | **a** | 2.17 | n.s. | 25.67 | ab | 0.93 | n.s. | 0.21 | n.s. | 1.14 | n.s. | 0.81 | n.s. | 0.08 | n.s. |
| GM2 | **31.34** | **a** | 3.11 | n.s. | **34.46** | **a** | 1.13 | n.s. | 0.23 | n.s. | 1.36 | n.s. | 0.83 | n.s. | 0.12 | n.s. |
| GM3 | **24.25** | **a** | 2.10 | n.s. | 26.36 | ab | 0.80 | n.s. | 0.21 | n.s. | 1.01 | n.s. | 0.79 | n.s. | 0.07 | n.s. |
| GM4 | **24.63** | **a** | 2.67 | n.s. | **27.26** | **a** | 0.91 | n.s. | 0.20 | n.s. | 1.11 | n.s. | 0.81 | n.s. | 0.08 | n.s. |
| GM5 | 15.28 | b | 1.61 | n.s. | 16.89 | b | 0.61 | n.s. | 0.15 | n.s. | 0.75 | n.s. | 0.81 | n.s. | 0.05 | n.s. |
| Average | 23.80 | | 2.33 | | 26.13 | | 0.87 | | 0.20 | | 1.07 | | 0.81 | | 0.08 | |

growth data of *H. illucens* obtained in this study are sparsely comparable to other published works using chicken manure as substrate, due to the different conceptualization or experimental conditions used (Oonincx et al., 2015; Rehman et al., 2017; Xiao et al., 2018). The high percentage of larval mortality in the preliminary trials could be attributed to the high content of lignin, a molecule that is not completely digested by BSFL. Rehman et al. (2017) observed a reduction of cellulose, hemicellulose and lignin in chicken manure treated with BSFL, although the decrease of lignin was lower than the other two polymers. The degradation of these polymers by BSFL was previously recorded also for rice straw, corncob (Li et al., 2015; Zheng et al., 2012a), and dairy manure (Li et al., 2011) and the role of gut microbiome symbioses is crucial to hydrolyze these molecules, helping the insects in the digestion process (Jeon et al., 2011; Lee et al., 2014). Likewise, the presence of microorganisms inside the substrate can help larvae in the decomposition process of waste, producing enzymes that cleave the different fibers (Douglas, 2010), thus making products of microbial activity available for larval growth. The soil improver from pruning shears of urban green proved to be a very unsuitable substrate for BSFL growth. Considering that it is extremely rich in fibers and it was already partly processed by microorganisms, the availability of nutrients was probably too scarce and unbalanced to guarantee BSFL growth. As recently demonstrated, the amount of non-fiber carbohydrates in the digestate after microorganism process is almost absent, and BSFL grown on biogas digestate have a lower yield compared to those grown on chicken feeds, vegetables or restaurant waste (Spranghers et al., 2017).

The application of chabazite on chicken litter reduces the emission of ammonia from the manure and has beneficial effects, such as the decrease of the salinity content of the final product, and a positive influence on chicken performance (Karamanlis et al., 2008; Li et al., 2008; Turan, 2008). In the second part of our study, only chicken manure was used as substrate for BSFL growth and two different sizes of chabazite were tested. To our knowledge, this work is the first to evaluate *in vivo* the biocompatibility of chabazite towards BSF larval growth. The results showed that coarse chabazite allows more insects to reach the pupae stage and leads to prepupae with a greater weight. However, to guarantee the optimal growth and survival of BSFL, chabazite should be lower than 8%. According to the fitted model, increasing the chabazite amount led to a lower production of prepupae with inferior weight, whereas both chicken manure and water have positive effect on the increase of prepupae weight. Concerning chabazite, although no data is available in literature to compare our results, we could assume that micronized chabazite, having a higher reactivity than coarse one, can "compete" in the absorption of nutrients thus negatively affecting BSFL growth. Nevertheless, physical clogging or physiological interference after the ingestion of chabazite particles cannot be excluded. The size of BSFL head capsule varies from 0.1 ± 0.02 mm (first instar) to 1.1 ± 0.05 mm (sixth instar) (Kim et al., 2010), making the ingestion of micronized chabazite (<10 μm) feasible. These aspects, however, should be further investigated.

The experimental design allowed the identification of the best mixture for optimizing the number of prepupae at 85.81%, with an average weight of 90 mg. These data referred to small-scale tests in which the substrate was administered only once, at the beginning of the test. This aspect could have partially negatively affected BSFL growth, as it was shown that an optimal daily feeding rate could improve BSFL growth performance (Diener et al., 2009).

In waste management, a very important parameter in terms of sustainability and convenience of the process is the overall reduction of the initial substrate. In the trial with the optimized mixture of chicken manure and coarse chabazite, substrate reduction was about 76%, which is considerably higher compared to rates between 37 and 55% obtained in previous trials with chicken manure (Oonincx et al., 2015; Rehman et al., 2017). These results indicate that optimization of substrate composition to maximize the larval performance also maximizes the reduction of substrate, despite the presence of a very high amount of straw in the mixture.

Regarding the agronomic evaluation of the frass obtained from the optimal substrate mixture, the values of pH and EC (Table A7, appendix) highlighted that BSFLF needs a dilution in order to be used as growing media, as suggested by other authors (Bugbee, 1996; Ronga et al., 2019a, 2016). The total N of BSFLF was more than five times compared to the peat, but the C/N value was ideal for soilless production, as suggested by Golueke (1981). According to Zucconi et al. (1981), both GI values of the BSFLF were above the phytotoxicity threshold of 50%. Considering the GI value of roots, that was greater than 100%, a probable biostimulant effect is favored. Besides, BSFLF showed values of heavy metal that allow its commercialization in the European Community, according to the European Regulation CE 2003/2003, except for the Cu, that is out of limit for 27 mg kg$^{-1}$. The EC values of the different GM, prepared for the agronomic test (Table A8, appendix), were under the threshold value of 3.5 dS m$^{-1}$ for organic substrates and the pH values were suitable for several horticultural crops (Bugbee, 1996; Lemaire et al., 1984; Noguera et al., 2003; Raviv et al., 1986). The FWC values shown by all GM confirmed the results of Abad et al. (2001).

To the authors' knowledge, this is the first study that figured out those data using BSFLF obtained from a real waste (chicken manure) applied in soilless production. Concerning the biometric traits (Table 1a), GM2 showed also the highest D value. By contrast, GM1, GM3, GM4 and GM5 behaved in a similar way for the stem H/D ratio, an important parameter for nursery production (Herrera et al., 2008), showing a balanced value. Regarding the visual quality of the lettuce, the greenness is an important indicator, also related with Chl (Fan and Thayer, 2001; Ronga et al., 2018). As expected, the best results about Chl and NBI traits were shown by the GM2 (Table 1b). Considering the agronomic traits (Table 2), the treatments from GM1 to GM4 showed the best results for SFW. Even for TFW, GM4 showed a value similar to GM2, confirming the use of BSFLF as GM (Kebli and Sinaj, 2017). In addition, all treatments showed a similar value regarding the dry weight.

In the germination assay, none of the treatments showed a phytotoxic effect, as long as all treatments showed values of emergence higher than the threshold of 50% (Zucconi et al., 1981). Moreover, GM5 showed the highest value of GI, suggesting a possible biostimulant effect, that might improve the agricultural sustainability (Ronga et al., 2019b). Even though the microbial population of the frass was not characterized, the pathogenic strains were likely reduced compared to the initial substrate, due to the antimicrobial activity of some peptides produced by BSFL, as previously demonstrated (Erickson et al., 2004; Lalander et al., 2015).

This work focused the attention on the sustainable management of chicken manure, in the perspective of a higher production of poultry waste consequent to an increased demand for eggs and meat (Xiao et al., 2018). The optimization of the yield of BSF prepupae was driven by the intent to maximize the biomass production, since different fractions of this insect might constitute raw material for different industrial purposes. Fats could be used for the production of biofuels (Li et al., 2011; Wang et al., 2017; Zheng et al., 2012a, 2012b), feed and food (Surendra et al., 2016; Wang and Shelomi, 2017; Xiao et al., 2018). Proteins, if not usable by the feed/food industry, could find application in other sectors as biodegradable plastics (Barbi et al., 2018), as purposed in the project driving this work.

The applicability of this study is currently limited in the European Union, due to specific regulatory issues that prevent the use of manure and any substrate formally recognized as “waste” as feed for animals (and farmed insects are subjected to this rule as well) (Regulation (EC) No 999/2001; Regulation (EC) No 767/2009) and, on the other side, restrict the use of the insects' protein fraction only for pet food and fish farming (Commission Regulation (EU) 2017/893). Nevertheless, the outcome of this study could be used in contexts where these restrictions do not apply, and provide hints for future developments. The conceptualization of the experiment could be applied to several other types of bio-waste (Spranghers et al., 2017) and the application of a mathematical approach in predicting BSFL growth on specific substrates could be the base to reduce the amount of *in vivo* experiments, thus facilitating the improvement of bio-based recycling plants.

## 5. Conclusions

This work demonstrated that BSFL represents a useful tool to gain a clean and sustainable management of chicken manure, by strongly reducing its initial amount and favoring its total recovery and exploitation as insect biomass and a compost-like leftover. This work is part of a project aimed at the sustainable valorization of livestock waste using BSFL in a circular economy perspective, intended to demonstrate the feasibility of organic waste management with BSFL at a pre-industrial scale by the development of a pilot plant for BSFL rearing as final achievement.

The outcome of the experiments led to the identification of an optimal substrate mixture for BSFL growth, and resulted in more than 75% reduction of the initial manure mass. These results not only improve the current knowledge about BSF growth, but also provide the operating conditions to run a pilot plant on a finest mix of chicken manure and chabazite. The agronomic trial performed with the LF obtained from the optimal mixture showed soil improver properties on baby-leaf lettuce, demonstrating the possibility to use this substrate, properly diluted, in both potted and soilless production, in agriculture or in a plant nursery. However, to put into practice the proposed strategy, further investigations are needed to: i) assess the microbiological quality of BSFLF in crop production; ii) evaluate the physiological effects of chabazite on BSFL; iii) verify the use of mixtures of various types of bio-wastes; iv) develop specific models for the scale-up of the technology.

Despite the regulatory restrictions in the EU, that currently preclude the use of insects as waste bio-converters and therefore the industrial development of the technology, this work indicates the way towards a cleaner and more sustainable management of livestock manure and other bio-waste, in agreement with the principles of circular economy.

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## Author contribution statement

Sara Bortolini: Writing - Original Draft, Conceptualization, Investigation; Laura Ioana Macavei: Investigation, Writing - Review & Editing; Jasmine Hadj Saadoun: Investigation; Giorgia Foca: Methodology, Formal analysis; Writing - Original Draft; Alessandro Ulrici: Methodology, Formal analysis, Writing - Original Draft; Fabrizio Bernini: Investigation; Daniele Malferrari: Writing - Original Draft, Conceptualization, Resources, Formal analysis; Leonardo

Setti: Writing - Original Draft, Investigation; Domenico Ronga: Writing - Review & Editing, Conceptualization, Resources; Lara Maistrello: Writing - Original Draft, Supervision, Conceptualization, Resources, Project administration, Funding acquisition.

## Declaration of competing 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.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2020.121289.

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