Sustainable protein sources from greenhouses

Desk study

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Report WPR-1045

Referaat
De afgelopen jaren is de vraag naar duurzame eiwitbronnen toegenomen. Eetbare insecten die gekweekt zijn op reststromen uit de glastuinbouw en eiwitrijke gewassen die in kassen geteeld worden, kunnen hierin voorzien. In een literatuurstudie heeft Wageningen University & Research de mogelijkheden verkend. Eetbare insecten zoals meelwormen, krekels en sprinkhanen bevatten naast hoogwaardige eiwitten ook onverzadigd vet, vitaminen en mineralen. Ze kunnen op verschillende reststromen of substraten worden gekweekt, maar het dieet zal aangevuld moeten worden met eiwitten en vetten. De belangrijkste plantaardige eiwitbronnen zijn peulvruchten en oliezaden, die vaak worden gebruikt om dierlijke eiwitachtige producten te maken. Verschillende gewassen zijn gescreend op potentie voor kasteelt. Daarbij is gekeken naar voedingswaarde, geschiktheid voor hogedraadteelt en afwezigheid van juridische problemen. Lupinesoorten en tuinbonen voldoen aan deze eisen.

Abstract
De demand for sustainable protein sources has increased in recent years. Rearing edible insects using side streams from greenhouses and high protein crops grown in greenhouses can contribute to this demand. Wageningen University & Research has explored the possibilities in a deskstudy. Edible insects such as mealworms, crickets and locust species contain quality proteins, but also unsaturated fat, vitamins and minerals. They can be reared on various waste streams from greenhouse crops, but proteins and lipids should be added to the diet. The major plant protein sources are legumes and oilseeds, which are often used to generate animal protein-like products. Several crops were screened on their potential for greenhouse production on the criteria high nutrition value, suitability for high-wire cultivation and absence of legal issues. Lupin species and faba beans meet these requirements.

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Summary

In order to meet the protein demand in the near future, developing alternative and sustainable protein sources is very important for the food industry. In this research, we aim to get an overview of two potential protein sources from greenhouses, (1) rearing edible insects using side streams from greenhouses; (2) introducing high protein-rich crops into greenhouses.

Edible insects contain not only high quality proteins, but also unsaturated fat, vitamins and minerals. Insect rearing also has a lower environmental impact compared to the traditional livestock, as edible insect species can be reared on organic side streams or on various kinds of substrates. Meanwhile, greenhouse emissions, the water usage and the place requirements are relatively low compared to the traditional livestock. In the western countries, insect species are reared for human food include the following species: mealworms (*Tenebrio molitor, Alphitobius diaperinus, Zophobas morio*), crickets (*Acheta domesticus, Gryllodes sigillatus, Gryllus bimaculatus*), locust species (*Locusta migratoria, Schistocerca gregaria*), others as by-products from the silk industry (*Bombyx mori*). Several insect species can be reared as feed, such as mealworms, the black soldier fly (*Hermetia illucens*) and the house fly (*Musca domestica*). The nutritional values of these insect species mentioned above were also listed, such as crude protein (% dry weight), crude fat content (% of dry weight), amino acid composition (AAC), unsaturated fatty acids, fibre content, vitamins and minerals.

The chemical composition of various waste streams from greenhouses and nutrient requirements of rearing edible insects were collected. The aim was to find out possible resources in the waste streams for rearing edible insects. It was found that waste streams, such as potato steam peelings, denatured soybean meal, beet molasses, carrots and carrot side products, wheat bran, oats, rice bran, cassava plant tops, water spinach, waste plant tissues, etc. were already used in rearing edible insects, but the minimally-processed waste streams could not support adequate growth of insects. Adding a suitable amount of proteins and lipids could benefit the growth of insects. Therefore, the development of an effective artificial diets is crucial for rearing a specific insect sort successfully. More waste streams could be tested and processed into the insect diets in the field experiment to gain a remarkable protein production.

Several plant-based protein sources have already been consumed by humans. Cereals (wheat, rice, millets, sorghum), pseudo-cereals (amaranth, quinoa), seeds (chia, hemp, flax, caraway), nuts (almond, walnut), legumes (soybeans, yellow pea, faba pea, cowpea, lentils, peas, lupins), and leaves (moringa, duckweed) are sources of proteins which can be sustainably produced. The major plant protein sources on the market are legumes and oilseeds, which are used to generate meat-like or animal protein-like product.

Several criteria were applied to screen the potential protein-rich legume crops: (1) high nutrition value, such as protein content, unsaturated fatty acid and other benefits of the seeds; (2) plants can grow taller than 150cm, being suitable for high-wire growing in greenhouses; (3) no legal issues to grow in the Netherlands. Lupin species (*Lupinus albus L.* and *L. mutabilis Sweet*) and faba beans (*Vicia faba L.*) meet all these requirements and could be introduced to the greenhouses as suitable protein crop candidates. Their nutrition compositions and protein products applications were mentioned in the study. Further, the growing conditions of these crops were briefly discussed.

This desk study offered an overview of two alternative and sustainable protein sources from greenhouses, and also analysed the possibilities to make use of them in practice, which provides a basis for the internal strategy projects on the same theme.

1 Introduction

One of the missions of the agriculture sector is to produce adequate protein for humans. With the population growth, the protein demand is expected to increase by 110 % in the year 2050 compared to 2005 (Tilman *et al.* 2011). Livestock production has become the major source of protein globally, and it is already requiring 70% of all agriculture land (Steinfeld *et al.* 2006). In order to meet the protein demand in the near future, developing alternative and sustainable protein sources is very important for the food industry. In this project, we try to have an overview of rearing edible insects using side streams from greenhouses and introducing new crops or plants rich in protein into greenhouses. Insects for feed will be also included. The nutritional value of these alternative protein sources will be summarized.

1.1 Objective

1. Evaluate existing side streams from greenhouses and their characteristics to find suitable insects to rear on those side streams;
2. To identity which crops are potential candidates for protein production in greenhouses;
3. Combination of literature and practical insights will result in a list of protein producing insects and potential crops to be screened.

1.2 Main questions

- What are the promising edible insect species for protein production?
- What are the nutritional values of these insect-based protein?
- What are the waste streams from greenhouses which be used to rear these insects?

- Which crops or plants are good sources of plant-based protein?
- What are the nutritional values of these plant-based protein?
- Which of these crops or plants are suitable to grow in greenhouses for protein production?

2 Insect-based protein sources

Edible insects are the insect species that can be not only used for human consumption but also for livestock feed as a whole, parts of them, and/or protein, and lipid extract. The interest of using insects as food and feed has been exponentially increasing in recent several years, because edible insects have some advantages for human nutrition, such as high protein, amino acid, lipids, energy and various micronutrients. Besides the nutritional values, insect rearing has a lower environmental impact compared to the traditional livestock, as various food wastes can be used to rear insects and the feed conversion rate is high, greenhouse emissions are low, the water usage and the place requirements are low (Varelas, 2019).

2.1 Promising edible insect species for protein production

The recorded edible insect species are more than 2000 in the world, mainly in tropical countries. As shown in Figure 2.1, these species include different groups in the orders of *Coleoptera* (beetles, especially the larvae), *Lepidoptera* (butterfly and moths), *Hymenoptera* (bees, wasps and ants), *Orthoptera* (grasshoppers, locusts & crickets), *Hemiptera* (true bugs, like cicadas, leafhoppers & planthoppers), Odonata (dragonflies), *Isoptera* (termites), Diptera (flies), Blattodea (cockroaches and spider) and others (Jongema, 2017).

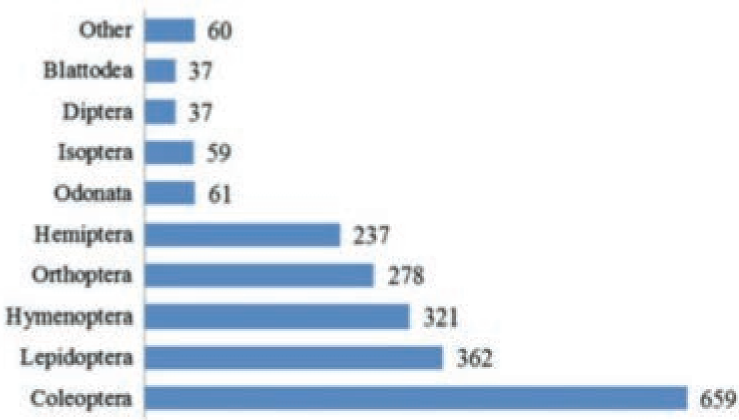


Figure 2.1 *Insect species in different groups (Jongema, 2017).*

Generally, there are three main strategies of rearing and gathering edible insects: wild harvesting (not farming), semi-domestication (outdoor farming) and farming (indoor farming). More than 90% of edible insect species are wild-harvested, but semi-domestication and farming are the more sustainable ways to provide a food supply (Varelas, 2019).

The edible insect species can be reared on organic side streams or on many kinds of substrates. In the western countries, insect species are reared for food include the following species (van Huis, 2020).

1. Mealworms: yellow meal worm (*Tenebrio molitor*), the lesser mealworm (*Alphitobius diaperinus*), the superworm (*Zophobas morio*)
2. Crickets: the house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*) and the two-spotted cricket (*Gryllus bimaculatus*)
3. Locust species: migratory locust (*Locusta migratoria*), desert Locust (*Schistocerca gregaria*)
4. Others as by-products from the silk industry: Pupae of the domesticated silk worm *Bombyx mori* (Tomotake *et al.* 2010) or honeybee drone brood from beekeeping (Ulmer *et al.* 2020).

Further, if we consider insect species as feed, these insect species are involved: mealworms, the black soldier fly (*Hermetia illucens*) and the house fly (Musca domestica).

Table 2.1 shows the summary of the edible insect species most commonly reared for food and feed in the western world. The developmental stage at which the insects are used, the type of farming system and commercial applications are also noted (Varelas, 2019; van Huis, 2020). To have an overview of the developmental stages, the life cycle of each insect sort was attached in Appendix 1.

Table 2.1
Summary of the edible insects which are commonly reared in the western world.

Insect species	Order	Common name	Developmental stage	Rearing ways	Applications
Tenebrio molitor	Coleoptera	Yellow mealworm	Larvae	Farming	Human food, feed for pets, zoo animals and fish, polystyrene degradation
Acheta domesticus	Orthoptera	House cricket	Adult	Farming	Human food, pet food, protein extraction
Locusta migratoria	Orthoptera	Locust	Adult, nymphs	Farming, wild harvesting	Human food, pet food and fish bait
Bombyx mori	Coleoptera	Mulberry silkworm	Larvae, pupae	Farming	Human food, animal feed
Apis mellifera	Hymenoptera	Honeybee drone brood	Adult	Farming, semi-domestication	Human food, medical uses (honeybee venom, propolis, royal jelly)
Gryllus bimacalatus	Orthoptera	Mediterranean field cricket	Adult	Farming	Animal Feed
Hermetia illucens	Diptera	Black soldier fly (BSF)	Larvae	Farming	Animal feed
Musca domestica	Diptera	Housefly	Larvae	Farming	Animal and fish feed

The following literature study will focus on the insect species listed in Table 1. In the following section, the nutritional values of these insect species will be discussed; After that, the nutrient requirements and the diet of rearing insects will be summarized. Finally we try to find out the good match of greenhouse waste streams and the potential feed source for insects.

2.2 Nutritional aspects of edible insects

The nutritional values of edible insects depend on many factors, such as the variety species, the metamorphic stage within the same group of species, diet and also their growing environment (temperature and humidity) (Zielińska *et al.* 2019). Moreover, the preparation and processing methods (e.g., frying , boiling and drying) used will also influence the nutritional compositions. The main components of edible insects are protein, fat and fibre. The nutritional value can be expressed as proteins, fatty acids, fibres, dietary energy, dietary minerals and vitamins.

2.2.1 Protein

Proteins are organic compounds consisting of various amino acids. They are important components of nutrition but also contribute to its physical and sensory characteristics. The nutritional value of insect-based protein, which depends on the following factors: protein content, amino acids compositions (AAC) and protein digestibility. Protein quality depends on the kind of amino acids compositions, essential amino acids and non-essential amino acids. Histidine, phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine and lysine are classified as essential amino acids, because the human body cannot synthesize them and must get them through food.

Proteins in edible insects are mainly available in the form of crude protein content, which is a percentage of dry matter (DM). Crude protein represents the approximate amount of protein in foods that is calculated from the determined nitrogen content by multiplying by a factor (6.25) derived from the average percentage of nitrogen in the food proteins. Table 2.2 shows the crude protein content range by insect orders, which varied in a wide range from 13 to 77% of dry weight (Food and Argriculture Organization of the United Nations, 2013).

Table 2.2
Crude protein content by different insect groups.

Insect order	Stage	Range (% protein)
Coleoptera	Adults and larvae	23-66
Lepidoptera	Pupae and larvae	14-68
Hemiptera	Adults and larvae	42-74
Homoptera	Adults, larvae and eggs	45-57
Hymenoptera	Adults, pupae, larvae and eggs	13-77
Odonata	Adults and nalad	46-65
Orthoptera	Adults and nymph	23-65

Accurate protein determination with an amino acid analyzer is more costly but gives useful information about the nutritional value and potential uses of the protein, for example as food ingredient. Amino acid composition (AAC) of different insect proteins is useful to determine their quality as an ingredient (Zhao *et al.* 2016).

Table 2.3

Crude protein (% of dry weight), crude fat and amino acid composition (AAC) of selected insect species from the recent studies comparing to common protein commodities.

Name	Stage/ Part	Crude protein (%)	Crude Fat (%)	Essential amino acid								Semi-essential amino acid			
				Ile*	Leu	Lys	Met	Phe	Thr	Trp	Val	Arg	His	Cys	Tyr
Coleoptera															
Tenebrio molitor (Yellow mealworm beetle)	Larva	48	38.0	4.0	6.9	4.9	1.2	3.2	3.6	1.0	5.9	4.5	2.7	0.6	5.4
Zophobas morio (superworm)	Larva	45	41.4	4.7	9.7	5.2	1.1	3.5	4.0	0.9	5.2	4.9	3.1	0.8	7.0
Diptera															
Hermetia illucens (soldier fly)	Larva	49	26.0	4.0	6.6	5.6	1.4	3.8	3.6	1.1	5.6	4.8	2.6	0.7	6.0
Musca domestica (house fly)	Pupa	62	15.5	3.5	5.3	5.2	2.6	4.2	3.2	-	3.4	4.2	2.6	0.4	4.8
Musca domestica (house fly)	Larva	54	24.3	3.2	5.7	6.9	2.2	5.0	3.3	3.2	4.4	4.9	2.8	0.4	5.1
Hymenoptera															
Apis mellifera (honeybee)	Adult	51	12.3	5.6	9.6	6.6	-	0.9	5.5	0.0	6.9	5.9	2.5	1.7	4.5
Apis mellifera (honeybee)	Pupa	49	20.1	5.6	7.7	7.3	-	0.5	4.6	0.0	5.9	5.6	2.7	1.0	4.9
Apis mellifera (honeybee)	Larva	42	18.9	6	9.4	7.0	-	0.8	6.1	0.0	6.2	6.0	2.6	1.3	2.0
Orthoptera															
Acheta domesticus (cricket)	Adult	62	-	2.6	4.5	3.5	0.9	1.4	2.2	0.4	3.7	3.7	1.6	-	2.5
Gryllus assimilis (cricket)	Adult	56	-	3.4	6.6	5	1.2	2.9	3.3	0.7	5.3	5.8	2.1	0.5	4.5
Gryllodes silligatus (cricket)	Nymph	56	-	3.7	6.9	5.3	1.6	3.1	3.5	0.9	5.2	5.7	2.2	0.9	4.2
Common Meat															
Beef				5.1	8.4	8.4	2.3	4.0	4.0	-	5.7	6.6	2.9	1.4	3.2
Pork				4.9	7.5	7.9	2.5	4.1	5.1	-	5.0	6.4	3.2	1.3	3.0
Chicken	Broiler, breast			4.2	6.9	7.8	2.1	2.5	3.7	-	4.6	6.4	4.4	-	3.5
Amino acid requirement in human nutrition (mg/kg/day)				30	59	45	16	-	23	6	39	-	15	16	-

* Isoleucine (Ile); Leucine (Leu); Lysine(Lys); Methionine(Met); Cysteine(Cys); Phenylalanine(Phe); Tyrosine (Tyr); Threonine(Thr); Tryptophan (Trp); Valine (Val); Arginine (Arg); Histidine (His). ** The data were summarized from existed literature research on edible insects from 1997 to 2016.

In Table 2.3, crude protein (% of dry weight), crude fat and amino acid compositions of selected insect species were compared to common protein commodities (Tang *et al.* 2019). However, in the study of Varelas, the crude protein content (% of dry weight) of yellow mealworm larvae was high up to 70-76%, while that of adult house cricket ranged only from 10.2% to 28.6%, which derivates quite a lot from de data shown in Table 3. This might be caused by the rearing environment, diets with different nutrition level (e.g. vegetables, grains or wastes) and preparation and procession methods, because some previous studies provided no details about the diets and the conditions used in rearing insects (Varelas, 2019).

Generally, edible insects provide essential amino acids at an ideal level, which are generally 76% to 96% digestible, although some insects are lacking or only contain very low levels of the essential amino acids or the semi-essential amino acid, such as methionine and cysteine (Tang *et al.* 2019).

2.2.2 Fat content

Fat is one of the most energy dense macronutrient in food, which facilitates the absorption of fat soluble dietary components such as vitamins. Fat enhances taste and acceptability of food and contribute to aroma, texture and flavour. Fat is classified into saturated fatty acids, unsaturated fatty acids and essential fatty acids(Aranceta & Pérez-Rodrigo, 2012).

Edible insects are also a considerable source of fat. Oils extracted from several insects are rich in polyunsaturated fatty acids and contain the essential linoleic and d α-linolenic acids, which are important for the healthy development of children and infants. The fatty acid composition of insects appears to be influenced by the diets used rearing insects. One disadvantage is that the presence of unsaturated fatty acids will cause rapid oxidation of insect food products during processing (Food and Argriculture Organization of the United Nations, 2013). Crude fat content (% of dry weight) of several selected edible insects are given in Table 2.4 (Tang *et al.* 2019).

Table 2.4

Crude fat content (% of dry weight) of several selected edible insects.

Name	Stage/ Part	Crude Fat (%)	Saturated fatty acids (SFA)				Monounsaturated fatty acids (MUFA)			Polyunsaturated fatty acids (EFA)		
			Total SFA	C14:0	C16:0	C18:0	Total MUFA	C16:1 n7	C18:1 n9	Total PUFA	C18:2 n6	C18:3 n9
Coleoptera												
Tenebrio molitor (Yellow mealworm beetle)	Larva	38.0	30.1	0.4	28.2	0.9	66.8	6.0	60.6	3.1	2.8	0.2
Zophobas morio (superworm)	Larva	41.4	36.7	1.5	27.7	5.7	40.3	0.9	36.3	20.5	20.2	0.2
Diptera												
Hermetia illucens (soldier fly)	Larva	26.0	67.9	9.9	13.3	2.0	17.4	4.1	12.8	14.7	13.9	0.5
Musca domestica (house fly)	Pupa	15.5	33.4	3.2	27.6	2.2	38.9	20.6	18.3	17.0	14.9	2.1
Musca domestica (house fly)	Larva	24.3	35.9	6.8	26.7	2.3	47.7	25.9	21.8	16.4	16.4	0.0
Hymenoptera												
Apis mellifera (honeybee)	Adult	12.3	25.2	0.6	14.4	9.3	67.0	2.6	45.2	7.8	7.8	-
Apis mellifera (honeybee)	Pupa	20.1	51.1	2.9	35.1	12.6	48.9	0.6	47.6	-	-	-
Apis mellifera (honeybee)	Larva	18.9	51.8	2.4	37.3	11.8	48.2	0.7	47.5	-	-	-
Orthoptera												
Acheta domesticus (cricket)	Adult	21.1	32.8	26.1	5.5	1.2	33.5	2.4	31.1	33.9	32.2	1.7
Common Meat												
Beef			32.3	0.8	16.7	9.5	18.8	-	10.5	49.1	36.1	6.2
Pork			41.0	3.4	21.7	12.7	43.0	2.9	39.4	16.0	7.3	1.7
Chicken			33.3	1.3	22.7	8.0	46.7	0.3	41.3	20.0	14.0	0.7
Amino acid requirement in human nutrition (mg)				26.9				30.9		16.9	15.0	1.5

SFA Saturated fatty acids: C14:0, myristic acid; C16:0, palmitic acid; C18:0, stearic acid. MUFA: C16:1 n7 – palmitoleic acid; C18:1 n9 – oleic acid. PUFA: C18:2 n6 – linoleic acid; C18:3 n3 – α-linolenic acid. The data were summarized by the following references from 2004 to 2016.

2.2.3 Fibre content

Chitin is the most common form of fibre in edible insects, which is an insoluble fibre derived from the exoskeleton. Fibre content is usually measured by crude fibre, acid detergent fibre and neutral detergent fibre. Although a lot of study provided the fibre content of insects, it is still difficult to compare them due to various methods were used. The chitin content of common insect species ranges from 11.6 mg to 137.2 mg per kg of dry matter (Food and Argriculture Organization of the United Nations, 2013).

2.2.4 Vitamins and minerals

Vitamins and minerals play and important role in biological processes in the body. Many insects contain high levels of vitamins and minerals, although their contents are variable across insect species and orders. Meanwhile, the development stages of insects and the diets used also influence their nutrition value (Food and Argriculture Organization of the United Nations, 2013).

Insects can provide vitamin A, B1-B12, C, D, E, K, which are necessary for normal growth and health. House cricket and mealworm both contained vitamin C, with 92 & 99 mg/kg respectively (Finke *et al.* 2015). Bee brood pupae is rich in vitamins A and D (Tang *et al.* 2019). The vitamin E content in freeze-dried silkworm powder, Bombyx mori is at a level of 9.65 mg per 100g. It is well worth to mention that vitamin B12 is also represented in different insect species. For example, mealworm larvae, *Tnebrio molitor* contains 0.47 ug per 100g vitamin B12, and house crickets, *Acheta domesticus* has a higher level of vitamin B12, which are 5.4 ug per 100g in adults and 8.7 ug per 100g in nymphs respectively (Food and Argriculture Organization of the United Nations, 2013).

A variety of minerals could be found in insects, including iron, magnesium, manganese, phosphorous, potassium, selenium, sodium and zinc. The mineral contents differ significantly in different insect species. The mineral compositions of common insect species are summarized in Table 2.5 (Tang *et al.* 2019).

Table 2.5

Mineral composition of common insect species (mg/100g of dry weight).

Species	Stage	Common elements					Trace elements					
		Ca	K	Mg	P	Na	Fe	Zn	Mn	Cu	I	Se
Coleoptera												
Tenebrio molitor (Yellow mealworm beetle)	Larva	47.2	895.0	210.2	748.0	140.9	5.4	13.7	1.4	1.6	0.0	0.1
Zophobas morio (superworm)	Larva	81.0	750.6	118.3	563.0	112.8	3.9	7.3	1.0	0.9	-	–
Diptera												
Hermetia illucens (soldier fly)	Larva	934.0	453.0	174.0	356.0	88.7	6.7	5.6	6.2	0.4	0.0	0.0
Musca domestica (house fly)	Adult	76.5	303.0	80.6	372.0	135.0	12.5	8.6	2.7	1.3	0.0	0.0
Musca domestica (house fly)	Larva	2010.0	-	-	1320.0	660.0	60.4	23.7	5.6	3.4	-	-
Hymenoptera												
Apis mellifera (honeybee)	Adult	222.9	1585.4	201.7	860.1	75.6	37.7	14.0	-	4.6	-	-
Apis mellifera (honeybee)	Pupa	97.0	2207.3	193.9	900.0	60.8	15.3	11.7	-	3.7	-	-
Apis mellifera (honeybee)	Larva	84.9	1871.9	177.0	782.5	59.4	13.3	11.6	-	3.6	-	-
Bee brood	Immature stages	59.5	1159.5	91.0	771.6	55.2	5.6	6.9	0.3	1.7	-	-
Lepidoptera												
Bombyx mori (domesticated silkworm)	Larva	102.3	1826.6	287.9	1369.9	274.6	9.5	17.8	2.5	2.1	-	0.1
Bombyx mori (domesticated silkworm)	Pupa	158.0	-	207.0	474.0	-	26.0	23.0	0.7	0.2	-	-
Orthoptera												
Acheta domesticus (cricket)	Adult	132.1	1126.6	109.4	957.8	435.1	6.3	21.8	3.7	2.0	-	0.1
Ruspolia differens (cricket)	Adult	24.5	259.7	33.1	121.0	229.7	13.0	12.4	2.5	0.5	-	-
Recommended daily intakes [mg/day] for adults		1300	4700	240	700	<=1500	33	8.5	2.2	1.1	-	0.03

2.3 Rearing insects using agri-food wastes

2.3.1 Waste streams from greenhouses

In the global food industry, approximately 1.3 billion tons of various food gets lost or wasted. For 2012, the estimated food waste for the EU alone was about 88 million tons, including fresh vegetables, fruits, milk, and grain products. Food wastes are difficult to utilize due to their biological instability, high water content, rapid autoxidation, and high level of enzymatic activity (Varelas, 2019).
In the report of 'Afval uit de Landbouw'(Bondt *et al.* 2010). The two main waste streams from greenhouses were identified as substrates/rock wool, and plant residuals, such as leaves and stems. The total plant related waste streams were 220,000 ton from greenhouses in 2005 (Bondt *et al.* 2010). The greenhouse area in the Netherlands are 10,200 ha (2011 from CBS), the waste streams will be approximately 21.6 tons per ha. However, the amount of waste streams from greenhouse vegetables was high up to 100 ton per ha according to another report of G. J.J. Smakman (Fytagoras BV, 2012).

In the Netherlands, the most important crops that grow in greenhouses are tomatoes, bell peppers, cucumbers, eggplants, rose and gerbera (Bondt *et al.* 2010). After harvest, vegetables and fruits with low quality, plant stems and leaves remain behind as waste streams from greenhouse horticulture. The waste streams comprise a huge nutrient stock that could be used through biodegradation by various edible insects in a large-scale production system (Varelas, 2019). Recycling the waste streams can be an excellent opportunity to reduce the disposal costs and bring added value for growers.

The chemical compositions of the plant-related waste flows include main components, such as cellulose, pectin and protein (besides water), and also the high-quality substances in small quantities, like alkaloids, terpenes, carotenoids, flavonoids and vitamins (Van Groenestijn *et al.* 2017).

Table 2.6 summarized the chemical composition of several food wastes which could be used for rearing edible insects, data of raw natural products are used in the table (Varelas, 2019).

The chemical composition the common wastes from greenhouse horticulture, such as tomato, bell pepper, cucumber, eggplant can be retrieved from the NEVO database.

Table 2.6

Summary of chemical composition of wastes from greenhouse.

Wastes	Chemical compositions
Carrot	Total N (0.11%), protein (0.7%), available carbohydrates (5.8%), dietary fibre (2.9%), total fat (0.4%), ash (0.7%), water (89.5%), vitamins (A, β-carotene, E, K1, B1, B2, B3, B5, B6, B7, B9, C), minerals and inorganics (Na, K, Ca, Mg, P, Fe, Cu, Zn, In, Mn, Cr, Se, Ni, Hg, As, Cd, Pb), carbohydrates (fructose, glucose, sucrose, hexoses, pentoses, uronic acids, cellulose, lignin), saturated fatty acids (C16:0, C18:0), monounsaturated fatty acids (C18:1 n-9), polyunsaturated fatty acids (C18:2 n-6, C18:3 n-3, C20:4 n-6), amino acids (isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan, valine, arginine, histidine, alanine, aspartic acid, glutamic acid, glycine, proline, serine)
Lettuce	Total N (0.204%), protein (1.3%), available carbohydrates (0.8%), dietary fibre (1.3%), total fat (0.4%), ash (0.8%), water (95.5%), vitamins (A, β-carotene, E, K1, B1, B2, B3, B5, B6, B7, B9, C), minerals and inorganics (Na, K, Ca, Mg, P, Fe, Cu, Zn, In, Mn), carbohydrates (fructose, glucose, sucrose, starch, hexoses, pentoses, uronic acids, cellulose, lignin), saturated fatty acids (C12:0, C16:0, C18:0), monounsaturated fatty acids (C16:1 n-7, C18:1 n-9, C20:1 n-11, C22:1 n-9), polyunsaturated fatty acids (C18:2 n-6, C18:3 n-3, C18:4 n-3, C20:4 n-6, C20:5 n-3, C22:5 n-3, C22:6 n-3), amino acids (isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan, valine, arginine, histidine, alanine, aspartic acid, glutamic acid, glycine, proline, serine)
Potato	Total N (0.324), protein (2.0%), available carbohydrates (15.9%), total fat (0.3%), dietary fibre (1.4%), ash (0.9%), water (79.5%), vitamins (A, B1, B2, B3, B5, B6, B7, B9, C), minerals and inorganics (Na, K, Ca, Mg, P, Fe, Cu, Zn, In, Mn, Cr, Se, Ni, Hg, As, Cd, Pb), carbohydrates (fructose, glucose, sucrose, starch, exoses, pentoses, uronic acids, cellulose), saturated fatty acids (C16:0, C18:0), monounsaturated fatty acids (C16:1 n-7, C18:1 n-9), polyunsaturated fatty acids (C18:2 n-6, C18:3 n-3), amino acids (isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan, valine, arginine, histidine, alanine, aspartic acid, glutamic acid, glycine, proline, serine)
Soy flour	Total N (6.520%), protein (37.2%), available carbohydrates (20.2%), dietary fibre (10.4%), total fat (22.2%), ash (5.1%), water (5.1%), vitamins (A, β-carotene, E, K1, B1, B2, B3, B5, B6, B9), minerals and inorganics (Na, K, Ca, Mg, P, Fe, Cu, Z, In, Mn, Cr, Se, Ni, Hg, Cd, Pb), carbohydrates (sucrose, starch, exoses, pentoses, uronic acids, cellulose, lignin), saturated fatty acids (C12:0, C14:0, C16:0, C18:0, C20:0, C22:0), monounsaturated fatty acids (C16:1 n-7, C18:1 n-9, C20:1 n-11), polyunsaturated fatty acids (C18:2 n-6, C18:3 n-3), amino acids (isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan, valine, arginine, histidine, alanine, aspartic acid, glutamic acid, glycine, proline, serine)
Tomato	Total N protein (0.7%), carbohydrates (2.9%),total fibre (1.3%), total fat (0.4%), ash (0.6%), water (95.4%), vitamins (A, β-carotene, E, K, B1, B3, B6, C), minerals(Na, K, Ca, Mg, P, Fe, Cu, Zn, In, Mn, Cr, Se), carbohydrates, etc.

2.3.2 Nutrient requirements of rearing edible insects

Food wastes comprise a potential source of ingredients for artificial diets used in rearing edible insects. The artificial diets can vary from liquid to solid, depending on the nutrient requirements of the insect species, the feeding adaptation of the insects and the pre-manufacturing the diet. The development of low-cost commercial diets is crucial for an industrial scale of edible insect production.

The macronutrients required for insect production are carbohydrates, lipids and various amino acids. Carbohydrates serve as an energy pool, and they are also required for configuration of exoskeleton of arthropods (chitin). Lipids are the main structural components of the cell membrane, and also store and provide metabolic energy and help conserve water in the arthropod cuticle. Some amino acids which insect cannot synthesize are needed, such as leucine, isoleucine, valine, threonine, lysine, arginine, methionine, histidine, phenylalanine, and tryptophan. While other amino acids which insects cannot synthesize, like tyrosine, proline, serine, cysteine, glycine, aspartic acid and glutamic acid are also needed in insufficient quantities at high energy consumption. The essential micronutrients involved in insect rearing are sterols, vitamins and minerals. Figure 2.2 provides an overview of the nutrient requirements of rearing edible insects (Varelas, 2019).

Macronutrients			Micronutrients		Minerals
Carbohydrates	**Lipids**	**Proteins**	**Sterols *****	**Vitamins**	**Elements *******
Glucose * Fructose * Galactose * Arabinose ** Ribose ** Xylose ** Galactose ** Maltose * Sucrose *	Linoleic (Pfa) *** Linolenic (Pfa) *** Phospholipids ****	Globulins Nucleoproteins Lipoproteins Insoluble proteins Amino acids: Leucine *** Isoleucine *** Valine *** Threonine *** Lysine *** Arginine *** Methionine *** Histidine *** Phenylalanine *** Tryptophan *** Tyrosine **** (major component of sclerotin) Proline **** (important during flight initiation) Serine **** Cysteine **** Glycine **** Aspartic acid **** Glutamic acid ****	Cholesterol Phytosterols (β-sitosterol, campesterol, stigmasterol) Ergosterol	A: Retinol + α-and β-carotene (Ls) B1: Thiamin (Ws) B2: Riboflavin (Ws) B3: Nicotinamide (Ws) B4: Choline (Ws) B5: Pantothenic acid (Ws) B6: Pyridoxine (Ws) B12: Cobalamine (Ws) C: Ascorbic acid (Ls) D: Cholecalsiferol and Ergocalsiferol (Ls) E: α-tocopherol (Ls) K: Phyloquinone (Ls)	Hydrogen Oxygen Carbon Nitrogen Calcium + Phosphorus +++ Chlorine Potassium +++ Sulphur Sodium +++ Magnesium +++ Iron ++ Copper +++ Zinc +++ Silicone Iodine Cobalt Manganese +++ Molybdenum Fluorine Tin Chromium Selenium Vanadium

*: Insects able to absorb and metabolize; **: Insects able to absorb but not metabolize; Pfa: Polyunsaturated fatty acids; ***: Insects unable to synthesize; ****: Insects able to synthesize; Ws: Water-soluble; Ls: Lipid-soluble; *****: Listed in order of importance as essential for living matter (from top down). Minerals consist of combinations of cations and anions of elements; +++: Important for insect growth; ++: Important in enzyme pathways including DNA synthesis; +: Important to a lesser extent, important role in muscular excitation.

Figure 2.2 *Summary of the nutrient requirements of rearing edible insects (Varelas, 2019)*

Lundy and Parrella suggested a feed quality model of substrates to determine the potential of a particular organic side-streams to support the growth of crickets. The feed quality index (y) equals the proportion of nitrogen (N) to the acid detergent fibre (ADF) content plus the proportional crude fat (CF) content in feed substrates [y = (N/ADF) + CF] (Lundy & Parrella, 2015).

2.3.3 From waste streams to insects feed

The application of agri-food wastes for insect production is an upcoming development. The big challenge is the proper selection of suitable wastes for a specific sort insect, which would assure a large-scale insect production and also have a beneficial nutrition composition. Another challenge is that many agri-food wastes are used for animal feeding, and replacing the feed materials could probably result in higher environmental impacts. Taking into account of these preconditions, the food wastes, such as mill brans, distiller's dried grains with soluble, brewery grains and milled pre-consumer waste, could be an excellent source for insect production. Proper application of food wastes could reduce the environmental impact of food substitutes production. The impact of insect-based food (1 kg) could be decreased to 2 kWh of energy use, around 1 kg CO_2 eq. of GWP, 1.5 m^2 of land use and 0.1 m^3 of water use (Smetana *et al.* 2018).

It is reported that a balanced diet composed of organic by-products was used by commercial breeders to rear mealworm species successfully. The growth, development, and feed conversion efficiency of yellow mealworm can be affected by different diets. Diets rich in yeast-protein appeared favourable regarding to reduced larval development time, reduced mortality and increased the weight gain of mealworms. Dry potato flour, dry egg white, soy protein, peanut oil, canola oil and salmon oil were added to the diet of yellow mealworm as nutritional supplements. Results showed that a diet with an a higher content of proteins and lipids increased most biological parameters significantly compared to a carbohydrate-rich diet (Morales-Ramos, 2013). Plant-derived wastes can be efficiently converted into animal feed rich in high-quality protein and fat by rearing mealworms in a relative short period (Varelas, 2019).

However, high lipid contents in the diet of insects can decrease their feed conversion rates; they are more likely to die, and if they survive their growth rate is lower. This is probably caused by impaired breathing as a result of oil accumulation in their trachea (Alves *et al.* 2016).

Table 2.7

Summary of various edible insect species reared on food wastes and their characteristics.

Order	Family	Species	Common name	Stage	Degraded Material
Coleoptera	Tenebrionidae	Tenebrio molitor L	Mealworm	Larvae	Spent grains and beer yeast, bread remains, biscuit remains, potato steam peelings, maize distillers' dried grains with solubles
Coleoptera	Tenebrionidae	Tenebrio molitor L	Mealworm	Larvae	Mushroom spent corn stover, highly denatured soybean meal, spirit distillers' grains
Coleoptera	Tenebrionidae	Tenebrio molitor L	Mealworm	Larvae	Beet molasses, potato steam peelings, spent grains and beer yeast, bread remains, cookie remains(Oonincx *et al.* 2015)
Coleoptera	Tenebrionidae	Tenebrio molitor L	Mealworm	Larvae	Carrots and carrot side product, mixed grains such as wheat bran, oats, soy, rye and corn supplemented with beer yeast(Oonincx & de Boer, 2012)
Orthoptera	Linnaeus	Locusta migratoria	Locust	Nymphs	Oat grass, ground oats(Clarkson *et al.* 2018)
Orthoptera	Gryllidae	Acheta domesticus	House cricket	Adult	Grocery store food waste after aerobic enzymatic digestion, municipal food waste heterogeneous substrate
Orthoptera	Gryllidae	Teleogryllus testaceus	Cambodian field cricket	Adult	Rice bran, cassava plant tops, water spinach, spent grain, residues from mung bean sprout production
Diptera	Stratiomyidae	Hermetia illucens	Black soldier fly (BSF)	Larvae	Waste plant tissues, garden waste, compost tea, catering waste, food scraps
Diptera	Muscidae	Musca domestica	Housefly	Larvae	Mixture of egg content, hatchery waste, and wheat bran
Diptera	Muscidae	Musca domestica	Housefly	Larvae	Maize bran, soybean bran mixed with maize grain pericarp. The same substrates were also provided for BSF(Ganda *et al.* 2019)

A large range of organic side streams via enzymatic digestion can be used to feed crickets, and they are easy to farm in a system producing 6 or 7 generations per year. Crickets fed the solid filtrate from food wastes via enzymatic digestion were able to reach a harvestable size and achieve feed an protein efficiencies (Varelas, 2019). However, the minimally-processed organic side streams, such as wheat and rice straw silage as a feed could not support adequate growth and survival of cricket populations (Lundy & Parrella, 2015). Grasshoppers were fed with brans which contains high levels of essential fatty acids, have almost double the protein content of those fed on maize (Food and Argriculture Organization of the United Nations, 2013). Therefore, the quality of the side streams would be a dependent factor for rearing edible insects.

Many studies regarding rearing edible meal worm, black soldier fly, housefly and crickets have been carried out using artificial diets based on food wastes. Food materials and wastes already used in rearing farmed edible insects are summarized in Table 2.7. In the study of Varelas in 2019, the chemical composition and nutritional value of various wastes were summarized based on the food dataset from Technical University of Denmark. These data could work as a reference to help adapt the diets to meet insects' corresponding nutrient requirements (Varelas, 2019; Oonincx *et al.* 2015).

2.4 Summary of insect-based protein sources

The most commercially interesting insects for food are mealworms, crickets and locust species, and black solider fly and house fly are usually cultivated for feed.

The macronutrients required for insect production are carbohydrates, lipids and various amino acids. The essential micronutrients involved in insect rearing are sterols, vitamins and minerals. Table 7 showed the summary of the nutrient requirements in detail.

The growth, development, and feed conversion efficiency of insects can be affected by different diets. Different studies reported that the minimally-processed waste streams could not support adequate growth of insects. Besides the enzymatic degraded waste streams, a balanced amount of proteins and lipids, such as dry egg white, yeast protein and peanut oil, could be added to the diets to improve the insect yield. Therefore, the development of an effective artificial diets is crucial for rearing a specific insect sort successfully.

When preparing artificial diets, several factors are worthy to be mentioned. Balanced diets composed of various organic waste streams via enzymatic digestion were able to reach a harvest size of cricket. Diets rich in yeast-protein appeared favourable regarding to reduced larval development time, reduced mortality and increased the weight gain of mealworms. Grasshoppers were fed with brans which contains high levels of essential fatty acids, have almost double the protein content of those fed on maize, however high fat content in the diet of insects can decrease their feed conversion rates.

3 Plant-based protein sources

3.1 Plant-based protein sources

Animals consume plant-based protein to produce meat or milk, but the conversion from plant-based protein to meat production is very inefficient. On the contrary, plant-based protein is produced in a more efficient way. To be exactly, less water, land, nitrogen, and fossil energy are required to produce a given amount of plant-based protein, comparing to animal protein. For instance, 20 times the amount of soy protein can be produced on the same amount of land, compared to a given quantity of beef production. Moreover, the same land can produce 10 times the amount of beans and legumes or 13 times the amount of rice. Consequently, consumption of a plant-based diet can feed 10-20 times more humans compared to the cultivation of crops for animal feed (Nadathur *et al.* 2017).

Globally, several plant-based protein sources have been consumed by humans. Cereals (wheat, rice, millets, sorghum), pseudo-cereals (amaranth, quinoa), seeds (chia, hemp, flax, caraway), nuts (almond, walnut), legumes (soybeans, yellow pea, faba pea, cowpea, lentils, peas, lupins), and leaves (moringa, duckweed) are sources of proteins which can be sustainably produced (De Ron *et al.* 2017).

Existing plant-based protein sources are usually converted to food products based on traditional knowledge or through new technological interventions. For example, soy can be used to make milk and tofu, and also textured vegetable proteins can be extracted from soy. The major plant protein sources are legumes and oilseeds, which are used to generate meat-like or animal protein-like product (Nadathur *et al.* 2017).

3.2 Nutritional values of plant-based protein sources

When it comes to nutritional values, animal-derived proteins are of higher quality than plant based proteins, due to better amino acid score (AAS), greater water solubility and their high digestibility. The drawbacks of plant-based protein can be adjusted by combining proteins from different plant sources based on their complementary AA profiles. For example, most of the pulses are rich in lysine and deficient in S-AA, but it can provide complementary AA profile when combined with lysine-limited cereals, such as corn, finger millet, rice, sorghum and wheat (Balandrán-Quintana *et al.* 2019).

Except for providing protein, plant-based food provides phytonutrients, vitamins, minerals, and fibre, which are also crucial for the body. Hemp and chia provide omega-3 fats, while pulses provide fibre for normal health and functioning. It is also confirmed that consumption of plant-based diets as a whole benefit in a variety of ways, including preventing cardiovascular disease and improved blood lipid profiles (Tuso *et al.* 2013), circumventing type 2 diabetes, providing bone health and diversifying intestine microbiota (Nadathur *et al.* 2017).

Other advantages of consuming plant-based food are those lipids associated with plant-based protein sources are composed of healthy fatty acid profiles and without cholesterol, and the associated cell walls comprise the dietary fibre and other important micro components. Protein is satiating and fibre is effective in inducing the satiating effect, therefore making plant-based diets ideal by controlling appetite and food intake (Nadathur *et al.* 2017).

Table 3.1

Nutritional values of plant-based protein sources, including protein content, amino acid profiles and other macro- and micro- components (Retrieved from nutritionvalue.org).

Nutrient	Chia seeds*	Lupin	Quinoa	Moringa leave	Cowpea	Lentil	Mung bean	Broad-bean / faba bean	Sorghum grain
Proteins (g/100g)									
Protein	16.54	36.17	14.12	9.4	23.85	24.63	23.86	26.12	10.62
Alanine	1.044	1.296	0.588	0.705	1.088	1.029	1.05	1.07	1.033
Arginine	2.143	3.877	1.091	0.532	1.652	1.903	1.672	2.411	0.355
Aspartic acid	1.689	3.877	1.134	0.92	2.881	2.725	2.756	2.916	0.743
Cystine	0.407	0.446	0.203	0.14	0.263	0.322	0.21	0.334	0.127
Glutamic acid	3.5	8.686	1.865	1.035	4.518	3.819	4.264	4.437	2.439
Glycine	0.943	1.539	0.694	0.517	0.985	1.002	0.954	1.095	0.346
Histidine	0.531	1.03	0.407	0.196	0.74	0.693	0.695	0.664	0.246
Isoleucine	0.801	1.615	0.504	0.451	0.969	1.065	1.008	1.053	0.433
Leucine	1.371	2.743	0.84	0.791	1.828	1.786	1.847	1.964	1.491
Lysine	0.97	1.933	0.766	0.537	1.614	1.72	1.664	1.671	0.229
Methionine	0.588	0.255	0.309	0.123	0.34	0.21	0.286	0.213	0.169
Phenylalanine	1.016	1.435	0.593	0.487	1.393	1.215	1.443	1.103	0.546
Proline	0.776	1.476	0.773	0.451	1.072	1.029	1.095	1.099	0.852
Serine	1.049	1.869	0.567	0.414	1.194	1.136	1.176	1.195	0.462
Threonine	0.709	1.331	0.421	0.411	0.908	0.882	0.782	0.928	0.346
Tryptophan	0.436	0.289	0.167	0.144	0.294	0.221	0.26	0.247	0.124
Tyrosine	0.563	1.36	0.267	0.347	0.771	0.658	0.714	0.827	0.321
Valine	0.95	1.51	0.594	0.611	1.137	1.223	1.237	1.161	0.561
Fats (g/100g)									
Fat	30.74	9.74	6.07	1.4	2.07	1.06	1.15	1.53	3.46
Saturated fatty acids (SFA)	3.33	1.156	0.706		0.542	0.154	0.348	0.254	0.61
Monounsaturated fatty acids (MUFA)	2.309	3.94	1.613		0.173	0.193	0.161	0.303	1.131
Polyunsaturated fatty acids (EFA)	23.665	2.439	3.292		0.889	0.526	0.384	0.627	1.558
Carbohydrates (g/100g)									
Carbohydrate	42.12	40.37	64.16	8.28	59.64	63.35	62.62	58.29	72.09
Fibre	34.4	18.9	7	2	10.7	10.7	16.3	25	6.7

Nutrient	Chia seeds*	Lupin	Quinoa	Moringa leave	Cowpea	Lentil	Mung bean	Broad-bean / faba bean	Sorghum grain
Vitamins (the unit is mg if not noted with IU or mcg)									
Betaine			630.4						
Choline			70.2			96.4	97.9	95.8	
Folate	49 mcg	355 mcg	184 mcg	40 mcg	639 mcg	479 mcg	625 mcg	423 mcg	20 mcg
Niacin	8.83	2.19	1.52	2.22	2.795	2.605	2.251	2.832	3.688
Pantothenic acid		0.75	0.772	0.125	1.511	2.14	1.91	0.976	0.367
Riboflavin	0.17	0.22	0.318	0.66	0.17	0.211	0.233	0.333	0.096
Thiamin	0.62	0.64	0.36	0.257	0.68	0.873	0.621	0.555	0.332
Vitamin A	54 IU		14 IU	7564 IU	33 IU	39 IU	114 IU	53 IU	
Vitamin A, RAE			1 mcg	378 mcg	2 mcg	2 mcg	6 mcg	3 mcg	
Carotene, beta			8 mcg			23 mcg	68 mcg	32 mcg	
Cryptoxanthin, beta			1 mcg						
Lutein + zeaxanthin			163 mcg						
Vitamin B6		0.357	0.487	1.2	0.361	0.54	0.382	0.366	0.443
Vitamin C	1.6	4.8		51.7	1.5	4.5	4.8	1.4	
Vitamin E (alpha-tocopherol)	0.5		2.44			0.49	0.51	0.05	0.5
Tocopherol, beta			0.08						
Tocopherol, delta			0.35						
Tocopherol, gamma			4.55			4.23			
Vitamin K						5 mcg	9 mcg	9 mcg	
Minerals (the unit is mg/100g if not noted with mcg)									
Calcium, Ca	631	176	47	185	85	35	132	103	13
Copper, Cu	0.924	1.022	0.59	0.105	1.059	0.754	0.941	0.824	0.284
Iron, Fe	7.72	4.36	4.57	4	9.95	6.51	6.74	6.7	3.36
Magnesium, Mg	335	198	197	42	333	47	189	192	165
Manganese, Mn	2.723	2.382	2.033	1.063	1.544	1.393	1.035	1.626	1.605
Phosphorus, P	860	440	457	112	438	281	367	421	289
Potassium, K	407	1013	563	337	1375	677	1246	1062	363
Selenium, Se	55.2 mcg	8.2 mcg	8.5 mcg	0.9 mcg	9.1 mcg	0.1 mcg	8.2 mcg	8.2 mcg	12.2 mcg
Sodium, Na	16	15	5	9	58	6	15	13	2
Zinc, Zn	4.58	4.75	3.1	0.6	6.11	3.27	2.68	3.14	1.67

*Plant-based protein sources mentioned in de list are raw and not processed.

Nutritional values of plant-based protein sources, including protein content, amino acid profiles and other macro- and micro- components were shown in Table 3.1. Based on the high protein level, high EFA level and other benefits of the plants/seeds, more attention will be paid on several legume species. Besides, several other criteria were also applied to screen the legumes: being suitable for high-wire growing in greenhouses and no legal issues to grow in the Netherlands. To be specific, the crop plant should grow taller than 150cm, the short plant sorts will be excluded in the further discussion. Oilseeds which are also rich in protein will not be included in this section, while two potential oilseeds sorts are listed in Appendix 2.

3.2.1 Lupins

Highlights of lupin seeds:

a. Contain 36.17 g/100g protein in seeds (30%-44%), highly digestible.
b. High in fibre (16%-18%), 18.9g/100g fibre content.
c. White lupins have higher fat content (8%-14%) than sweet lupins.
d. White lupin may grow up to 150 cm (Kohajdová *et al.* 2011), while *lupin mutabilis* grows up to 200 cm high with almost no branching(Falconí, 2012).

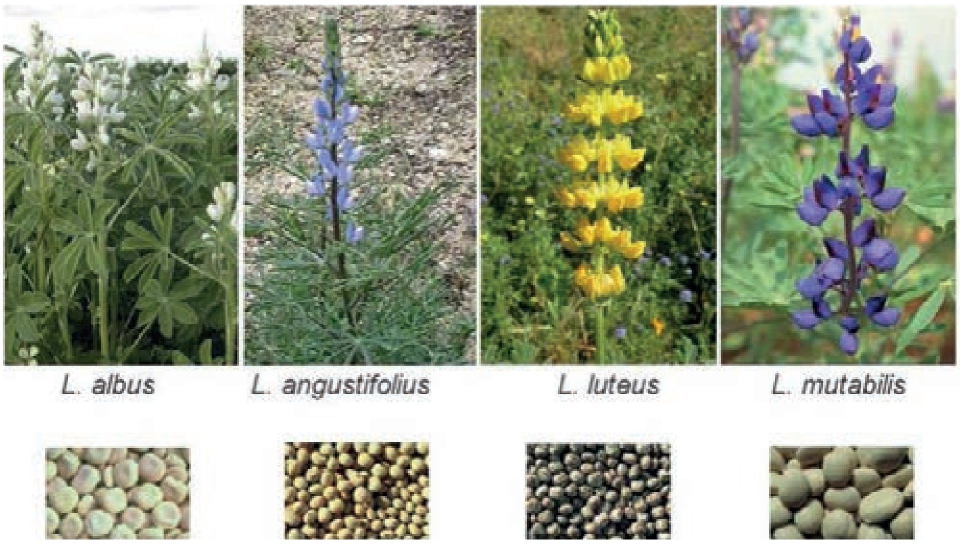


Figure 3.1 *Four common lupin species and their seeds (Retrieved from toxinology.nilu.no).*

Europe depends on soya bean imports for 70% of its plant protein requirements. Lupin, as an economically and agriculturally valuable plant, can become alternative to soya beans. The native European lupins are white lupin (Lupinus albus L.), narrow-leafed lupin (L. angustifolius L.) and yellow lupin (L. luteus L.). White lupin is the lupin species that most used for human consumption in Europe (Oliveira *et al.* 2014).

The andean lupin (L. mutabilis Sweet) is not present in Europe at a commercial scale, although it has been cultivated South America (Kohajdová *et al.* 2011). *L. mutabilis* is also not well studied, and its use was always hampered due to its low yield and the presence of the bitter alkaloids (Falconí, 2012). The predominant alkaloids in Andean lupin seeds are 4-hydroxylupanine and lupanine (Galek *et al.* 2017). The sweet lines of *L. mutabilis* was mainly based on natural or induced mutants, for instance, the first stable sweet variety, Inti, was characterized by an alkaloid content of 0.0075% with no reported detrimental effect on the protein or oil content (Gulisano *et al.* 2019). However, the seeds of *Lupinus mutabilis sweet* are characterized by a high protein and oil content (44% and 18% dry weight respectively), which exceeds that of other lupin species (Gulisano *et al.* 2019).

Lupin beans have a high protein content with good quality, which is high up to 44%. Lupin seeds are devoid of starch, and the major carbohydrates are oligosaccharides, specifically stachyose and raffinose, and cell wall storage polysaccharides (Gulisano *et al.* 2019). They also offer several potential health benefits. Besides, they are suitable for sustainable production. Although lupin is already grown in Europe, its production is still insufficient to guarantee a stable supply for its in food and feed industry. Lupin ingredients, such as flour, protein isolates and concentrates, are usually used in bakery and gluten-free products as minor components in Europe (De Ron *et al.* 2017).

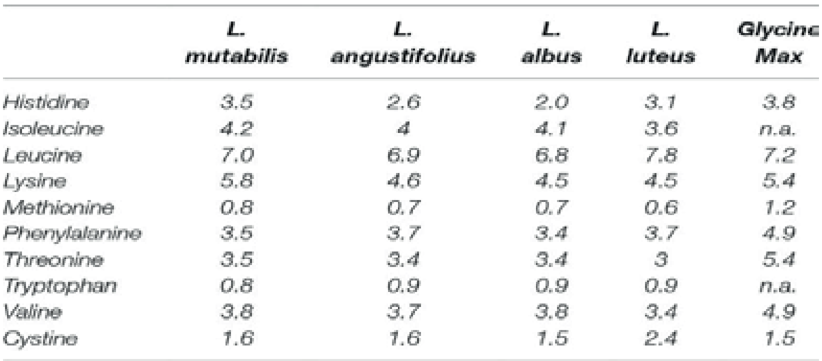
Lupin seeds are produced in pods which grow on the main stem of the lupin plant. White lupin seeds are 8-14 mm in diameter, flattened and of cream color. Blue lupins have beige or brown-speckled, round and relatively light seeds, whereas yellow lupin seeds resemble soybeans (Retrieved from toxinology.nilu.no). Figure 2 shows the four common lupin plants and their seeds.

The nutritional value of lupin is comparable of soybeans, and it contains the essential amino acids lysine, leucine, and threonine. Extra attention are needed on quality traits of lupins when using lupin for food and feed (De Ron *et al.* 2017), due to the following reasons:

- Selecting sweet lupin cultivars when using lupin for either food or feed to avoid the toxic alkaloids. The current Food Standard sets a maximum threshold of 200 mg/kg of lupin alkaloids (Vineberg, 2011).
- The conglutin-γ protein fraction in lupin beans could be used to control insulin resistance and diabetes.
- The oil content of white lupin ranges from 8% to 14%, which also has an excellent quality.
- Ferritin, the Fe-rich protein, is abundant in lupin, which making lupins a safe way to increase dietary iron intake.
- Lupin proteins might influence lipid and glucose metabolism, as well as blood pressure levels. Amino acid sequences detected in lupin proteins may be related to hypotensive and lipid-lowering activities.
- Lupin proteins have possibly some effects on inflammatory processes and changes in the gut microbiome.
- The cross-reactivity between peanut and lupin exists, therefore lupin products should be properly labelled according to the allergen list of EU directive 2006/142/EC.

3.2.1.1 Nutrition compositions of lupins

In summary, lupin protein are characterized by a high amino acid sore (AAS), biological value (BV), protein digestibility corrected amino acid acid score (PDCAAS). Figure 3.2 shows the essential amino acids profile from *L. luteus*, *L. angustifolius*, *L. albus*, *L. mutabilis sweet* and soybean (Glycine Max) (Gulisano *et al.* 2019).



	L. mutabilis	L. angustifolius	L. albus	L. luteus	Glycine Max
Histidine	3.5	2.6	2.0	3.1	3.8
Isoleucine	4.2	4	4.1	3.6	n.a.
Leucine	7.0	6.9	6.8	7.8	7.2
Lysine	5.8	4.6	4.5	4.5	5.4
Methionine	0.8	0.7	0.7	0.6	1.2
Phenylalanine	3.5	3.7	3.4	3.7	4.9
Threonine	3.5	3.4	3.4	3	5.4
Tryptophan	0.8	0.9	0.9	0.9	n.a.
Valine	3.8	3.7	3.8	3.4	4.9
Cystine	1.6	1.6	1.5	2.4	1.5

Figure 3.2 *Essential amino acids profile from lupin species and soybean (Glycine Max) (Data were expressed as g/100g of proteins)(Gulisano et al. 2019).*

Since L. Albus is the most consumed variety, the essential amino acids from *L. Albus* were compared with the Recommended Daily Allowances (RDA) for an individual with 80 kg (Oliveira *et al.* 2014). Tryptophan is the limiting amino acid to these three lupin species, which reaches 54% of the RDA, while the valine content is quite high, which reaches 465% of the RDA.

Figure 3.3 provides an overview of the crude protein, crude fat, crude fibre, saturated fatty acids and unsaturated fatty acids of these four lupin species. The fibre in lupins is primarily composed of cellulose and lignin (Gulisano *et al.* 2019).

	Crude protein	Crude lipids	Crude fiber	FA saturated/ unsaturated	Unsaturated fatty acids (g/100 g DW)			
					C18:1 (Oleic)	C18:2 (Linoleic)	C18:3 (Linolenic)	C22:1 (Erucic)
L. mutabilis	43.3	18.9	8.2	0.17	46.4	33.1	2.5	–
L. albus	38.2	11.2	8.9	0.5	54.0	18.7	8.6	0.4–2.7
L. luteus	42.2	5.5	15.8	0.13	28.5	48.2	6.3	tr-1.5
L. angustifolius	33.9	6.3	16	0.23	33.9	40.3	5.6	0.1–0.5
Glycine max	42.9	19.8	5.1	0.18	22.8	50.8	5.9–8.3	–

Figure 3.3 *Nutritional composition of lupin species and soybean (Glycine Max) (Data were expressed as g/100g of proteins)(Gulisano et al. 2019)*

3.2.1.2 Lupin ingredients applications

Given the high protein content, lupin flour could be used an excellent raw material to supplement different food products and can substitute egg in cakes, pancakes, biscuits, pasta or bread. Lupin fibre can be used as a source of dietary fibre. It can also incorporated into wheat flour to improve the nutritional value of the final products, because lupin is rich in lysine and poor in methionine and cysteine, while wheat flour is lysine-poor and sulfur amino acids-rich. In general, adding 10% lupin flour can improve the water binding, texture, shelf life and aroma of bread (Lucas *et al.* 2015).

Lupin protein powder can also be mixed with fruit juice, smoothie, or being added to the muesli breakfast or soup.

Figure 3.4 *lupin protein (36%-43%) applications on the market (Products are priced at €21.64/kg and €34.15/kg respectively) (Retrieved from amazon.de and vitaminestore.nl).*

3.2.2 Faba beans

Highlights of faba beans:

a. Contains 26.12 g/100g protein in raw seeds, highly digestible.
b. Contains all 9 essential amino acids, high arginine, glutamine and BCAAs (Branched-chain amino acids: leucine, isoleucine and valine) than pea protein.
c. Low fat level.
d. High fibre, 25 g/100g fibre content.
e. Plants can reach 40-210 cm in height.

Figure 3.5 *Faba bean plants with flowers, various color and pattern of faba beans (De Ron, 2015).*

The faba bean (*Vicia faba L.*) is a protein-rich grain legume which belongs to the Fabaceae family and has the ability to grow in various climate zone. It has a long history of cultivation in the temperate zone of the northern hemisphere. It is also known as horse bean, faba bean, filed bean or broad bean. The plant height ranged from 40-210cm, and 20% of accessions had a single main stem, whereas 70% carried several basal branches. The number of effective pods ranged from 1.1 to 93.7, and the number of mature seeds per pod is from 0.8 to 6.1. The 100-seed weight of dry grain ranged from 6 to 240 g, while the dry grain yield per plant from 1.2 to 127.0 g. The dry pod length ranged from 1.2cm to 18.8 cm, and the width ranged 0.7cm to 3.5 cm (De Ron, 2015). In the human diet faba bean is mostly the seed grain that is consumed, while the pods are used as feed for livestock. Figure 3.5 shows the faba bean plants with pure white flowers, and the large variation in seed coat color and pattern, such as hilum color and cotyledon color (yellow or green).

In sustainable agriculture, faba bean provides valuable ecological and environmental services. For instance, it hosts various associated organisms, including pollinating insects. Faba bean species has the ability to adapt to diverse climates, but it might have a low and unstable yields. China, Ethiopia, the UK, Australia and France were the five main producing countries, which harvested 72 % of the world production in 2011. In Europe, faba bean covers 21.3 % of the 1.6 million ha of grain legume cultivation, ranking second behind pea and ahead of soya bean (De Ron, 2015).

3.2.2.1 Nutrition compositions of faba beans

Faba bean is an excellent candidate crop to provide nitrogen input into temperate agricultural systems(Robson *et al.* 2002). The nutritional importance of faba bean is prominent (approx. 25% protein) and it also offers a valuable amount of energy: 320 kcal/100 g dry weight(Multari *et al.* 2015). In the study of de Ron, the main nutritional composition was determined on a large faba bean collections from China and Europe. The crude protein content ranged 17.6% to 34.5% on a total seed dry matter basis. The total starch content ranged 33.2% to 53.4%, while amylose content of starch ranged 6.% to 27.9%, and the lipid content ranged 0.52% to 2.8%(De Ron, 2015).

Faba beans also have therapeutic potential, because it provides L-3,4-dihydroxyphenylalanine (L-DOPA), which is the precursor to the neurotransmitter catecholamine and can also be used to treat Parkinson's disease(Ramya & Thaakur, 2007). Faba bean also contains anti-nutritional factors, such as saponins, protease inhibitors, , α-galactosides, and phytic acid, but even simple domestic processes methods, soaking and cooking, can remove the presence of these anti-nutritional contents(Multari *et al.* 2015).

During the seed development, faba bean accumulates large amounts of proteins. The major storage proteins are globulins (69%-78%), which contain 2 high-molecular-weight proteins, legumin and vicilin, respectively. Legumin and vicilin are known as 11S and 7S and defined by analytical centrifugation (Duranti & Gius, 1997). Table 3.6 shows the amino acid composition of legumin and vicilin fractions. Both fractions have similar amino acid compositions (Multari *et al.* 2015). The *in vivo* digestibility of faba bean-isolated globulins in the rat small intestine has been demonstrated to be over 90% (Rubio *et al.* 1995).

Amino acids	*V. faba* 11S (%)	*V. faba* 7S (%)
Aspartic	10.60	11.60
Threonine	4.28	3.27
Serine	6.50	6.59
Glutamic	16.40	15.30
Glycine	7.40	5.00
Alanine	6.10	4.87
Valine	4.91	4.90
Cystine	0.80	0.31
Methionine	0.59	0.31
Iso-leucine	3.98	5.12
Leucine	7.84	9.21
Tyrosine	2.61	2.59
Phenylalanine	3.56	5.20
Lysine	4.57	7.13
Histidine	2.44	1.95
Arginine	7.95	5.59

Figure 3.6 *Amino acid profile of legumin and vicilin fraction in faba beans (Jackson et al. 1969; Multari et al. 2015)*

Besides globulins, faba bean also contains other forms of proteins, such as glutelins (12.0%-18.4%), prolamins (1.83%-3.57%) and albumins (1.41%-3.01%), which are related to the seed nutritional quality (Alghamd, 2009). Glutelins are soluble in sodium hydroxide and have an amino acid composition similar to that of prolamins, but with higher levels of glycine, methionine, and histidine (El Fiel *et al.* 2002). Prolamins are alcohol-soluble proteins with the highest levels of leucine, proline, and glutamic acid but lack of lysine and tryptophan. Albumins are biologically active water-soluble proteins, with a higher amount of methionine and cysteine compared to globulins (El Fiel *et al.* 2002). The proportion of different protein fractions of faba beans could vary according to their cultivars, fertilization, season of growth, method of investigation (Multari *et al.* 2015).

3.2.2.2 Faba beans protein applications

Faba bean protein powders can be easily mixed with sweet or savory dishes to boost protein intake, the power is also heat stable, making it good for hot soups and casseroles (Retrieved from pulsin.co.uk and tophelathingredients.com). The textured protein can also be used as meat alternative (Retrieved from roquette.com).

Figure 3.7 *Faba bean protein applications on the market (Pulsin faba bean protein as shown in the figure is priced at £33.45/kg).*

3.3 Growing conditions of high protein crops

3.3.1 *Lupin mutabilis*

To grow Lupin mutailis commercially, varieties with high-yielding, low alkaloid and earlier flowering are needed to be development. The growth and development of the lupin plant are complex and overlapping (Figure 3.8). The growth and development of a lupin plant is directly related to water use, light interception, photoperiod and temperature (Walker, 2011).

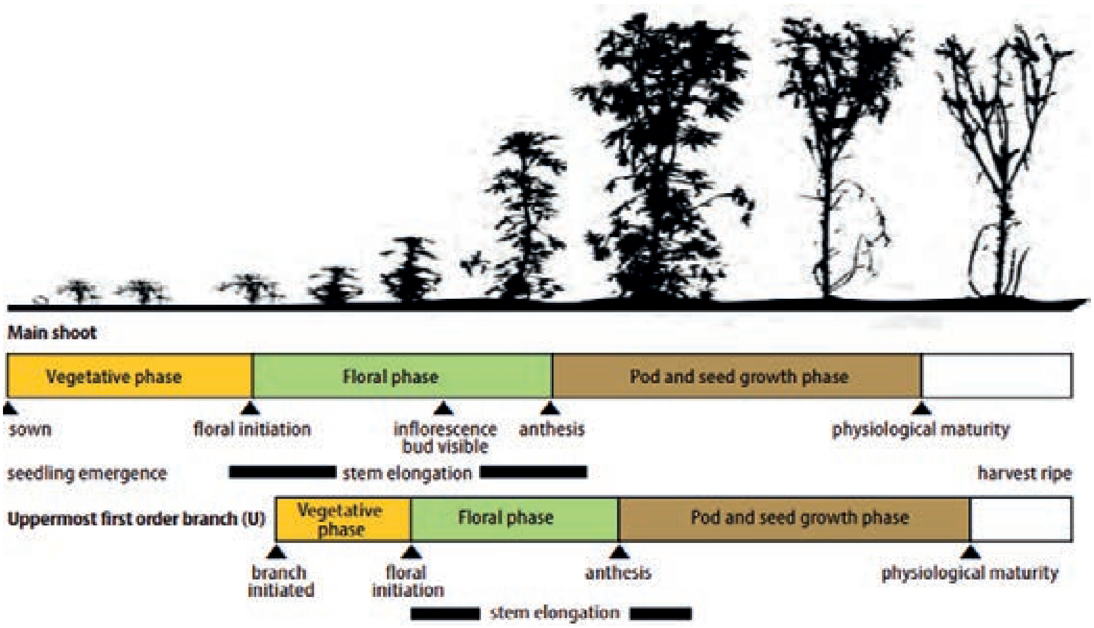


Figure 3.8 *Life cycle of the lupin plant (Walker, 2011).*

Developmental phase	Growth stage (GS)	Description
00 Germination and emergence	GS000	Dry seed
	GS001	Imbibed seed
	GS002	Radicle apparent
	GS003	Plumule and radicle apparent
	GS004	Emergence
	GS005	First leaf unfolding
	GS006	First leaf unfolded
10 Vegetative	GS101	First node (i.e. first leaf fully unfolded with one pair of leaflets)
	GS10(x)	x = node x leaf fully unfolded with more than one pair of leaflets
	GS1(n)	n = last recorded node n – any number of nodes on main stem with fully unfolded leaves, according to variety
20 Reproductive	GS201	Flower buds visible and still green
	GS203	First open flowers on first flower cluster (raceme)
	GS204	First pod visible at first fertile node
	GS205	Green pods fully formed, small immature seeds within.
	GS207	Pod-fill, pods green
	GS209	Seeds rubbery, pods still pliable, turning black
	GS210	Pods dry and black, seeds dry
30 Pod senescence	GS301	10% of pods dry and black
	GS305	50% of pods dry and black
	GS308	80% of pods dry and black
	GS309	90% of pods dry and black
	GS310	All pods dry and black
40 Stem senescence	GS401	10% stem brown/black or most stem green
	GS405	50% stem brown/black or 50% stem green
	GS410	All stems brown/black, all pods dry and black, seed hard

Figure 3.9 *developmental phases and growth stages of faba bean plants (faba bean: the Ute Guide 2009) (GRDC Grownotes, 2018).*

Faba bean developmental stages can be divided into five principal phases, with each phase sub-divided into secondary stages (Table 3.9). The stages of development allow for better understanding of farm chemical labels for correct timing of applications (GRDC Grownotes, 2018).

Faba bean development is mostly influenced by daily average temperature, longer days and to a lesser extent, a cold requirement. Honey bees can increase pollination and, and therefore improve the yield of faba bean crops. Low temperatures can cause flower and pod abortion. Low light can also reduce podset. Breeders aim to overcome these issues in the future with new varieties. Faba bean have poor tolerance to drought and high temperatures (GRDC Grownotes, 2018).

3.3.2.1 Growth conditions related to protein content

In the study of Alghamd, 13 faba bean genotypes were grown at three water regimes (13200, 7600 and 4800 m^3/ha). Irrigation and the variability of seed nutrition composition was examined. The results showed that the protein contents in all faba bean genotypes increased at a lower level of irrigation (Alghamd, 2009).

Protein content is also related to the genotypes of faba beans. Higher seed concentrations of protein content (1.9%) was observed for low-tanin genotypes of faba beans to tanin-containing genotypes, meanwhile the mineral contents of Ca, Mn, Mg, and Cd were also higher in low-tanin genotypes (Khazaei & Vandenberg, 2020). This study used an NIR sensor (DA 7440TM, Perten, Stockholm, Sweden) to determine the seed protein content.

4 Conclusions and recommendations

4.1 Conclusions

The most commercially interesting insects for food are mealworms, crickets and locust species, and black solider fly and house fly are usually cultivated for feed. The growth, development, and feed conversion efficiency of insects can be affected by different diets. Different studies reported that the minimally-processed waste streams could not support adequate growth of insects. Therefore, the development of an effective artificial diets is crucial for rearing a specific insect sort successfully. Several factors should be taken into account when preparing the artificial diets for insects. Waste streams are suggested to be enzymatic degraded before using; adding suitable amount of proteins and lipids, such as essential fatty acids and yeast-protein, which will be very beneficial for the growth of insects.

The major plant protein sources on the market are legumes and oilseeds, which are used to generate animal protein-like product. There criteria were applied to screen the potential protein-rich legume crops: (1) high nutrition value, such as protein content, unsaturated fatty acid and other benefits of the seeds; (2) plants can grow taller than 150cm, being suitable for high-wire growing in greenhouses; (3) no legal issues to grow in the Netherlands. Lupin species and faba bean plants meet all these requirements. To sum up, the white lupin (*Lupinus albus L.*), the andean lupin (*L. mutabilis Sweet*) and the faba bean (*Vicia faba L.*) could be suitable crop candidates to the greenhouses for protein production for further research.

4.2 Recommendations

Very few studies about greenhouses horticultural waste streams for rearing edible insects were found. The common wastes from greenhouse horticulture, such as tomato, bell pepper, cucumber, eggplant contain a great amount of nutrition. They could possibly be processed as ingredients of artificial diets for rearing insects, such as mealworms, crickets and locust species.

Lupin species and faba beans are suitable crop candidates to grow in the greenhouse for protein production. To introduce these crops into greenhouses, small field per-test should be carried out in the first experimental phase after collecting legume seeds. Growing conditions of these crops, such as temperature, light, pollination etc., should be charted in detail. This is to find the best growing condition for producing a remarkable protein yield. Also, different growing conditions influence the protein production, which could also be an interesting research area in the field experiment.

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Annex 1 Life cycles of edible insects in Table 2.1

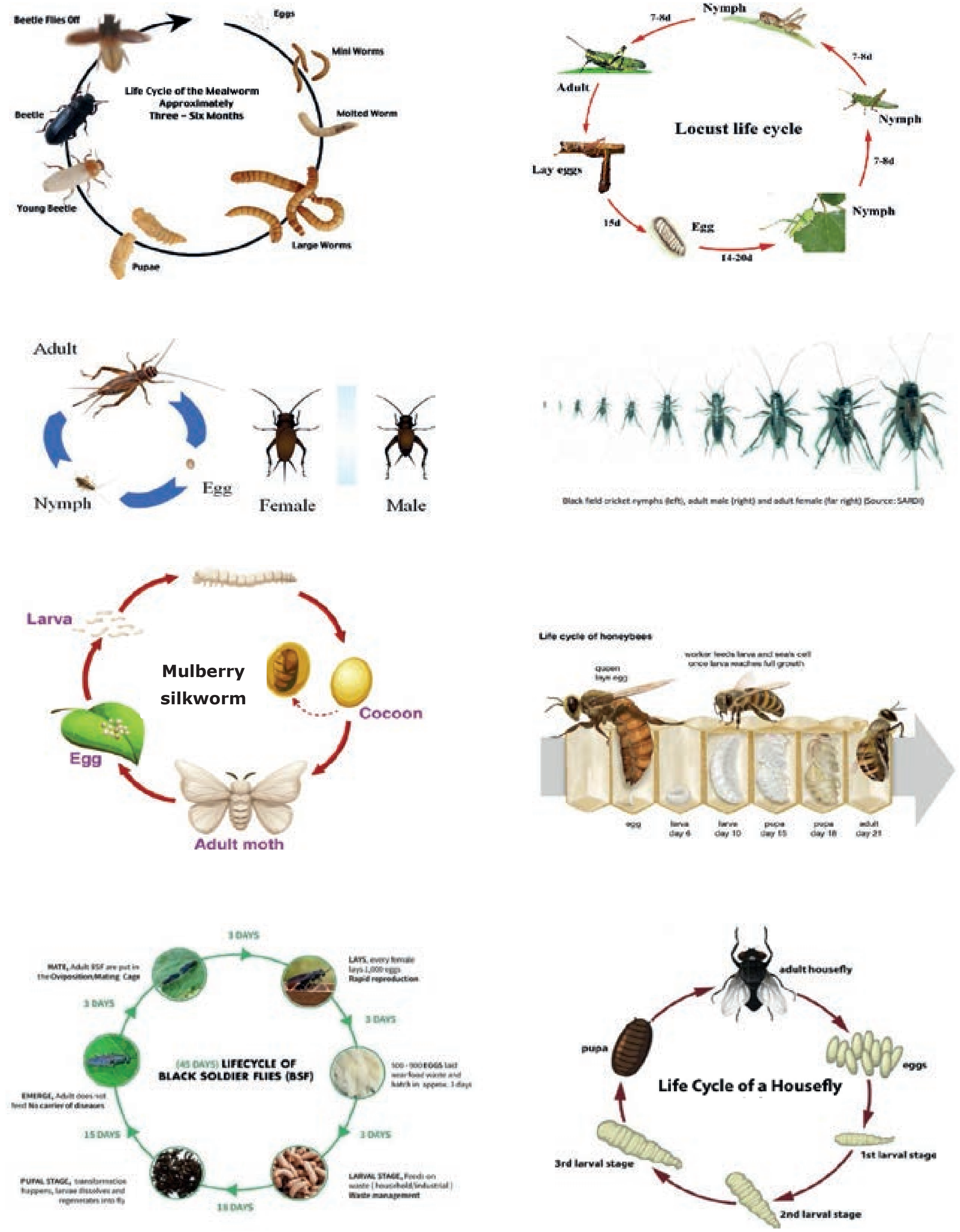

Figure 1 *Life cycles of the edible insects listed in Table 1 (yellow mealworm; Locust; House cricket; Field cricket; Mulberry silkworm; Honeybee; Black soldier fly; Housefly).*

Annex 2 Oilseeds with high protein content

- **Chia plants and beans**
  a. 16.54 g/100g protein in seeds.
  b. Contain all 9 essential amino acid.
  c. 23.665 g/100g EFA.
  d. 34.4 g/100g fibre content.
  e. High minerals level.
  f. 90 cm at maturity, high planting can reach 150-180 cm.

- **Sacha inchi seed**
  a. 30 g/100g protein in seeds.
  b. Contains all 9 essential amino acid.
  c. Sacha inchi oil is usually used in cosmetic industry, but only reply on imports.
  d. 200cm in height.
  e. In 2013, the European Union approved the use of sacha inchi oil for food products. (novel food). FSAI conlcuded ina inchi oil was similar to linseed oil.
  f. The plants have not been cultivated in Europe yet.
  g. The sacha inchi plant only grows in tropical climates at 5577 feet (1,700 m) above sea level.

To explore the potential of nature to improve the quality of life

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