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BSF-colony management efficiency at a large scale fly larvae composting company in Kenya – A field study

Utvärdering av flugkoloni-effektiviteten på ett storskaligt fluglarvskomposterings-företag i Kenya – En fältstudie

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ABSTRACT

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The majority of organic waste globally is either dumped or placed on landfills, which can result in spreading of disease and pests, methane gas emissions, the deterioration of landscapes and odour pollution. One of the solutions to poor organic waste management is to create value from waste. A way to do this is with black soldier fly (*Hermetia illucens*) larvae (BSFL) composting. The interest for BSFL composting has increased the past 10 years mainly because it is a technology with relatively low investment costs. The part of the process where the seed larvae that are used in the treatment are produced is called BSF-colony. Producing seed larvae in an efficient way is an important part of the technology's feasibility. However, there is limited research published on BSF-colony management, especially on a larger scale. The aim of the study was to investigate the factors that impact the efficiency of the BSF-colony on a large scale BSFL composting company in Kenya in a semi-open setting. The efficiency of the BSF-colony management was assessed in terms of emergence rate (percentage of pupa that emerge as flies), hatching rate (percentage of eggs that hatch and survive to 5 day old larva) and number of eggs laid per female BSF. The result of the study suggests that parasitic wasps (*Dirhinus giffardii*) can reduce the emergence rate significantly, and high temperatures and water shortage for the adult BSF can reduce the egg production. Observations made during the study indicate that personnel routines and how the BSF-colony is arranged also could affect the egg production significantly. The overall variation in the results suggests that other factors, beyond the ones investigated in the study, impact the efficiency of the BSF-colony, and further research regarding BSF-colony management is recommended.

Keywords: Black soldier fly composting, BSF-colony, industrial scale, parasitic wasps

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REFERAT

Utvärdering av flugkolonieffektiviteten på ett storskaligt fluglarvskomposteringsföretag i Kenya – En fältstudie

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Merparten av det organiska avfallet i världen dumpas eller läggs på deponier, vilket kan leda till spridning av sjukdomar och skadedjur, utsläpp av metangas, förstörelse av landskap och luktföroreningar. En av lösningarna på bristfällig hantering av organiskt avfall är att skapa värde av avfallet. Ett sätt att göra det är med hjälp av fluglarvskompostering med amerikansk soldatfluga (*Hermetia illucens*). Intresset för fluglarvskompostering har ökat under de senaste tio åren, främst på grund av att det är en teknik som inte behöver innebära höga investeringskostnader. Flugkolonin är den del av processen där sättlarverna som användes i komposteringen produceras. Att sättlarver produceras på ett effektivt sätt är en viktig del av teknikens genomförbarhet. Det finns dock begränsad forskning publicerad om effektivisering av flugkolonier, speciellt sådan som berör storskaliga kolonier. Syftet med studien var att undersöka vilka faktorer som påverkar flugkolonins effektivitet på ett storskaligt fluglarvskomposteringsföretag i Kenya i en halvöppen miljö. Flugkolonins effektivitet bedömdes i termer av metamorfosfrekvens (den andel av pupporna som blir flugor), antalet lagda ägg per fluga och kläckningsfrekvens (den andel ägg som kläcks och överlever till 5-7 dagar gamla larver). Resultatet av studien tyder på att parasitsteklar kan minska metamorfosfrekvensen avsevärt, och att höga temperaturer samt vattenbrist kan minska äggproduktionen. De observationer som gjordes under studien tyder på att personalens rutiner och hur flugkolonin är anordnad också kan påverka äggproduktionen avsevärt. Den relativt stora variationen i alla resultat tyder på att andra faktorer än de som undersökts i studien påverkar flugkolonins effektivitet, fortsatta studier inom effektivisering av flugkolonier rekommenderas.

Nyckelord: Amerikansk vapenfluga, fluglarvskompostering, storskaligt, parasitsteklar

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PREFACE

This master thesis constitutes the last part of the master's programme in environmental and water engineering at Uppsala university. The thesis comprises 30 credits and has been carried out through a collaboration between Biobuu Limited and the Swedish University of Agricultural Sciences. Cecilia Lalander has been supervisor and Björn Vinnerås subject reviewer, both at the Department of Energy and Technology, Swedish University of Agricultural Sciences. The examiner was Johan Arnqvist at Department of Earth Sciences, Uppsala university.

Special thanks to Kigen Compton and everyone at the company for a warm welcome and for helping make this thesis possible. I would also like to extend my sincere thanks to Cecilia Lalander and Viktoria Wiklicky for their guidance, support, and encouragement throughout the study. Lastly, I would like to acknowledge that this project would not be possible without the financial support from Åforsk travel grant.

Hilda Anderberg

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POPULÄRVETENSKAPLIG SAMMANFATTNING

Fluglarvskompostering - ett sätt att omvandla avfall från problem till resurs? En fältstudie om storskalig uppfödning av sättlarver

Hilda Anderberg

Fluglarvskomposteringen kan omvandla matavfall från ett problem till en resurs som skapar arbetstillfällen, hållbart djurfoder och organiskt gödselmedel. Avgörande för teknikens ekonomiska genomförbarhet är hur effektivt sättlarver kan födas upp på ett storskaligt vis. Den här studien visar att det finns många potentiella fallgropar, varav två är parasitangrepp och för höga temperaturer. Båda faktorerna skulle kunna dra ner effektiviteten till den grad att processen blir ekonomiskt olönsam och problematisk i relation till den stora mängd inkommande avfall som ska behandlas.

Globalt sett hamnar 67 % av organiskt avfall på deponier eller dumpas öppet, vilket bland annat kan leda till spridning av sjukdomar, förorenat ytvatten och metanutsläpp. En viktig aspekt för att driva på positiv förändring inom avfallshantering är att omvandla avfallet från problem till resurs, vilket leder oss till fluglarvskompostering. I fluglarvskompostering omvandlas organiskt avfall till en kompost, som säljs som organiskt gödselmedel, och den vanligaste flugarten som används är den amerikanska vapenflugan. Larverna, som omvandlar avfallet, skördas och säljs som djurfoder, till fisk, hönor och grisar, vilket kan ersätta problematiska djurfoder som soja- och fiskmjöl vilka bidrar till skövlingen av regnskogen respektive överfiske av haven. Intresset för storskalig fluglarvskompostering ökar globalt sett, mycket på grund av att det är en teknik som inte behöver innebära höga investeringskostnader, vilket är speciellt viktigt i låg- och medelinkomstländer.

För att kunna behandla flera ton organiskt avfall per dag och driva en storskalig fluglarvskompostering lönsamt, så behöver alla steg i processen ha en god effektivitet. En av luckorna i forskningen om fluglarvskompostering rör storskalig uppfödning av flugan för produktion av sättlarver. Den delen av processen kallas för flugkoloni, och innebär att en del av larverna från behandlingen utvecklas till puppor och från pupporna fås flugor, som parar sig och lägger ägg, som kläcks till sättlarver som används i avfallsbehandlingen.

Den här studien undersökte effektiviteten i en storskalig flugkoloni på ett fluglarvskomposteringsföretag i Kenya. Det visade sig att andelen puppor som blev till flugor skulle kunna vara 66 %, men att det nuvarande system var angripet av parasitsteklar vilket resulterade i att endast 26 % blev till flugor. Det visade sig också att temperaturer över 37 °C påverkade äggproduktionen negativt och att det gick att fördubbla äggproduktionen genom att flytta flugorna till en zon med lägre temperatur. Den stora variation i studiens resultat pekar på att det finns fler faktorer än de som undersöktes som påverkar effektiviteten. Vidare studier om flugkolonieffektivitet behövs för att öka kunskapen kring var och hur övervakning av processen ska gå till. Bättre övervakning och kunskap om flugkolonin möjliggör felsökning och optimering av processen. Mer forskning och kunskapsutbyte kring hur en storskalig flugkoloni ska skötas på bästa sätt är en viktig del i det organiska avfallets färd från problem till resurs.

TABLE OF CONTENT

DEFINITIONS

Attractant substrate	Substrate that should attract female BSF to a certain egg laying area.
BSFL	Black soldier fly larva.
BSF-colony	The part of BSFL-composting where seed larvae are reared. The colony include BSF at the stages: prepupae, pupae, flies, eggs, neonates, and seed larva.
BSFL-composting	Composting where BSFL are used to process organic waste.
BSF-net	A mesh cage where BSF mate and lay eggs.
Puparium	Cages where prepupa evolve into pupa and pupa emerge into fly. The cage does not let in any light to prevent mating.
Egg shower	Egg showers are located in the nursery. It is where eggs hatch and neonates drop into starter substrate.
Egg traps	The object where BSF should lay their egg, which make the eggs easy to harvest.
Emergence rate	The ratio between the total number of emerged flies and the total number of prepupae/pupae.
Frass	The compost/treatment residue from BSFL composting.
Hatching rate	The ratio between total number of eggs and the number of the eggs that hatch and survive to the seed larval stage.
Neonates	Newly hatched BSFL.
Nursery	Where eggs hatch and neonates grow to seed larvae.
Prepupa	The life stage BSFL evolve into when finishing the larval stage, before evolving into a pupa.

Pupa	The life stage BSFL evolve into after the prepupal stage, before emerging into a fly.
Seed larva	5 – 7 day old BSFL that are reared in the BSF-colony to be used in the composting process.
Starter substrate	The feed given to the neonates until they have grown into seed larvae.
Treatment area	The process area where the BSFL process the organic waste and the BSFL grow from seed larvae to prepupa.

1. INTRODUCTION

In 2019, the world generated approximately 931 million tons of food waste (Statista 2023a). With predicted population growth, urbanization and economic development, the global municipal waste generation is expected to increase with 70 % by 2050 (Statista 2023b). Globally, around half of municipal waste is organic (World Bank n.d.). Despite the existence of alternative management options the majority of organic waste globally is either openly dumped (31 %) or placed on landfills (37 %) (World Bank n.d.). Disposing organic waste on open dumps or on landfills can result in spreading of disease and pests, methane gas emissions, the deterioration of landscapes and odour pollution (Siddiqua et al. 2022).

Organic waste management is a multi-dimensional problem involving technical, economic, political, socio-cultural, organizational, and environmental issues (Guerrero et al. 2013). According to Kharola et al. (2022), there exist several barriers for sustainable management of organic waste, where lack of communication, planning, financial resources, technology and infrastructure are some of them. On the top of their list of solutions to end poor organic waste management is to create value from waste.

There are several examples of waste management systems that creates value from waste (Ki Lin et al. 2013; Lalander et al. 2018b). Conventional composting produces an organic fertilizer and anaerobic digestion creates both an organic fertilizer and biogas, which can be used to generate electricity or as vehicle fuel (Tominac et al. 2021). Replacing synthetic fertilizers with organic fertilizers is interesting in relation to the resource-intensive production of synthetic fertilizers (Capdevila-Cortada 2019; Sveriges geologiska undersökning 2020) and the recent price increase of synthetic fertilizer (Crespi et al. 2022).

The challenges for conventional composting are risk of methane emissions and odour pollution (Ayilara et al. 2020), and anaerobic digestion include high financial cost and the need for highly trained personal to insure process control (Xu et al. 2018). Black soldier fly (*Hermetia illucens*) larvae (BSFL) composting is another option for creating value from waste (Tomberlin & van Huis 2020). The process produces larvae, which can be used in animal feed or processed into fuel, and an organic fertilizer.

The interest in the waste management technique black soldier fly larvae (BSFL) composting has increased over the past 10 years (Tomberlin & van Huis 2020). This is mainly due to its financial viability when used as an organic waste management strategy, with relatively low investment costs, which is particularly important in low- and middle- income countries (Diener et al. 2011; Purkayastha & Sarkar 2022). Lalander et al. (2018b) found that BSFL composting had the highest economical product-value in comparison to conventional composting and anaerobic digestion. Compared to conventional composting, BSFL composting is a faster process (Amrul et al. 2022), the larvae can digest a wider range of organic waste and are more resilient to environmental changes than worms (da Silva & Hesselberg 2020). Few studies have assessed the environmental impact of BSFL composting and compared it to other organic waste management systems. The studies that have been conducted indicates that BSFL composting has a lower global warming potential than conventional composting (Mertenat et al. 2019; Liu et al. 2022).

Given that BSFL composting is relatively new on an industrial scale, further research is necessary to identify safe, efficient, environmentally sustainable, and profitable ways to carry out the technology (Diener et al. 2011; da Silva & Hesselberg 2020). It is crucial to have control and knowledge of all stages in the process for an industry that handles several tons of food waste every day (Diener et al. 2011). In order to making the process feasible it is also important to determine efficient treatment techniques, optimal environmental conditions for the larvae and improve methods of BSF-colony management (Singh & Kumari 2019).

Despite the large interest for BSFL composting, limited progress has been made to advance the knowledge of the biology of the BSF and there are research gaps relating to the biological mechanisms and operating procedures affecting mating and egg laying (Joly & Nikiema 2019; Lemke et al. 2022). Most of the studies on BSF-colony management has been done in a small scale laboratory setting (Singh & Kumari 2019) which may differ significantly from how large scale companies operates. This study aims to contribute to knowledge on efficiency of BSF-colony management on a large scale BSFL composting company.

1.1 AIM

The aim of the study was to investigate factors that could impact the efficiency of a BSF-colony run at a large scale BSF-company located in Kenya, in order to enable improvement of the efficiency. The efficiency of the colony management was assessed in terms of emergence rate (percentage of pupae that emerge as flies), number of eggs laid per female BSF, and hatching rate (percentage of eggs that hatch and survive to 5 day old larva).

1.2 RESEARCH QUESTION

A. What factors impact the efficiency of the BSF-colony, in terms of emergence rate, on a large scale BSFL composting company?

B. What factors impact the efficiency of the BSF-colony, in terms of number of eggs laid per female BSF, on a large scale BSFL composting company?

C. What factors impact the efficiency of the BSF-colony, in terms of hatching rate, on a large scale BSFL composting company?

1.3 DELIMITATIONS

The study was conducted at a BSFL composting company located in the coastal region in Kenya near the equator. Data was collected over a 10 week period from January to March 2023, during the dry season, with few cloudy and rainy days. The study was conducted in parallel with normal production, and some of the set-up details were conducted by working personnel. The study investigated the production of seed larvae, including the steps of pupae to fly, fly to egg and egg to seed larvae. However, the study did not examine the survival rate from seed larvae to prepupae, the bioconversion performance of the BSFL, nor the qualities of the fertilizer produced.

It is worth noting that the number of eggs laid per female only included the eggs that were laid inside or on top of the egg traps and not misplaced eggs. If the majority of the eggs were laid somewhere else in the net, that net was excluded from the results. This was determined on the basis of a visual examination of the nets.

2. BACKGROUND

2.1 BSFL COMPOSTING

Black soldier fly larva (BSFL) composting is a waste management technique suitable for a variety of organic wastes, such as food waste, abattoir waste, and animal and human manure (Lalander et al. 2018a; Liu et al. 2022). The black soldier fly (*Hermetia illucens*) (BSF), a tropical fly originally from the American continent (Fachin et al. 2021), lays its eggs in the vicinity of decomposing organic material to provide feed for the larvae when they hatch (Lemke et al. 2022). The BSFL's natural behavior to consume decomposing organic material is utilized by the waste management industry to convert organic waste into larvae rich in protein and fat (Siddiqui et al. 2022). Under optimal conditions, the BSFL can double its weight approximately 200 times during the two to three weeks it takes for it to grow from hatchling to fully developed larva (Lalander et al. 2018a). According to Purkayastha & Sarkar (2022), BSFL can degrade the organic, on a wet weight basis, by a range of 14 to 95 %, depending on the type of waste.

The BSFL are separated from the treatment residue, called frass, and can be fed directly or as larvae meal to chickens, fish, and pigs, or be processed into biodiesel (Purkayastha & Sarkar 2022). Another possible product is chitin, a polymer which can be extracted from the exoskeleton from prepupae, pupae shells and flies and used in tissue engineering, the textile industry and as adsorbent in wastewater treatment (Purkayastha & Sarkar 2022). The frass, produced by BSFL, can potentially be used as an organic fertilizer, soil amendment or further processed as biochar or in biogas plants (Basri et al. 2022; Lopes et al. 2022).

For an industry scaled BSFL composting facility, six main process areas are required (Figure 1) (Dortmans et al. 2017): 1)Waste pre-processing area, where the organic waste is received, processed (e.g grounding and mixing) and stored; 2) Treatment area, where the larvae consume the organic waste; 3) BSF-colony, where new larvae are produced 4) Harvest area, where larvae and frass are separated; 5) Larvae refining area, where larvae are processed and packaged; 6) Composting processing, where the frass is processed and packaged.

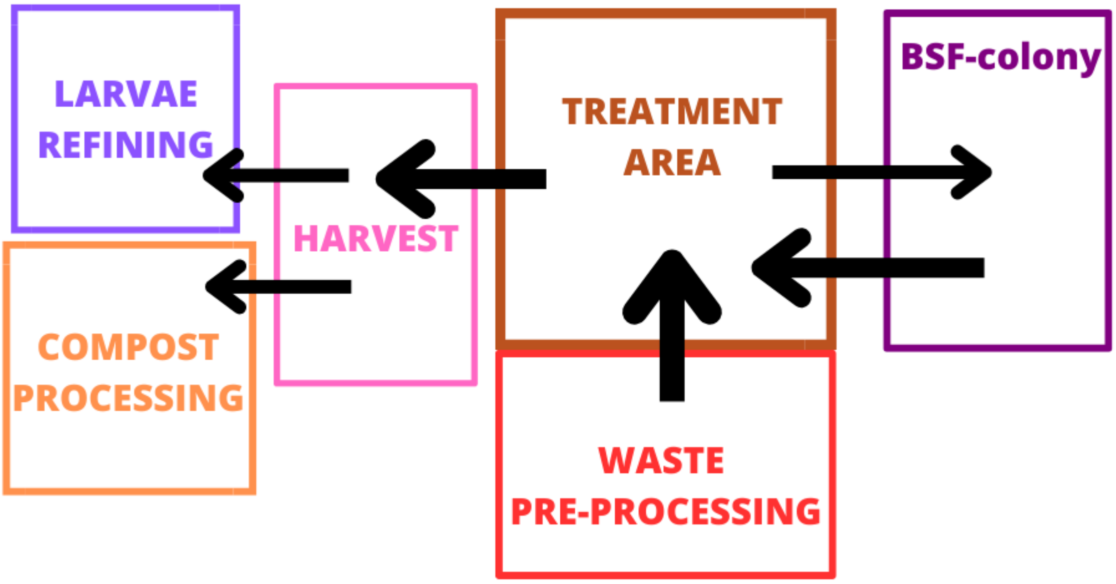

Figure 1. Schematic scheme of a BSFL composting facility: Larvae refining, compost processing, harvest, treatment area, waste pre-processing, BSF-colony.

2.2 CHALLENGES WITH LARGE SCALE BSFL COMPOSTING

Given that BSFL composting is relatively new on an industrial scale, further research is necessary to identify safe, efficient, environmentally sustainable, and profitable ways to carry out the process (Diener et al. 2011; da Silva & Hesselberg 2020). The BSFL composting industry faces numerous challenges, including concerns related to the safety of workers (Liu et al. 2022), legislative hindrances and the lack of an existing market for the products (da Silva & Hesselberg 2020). Connected to legislative hindrances and the expansion of the BSFL market is the need for further research on consumer safety, particularly in relation to bioaccumulation of heavy metals, pharmaceutical and other potentially hazardous chemicals, as well as the risk of spreading pathogens (Joosten et al. 2020; Lievens et al. 2021).

In order to make the process feasible, it is crucial to determine efficient treatment techniques, identify optimal environmental conditions for the larvae, limit the risk of process failure posed by parasites, pathogens and pests (Barrett et al. 2022) and improve BSF-colony management (Singh & Kumari 2019).

2.3 BSF-COLONY MANAGEMENT

The black soldier fly has a life cycle consisting of five main stages: egg, larva, prepupal, pupal and fly (Purkayastha & Sarkar 2022) (Figure 2). In order to produce new larvae, called seed larvae, for the treatment, a BSFL composting facility needs to manage all the stages of the life cycle. This is called BSF-colony management which can be divided into three fractions: 1) Puparium management, where pupae emerge into flies; 2) BSF-net management, where flies mate and lay eggs; 3) Nursery management, where eggs hatch and newly hatched larvae, called neonates, grow to seed larvae.

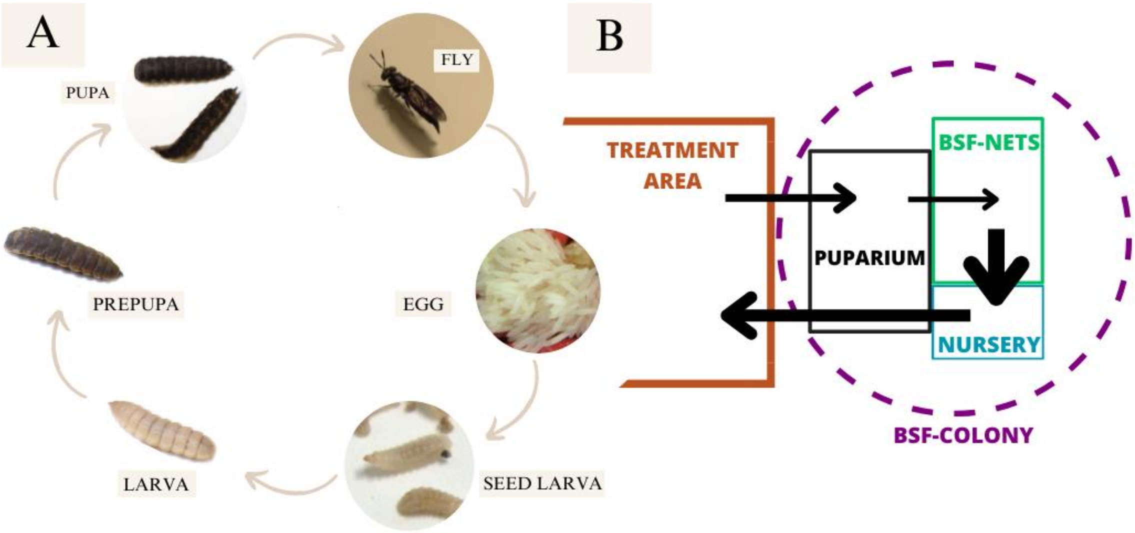

Figure 2. A) Photographs of the different stages in the black soldier fly (Hermetia Illucens) lifecycle: Egg, seed larva, larva, prepupa, pupa, fly; B) Schematic scheme of BSF-colony facility areas: Treatment area, BSF-colony, puparium, nursery, BSF-nets.

In order to identify potential problems when managing an industrial scaled BSF-colony, it is important to monitor performance parameters such as the emergence rate (percentage of pupa that emerge as flies), hatching rate (percentage of eggs that hatch and survive to 5 day old larva) and number of eggs laid per female BSF (Figure 3) (Dortmans et al. 2017) . To the authors knowledge, there is little published research on what could be considered normal baseline values for these performance parameters in an industrial setting. There are also limited knowledge on operating procedures and biological mechanisms affecting the pupation, emergence rate and egg hatching.

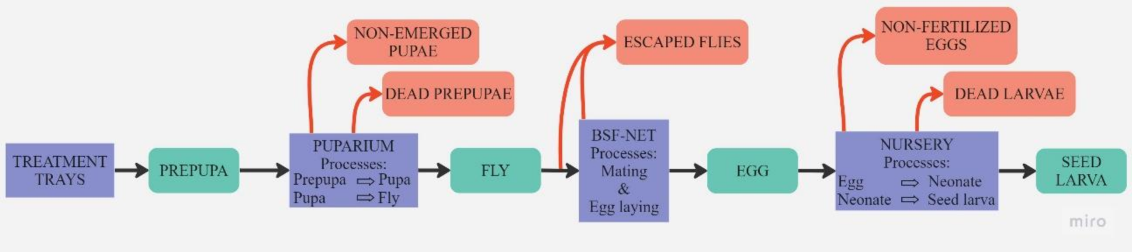

Figure 3. Schematic scheme of the process flow of the BSF-colony management system. The red boxes represent where losses are generally expected.

There are however several known factors that can affect the efficiency of the BSF-colony. Two parameters that affect the whole process is temperature and relative humidity (Holmes et al. 2012; Chia et al. 2018a). It affects both the survival of egg, larva, pupa and fly, and the duration of the different stages (Holmes et al. 2012; Chia et al. 2018a). The life cycle of the BSF can vary from 34 days at 30 °C to 89 days at 20 °C (Fazli Qomi et al. 2021). Another factor affecting the whole life cycle is the feed given to the larvae, which affects survival rate of larva, prepupa and pupa, the speed of reaching prepupal and pupal stage, the longevity of the flies and the number of eggs laid per female fly (Chia et al. 2018b; Miranda et al. 2019).

2.3.1 Puparium management: From larva to fly

When a BSFL has reach the end of the larva stage it changes into a prepupa stage and searches for a drier place to evolve into a pupa (Dzepe et al. 2020). Therefore, it is important to ensure that the prepupae kept for the BSF-colony is removed from potentially wet feed and placed in a drier environment, called puparium (Figure 3). The puparium is a dark environment, which it is to prevent mating inside the puparium (Dortmans et al. 2017). It takes two to six days for the prepupa to evolve into a pupa and 14 – 20 days for the pupa to evolve into a fly (Purkayastha & Sarkar 2022). When the puparium starts producing flies, it can regularly be emptied into mesh cages, called BSF-nets.

Some studies have been conducted on different pupation substrates, which have concluded that wood shavings or corrugated cardboard are materials that can increase the pupation rate from 77 % (no substrate) to 92 % (Dzepe et al. 2020; Purkayastha & Sarkar 2022). In a study by Chia et al. (2018) it was demonstrated that the optimal temperature for this stage is between 25 – 35 °C and that the survival rate goes down from 75 % at 35 °C to 24 % at 37 °C. Fazli Qomi et al. (2021) reported a maximal survival rate of 83 % at 25 °C. The prepupae can survive at temperatures down to 16 °C for several weeks, but the pupation rate will slow down (Holmes et al. 2016). According to Holmes et al. (2012), the optimal relative humidity (RH) is 70 %. The survival rate goes down from 98 % at 70 % RH to 35 % at 25 % RH.

When the BSFL has reached the pupa stage, various factors influence if and when a fly is going to emerge from that pupa. According to Dortmans et al. (2017) and the technical handbook by Caruso et al. (2014), a normal emergence rate for an industrial BSF-colony is between 80 – 90 %. Two field studies conducted at open environment BSFL composting facilities in Pakistan and Indonesia, found emergence rates of 59 and 82 %, respectively (Mahmood et al. 2022).

The optimal temperature for the pupa is 30 °C according to two studies that reached a maximal emergence rate of 77 % at 30 °C (Chia et al. 2018a; Fazli Qomi et al. 2021). The pupa stage is more sensitive to high temperatures than low temperatures, according to Chia et al. (2018). At 15 °C, the emergence rate was down to 49 %, while it at 37 °C was 5 %. Holmes et al. (2012) have suggested that the optimal RH is 70 %, as the emergence rate at 70 % was found to be 93 %, while it was lowered to 16 % at an RH of 25 %.

Pupation material could affect the emergence rate according to Dzepe et al. (2020). The emergence rate increased from 64 % with no substrate to 92 % when adding wood shaving as pupation material. According to a study by Lin (2016), the pupation material should contain 50 – 55 % moisture content, a moisture content of 85 % increased the number of flies with malformed wings.

The pupa stage can be infected by different parasites (Barrett et al. 2022). One parasite is the parasitic wasp (*Dirhinus giffardii*), which lay their eggs inside the pupa. In a study by Devic & Maquart (2015), the emergence rate was reduced from 99 % to 9 % when infected by parasitic wasps (*Dirhinus giffardii*).

2.3.2 BSF-net management: Mating and egg laying

The purpose of BSF-nets is to ensure mating, egg laying, and fast and effortless harvest of eggs (Figure 3) (Joly & Nikiema 2019). Therefore, the BSF-net management need to enable and maximize mating and egg laying, which has been found to be impacted by various factors. In the wild, BSF live for seven to nine days and egg laying takes place within four days after emerging from the pupa (Purkayastha & Sarkar 2022). A study, conducted in a semi-open environment in the tropics, found that BSF started mating two to eight days after emerging from the pupa (Julita et al. 2020). Peak mating occurred from 9 AM to 2 PM, and the egg laying period started around two days after the mating period, meaning four to ten days after emerging from the pupa, with peak egg laying occurred from 1 PM to 2 PM.

It has been found that the lifespan of BSF can be prolonged to 14 days when providing the flies with water or, up to 27 days when providing the flies a sugar solution (Chia et al. 2018b; Macavei et al. 2020). According to Romano & Fischer (2020), BSF prefer a white container during feeding. Also temperature affects the lifespan of BSF: they can survive up to 16 days at the optimal temperature of 20 °C, but only five days at 35 °C (Chia et al. 2018a). Also in this life stage, the optimal RH has been suggested to be 70 %, at which the lifespan of BSF was found to be prolonged by three days comparing to at a RH of 25 % (Holmes et al. 2012).

BSF requires direct sunlight, or artificial lights that mimics natural sunlight, to mate (Tomberlin & Sheppard 2002; Purkayastha & Sarkar 2022). Female flies can lay eggs without light, but there is a higher risk of unfertilized eggs (Tomberlin & Sheppard 2002). However, placing BSF-nets in direct sunlight in tropical regions is not recommended, due to the risk of dehydrating the flies (Dortmans et al. 2017). The sex ratio in the BSF-net affect the mating according to Putra & Safa'at (2020), which concluded that the best male-to-female ratio for reproduction success was 40:60. Joly & Nikiema (2019) pointed out that different studies has reached different conclusions regarding the optimal density in BSF-nets. According to Hoc et al. (2019), mating occurred in BSF-nets with densities ranging from 500 to 8 500 BSF/m^3, and Gougbedji et al. (2021) found that mating also was achieved in BSF-nets with a density of 10 500 BSF/m^3.

When female BSF lay their eggs, it is favorable, in an industrial setting, if they lay them in a way that make them easy to harvest (Purkayastha & Sarkar 2022). Therefore, egg traps are used in the BSF-nets. An egg trap should have 1 – 2 cm gaps directed away from light, where the female BSF can lay their eggs. A study by Julita et al. (2021) recommended wood and corrugated cardboard as materials for egg traps. According to Romano & Fischer (2020), BSF prefer to lay their eggs on blue surfaces. The egg traps are placed on an attractant substrate, which is a substrate used to attract female BSF to the egg traps (Purkayastha & Sarkar 2022). The attractant substrate should have a strong odor and 60-70 % moisture content. Examples of attractant substrate are manure, residue from BSFL treatment, fermenting fruit or vegetable, or household waste. Relative humidity could affect egg laying, and Tomberlin & Sheppard (2002) concluded that egg laying increased at RH above 60 %.

According to Dortmans et al. (2017), a normal number of eggs laid per female BSF for an industrial BSF-colony is 350. Two field studies conducted on open-environment

BSFL composting facilities in Pakistan and Indonesia resulted in an average of 96 and 370 eggs laid per female BSF, respectively (Mahmood et al. 2022).

Various studies have examined the relationship between the number of eggs laid per female BSF and the diet given to BSF. Macavei et al. (2020) found that giving the BSF water increased the egg production 100 – 200 %, and adding sugar to the water increased the egg production up to 930 eggs per female BSF. However, Chia et al. (2018b) reported that the number of eggs did not vary when BSF was given a sugar solution. Klüber et al. (2023), showed that the maximal egg production occurred when the BSF were given a honey solution, resulting in 630 eggs per female BSF. They also noted that the egg laying occurred fewer days after emergence when the BSF were given a protein solution, compared to when they were given water or honey solution.

Regarding fly densities, Hoc et al. (2019) investigated fly densities ranging from 500 to 8 500 BSF/m^2 and found that the optimal density for egg laying was 6 500 BSF/m^2. Additionally, according to Gougbedji et al. (2021), a density of 8 500 BSF/m^2 resulted in a higher number of eggs per female compared to a density of 10 500 BSF/m^2.

2.3.3 Nursery management: From egg to seed larva

When the eggs are harvested from the BSF-nets, they are placed in a nursery where they hatch, two to four days after being laid, and grow five days before being used in the treatment process (Figure 3) (Holmes et al. 2012; Dortmans et al. 2017; Purkayastha & Sarkar 2022). Low technological facilities normally remove the eggs manually from the egg traps and place them into a hatching container, or place them directly in the nursery (Joly & Nikiema 2019). The newly hatched larvae (neonates) drop into a container with a high-quality feed called starter substrate where they generally grow for five days before being used in the treatment process (Dortmans et al. 2017). Dortmans et al. (2017), recommend the use of chicken feed with 70 % moisture content as starter substrate.

Several factors affect when and if the eggs will hatch, as well as how many of the neonates that will survive to the seed larval stage (Joly & Nikiema 2019). According to Dortmans et al. (2017) and the technical handbook by Caruso et al. (2014), a normal hatching rate for an industrial BSF-colony is 70 – 80 %. Two field studies were conducted on open-environment BSFL composting facilities in Pakistan and Indonesia, resulting in a hatching rate of 45 and 59 %, respectively (Mahmood et al. 2022).

Provided that the eggs are fertilized, their hatchability depends on the relative humidity and temperature. The optimal RH is 70 %, and the optimal temperature is 30 °C (Holmes et al. 2012; Chia et al. 2018a). Chia et al. (2018a) found that at 30 °C, 80 % of the eggs hatched, while only 10 % of the eggs hatched at 37 °C. Holmes et al. (2012) concluded that the hatchability increased from 5 % at 25 % RH to 87 % at 70 % RH. A higher RH also decreased the time from egg laying to hatching by 2,5 days.

To the authors knowledge, few published studies have investigated the environmental conditions affecting the survival rate of neonates, as most research has focused on the larval stage during the treatment step (Figure 1). The optimal temperature and RH for the larval stage is 25 – 35 °C and 70 %, respectively (Holmes et al. 2012; Chia et al. 2018a). The survival rate has been found to decrease from 92 % at 35 °C to 28 % at

40 °C, and from 97 % at 70 % RH to 74 % at 40 % RH. Kortsmit et al. (2022), reviewed the published studies regarding BSF larval behavior and the possible effects it could have on productivity. They concluded that overcrowding of larvae could cause thermal stress, escape behavior, and cannibalism. Parra Paz et al. (2015) recommended a larval density of 1,2 larvae/cm^2 for mass rearing purposes.

3. MATERIAL & METHOD

3.1 MATERIAL

Three different balances and five temperature and relative humidity sensors were used for the data collection in this study. Balance B1 had an accuracy of one gram, balance B2 had an accuracy of one-tenth of a gram and balance B3 had an accuracy of one-hundredth of a gram. Tinytag sensors were used to record the relative humidity (± 3 %RH) and temperature (± 0.6 °C) in the different facility areas every 10 minutes. A tweezer was used to scrape the egg traps and manually count samples.

The starter substrate, that were given to the neonates, contained a mixture of 360 g of wheat bran, 180 g of coconut bran, 60 g of chicken feed and 2 400 g of dairy waste (cow milk). The fat content in the dairy waste varied throughout the study, but the average moisture content of the starter substrate was 75 ± 2 %. Aluminum molds and a convection oven were used when establishing the moisture content of the starter substrate. The attractant substrate, used to attract the BSF to the egg traps, contained a mixture of fruit, vegetable and dairy waste that had been stored in the sun for a couple of days.

3.2 FIELD TRIAL SETTING

The study took place at a BSFL composting company located outside Mombasa, Kenya. The facility processed around 20 tons of organic waste per day, mostly fruits, vegetables, and dairy waste. The temperature, RH, and direct sunlight was mainly regulated by the current weather condition, due to the semi-open construction. The facility was divided into following areas (Figure 4): waste pre-processing, treatment, BSF-colony, harvest, larvae refining and compost processing. The BSF-colony included areas for puparia, where the flies emerge from pupae, and a greenhouse where mating, egg laying and hatching took place. All areas were partially covered with mesh on the sides to protect insect production from birds and other pests. The treatment and puparium areas had metal roofs to protect against rain and direct sunlight and the greenhouse had a plastic and net roof to protect against rain while allowing direct sunlight. The mesh cages with BSF (BSF-nets) and the nursery were both placed inside the greenhouse, the BSF-nets received direct sunlight and the nursery was placed in a shaded area.

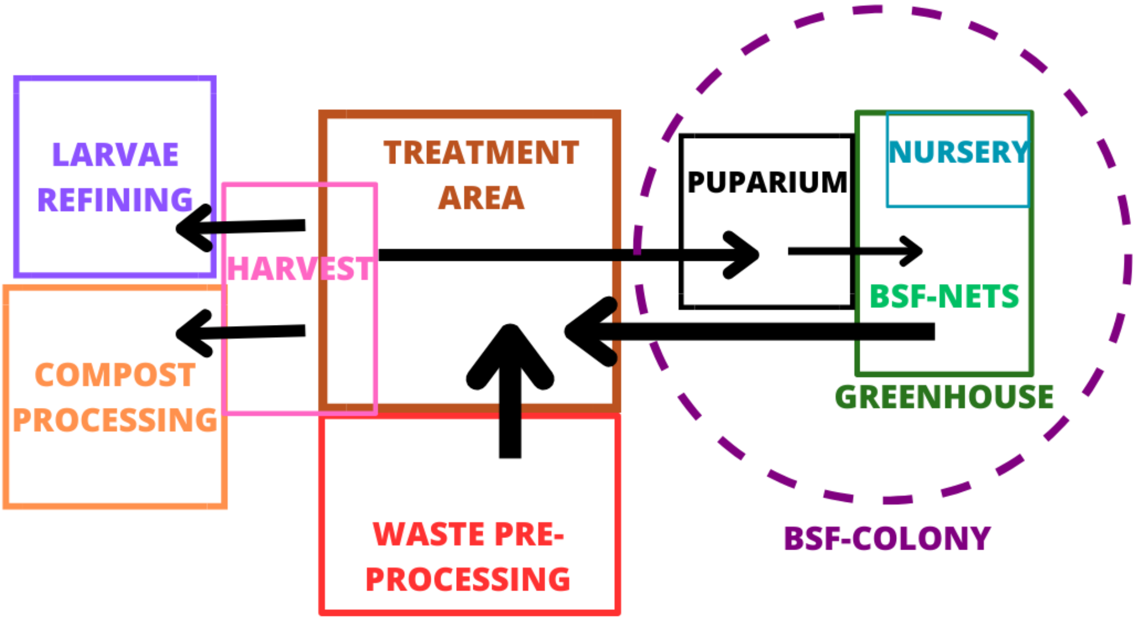

Figure 4. Schematic view of the facility areas: Larvae refining, compost processing, harvest, treatment area, waste preprocessing, BSF-colony, puparium, greenhouse, nursery, BSF-nets.

The incoming waste was grounded and mixed in the waste pre-processing area. Around 10 000 seed larvae were added to trays with 5 kg of mixed waste in the treatment area (Figure 5). After two to three weeks, the larvae were harvested through a mechanical sieve and refined for selling. The frass was processed to an organic fertilizer.

Figure 5. Picture of the treatment area.

3.2.1 BSF-colony management

Puparium management: From larva to fly

Roughly 15 % of the treatment trays were kept for the BSF-colony. The larvae, purposed for the BSF-colony, processed the waste for four weeks, until reaching prepupal or pupal stage, and were harvested with a mechanical sieve, to separate the pupae and prepupae from the frass, during the fifth week. At the end of the fifth week, the harvested prepupae and pupae were placed into puparia (Figure 6). Due to fluctuations in the treatment and an imperfect sieving process, the puparium content was a mixture of pupae, prepupae, dead larvae, shells, and frass. Each puparium measured 2,7 m^3 (140 cm x 140 cm x 140 cm) and was filled with 30 trays which each contained 20 x 85 ml of the puparium content, which was intended to correspond to approximately 7 000 prepupae and pupae combined. The puparium was active for three weeks.

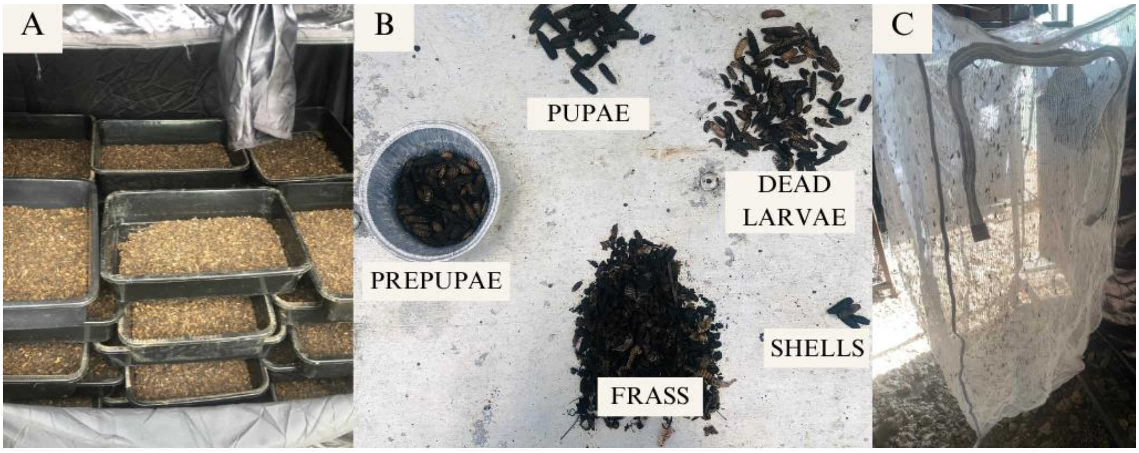

Figure 6. Picture of the puparium management: A) Filled puparium; B) Fractions of the puparium content, prepupae, pupae, dead larvae, shells, and frass; C) BSF-net attached to a puparium during harvest of flies.

The puparium started to produce flies after two to three days and after that, the emerged flies inside a puparium were emptied once a day or every other day into BSF-nets measuring 0,69 m^3 (140 cm x 70 cm x 70 cm). The BSF-net was weighed after filling, with balance B1, and was filled based on eye measurement until there seemed to be approximately 3000 flies in it. Depending on how many flies that had emerged inside the puparia, one to three puparia were emptied into one BSF-net which took 15 minutes to 2,5 hours of waiting time.

BSF-net management: Mating and egg laying

The nets were mounted inside the greenhouse at two levels on metal frames (Figure 7). A bowl with attractant substrate was placed inside the net at the bottom to attract female BSF to that area for egg laying. To make it easier to harvest the eggs, egg traps were placed on top of the attractant bowl to make the female BSF lay their eggs inside the egg traps. The eggs traps consisted of four wooden boards tied together with two rubber bans at the ends. Every other board had bent nails in them to create gaps of a couple of millimeters where the flies could lay their eggs. The egg traps were placed so that the gaps were parallel to the ground to avoid eggs falling out and protect them from direct sunlight. Three egg traps were placed on top of the attractant bowl, or four if the BSF had a high fly density.

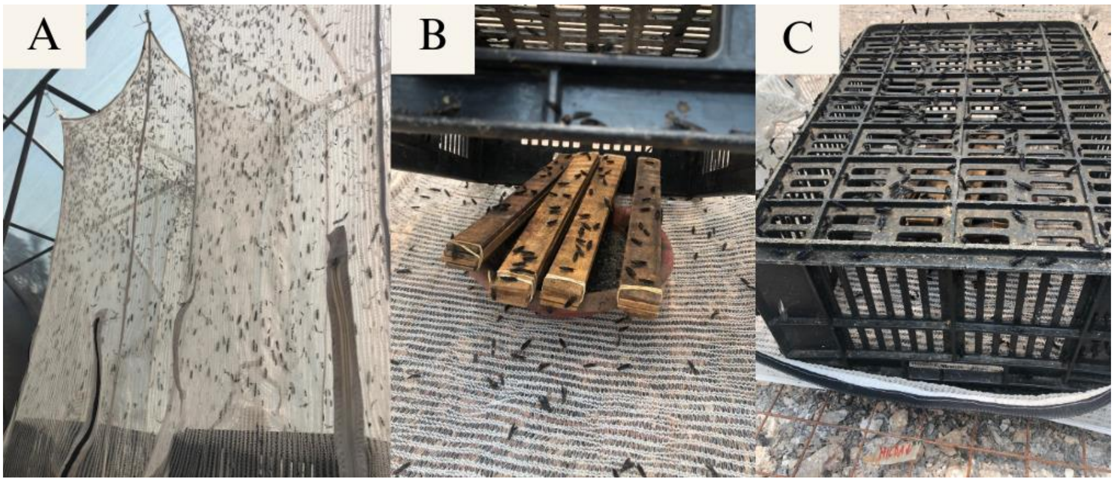

Figure 7. Picture of baseline BSF-net management: A) BSF-net inside greenhouse; B) Egg traps on top of attractant bowl: C) Protective crate inside BSF-net.

A protective black plastic crate with 1- 2 cm wholes was placed, upside down, on top of the attractant bowl and the egg traps, to create a shaded and protected environment for the female BSF and the egg traps. The nets were sprayed with water at 9 AM and 2 PM every day to as water source for the BSF. The factory was closed for half of Saturday and all of Sunday, therefore the BSF-nets were only sprayed in the morning on Saturdays and not at all on Sundays. The BSF mated and laid eggs for four days and then the egg traps were collected.

Nursery management: From egg to seed larvae

The egg traps were collected, taken apart, and the eggs were carefully scraped off with a tweezer into tea strainers (Figure 8). Each tea strainer was filled with 9 g of eggs using balance B2. One egg weigh 29 µg[1] which means that one tea strainer contained 310 000 eggs. The nursery was a metal frame that was covered with a net for protection from pest such as birds, rats, and houseflies. Inside the nursery were egg showers and trays with neonates. An egg shower contained six filled tea strainers that were hung above a tray with 3 kg of starter substrate. The eggs started to hatch inside the tea strainer and dropped into the starter substrate. The egg showers were continuous systems, meaning that the starter substrate and one tea strainer were changed every day. The neonates remained in the nursery and fed on the starter substrate for 5 -7 days, depending on how fast the larvae grew to a size when they could be enumerated, and the starter substrate had dried enough to make the larvae separable from the substrate. The larvae were manually separated from the starter substrate residue and added to the treatment process.

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[1] Unpublished data from SLU BSF-colony.

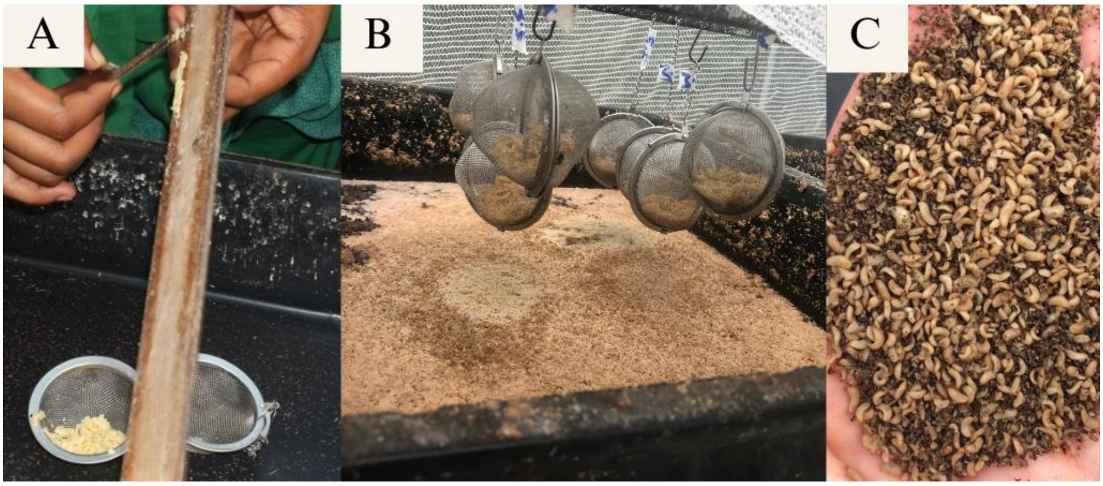

Figure 8. Picture of nursery management: A) Scraping egg of egg traps; B) Egg shower; C) seed larvae.

3.3 EXPERIMENTAL SET-UP

Three sets of experiments were conducted to determine
A) Emergence rate;
B) Number of eggs laid per female BSF;
C) Hatching rate.

Set A comprised of two set-ups
A0) Baseline puparium management (semi-open environment, both prepupae and pupae);
A1) Puparium management (closed environment, only pupae).

Set B comprised of four set-ups
B0) Baseline BSF-net management (inside greenhouse, protective crate, spraying);
B1) BSF-net management (inside greenhouse, no protective crate, spraying);
B2) BSF-net management (inside greenhouse, no protective crate, no spraying, container of water);
B3) BSF-net management (outside greenhouse, no protective crate, no spraying, container of water).

Set C comprised of four set-ups
C0) Baseline nursery management (egg laying and hatching inside greenhouse, egg scraping);
C1) Nursery management (egg laying and hatching inside greenhouse, no egg scraping);
C2) Nursery management (egg laying inside greenhouse, hatching outside greenhouse, egg scraping);
C3) Nursery management (egg laying and hatching outside greenhouse, egg scraping).

The set-ups marked with 0 at the end were part of the baseline study and were conducted as similar to the procedure described in 3.2.1 as possible. The other set-ups were conducted to investigate parameters that could affect the BSF-colony efficiency.

During the first weeks of investigating the baseline, some observations were made:

1. A parasitic wasp (*Dirhinus giffardii*), that infects the pupae, was discovered in the treatment area and inside the puparia.
2. BSF escaped from the BSF-net while the protective crate was placed inside the net, and some of the eggs were misplaced on the protective crate instead of the egg traps.
3. The BSF-nets dried out quickly after being sprayed with water.
4. The temperature in the greenhouse was above the recommended value.
5. The egg scraping process appeared harsh for the eggs.

3.3.1 Emergence rate

A0: Baseline

The baseline set-up for puparium management was the same as the normal production (Figure 6). A Tinytag sensor was attached to the roof inside the puparium to track the temperature and RH.

A1: Closed environment, only pupae

In response to observation 1, a more closed environment set-up for the puparium was conducted to investigate if the parasite was affecting the BSF-colony efficiency. This set-up contained only pupae that were picked manually from the treatment trays. Three containers, each containing 100 pupae, were sealed with mesh on top and placed inside a puparium (Figure 9). The puparium was opened after three weeks, the containers were placed in a freezer for one hour to kill the flies, and then examined one by one. A Tinytag sensor was placed inside the puparium to track the temperature and RH.

Figure 9. Picture of A1 set-up. Three containers, with 100 pupae in each, with mesh net on top inside a puparium.

3.3.2 Number of eggs laid per female BSF

B0: Baseline

The baseline set-up for BSF-net management was the same as the normal production (Figure 7). A Tinytag sensor was hanged at the top of the first level of BSF-nets (1,5 m up) and at the top of the second level of BSF-nets (3 m up), to track the temperature and RH inside the greenhouse. An empty BSF-net was weighed before and after being filled using balance B1. The egg traps were weighed using balance B2 before and after egg laying.

B1: No protective crate

In response to observation 2, it was decided to investigate the effect of the BSF-colony efficiency when excluding the protective crate from the BSF-net management. Except for the absence of the protective crate, the set-up was the same as B0 (Figure 7).

B2: Water container instead of spraying and no protective crate

In response to observation 3, a container filled with water was placed below the net when the net was mounted (Figure 10), instead of spraying water on the BSF-nets two times a day. The attractant bowl was also placed below the net and the egg traps were placed inside the net on top of the attractant bowl. The water container was placed below the net to lower the risk of flies drowning when drinking and filled to the brim so that the flies could access the water. Due to spatial limitations, there was no protective crate.

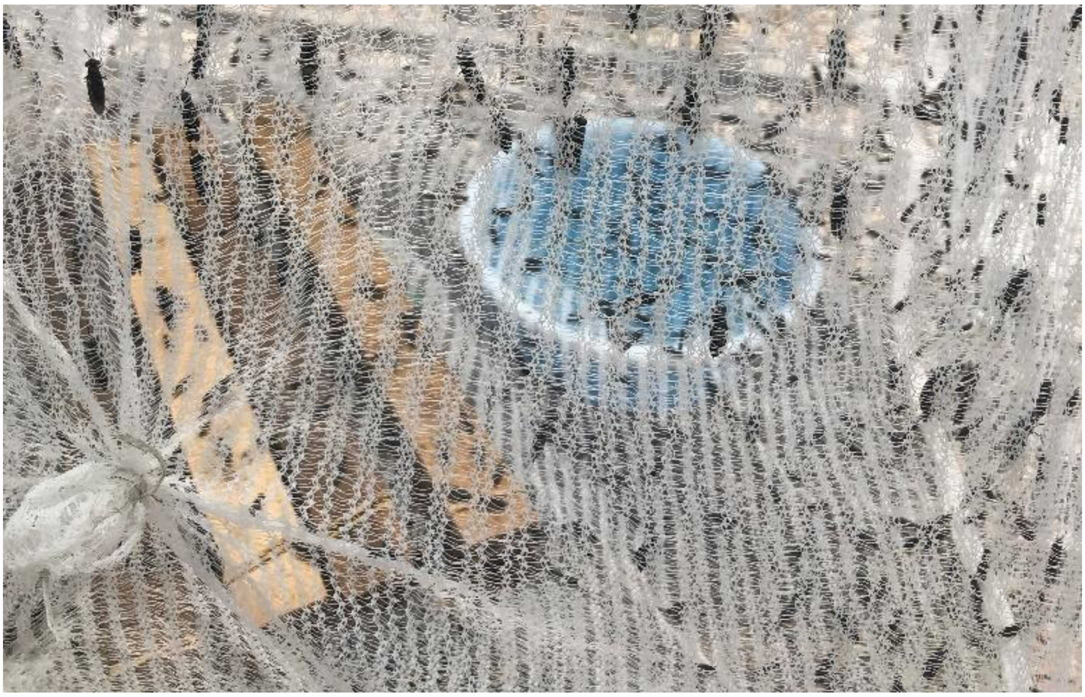

Figure 10. Picture of B2 set-up. Water container and attractant bowl below BSF-net and egg traps inside BSF-net.

B3: Lower temperature, water container and no protective crate

In response to observation 4, two BSF-nets were moved outside the greenhouse where the temperature was lower (Figure 11). The nets were mounted in an area with same access to direct sunlight as the greenhouse and with the same protective net around the metal frames. The set-up was otherwise the same as B2. Tinytag sensors were placed inside the greenhouse and in the BSF-nets outside the greenhouse to track temperature and RH.

Figure 11. Picture of B3 set-up. Two BSF-nets outside the greenhouse, with protective green mesh covering them.

3.3.3 Hatching rate

C0: Baseline

The baseline set-up was the same as the normal procedure except for being a continuous system were one tea strainer and the starter substrate were changed every day, see section 3.2.1. Instead of a continuous system, six tea strainers with 9 ± 0,1 g of eggs in each were placed in one egg shower at the same time (Figure 8). The starter substrate was changed once after two days and after five days the egg shower was finished.

C1: No scraping of the eggs

In response to observation 5, a set-up without scraping was conducted. Instead of scraping the egg traps the egg traps were placed directly in the egg shower with the gaps perpendicular to the ground to make it easier for the larvae to fall into the starter substrate (Figure 12). The egg traps were weighed with balance B2 before and after being placed into BSF-nets. Approximately seven egg traps were needed to obtain 54,0 ± 1,0[2] g of eggs. Apart from this, the set-up was the same as for C0.

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[2] The accuracy differed from the scraping set-ups because of the lack of control of how many grams of eggs there were in the egg traps.

Figure 12. Picture of C1 set-up. Egg shower with egg traps instead of tea strainers.

C2: Nursery with a lower temperature

In respons to observation 4, two experimental set-ups were investigated (C2 & C3) to establish if the hatching rate was affected by the temperature. In the first set-up, the BSF-nets remained inside the greenhouse and the egg shower was placed in the treatment area instead of the greenhouse (Figure 4), because the treatment area had a lower temperature. After two days of hatching, the first tray was moved to the nursery in the greenhouse, and after the fifth day, the second tray was moved there as well. Apart from this, the set-up was the same as for C0. Tinytag sensors were placed in the nursery inside the greenhouse and the egg shower in the treatment area to track temperature and RH.

C3: BSF-nets and nursery with a lower temperature

This experimental set-up investigated the potential effect that the high temperature in the greenhouse had on the eggs during egg laying and hatching. The BSF-nets were placed outside the greenhouse, the same set-up as B3 and apart from this, the set-up was the same as for C2.

3.4 DATA COLLECTION AND ANALYSIS

Temperature and RH data was collected and analysed for the areas where the experimental set-ups took place: The egg shower at the treatment area, the nursery inside the greenhouse, inside the puparia, the BSF-net area inside the greenhouse at two levels and the BSF-nets outside the greenhouse (Figure 4). The sensors measured the temperature and RH every ten minutes. An average for each time point per facility area was calculated. From that, the daily minimal and maximum average temperature and RH was received. Data for the daily maximum temperature measured throughout the study per facility area was also received from the data.

3.4.1 Emergence rate

A0: Baseline

To determine the efficiency of the puparium management, the emergence rate of the puparia was analysed. The emergence rate is the ratio between the sum of emerged flies and the sum of prepupae and pupae in a puparium. Therefore, it was necessary to determine how many prepupae and pupae the puparia contained and how many flies that had emerged.

Because the content in the puparium was a mixture of pupae, prepupae, dead larvae, pupae shells, and frass (Figure 7), samples were taken to analyse how large fraction of the content that was pupae and prepupae. The puparium content was divided evenly into five trays that were weighed, using balance B2. The puparium content was thoroughly mixed to get even samples, and one sample of approximately 85 ml was manually collected from each tray. The five samples were weighed separately using balance B3 and separated manually into fractions: pupae, prepupae, dead larvae, pupae shells, and frass. The fractions were weighed using balance B3 and the prepupae, pupae, dead larvae and shells were enumerated.

To determine the total number of flies that emerged, the total weight of flies emerged and the weight of one fly was collected. Samples of flies were taken from several nets throughout the study to determine the weight of one fly. A small empty plastic bag was weighed using balance B3 and filled with approximately 10 flies. The bag with the flies was weighed and the flies enumerated.

A1: Closed environment, only pupae

To determine the effect that the parasitic wasp (*Dirhinus giffardii*) had on the efficiency of the puparium management, the emergence rate was analysed in a more controlled environment. The containers were emptied and examined one by one to count the number of flies, shells, and pupae. If there were more shells than flies, it was assumed that the excess shells had contained parasites. All the non-emerged pupae were dissected and separated into infected and non-infected pupae, depending on if the pupae contained a parasitic wasp or not, Figure 13. The different fractions were enumerated.

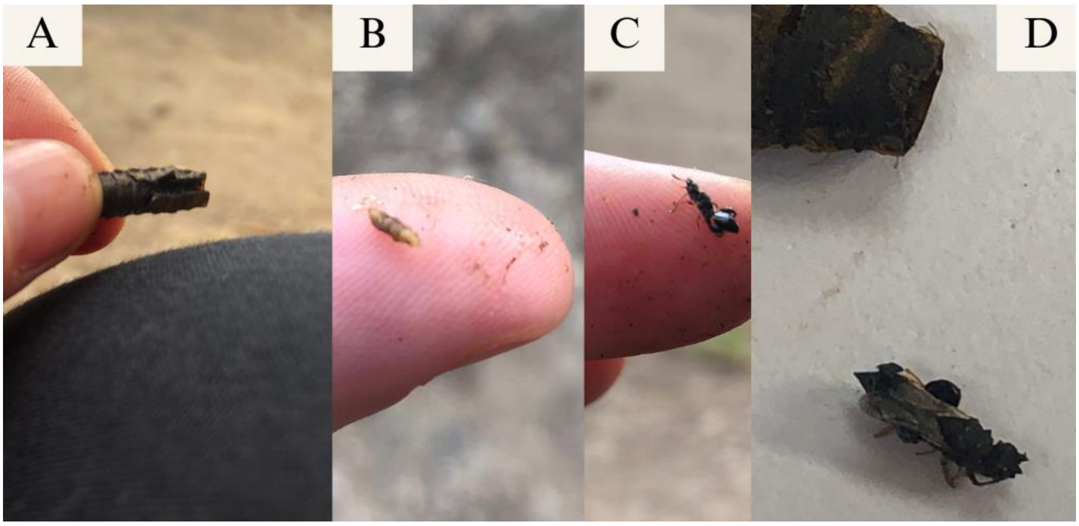

Figure 13. Picture of parasitic wasp (Dirhinus giffardii) at different stages: A) Dissected BSF-pupa; B) Parasitic wasp, pupal stage, found inside BSF-pupa; C) Parasitic wasp, adult stage, found inside BSF-pupa; D) Parasitic wasp, adult that emerged from BSF pupa.

3.4.2 Number of eggs laid per female BSF

To determine the efficiency of the BSF-net management, the number of eggs laid per female BSF was analysed. The weight of BSF per net, the weight of one fly and the weight of the eggs laid per net were measured using balance B2. Only the eggs laid in the egg traps were considered and it was assumed that the sex ratio of female and male BSF was 50:50. During collection of the egg traps, the BSF-net was examined visually to estimate the amount of misplaced eggs inside the net. If there was significant fraction of misplaced eggs, the net was excluded from the results.

3.4.3 Hatching rate

To determine how efficient the nursery management was, the hatching rate was analysed. The hatching is the ratio between total number of eggs and the number of the eggs that hatch and survive to the seed larval stage, per egg shower (Figure 8 & Figure 14). The weight of the eggs was measured using balance B3.

Five to seven days after hatching, the seed larvae were separated manually from the starter substrate residue/frass (Figure 14). This was done by repeating following steps until there were two fractions that contained mainly larvae and frass:

1. Mixed the tray evenly.
2. Waited 5 – 10 minutes until the larvae had crawled down to the bottom of the tray.
3. Scraped of the frass on the top.

Figure 14. Picture of fractions after separating seed larvae. Frass to the left and seed larvae to the right.

As some seed larvae remained in the frass fraction and some frass remained in the seed larvae fraction, an estimation on how many seed larvae there were in each fraction was needed. Therefore, both fractions were weighed, using balance B2, and sampled, respectively. Because of the mixture of frass and seed larvae in both fractions, the content of the fractions was thoroughly mixed before the sampling. Three samples, 0,5 – 1 ml each, were taken per fraction. Each sample were weighed, using balance B3, and the seed larvae in the sample enumerated.

3.5 CALCULATIONS

All calculated averages in the study are arithmetic means. The values after the plus-minus sign (±) for some of the mean values are the standard deviation. The standard deviations were calculated to show the dispersion of the data points that the means were based on.

3.5.1 Emergence rate

A0: Baseline

The emergence rate, $ER = Fn/(PPn+Pn)$, was defined as the ratio between the total number of emerged flies, Fn, to the total number of pupae, Pn, and prepupae, PPn, in the puparium.

The total number of pupae, $Pn = fp*mt/P1m$, in the puparium was calculated as the average proportion of the weight made up of pupae, fp, times the total weight of the puparium content, mt, divided by the average weight of one pupa, $P1m$. The weight proportion, $fp = Pms/ms$, was calculated as the sample pupae weight sample, Pms, divided by the total sample weight, ms. The weight of one pupa, $Pm1 = Pms/Pns$, was calculated as the sample pupae weight, Pms, divided by the number of pupae in the sample, Pns. The number of prepupae, PPn, was calculated in the same way.

The number of emerged flies, Fn, was calculated as the sum of emerged flies per BSF-net divided by the average weight of one fly. The weight of one fly was calculated as the total weight of flies in a sample divided by the number of flies in that sample.

A1: Closed environment, only pupae

The emergence rate without the influence of parasitic wasps, $ERp = Fn/(Pn-PIn)$, was defined as the ratio between the total number of emerged flies, Fn, to the total number of pupae, Pn, in the puparium, minus the number of parasite infected pupae, PIn.

3.5.2 Number of eggs laid per female BSF

The number of eggs laid per female fly, $EPFF = En/(0{,}5*Fn)$, was defined as the ratio between the total number of eggs laid inside the egg traps in a BSF-net, En, to 50 %[3] of the total number of flies in the net, Fn.

The total number of eggs, En, laid in the egg traps in a BSF-net was calculated as the weight of the eggs divided by the weight of one egg (29 µg/egg)[4]. The total number of flies per net was calculated as described in section A0.

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[3] A sex ratio of 50:50 in the BSF-nets was assumed.

[4] Unpublished data from SLU BSF-colony.

3.5.3 Hatching rate

The hatching rate, $HR = Ln/En$, per egg shower was defined as the ratio between the total number of seed larvae, Ln, to the total number of eggs, En.

The total number of seed larvae per egg shower, Ln, was calculated as the sum of larvae from the larvae fraction, LLn, and the larvae from the residue fraction, LFn, (Figure 14). The total number of larvae per fraction, $LLn = LLm/L1m$, was calculated as the total weight of the fraction, LLm, divided by the average number of larvae per fraction weight, $LL1m$. The number of larvae per fraction weight, $LL1m = LLms/LLns$, was calculated by dividing the sample weight, $LLms$, by the number of larvae in the sample, $LLns$.

4. RESULT

The highest daily average maximum and maximum temperature were observed inside the greenhouse, at the second level of the BSF-nets, reaching 42 and 45 °C, respectively (Table 1). The first level of the BSF-nets inside the greenhouse was the second warmest area, with a daily average maximum temperature of 37 °C and a daily maximum temperature of 41 °C. The BSF-nets outside the greenhouse were 1 °C cooler on daily average maximum temperature and 3 °C cooler in daily maximum temperature than level one inside the greenhouse. The nursery at the treatment area had the lowest daily average maximum temperature, at 32 °C, and the nursery inside the greenhouse had an daily average maximum temperature of 35 °C.

The second level inside the greenhouse had the lowest daily average minimum RH, at 36 % (Table 1). The first level had the second lowest daily average minimum RH at 47 %. The highest average minimum RH was observed at the nursery in the treatment area, at 63 %. In all areas, the daily average maximum RH was above 80 %.

Table 1.The daily maximum temperature and the daily average minimum and maximum temperature and relative humidity in the different facility areas: Puparium, Greenhouse (Level 1, level 2, nursery), Nursery in the treatment area, and BSF-nets outside greenhouse.

Facility area	Daily average minimum temperature [°C]	Daily average maximum temperature [°C]	Daily maximum temperature [°C]	Daily average minimum RH [%]	Daily average maximum RH [%]
Puparium area	25	33	40	57	86
Greenhouse, Level 1	24	37	41	47	89
Greenhouse, Level 2	24	42	45	36	93
Greenhouse, Nursery	25	35	38	53	88
Treatment area, Nursery	25	32	38	63	90
BSF-nets outside greenhouse	24	36	38	50	97

The daily peak temperature was above the optimal temperature every day in all the BSF-nets (Figure *15*). The daily peak temperature inside the greenhouse was higher than in the BSF-nets outside the greenhouse. The graphs in Figure *15* partly show the temperature for different time periods because there was limited number of sensors and the experimental set-ups were not all carried out in parallel.

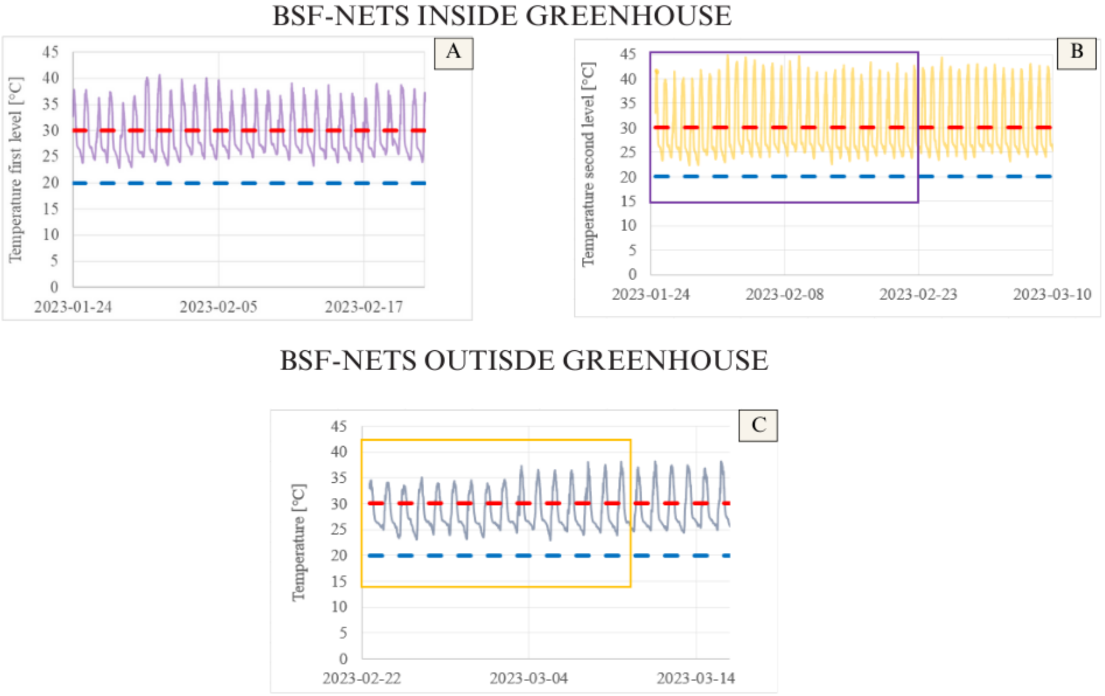

Figure 15. Temperature in the BSF-nets: A) First level, inside the greenhouse; B) Second level, inside the greenhouse. The purple box shows the overlap period for the temperature data in A); C) Outside the greenhouse. The orange box shows the overlap period for the temperature data in B). The temperature between the blue and red dashed line are the optimal temperature according to current scientific literature.

The daily peak temperature was above the optimal temperature the most days in the nursery inside the greenhouse (Figure 16). The daily peak temperature was above the optimal temperature a couple of times, at the end of the study, in the egg showers outside the greenhouse. The graphs in Figure 16 partly show the temperature for different time periods because there was limited number of sensors, and the experimental set-ups were not all carried out in parallel.

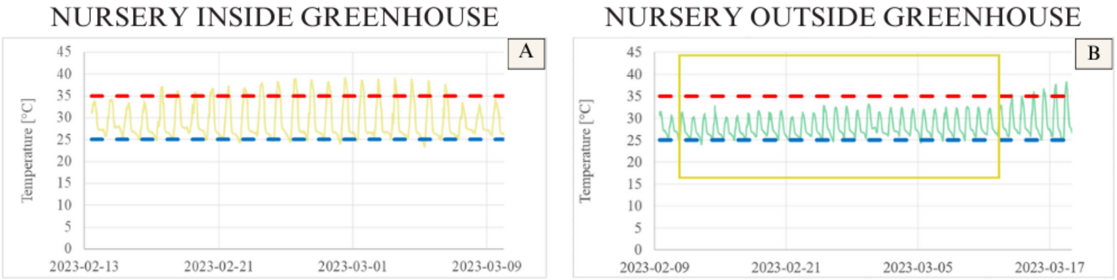

Figure 16. Temperature in the nursery: A) Inside the greenhouse; B) Outside the greenhouse, in the treatment area. The yellow box shows the overlap period for the temperature data in A). The temperature between the blue and red dotted line are the optimal temperature according to current scientific literature.

The temperature in the greenhouse was mostly within the limits of the optimal temperature (Figure 17). On February 16, is a clear temperature peak of 40 °C.

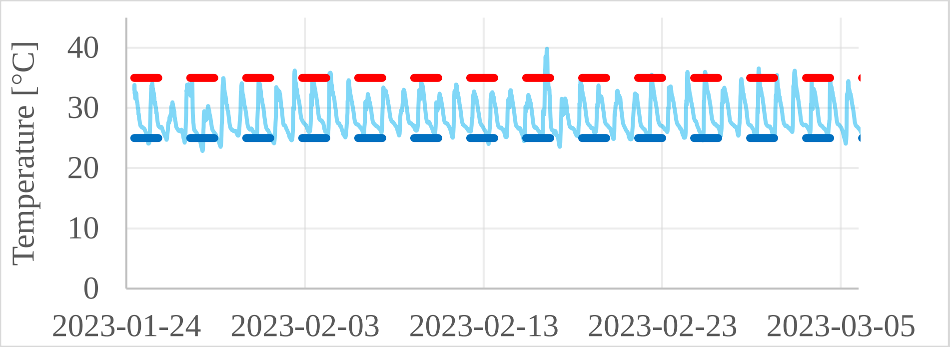

Figure 17. Temperature inside the puparia. The temperature between the blue and red dotted line are the optimal temperature according to current scientific literature.

4.1 EMERGENCE RATE

Three puparia were monitored to establish the baseline emergence rate (set-up A0). The initial content in the puparia was divided into fractions, and the prepupal stage was the most common, making out 41 % of the total BSFL found (Table 2). The average total number of prepupae and pupae per puparium was 82 000 ± 14 000 and the frass represented 39 ± 5 % of the total weight.

Table 2. Sample weight: frass, prepupa, pupa, dead larva, pupa shells, total; number of BSFL: prepupa, pupa, and dead larva per sample; calculated total number of BSFL fractions per puparium (set-up A0).

	Weight per sample [g]	BSFL per sample [#]	BSFL per puparium [#]
Frass	7 ± 1	-	-
Prepupa	5 ± 2	57 ± 29	46 000 ± 12 000
Pupa	5 ± 1	42 ± 10	36 000 ± 2 000
Dead larva	2 ± 2	41 ± 34	31 000 ± 19 000
Pupa shell	0,2 ± 0,1	7 ± 4	7 000 ± 5 000
Total	19 ± 6	147 ± 77	120 000 ± 38 000

The average weight of one adult BSF was 0,068 g and the total number of emerged BSF per puparium was 23 000 ± 2 000. The results for A0 and A1 are based on three data points per set-up. The emergence rate for A0 was 26 ± 4 % (Figure 18). The emergence rate for A1, puparium management in a more controlled environment, was 42 ± 5 %, and when excluding the parasite infected pupae, the emergence rate for A1 increased to 66 ± 4 %.

Figure 18. Emergence rate per set-up: A0) Baseline, both prepupae and pupae; A1) Closed environment, only pupae; A1 no parasites) Closed environment, only pupae, without parasite infected pupae.

4.2 NUMBER OF EGGS PER FEMALE BSF

The BSF-nets were filled with 4 600 ± 1 500 BSF per net, with an average weight of 0,068 g per adult BSF. The number of monitored BSF-nets used in the results were, 18 nets for set-up B0, 12 for set-up B1, 10 for set-up B2, and 9 for set-up B3. The maximum number of eggs laid per female BSF was observed for set-up B3, at 832 eggs/female BSF (Figure 19). The average number of eggs per female BSF ranged from lowest to highest in the following order: B1 (273 ± 96), B0 (383 ± 128), B2 (439 ± 189) and B3 (581 ± 140). The results from set-up B2 had the largest variation, ranging from 104 to 778 eggs/female BSF, while set-up B3 had the smallest variation, ranging from 328 to 832 eggs/female BSF.

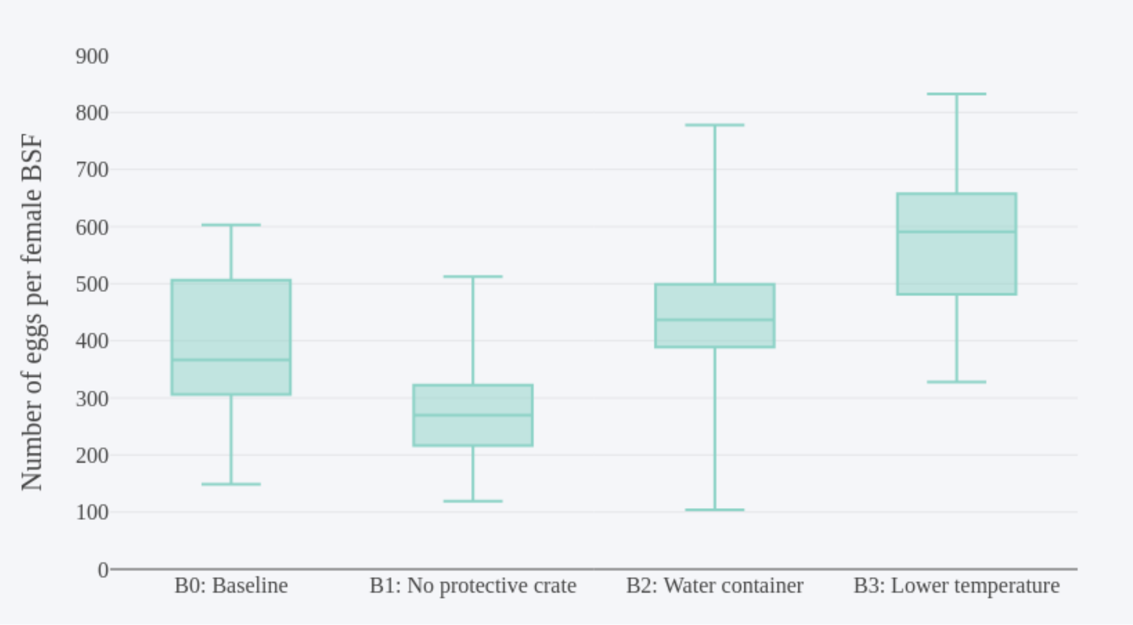

Figure 19. Number of eggs per female BSF per set up: B0) Baseline, inside greenhouse, protective crate, spraying; B1) Inside greenhouse, no protective crate, spraying; B2) Inside greenhouse, no protective crate, no spraying, water container; B3) Outside greenhouse, no protective crate, no spraying, water container.

4.3 HATCHING RATE

Each egg shower contained 1 864 000 ± 4000 eggs for set-up C0, C2 and C3, while set-up C1 contained 1 863 000 ± 33 000 eggs. The number of egg showers monitored that was used in the result was 7 for C0, 3 for C1, 3 for C2, and 4 for C3. The lowest achieved hatching rate was observed for the baseline (C0) set-up, the highest observed hatching rate was observed in set-up C3, where the egg laying and hatching were done at lower temperature (Figure 20). The average hatching rate ranged from lowest to highest in the following order: C0 (40 ± 9 %), C2 (44 ± 3 %), C1 (45 ± 7 %) and C3 (51 ± 20 %). Set-up C3 had the largest variation, ranging from a hatching rate at 28 to 71 %.

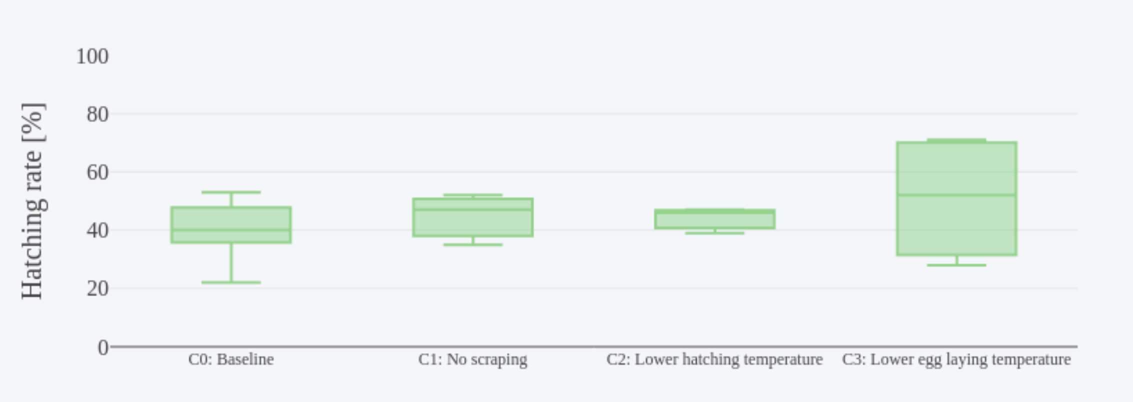

Figure 20. Hatching rate per set-up: C0) Baseline, BSF-nets and nursery inside greenhouse, scraping; C1) BSF-nets and nursery inside greenhouse, no scraping; C2) BSF-nets inside greenhouse, nursery outside greenhouse, scraping; C3) BSF-nets and nursery outside greenhouse, scraping.

5. DISCUSSION

The study was conducted to investigate possible factors that can impact the efficiency of the BSF-colony, in terms of emergence rate, number of eggs laid per female BSF, and hatching rate, at a large scale BSFL composting company. In order to do that, a baseline study was first carried out to establish the current efficiency at the BSFL composting company where the study took place. Observations were made on the puparium-, BSF-net-, and nursery management, which resulted in five possible factors that were impacting the efficiency: High temperature in the BSF-nets and nursery, water shortage for the adult BSF, damage of the eggs while scraping them off the eggs traps, and parasitic wasps in the puparium area.

5.1 EMERGENCE RATE

The emergence rate for the baseline puparium management was 26 ± 4 % (Figure 18) which is significantly lower than 80 – 90 % that could be expected according to Dortmans et al. (2017) and Caruso et al. (2014). The emergence rate increased from 42 ± 5 % to 66 ± 4 % when excluding the parasite infected pupae from the results in A1, which suggest that parasitic infestation is a major reason for the low emergence rate in the baseline system. However, the emergence rate was still higher for A1, even when the parasites were included in the result. This could be explained by the different input in the puparia in the two set-ups. Set-up A0 had both prepupa and pupa as input, while A1 only had pupa. The puparia were kept up for three weeks, and upon a visual inspection at that time, in set-up A0, a significant proportion of the prepupae were still moving. Possible reasons could be insufficient energy or nutrition in the feed during the treatment step (Chia et al. 2018b).

A visual examination was conducted during the set-up and takedown of each puparium. Initially, the parasitic wasps (*Dirhinus giffardii*) were not noticeable in the content of the puparia (Figure 6). However, when the puparia were taken down after three weeks, multiple parasitic wasps were observed in the trays, flying around, and on the walls inside the puparia. Even if no adult wasps were noticed in the A0 puparia at the time of set-up, it is likely that there were some present, due to the large volume of the puparium content and the observation of adult wasps in the treatment area. The A1 set-up was more controlled, and care was taken to ensure that no adult parasitic wasps were included in the puparium content. *Dirhinus giffardii* lifecycle ranges from 16 to 20 days (Devic & Maquart 2015), and one female wasp can infect 13 – 58 BSF pupae. This indicates that the adult parasitic wasps in the puparium content (set-up A0) and the wasps inside BSF pupae at the time of set-up had time to emerge, mate and infect new pupae during the active period of the puparium. Therefore, the parasite infestation may have accumulated inside the A0 puparia.

Wang & Messing (2004) found that *Dirhinus giffardii* mainly infect pupae that are less than two days old. The baseline (A0) set-up contained a high proportion of prepupae that were evolving into pupae during the time the puparium was active, providing young pupae for the parasitic wasps to infect. This suggests that set-up A0 was a more favourable environment for the parasitic wasps than A1, which only contained BSFL already in the pupal stage at the time of the set-up. These observations combined with the difference in set-up and emergence rate between A0 and A1 suggest that there was a higher parasitic infestation rate in the A0 puparia than the A1 puparium.

Unpublished data from SLU's BSF-colony suggest that there is a correlation between the dimensions of the puparium and the emergence rate, in relation to how many flies that are able to exit the puparium. The study's puparium measured 140x140x140 cm with an opening that measured 30x20 cm and was placed relatively high up on the puparium (Figure 21). The unpublished study by SLU found that it is favourable to have a puparium with an opening that is relatively big in relation to the length and height to ensure that the adult BSF can see the light, outside the puparium, and find the opening when emptying the puparium of BSF. This suggest that the dimensions of the puparia in this study were non-optimal and contributed to the low baseline emergence rate.

Figure 21. Closed puparium. Opening for emptying adult BSF, tied with a knot, at the top of the puparium.

5.2 NUMBER OF EGGS LAID PER FEMALE BSF

The results from the BSF-nets for the different set-ups indicate that the number of eggs laid per female BSF were influenced by both water access and temperature (Figure 19). Adding a water container resulted in an increased egg production, compared to the baseline value, by 15 %. Adding a water container and moving the BSF-nets outside the greenhouse resulted in an increase in egg production, compared to the baseline value, by 52 %. This suggests that the temperature influenced the egg production in a higher rate than the water access in this case.

Chia et al. (2018a) determined that the longevity of BSF was significantly reduced at 35 °C and above. This suggest that the BSF maybe did not survive long enough to lay eggs in the BSF-nets inside the greenhouse due to high temperature. The daily maximum temperatures in the BSF-net inside and outside the greenhouse were potentially all above the threshold (Figure 15). The daily average maximum temperature in the greenhouse was 37 - 42 °C, with peaks up to 41 – 45 °C, and the daily average maximum in the BSF-nets outside the greenhouse was 36 °C, with peaks at 38 °C. However, the results suggest that even at these high temperatures, lowering the temperatures with a couple of degrees can increase the egg production significantly (Figure 19).

When the protective crate was removed, there was a decrease in egg production, compared to the baseline value, by 29 % (Figure 19). The initial motivation for usage of a protective crate was to create a protected and shaded environment for the female BSF to lay their eggs, as suggested by Dortmans et al. (2017). Observations during the study suggested that the protective crate also served as an alternative water source due to water condensation on the crate. The BSF-nets with a protective crate were covered with condensed water in the mornings, providing BSFs water access during the nights until the water spraying at 9 AM. The increase in egg production in the other set-ups which did not have a protective crate, indicates that the main reason for the loss in egg production is due to the loss in water access or other factors that were not explored.

Providing water to BSFs during mating and egg laying has been observed to significantly increase egg production (Dortmans et al. 2017; Chia et al. 2018b; Macavei et al. 2020). As seen in the results, it may be beneficial to stop spraying the BSF-nets with water and instead add a water container to increase egg production (Figure 19). However, there is uncertainty regarding this due to the of lack of routine relating to the spraying. The spraying of water was carried out by working personnel, and it was observed that there were many days with only one or no spraying throughout the day. This strengthens the suggestion that the BSF-nets with a protective crate produced more eggs than the ones with no crate because of the increased water access from the condensed water on the crate. The variation in spraying frequency may also explain some of the variation seen in the results for the baseline (B0) and the non-protective crate (B1) set-up.

Egg laying outside the egg traps, from here on called misplaced eggs, occurred at some rate in all the BSF-nets monitored, but there was a relatively high variation which was difficult to assess visually (Figure 22). This could explain part of the relatively high variation in the results (Figure 19). It was observed that the BSF-nets placed closest to the nursery had a higher rate of misplaced eggs. The female BSF in these BSF-nets mainly misplaced the eggs on the same side as the nursery, suggesting that the odour from the starter substrate in the nursery could have attracted them. Other factors affecting the rate of misplaced eggs were the cleaning and handling of the BSF-nets and the protective crate. The routine was to wash the BSF-nets and protective crate after every use and handle of the BSF-nets during filling and mounting in a way that did not contaminate the net with dirt. It was observed that the BSF-nets were washed after every use, but the handling of the nets could have contaminated them. The protective crates were rarely washed throughout the study and were observed to be a source of misplaced eggs. This suggests that in order for the female BSF to lay their eggs in the egg traps (encouraged by the smell of the attractant), the nets, protective crate and, the surrounding vicinity should be clean and not submit smells that could attract the female BSF.

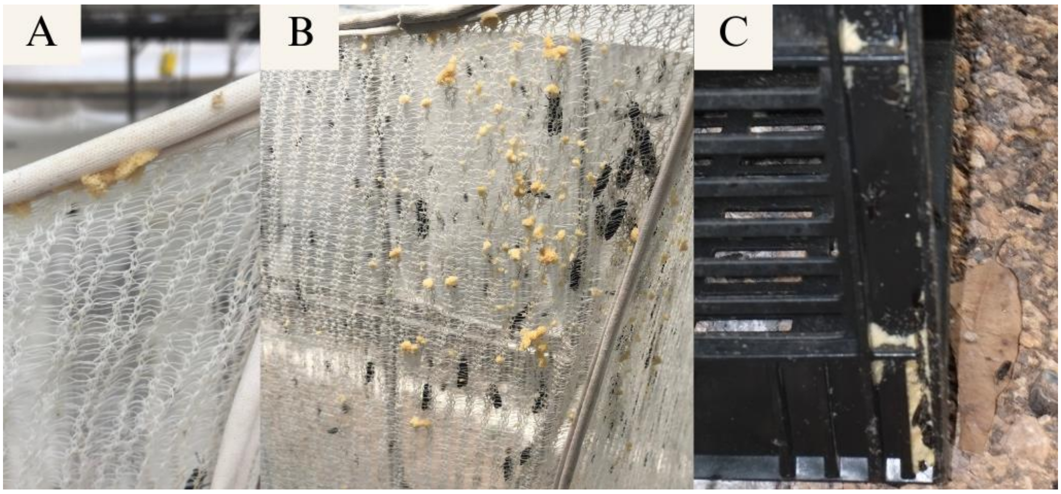

Figure 22. Misplaced eggs: A) Misplaced eggs on a BSF-net; B) Misplaced eggs on a BSF-net close to the nursery; C) Misplaced eggs on the protective crate.

5.3 HATCHING RATE

The low number of data points per set-up in combination with a significant variation in the results, should be taken into consideration when interpreting the hatching rate results (Figure 20). However, based on the results and visual observations, it is suggested that the temperature during egg laying was the factor that had the greatest impact on the hatching rate. The highest hatching rate, 71 %, were achieved in set-up C3, where the BSF-nets were placed outside the greenhouse and the egg showers were placed in the treatment area (Figure 4).

A possible explanation that the hatching rate was higher for set-up B3 is that the BSFs may had better access to direct sunlight. Although the BSF-nets outside the greenhouse were covered with the same green mesh as the BSF-nets inside the greenhouse, the greenhouse mesh was dirtier, and a part of the greenhouse was shaded by a tree during a few hours each day. This could have led to more successful mating in the BSF-nets outside the greenhouse, resulting in a higher frequency of fertilized eggs.

Other possible reasons for the eggs to be damaged were observed throughout the study. A couple of the egg traps were contaminated with powdered lime, which was used around the BSF-nets to repel ants. To the authors knowledge, the possible effect lime has on BSF eggs has not been studied, but lime has been observed to repel a wide variety of insect species (Boucher & Adams 2012). There were also a couple of egg traps in the BSF-nets outside the greenhouse that were attacked by ants.

Zenyr Garden sells BSF eggs and recommend a maximum temperature of 29 °C and keeping the eggs in a shaded and humid environment to prevent moisture loss. Chia et al. (2018a) saw that 80 % of BSF eggs hatched at 30 °C, while only 10 % hatched at 37 °C. The daily average maximum temperatures in the BSF-nets and in both nurseries were all above 30 °C (Figure 16). However, the nursery in the treatment area had the lowest daily average maximum temperature of 32 °C, and was not warmer than 35 °C more than four days throughout the study. The nursery inside the greenhouse had a peak temperature above 35 °C the majority of the days throughout the study.

The optimal RH for the eggs is 70 % according to Holmes et al. (2012). The daily average minimum RH in the nursery, inside the greenhouse, was 53 %, while the minimum RH in the nursery, in the treatment area, was 63 %. Visual examination of the hatched eggs was made after the egg showers were finished, and it was observed that the eggs from the nursery in the treatment area were fluffier and drier than the eggs from the nursery inside the greenhouse (Figure 23). According to Zenyr Garden (2022), successfully hatched BSF eggs should be fluffier, lighter and drier than newly laid eggs. This strengthens the suggestion that the eggs hatched better in the zone with lower temperature and higher RH.

Figure 23. Picture of eggs after five days in the egg shower: A) Eggs from nursery in the treatment area; B) Eggs from nursery inside the greenhouse.

All the eggs from the nursery in the treatment area were observed to be relatively well hatched, as described above. However, for some of these egg showers, the hatching rate was below 30 %, which suggests that the visual observation may have been misleading, a high degree of unfertilized eggs, or that there was a low survival rate of the neonates.

The neonates were given a starter substrate consisting of wheat bran, coconut bran, chicken feed and cow milk in the same ratio. However, the cow milk came from the same dairy waste used in the treatment process, and there was variation in its fat content. Joly & Nikiema (2019) suggest in their review, that neonates are sensitive to changes in environmental conditions and food competition. In a review by Kortsmit et al. (2022), three behavioral indicators for BSFL welfare were presented that could affect the efficiency of the rearing process: escape behavior due to unfavorable conditions in the feed, larval aggregation leading to temperatures that prevent optimal growth, and cannibalism due to food competition or excessive larval density. According to Barragan-Fonseca et al. (2021), the nutritional composition of the feed can affect the survival rate of BSFL. If given too little feed, BSFL may exhibit cannibalistic behavior, as per unpublished data by J.J.A von Loon (Kortsmit et al. 2022). The neonates may also suffocate if provided with feed having too fine particles or high moisture content, as they may struggle to create pore space for breathing (Yang 2017). Fluctuations in the starter substrates fat and moisture content could therefore be part of the variation in the results.

5.4 SOURCES OF ERROR IN DATA COLLECTION

There are some overall insecurities regarding the results, specifically related to disturbances of the balances used throughout the study. The balances were placed in a semi-open environment that was affected by wind and vibrations from passing vehicles and working personnel.

It was observed that some of the BSF-nets had been worn down to the point where BSFs could escape the net. This was especially noticeable in BSF-nets with high fly density, around 10 000 BSF/m^2. This implies that some of the data points may have been higher since the number of eggs laid was related to the number of BSFs added initially to the BSF-net and did not account for losing flies during the process.

There was no significant increase in the hatching rate for set-up C1, in which the eggs were not scraped from the eggs traps. However, part of the variation in the results for the other set-ups could be related to the scraping of the eggs. All the scraping was done manually and is likely to have been done with varying degrees of care, depending on factors such as stress or other human factors.

5.5 FURTHER RESEARCH

To the authors knowledge, there are no established normal baseline values for performance parameters, such as emergence rate, number of eggs per female BSF, and hatching rate, on an industrial scale. As mentioned by Joly & Nikiema (2019), monitoring performance parameters in every stage when mass producing BSFL could help track potential problems. The question of feasibility for BSFL composting companies may be closely connected to this. The BSFL composting company in this study used approximately 15 % of the produced larvae in the BSF-colony, and according to Dortmans et al. (2017), only 2 -5 % should be needed for a well functional BSF-colony. The results in this study suggest that numerous factors affect how many percentages of the production are needed in the BSF-colony. Therefore, further research and knowledge exchange among stake holders are needed to determine if, where and, why certain values for performance parameters, such as emergence rate, number of eggs per female BSF, and hatching rate, indicate problems in the process.

In the course of the study, several observations were made that raised questions about other potential factors that could have impacted the efficiency of the BSF-colony. However, answers to these questions could not be found in the current scientific literature, which opens up the possibility of further research in BSF-colony management.

When the puparia in the baseline study was taken down, a visual examination of the content was made. The examination showed a relatively large fraction of prepupae that still had not pupated. This raised the question of whether the high number of adult parasitic wasps inside the puparia agitated the prepupae, preventing them from pupating.

The results in the study suggest that high temperatures have a significant negative impact on the egg production. As mentioned, this could be explained by the possible shortened longevity of the female BSF, if they died before they could lay their eggs. However, the result raised the question about the possible correlation between temperature and egg laying, although no study found has been conducted regarding this. It would therefore be of interest to do further research to investigate whether the adult female BSF has a biological trigger that prevents it from laying eggs at unfavourable temperatures.

The dairy waste used in the starter substrate was stored in different ways and for different lengths of time. This raised the question whether the cow milk may have contained different kind of pathogens throughout the study, depending on its age, source and storage, and if this could have affected the survival rate of the neonates. According to Kortsmit et al. (2022), there are few reports of BSFL being affected by pathogens, but according to Joly & Nikiema (2019), neonates are more sensitive than seedlarvae. It would therefore be of interest to do further research regarding neonates survival rate related to pathogens.

6. CONCLUSION

The study’s aim was to investigate what factors affect the efficiency of the BSF-colony, in terms of emergence rate, number of eggs laid per female BSF, and hatching rate, on a large scale BSFL composting company.

The current BSF-colony was found to be infected by parasitic wasps (*Dirhinus giffardii*) and the results suggest that the parasitic wasps can reduce the emergence rate significantly. Further research on preventing and combating parasitic infestations in a BSF-colony is recommended.

When it comes to the number of eggs laid per female BSF, several factors were found to impact the efficiency. The results suggests that high temperatures and water shortage for adult BSF can reduce the number of eggs laid per female BSF. In addition, observations made during the study indicate that personnel routines and how the BSF-colony is arranged also could affect the egg production significantly.

However, in the case of the hatching rate, it was not possible to draw any conclusion, due to the great variation in the results. Further research regarding eggs hatchability and the survival rate of neonates is recommended.

The overall results indicate that the way BSF-colony management is conducted can affect the efficiency considerable. The high variations in the results suggest that there are additional factors that impact the efficiency, beyond the parameters explored in the study. Further research is needed to establish baseline values for emergence rate, number of eggs laid per female BSF, and hatching rate, on large scale BSFL-composting facilities.

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