

# Growth of Bitter Gourd in Dutch Greenhouses

Cultivation manual Momordica charantia

This project is made possible by the European Fund for Regional Development of the European Union and a contribution from the province of South Holland and the municipality of Almere











# Growth of Bitter Gourd in Dutch Greenhouses

Cultivation manual Momordica charantia

Composition:	D. Laanen	Aeres Hogeschool Dronten
In collaboration with:	Z. Suleiman-Alkadour B. Gehner R. Severens A. Ruijsenaars S. Meewisse	Aeres Hogeschool Dronten Aeres Hogeschool Dronten Aeres Hogeschool Dronten Aeres Hogeschool Dronten Grower Bleiswijk – Fresh Farma
Other:	R. van Zwet R. Schilperoord	Grower Almere Grower Mijnsheerenland
Editor:	C.E. Westerdijk	Aeres Hogeschool Dronten
Commissioned by:	Green Health Solutions Horizon Flevoland	5
Date:	29 November 2022	



This work is licensed under the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

The information contained in this growing manual is given to the best knowledge of all partners and is believed to be accurate. The terms of your use and application of the suggested actions and recommendations are beyond our control. No warranty is made as to the accuracy of any information or statement contained herein. Partners specifically disclaim any responsibility or liability with respect to the use of the suggested actions and in no event shall they be liable for any special, incidental or consequential damages arising out of such use.

# Content

1	I	ntro	oduction	4				
2	Ş	Soil	and substrate	5				
	2.1		Cultivation in soil	5				
	2.2		Cultivation in substrate	5				
3	I	rrig	ation and fertilisation	5				
	3.1		Irrigation	5				
	3.2		Fertilization	6				
4	١	/ari	eties	9				
	4.1		Bilai F1	9				
	4.2		Other varieties	9				
5	ç	Sow	ing and planting	10				
	5.1		Sowing and nursing methods	10				
	5.2		Cultivation method and planting	10				
6	(	Cult	ivation and crop care	13				
	6.1		Climate	13				
	6.2		Pruning and binding	13				
	6.3		Pollination and fruit set	14				
7	I	PM		16				
	7.1		Cultivation preparation	17				
	7.2		During the Cultivation	17				
	7.3		Diseases and pests	18				
8	ł	Harv	vest	22				
9	F	Proc	cessing and storage	23				
1	0	С	ultivation for compounds	24				
	10.	1	Secundairy metabolites	24				
	10.	2	Effect on strategy	26				
Li	tera	tur	e	27				
A	ppe	ndix	٢	31				
	Ap	pen	dix 1: Research results substrate	31				
	Ap	pen	dix 2: Fertilizer schedule bell pepper	32				
	Ap	pen	dix 3: Pests scouting record table (greenhouse)	33				
	Ap	pen	dix 4: Overview biological control	34				
	Ap	pen	dix 5: IPM according to cropstage bitter gourd in open soil cultivation	35				
	Appendix 6: Content polypeptide-p per accession							

# 1 Introduction

Bitter gourd (*Momordica charantia*) is also called sopropo, balsam-pear, karela or bitter melon and is a member of the cucumber family (Cucurbitaceae). It is a monoecious, annual, fast-growing and herbaceous creeping plant. The wrinkled fruit of the bitter gourd is consumed as a vegetable and medicine in Asia, East Africa, South America and India. *Momordica* means "to bite", referring to the serrated edges of the leaf, which look as if they have been bitten (Grover & Yadav, 2004). The entire plant is edible and bitter in taste, with the ripening fruits changing from green to orange-yellow. Nutrient analysis shows that bitter gourd is rich in fiber, calcium, potassium, iron and vitamins C and A (Lucas, Dumancas, Smith, Clarke, & Arjmandi, 2010).

The fruits contain compounds that can be used against diabetes, obesity, high blood pressure, dyslipidemia and cancer. These substances are antibacterial and antiviral as well as anti-aging (Grover & Yadav, 2004; Raina, Kumar, & Agarwal, 2016; Lucas, Dumancas, Smith, Clarke, & Arjmandi, 2010; Basch, Gabardi, & Ulbricht, 2003; JinYanga, et al., 2015; Saeed, et al., 2018; Lee-Huang, et al., 1995; Xiong, et al., 2009; Ray, Raychoudhuri, Steele, & Nerurkar, 2010; Kaur, et al., 2013). This medicinal effect has been proven in laboratory experiments, but more clinical studies in humans are needed to prove its use (MSKCC, 2021).

The aim of this bitter gourd cultivation manual is to make this cultivation accessible to Dutch growers and in this way be able to meet market demand. In addition, this cultivation manual aims to provide insight into the standardized production of the medicinal ingredients in the fruit.

# 2 Soil and substrate

### 2.1 Cultivation in soil

Bitter gourd can be grown in soil and in many greenhouses the soil usually consists of a combination of peat, sand and clay. Sandy loam with an organic matter content of 4% to 6% and a pH between 5.5 and 6.5 is most suitable for cultivation. A higher pH means less yield. Rototilling is necessary to stimulate the rooting of the plants. A soil with a higher groundwater level has a negative influence on the growth of the plants (Schilperoord, 2021). Adding sand to the soil can help with drainage, as the plants prefer not to be too wet.

### 2.2 Cultivation in substrate

It is also possible to grow the plants on substrate slabs. Small-scale research (APPENDIX 1) has shown that a combination of plants in stone wool blocks on a stone wool slab or container filled with pumice is better for the growth of bitter gourd. The plants should preferably not stand in too wet substrate. Pumice produces fruits with a higher weight, whereas stone wool produces more fruits. Potting soil results in less fruit growth, but the higher amount of retained nutrients makes the plant more resistant to diseases (Remijn, Veenkamp, & Ruijssenaars (o.l.v.), 2022).

When using stone wool, it is important to pay attention to the pH value. The pH target value of the slab is 6, as the plant prefers a lower pH.

When the bitter gourd plants are not placed on a slab, but in a pot or bucket, this limits the root volume (Plagron, z.d.). Although this ensures a more compact growth of the plant and is therefore more manageable, the plant produces less fruit. The plant produces flowers earlier, which may be a result of stress (Severens, 2021). The advice is therefore to give the plant enough space to keep the root volume high. This is not only better for the absorption of nutrients and water, but also ensures a better vitality of the plant.

# 3 Irrigation and fertilisation

### 3.1 Irrigation

For soil cultivation, it is recommended to carry out the watering with a dripper system. A dripper is placed with each plant, regardless of whether it concerns a vertical or horizontal cultivation. Depending on the season, the plants are watered 1 to 3 times a day. This increases from 3 litres per square meter to 5 litres of water per m2 in the summer (Meewisse, 2021; Schilperoord, 2021).

For cultivation on substrate slabs, the use of drippers is also a logical choice.

#### Figure 1: Drip irrigation vertical soil cultivation



#### 3.2 Fertilization

Depending on the type of cultivation and the watering method, the application of fertilizer is necessary. The alternation of rainwater with nutrient-rich ditch water ensures sufficient fertilization. When using tap water, the following fertilization regime is desirable for growin in stone wool:

- pH target value: 5,3 to 5,5
- EC target value: 3 to 4

For soil cultivation it is recommend to have a higher pH target value and a lower EC target value in comparison to the values above. A high nitrogen content in the fertilizer provides more leaf surface and counteracts aphids, pathogens and other pests. When growing in an inert substrate, like stone wool, a standard cucumber schedule can be followed as fertilizer recipe (TABLE 1). From experience, a bell pepper fertilization schedule also works well for the bitter gourd plants. The recipe from APPENDIX 2 is used for this.

Table 1: Fertilizer schedule cucumber (van der Lugt, 2017). The amount of fertilizer to be applied in the A or B tank is calculated for a volume of 1000 L and results in a 100x concentrated nutrient solution.

#### CROP: CUCUMBER (Cucumis sativus)

#### INERT SUBSTRATE

	-	Target	Nutrient		Target	Nutrient		Adjustments						
		values	solution		values	solution	Sta	Start		set	High water		End season	
рН		5.2-6.0	5.3		5.2-6.0	5.3			*		**		***	
EC	mS/cm	3	2.2	mS/cm	3	2.2								
Na	mmol/l	<6		ppm	<184									
Cl		<6			<284									
HCO <sub>3</sub>		<0.5			<6									
N-NH <sub>4</sub>	mmol/l	<0.5	1.25	ppm	<2	18	-0.5	-7					-1	-14
К		8	8		313	313	-1	-39	1	39	.1	39		
Ca		6.5	4		260	160	0.5	20			0.5	20		
Mg		3	1.375		73	33	0.25	6						
N-NO₃	mmol/l	18	16	ppm	252	224			1	14				
S		3.5	1.375		112	44								
Р		0.9	1.25		28	39							-1	-31
Fe	µmol/l	30	15	ppb	1680	840	10	560						
Mn		7	10		385	550								
Zn		7	5		458	327								
В		50	25		540	270	10	108						
Cu		1.5	0.75		95	48								
Мо		0.5	0.5		48	48								

\* The adjustments for fruit set may vary from 0.25 to 2 mmol/l for K and 0.2 tot 0.75 mmol/l for Ca.

\*\* Adjustments for high water supply are recommended when water supply exceeds 5 I/m<sup>2</sup>/day.

\*\*\* End of the crop, after the removal of the growth point of the ranks. This is mostly in autumn, when the ranks are no longer growing and the last fruits are ripening.

A		
Calcium nitrate solid	86	kg
Potassium nitrate	18	kg
Iron DTPA 6% or EDDHA 6% or HBED 6%	1396	g
Manganese EDTA 12.8%	429	g
Zinc EDTA 14.8%	221	g
Copper EDTA 14.8%	32	g

В		
Potassium nitrate	55	kg
Monopotassium phosphate	11	kg
Magnesium sulphate 16% MgO	34	kg
Monoammonium phosphate	5	kg
Borax 11.3% B	239	g
Sodium molybdate 39.6%	12	g

#### 3.2.1 Nutrient deficiencies

The table below describes a number of deficiency symptoms of the bitter gourd and also describes how to correct these symptoms.

Table 2: Nutrient deficiency symptoms and correction

#### Nitrogen

**Deficiency symptoms:** Both vegetative growth and fruit production are severely restricted plants appear pale and spindly. New leaves are small but remain green, whereas the oldest leaves turn yellow and die. The yellowing spreads up the shoot to younger leaves. Yield is reduced and fruit are pale, short and thick.

**Correction measure:** Side-dress deficient in-ground crops with 20-50 kg N/ac, or apply fortnightly foliar sprays of 2% urea at high volume.



#### Potassium

**Deficiency symptoms:** Potassium deficiency causes yellowing and scorching or older leaves. These symptoms begin at the margins of the leaf and spread between the veins towards its centre. Large areas of tissue around the major veins remain green until the disorder is well advanced. A brown scorch develops in the yellow areas and spreads until the leaf is dry and papery. Potassium from a fertilizer will move from the soil surface to the roots only if the soil is very sandy. Potassium fertilizers are therefore best incorporated in the soil before planting. Fertigation or drip feeding can also be used to treat a deficient crop.



Correction measure: Foliar spray of KCl 1% at weekly interval.

#### Calcium

**Deficiency symptoms:** Emerging leaves appear scorched and distorted and may cup downwards because the leaf margins have failed to expand fully. Mature and older leaves are generally unaffected. With a severe deficiency, flowers can abort, and the growing point may die. Fruits from calcium-deficient plants are smaller and tasteless, and may fail to develop normally at the blossom end.

**Correction measure:** Soil application of gypsum as per gypsum requirement based on soil test report or by foliar spray of CaSO<sub>4</sub> at 2% solution in water.



#### Magnesium

**Deficiency symptoms:** Magnesium deficiency causes yellowing of older leaves. The symptom begins between the major veins, which retain a narrow green border. A light tan burn will develop in the yellow regions if the deficiency is severe. Fruit yields are reduced.

**Correction measure:** Incorporate magnetite (300 kg/ac) or dolomite (800 kg/ac) into deficient soils before planting. Fortnightly foliar sprays of MgSO<sub>4</sub> (2 kg/100 L) at high volume (500-1000 L/ac).

#### Boron

**Deficiency symptoms**: Distortion of newer leaves (in severe cases the growing point dies) and the appearance of a broad yellow border at the margins of the oldest leaves. Young fruit can die or abort; abortion rates are high. Stunted development and mottled yellow longitudinal streaks, which develop into corky marking (scurfing) along the skin.

**Correction measure**: Foliar spray of 0.2% Borax at forthrightly interval. Application of 10 kg borax per hectare to deficient soil before will prevent boron deficiency.

#### Iron

**Deficiency symptoms:** Iron deficiency causes a uniform pale green chlorosis of the newest leaves; all other leaves remain dark green. Initially, the veins remain green, which gives a net-like pattern. If the deficiency is severe, the minor veins also fade, and the leaves may eventually burn, especially if exposed to strong sunlight. Good drainage and soil aeration favour iron availability. Foliar sprays of iron sulphate (150 g/100 L) can be used to treat symptoms.

**Correction measure:** Foliar spray of 0.5% FeSO<sub>4</sub>.

#### Manganese

**Deficiency symptoms:** The veins of middle to upper leaves of manganese-deficient plants appear green against the mottled pale green to yellow of the blade.

**Correction measure**: Spray the foliage with MnSO<sub>4</sub> at 0.1% (100 g/100 L water).











#### Cultivation manual bitter gourd

## 4 Varieties

The *Momordica charantia* has several varieties. Comparative research has shown that the Bilai F1 variety is best suited for cultivation in an unheated greenhouse. In addition, other varieties, such as Palee or Benkian, are also suitable for cultivation, for both unheated as heated greenhouses (PARAGRAPH 6.1).

### 4.1 <u>Bilai F1</u>

Bilai is a very early maturing hybrid. The fruits are very attractive, glossy light green and have good uniformity. The vigour of the plant is not strong, therefore it is recommended to grow Bilai as a vertical crop (PARAGRAPH 5.2). Due to the plant origin, the variety is also suitable for greenhouse cultivation (Meewisse, 2021).

#### Features Bilai F1 (East West Seed ROH Limited, 2019):

- Type: Vietnam-type, medium
- Colour: Glossy green
- Days to maturity: 37-40
- Shape: spindle
- Diameter: 6.0-7.0
- Length (cm): 18-23
- Vigour: moderate

#### Figure 2: Bilai (East West Seed ROH Limited, 2019)



PaleeF1

#### 4.2 Other varieties

In addition to Bilai, it is also possible to choose a different variety, depending on the cultivation strategy of the company and the market demand.

Benkian F1

#### Bitter gourd varieties from East West Seeds compared to Bilai:

- Benkian/Benqiang: grows later than Bilai, fruit similar to Bilai, shorter and stronger plant.
- Best: large fruits, a lot of variation in fruit size, which can cause them to ripen too quickly.
- **Palee:** large fruits, most bitter, healthier.
- **Preti:** rounder fruit, smaller than Palee.
- **High Moon (Vreeken's zaden):** pale to white fruit, easily tainted.

Figure 3: Fruit types Benkian, Best en Palee (East West Seed ROH Limited, 2019)



Best F1



When choosing one of these varieties, it is necessary to maintain a larger planting distance or to prune more frequently to stop lateral growth (Schilperoord, 2021; East West Seed ROH Limited, 2019).

# 5 Sowing and planting

### 5.1 Sowing and nursing methods

The seeds of the bitter gourd resemble pumpkin seeds and are located in the centre of the poisonous fruit, surrounded by white threads. Seeds are purchasable in the Netherlands from East West Seeds among others. This company imports seeds from Asia. Seed can be nursed at a plant nursery or by yourself.

Seeds are sown in seed trays at a depth of 1 to 2 centimetres. When sowing, the pointed side of the seed, from which the root will emerge, is put down and the other side half above the ground. After sowing, cover the top layer with a layer of potting soil until the seed is covered (East West Seed Knowledge Transfer, 2019).

The germination time is 2 to 7 days. Seed emergence is highest when sowing at a high temperature (between 22 °C and 30 °C) and high humidity. The emergence is variable depending on the quality and age of the seed lot. The turnout is usually around 90%. Figure 5: Seedlings



Transplanting the seedlings into the ground is possible when the seedlings are 5 to 8 cm tall, this is after approximately 8 days. When growing in substrate, the advice is to germinate the seeds in stone wool blocks.

### 5.2 <u>Cultivation method and planting</u>

The plants can grow both vertically and horizontally. With vertical growth, the plantings are closer together and the plants are guided over a vertically placed trellis (FIGURE 6). With horizontal growth, the planting distance is wider and the plants are guided over a horizontal mesh that is hung from the greenhouse cover. This allows optimal use of the greenhouse space, because other types of cultivation can also take place under the bitter gourd plants (FIGURE 7). It is necessary to take in account a reduction in light incidence, because the bitter gourd plants absorb this light. With these plants, the side shoots are pruned down to the mesh.

Figure 6: Vertical plant growth



Figure 7: Above the plants on the trellis (left) and horizontal plant growth (right)



#### 5.2.1 Vertical cultivation

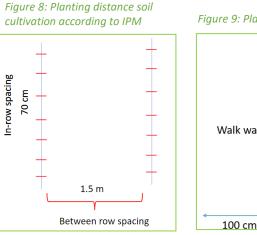
Before planting, a wire mesh panel, mesh mat or trellis must be secured between the plants. This mesh panel should be about 180 to 250 centimetres high.

There are two ways to plant the bitter gourd seedlings in this vertical growth:

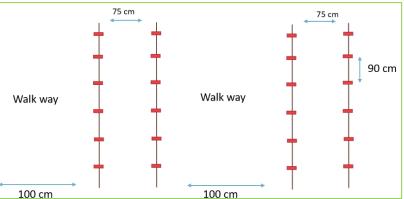
- On both sides of the mesh panel;
- Alternately of the mesh panel;

#### Plant distance

In many crops it is generally assumed that about 8,800 plants per hectare can grow, which amounts to about 9 plants per 10 m2. The planting distance depends on the variety, the growth habit, the crop protection strategy (IPM) and the season and is usually between 0.5 to 1 meter between plants per row. To ensure sufficient air circulation, it is recommended to maintain a planting distance of 90 cm. Figure 8: Planting distance soil cultivation according to IPMFIGURE 8 shows the planting distances for open ground cultivation. When growing on substrate, it is recommended to keep the planting distances as shown in FIGURE 9.







### 5.2.2 Horizontal cultivation or high-wire cultivation

The planting distances and planting method differ when choosing horizontal growth. A larger planting distance can be used here, whereby a mesh panel is hung high near the greenhouse roof and the plants grow over it (Schilperoord, 2021).

The possibility of using high-wire cultivation, as is usually the case with cucumbers, has not yet been extensively researched. This cultivation variant ensures that there are more cultivation operations, since the bitter gourd has to be pruned more often to achieve height growth. A variety with larger leaves and fewer branches means that fewer cultivation operations are required. However, a new variety will have to be bred for this. Cultivation operations are further described in PARAGRAPH 6.2.

A hedge roof system is also sometimes used abroad and sporadically for cultivation in the Netherlands. This fits well with the wild way of growing bitter gourd (Hertog, 2020). This also implies that less cultivation procedures are required with this cultivation system.

# 6 Cultivation and crop care

### 6.1 <u>Climate</u>

Since it concerns a cultivation of a tropical plant, cultivation in a greenhouse is recommended. In the spring, the amount of light is sufficient, so that extra lighting is not necessary. When growing in the autumn, it is advised to use lighting.

#### 6.1.1 Cultivation in an unheated greenhouse

It is possible to grow bitter gourd in an unheated greenhouse, where the temperature is around 25 °C during the day and around 18 °C at night. The optimum temperature for plant growth is around 25 °C, below this point the growth slows or stops. The advantage of growing in a cold greenhouse is the low energy costs. The disadvantage of growing in a cold greenhouse is the limited control of temperature, irradiation and humidity.

### 6.1.2 Cultivation in a heated greenhouse

It is easier to control the cultivation in a high-tech or heated greenhouse. The plant grows well at a temperature between 25 and 30 °C. The photosynthesis efficiency can be increased by maintaining a higher temperature in the greenhouse. However, for this it is necessary to have a high light sum, a lot of CO2 supply and sufficient humidity (Het Nieuwe Telen, z.d.). PARAGRAPH 10.2 explains how "Het Nieuwe Telen" can be used in order to produce a stable amount of ingredients in the product.

#### 6.2 Pruning and binding

Bitter gourd plants have a massive vegetative growth, which makes it important to prune and bind the plants timely.

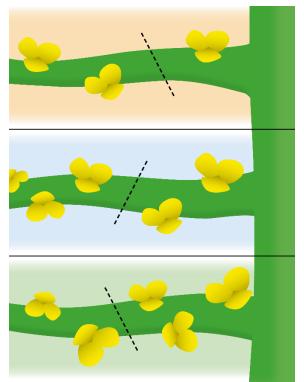
First of all, it is important to prune the side shoots, which are not productive, at the first ten axillary buds, around 50 to 75 cm above the ground or substrate (East West Seed Knowledge Transfer, 2019).

Pruning the plant has a positive effect on photosynthesis. It provides an increase in cumulative light, so that the plant can absorb more light. During pruning, the poorly pollinated and crooked fruits can also be removed, which can be recognized by the small and strange shape. This restores the balance in the plant and the sugars formed can go to the other fruits. The pruning of both plant parts and fruits ensures a more efficient use of the assimilation and a doubling in the cumulative light (Severens, 2021).

Brown leaves should also be removed during pruning to reduce the spread of disease, see also CHAPTER 7.

In addition, when pruning, the tendrils (lateral shoots) are removed at 15 cm from the stem. This is done so that fruit growth can take place on the remaining piece of stem and the plant can put more energy into the fruit on the shoot. The place where the side shoot should be pruned depends on the timing and the climate conditions (Zwet, 2021). In good conditions, more ovaries can be left on the side shoot than in less favourable conditions. This can be seen in a schematic representation in FIGURE 10.

Figure 10: Pruning of lateral shoots.



#### Red

In a week with less favourable conditions, more ovaries can be pruned and more of the lateral shoot.

#### Blue

The conditions are normal (enough light, water and warmth). Lateral shoots are pruned after two ovaries are produced.

#### Green

When the conditions in a week are better than normal (more warmth, more nutrients etc.), more ovaries can stay on the shoot.

Binding the plant is also necessary, since it grows massively. Binding prevents stems from tangling, improves aeration and light transmission for the crop. The best technique for binding the plant is to use wire mesh and twisting them. In this way the plant is guided in the desired growth direction.

#### 6.3 Pollination and fruit set

Thirty to fifty days after germination, the plant will flower and flowers will appear in the leaf axil of about three centimetres in size. The bitter gourd is monoecious, meaning that it has female and male flowers on the plant (FIGURE 11). The female flowers, easy to spot because there is already an ovary underneath, should preferably be pollinated by hand. This is quite simple, by holding a male flower with pollen on top of the female flower (FIGURE 12). The best time for this is between 08:00 and 10:00. In these two hours, an employee can pollinate about 200 flowers manually. A male flower can pollinate about three female flowers. Using this technique increases the chance of fruit set.

The use of bees or bumblebees for pollination is also possible, but this results in a lower pollination rate. In addition, the activity of bees is lower at a higher greenhouse temperature and this also causes the flowers to disappear, which are therefore unable to develop into fruit (Schilperoord, 2021). Depending on the type of greenhouse and the options for climate control, an optimum temperature for the pollinators can be ensured during the pollination period.

Poor pollination results in lesser fruits at the beginning of the fruit growth stage (Severens, 2021). In addition, it also causes fruit falling from the plant prematurely. It takes about 14 days from pollination to full-grown fruit.

The Bilai variety is known to have insufficient male flowers in very cold weather. The length of the photoperiod does not affect the flowering period of the plant, but it does affect the proportions between male and female flowers. The shorter the day, the more male flowers will grow on the plant. As the day gets longer, more female flowers grow on the plant (Meewisse, Contact per mail and Feedback, 2021; Zwet, 2021). As soon as more male flowers appear, it can be interesting to give the plant less water or heat, for example. Due to this stress, more female flowers are produced (Zwet, 2021). The plant hormones auxin, gibberellic acid and cytokinin play an important role in fruit setting (Kumar, Khurana, & Sharma, 2014).

Figure 11: Female flower (left) and male flower (right) (Meewisse, Contact per mail and Feedback, 2021)



Figure 12: Pollination of flowers by hand (Rinki's Home Garden, z.d.)



# 7 IPM

A successful greenhouse integrated pest management (IPM) program incorporates sustainable and cost-effective management strategies that ultimately deliver high-quality plants for retailers and consumers (FIGURE 13).





As you see in FIGURE 13 is cultural methods one of the IPM components of preventing the occurrence of the problem, such as using resistant varieties. Cultural methods also include adequate adaptations to manage fertility, reduce cultural stress, and control soil moisture and leaf wetness to minimize favorable conditions for pests and diseases such as downy mildew and powdery mildew.

Scouting and monitoring, as a preventive and curative method, in which each plant or plant material is inspected before it enters the greenhouse. Monitoring is very important because you can take action at the right time and prevent the pest population from increasing and becoming a real problem.

In PARAGRAPH 7.1 is described how IPM is applied during cultivation preparation and in PARAGRAPH 7.2 during cultivation. An overview of common pests and diseases can be found in PARAGRAPH 7.3.

The integrated crop protection described is applied in combination with the components described in previous and subsequent chapters of this cultivation manual. Appendix 5 can also be used for an overview of the IPM per cultivation stage for open field cultivation.

# 7.1 Cultivation preparation

#### **Greenhouse preparation**

- Walkways must be free of potting soil, organic matter and weeds
- Gutters, pots and trays must be disinfected
- A foot bath will be installed at the entrance of the greenhouse
- Fine mesh screens on ventilation openings to prevent pest invasion.
- The crop must be planted following advised planting distances, see PARAGRAPH 5.2

#### Soil and substrate preparation

- Soil is a host for diseases and pests. This is a major disadvantage, especially in a greenhouse. Most greenhouse glazing panels filter UV light which sterilizes the soil naturally.
- If there is no crop rotation, the soil cannot recover from diseases. By sterilizing the soil or choosing a different crop, the soil remains clean.
- Substrate must be clean and disinfected to prevent soil pathogens such as Cercospora.
- Use clean substrate mix, plant material should not enter the room where substrate is mixed.
- Use a slow release fertilizer
- Adjust the Nitrogen level to prevent aphids, pathogens and other pests.

#### Seed treatment

- Most fungal, bacterial and viral plant diseases are spread naturally by infected and contaminated seeds of other species in soil or potting soil.
- Use resistant varieties against Powdery mildew and Downy mildew.
- Remove the seed coating and soak the seeds for 30 minutes in warm water (52°C), against Cercospora.
- Separate new plantings from old plantings.

### 7.2 During the Cultivation

#### **Quarantine plants**

- Staff should make it a habit to care for plants in infested or quarantine areas last to avoid carrying small insects or pathogenic spores on their clothing to the rest of the greenhouse.
- Remove weeds and host plants that can be a source of aphids and thrips. PET plants are plants that are more interesting for the pests than the crop.
- It is recommended to grow some Banker plants in the greenhouse. Banker plants are a host for natural enemies, when cultivation is over the natural enemies transfer to these host plants, giving them enough food to survive to the next season. This saves costs.

#### Scouting and monitoring

- Scouting and monitoring should take place weekly during the entire production season.
- Scouting starts at the major doorway (entrance point of disease and pests).
- Scouts should walk through each aisle and move from gutter to gutter in a snake-like manner within 10 minutes and a minimum of 20 plants per 300 m2. Make sure the scouts don't touch anything to prevent them from being the spreader themselves.
- Start scouting from the bottom of the plant by checking the soil for insects, mites and diseases looking upwards at older leaves, younger leaves and new growth.
- Yellow grid cards (aphids and whiteflies) and blue grid cards (thrips) for catching and monitoring must be placed. One card per 300 m2 and placed above the canopy. Traps should be replaced and insects should be counted weekly. APPENDIX 3 can be used for this.
- Use indicator plants to determine the effectiveness of pest management tactics and to check sensitive crops for viruses (tospoviruses).

#### Irrigation

- Choose irrigation instead of sprinkling or misting.
- Wetting the foliage should be avoided (moist leaves provide ideal conditions for pathogens).

#### 7.3 Diseases and pests

The following diseases and pests are common in the cultivation of *Cucurbitacae* and bitter gourd.

#### 7.3.1 Common diseases

#### Powdery mildew (Sphaerotheca fuliginea)

- White powdery residue primarily on the upper leaf surface.
- Circular patches or spots appear on the lower surface of the leaves.

Figure 14: Symptoms Powdery mildew



#### Downy mildew (Pseudoperonospora cubensis)

- Yellow angular spots on the upper surface of the leaves.
- Rapid defoliation occurs.

#### Figure 15: Symptoms Downy mildew



#### Bitter gourd mosaic virus

- Small irregular yellowish patches are on the leaves
- Some leaves show vein clearing in one or two lobes of the leaf
- Transmitted by five species of aphids

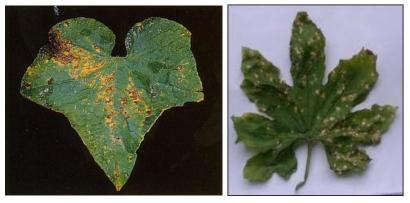
Figure 16: Symptoms bitter gourd mosaic virus



#### Angular leaf spot on bitter gourd (Pseudomonas syringae)

- It is a bacterial disease
- Symptoms first appear as water-soaked lesions on underneath surface of leaves that occur between the minor veins of the leaves.
- As the disease progresses, the infected spots turn brown and the leaf material falls out, leaving angular holes in the leaves.

Figure 17: Symptoms angular leaf spot



#### Cercospora leaf spots (Cercospora citrulline)

- Starts as small, round, whitish or dark brown leaf spots, but when favourable conditions arise these spots enlarge and merge to form irregular lesions with a whitish centre and dark brown edges.
- In the advanced stage, the fructifications of the fungus.
- Severe infection of the disease can cause significant defoliation and death of entire stems.

#### 7.3.2 Management program for diseases

- Thoroughly screen the nursery bed for infected seedlings and remove them carefully. Only transplant healthy seedlings and remove the diseased plants in the main field.
- Weeds that can serve as additional hosts should be removed.
- Low humidity and good air circulation 15-20°c 77% humidity.
- A correct balance in fertilization, the use of resistant varieties, a good planting distance and the use of underground irrigation ensures less disease pressure.
- Polyethylene sheets.
- For viral diseases, follow the pest control procedures to manage the virus vectors.
- Several biological agents can be used to combat fungal and bacterial diseases, such as: *Trichoderma viride* and *Pseudomonas fluorescens.*

#### 7.3.3 Pests

#### Red pumpkin beetle (Aulacophora foveicollis)

- Attacks the melons at the seedling stage
- They make holes in cotyledonary leaves of melon
- As a result, the seedlings in the young stage die

#### Aphids (Aphis sp.)

- Aphids damage the plants by sucking the leaf sap in young stage
- Cotyledonary leaves crinkle and in severe cases the plants wither

#### Spider mite (Tetranychus urticae)

- Spider mites belong to the group of sucking feeders.
- The cells are sucked out which results in chlorotic lesions (reductions in chlorophyll concentration) on the leaves of the plant.
- Less plant development and it can be devastating for the plant.

#### Fruit fly (Dacus cucurbitae and Dacus dorsalis)

- The female fly oviposits on soft fruits and causes necrosis around the puncture mark visible on the fruit.
- Feeding by the larvae causes yellowing and rotting of the fruit.

#### Thrips (Thrips palmi)

- The adults tend to feed on young growth, and so are found on new leaves.

#### White fly (Trialeurodes vaporariorum)

- If the population is very large, feeding on plant sap can affect the physiology of the plant, leaves can wilt and fall. Such leaf damage can in turn influence the development of fruit and lead to a reduction in yield.
- Viruses can be transmitted.

#### Cucumber moth (*Diaphnai indica*)

- External feeding on leaves and fruits Early symptoms of infestation are the development of lace-like patches of networks of intact small leaf veins.
- Damage is most serious in the early stages of fruit formation when the pests feed on and puncture the skin of young fruit, particularly where they touch leaves or the soil.

#### 7.3.4 Management program for pests

- Weekly monitoring through pest scouting using monitoring devices such as pheromone traps and coloured sticky traps. APPENDIX 3 contains a tool for recording this.
- The recorded monitoring and the thresholds used as a basis for decision-making in tackling the pest.
- The action threshold is the point at which action must be taken for a particular pest population.
   For example, for aphids this is 1000 aphids per plant and for thrips this is 15 thrips per poster per week per 100 m<sup>2</sup>.
- From fifth leaf to harvest, aphids are monitored weekly by direct observation of 10 mature leaves from 5 representative sites (50 leaves) of the field.
- Vacuum cleaner to remove whiteflies from plants
- Neem Oils
- Use biological control agents, such as parasites and predators. APPENDIX 4 contains an overview of the various pests and their biological control agents. It is important to take note of the life cycle of these pests, so that appropriate action can be taken.

#### Specific for fruit fly

- Pest monitoring for fruit flies using Cue-lure traps should be performed regularly from the time fruits appear on the plant.
- IPM strategies: (sanitation) by collecting and destroying fallen fruit and infected fruit on plants helps to control fruit flies. Collect and destroy the pest when the incidence is low.
- Wrap the fruit with newspaper or plastic bags.
- Rake the soil around the plant to expose the fruit fly pupae to natural enemies.
- Mulching around the plant helps prevent fruit fly larvae from entering the soil for pupation and exposing them for natural predation.
- Preservation of pupal parasitoids such as *Opius fletcheri* helps in the long-term management of fruit flies.
- Prepare organic insecticides (a few drops of dishwashing liquid + a squeeze of lemon juice), put them in a spray bottle and apply once every 4 days.

# 8 Harvest

About 15 to 20 days after the ovary emerges, the fruits are large enough to harvest. The fruits can be harvested when they are about 20 centimetres long (Perez, Jayaprakasha, Crosby, & Patil, 2018).

The fruit is ready to harvest when the ridges on the fruit become less deep (FIGURE 18, STAGE 3). Harvesting must take place at the right time, otherwise the fruits will turn orange or yellow and then they can no longer be used. The ripe fruits open with three flaps at the bottom, revealing the red placenta and seeds. This is the natural way of seed dispersal, with the seeds releasing and falling to the ground (FIGURE 18, RIGHT).

Figure 18: Left: Four stages of fruit development. The white bar has a scale of 2 cm (Cuong, et al., 2018). Right: (over)ripe fruits



About 30 fruits can be harvested per plant. An estimate of the number of fruits comes down to 3 to 6 kilos per  $m^2$ . This depends on the season and weather conditions. In warm conditions, the fruits ripen too quickly and are no longer suitable for marketing.

The harvest of the fruits should be spread over several moments, for example every three to four days. This not only ensures that fruits do not become overripe, but also ensures that plants are stimulated to continue growing and produce more fruits (Zwet, 2021; HGVJ, 2021). The fruits can be cut from the mother plant with a sharp knife. Harvest carts can be used for large-scale harvests and when space on the path and/or between the plants is sufficient. For the small-scale harvest, baskets can be used that can be hung around the neck to walk through the paths.

# 9 Processing and storage

After harvesting, the fruits can be stored for a few days at 8 °C in a cooling cell. If the fruits are not kept refrigerated, they will ripen further and turn orange. Orange fruits are no longer usable, so they cannot be sold.



Figure 19: Fruits in cardboard boxes, before they are in cold storage

The fruits are preferably eaten fresh, but can also be frozen or processed to prevent loss. In addition to pickling or drying the product, there are also other developments to extend the shelf life. For example, there is a sticker that provides a coating around the product, which prevents further ripening and increases the shelf life (Apeel Sciences, 2020). This sticker is not yet used in the Netherlands, but it can offer extension of the shelf life of bitter gourd. The use of ozone can also ensure better storability, as this limits the dissimilation or respiration of the product.

# 10 Cultivation for compounds

### 10.1 <u>Secondary metabolites</u>

The fruits of *Momordica charantia* contain components that can be used against diabetes, obesity, high blood pressure, dyslipidemia and cancer. These substances are antibacterial and antiviral as well as anti-aging (Grover & Yadav, 2004; Raina, Kumar, & Agarwal, 2016; Lucas, Dumancas, Smith, Clarke, & Arjmandi, 2010; Basch, Gabardi, & Ulbricht, 2003; JinYanga, et al., 2015; Saeed, et al., 2018; Lee-Huang, et al., 1995; Xiong, et al., 2009; Ray, Raychoudhuri, Steele, & Nerurkar, 2010; Kaur, et al., 2013).

Concentrated bitter gourd extracts have a hypoglycemic effect in both pre-diabetic and diabetic patients and can therefore be used in the treatment of diabetes (Wang, et al., 2011; Lo, Ho, Lin, Li, & Hsiang, 2013; Chen, Chan, & Li, 2003; Krawinkel, et al., 2018; Fuangchan, et al., 2011; Peter, et al., 2019). It is best to consume bitter melon in raw or juice form, taking it in capsule or tablet form does not significantly lower blood glucose levels (Bachok, Yusof, Ismail, & Hamid, 2014). However, the bitter gourd does not replace diabetes drugs, but can be a natural adjunct to drug use (in type-2 diabetics) (MSKCC, 2021; Wang, et al., 2014). In addition, too much bitter gourd, from consuming the seeds, extracts or large amounts of juice, can also have negative effects.

Nutrient analysis shows that bitter gourd is rich in fiber, calcium, potassium, iron and vitamins C and A (Lucas, Dumancas, Smith, Clarke, & Arjmandi, 2010). In addition to these components, the plant also produces secondary metabolites under the right growing conditions, which provide the medicinal effect. The bitterness of the fruit is also attributed to the presence of these alkaloids, momordicosides and momordicines.

Some of the secondary metabolites in bitter gourd are explained below.

#### Saponins

Saponins are triterpene glycosides and taste bitter or pungent, are inedible to insects and act to protect the plant against damage. These substances have lipid-lowering properties. This means that they can lower cholesterol, and therefore may be useful for the treatment of dyslipidemia (Ejelonu, Elekofehinti, & Adanlawo, 2017). Dyslipidemia is an abnormality in the metabolism of fats, which causes abnormalities in, among other things, cholesterol and triglycerides.

In addition to the cholesterol-lowering effect, saponins also show a cytotoxic effect on cancer cells, because they initiate programmed cell death (apoptosis). Furthermore, they can also be used in chemotherapy, as they influence protein expression in the cell cycle, cancer progression and metastasis (Moses, Papadopoulou, & Osbourn, 2014; Elekofehinti, Iwaloye, Olawale, & Ariyo, 2021). The effect of saponins has also been widely proven as the blood sugar lowering component of medicinal plants (Elekofehinti O. O., 2015).

#### Momordicin

The active saponins (triterpenoids) in the fresh fruits of *M. charantia* are (Begum, et al., 1997) :

- Momordicin-28
- Momordicinin
- Momordicilin

Momordicosides are found in unripe fruits (Toshihiro Akihisa, 2007; Liu, Chen, Wang, & Qiu, 2009; Liva Harinantenaina, 2006; Liu, Chen, Wang, & Qiu, 2010). Other interesting metabolites in the fruits are momordenol (a sterol), momordol and momordicin I (Begum, et al., 1997).

#### **Bioactive components**

In the fruits of bitter gourd, in addition to these momordicins, there are also other important bioactive components.

For example, in a recent study by Mahwish, et al., 2021, the substances charantin and vicine have been shown to be useful in treating and limiting the development of diabetes. Charantin occurs mainly in the flesh (0.16  $\pm$  0.02 mg/g), while vicine was abundant in the whole fruit (0.21  $\pm$  0.01 g/100 g). Consumption of the bitter gourd showed significant improvements in hyperglycemic symptoms. The most potent dose was 300 mg/kg whole fruit, resulting in a 31.64% decrease in blood glucose and a 27.35% increase in insulin in hyperglycemic rats (Mahwish, et al., 2021).

Charantin is a 1:1 mix of saponins and glucosides (Parkash, Ng, & Tso, 2002). Vicine is an alkaloid glycoside, also found in *Vicia faba* beans for instance among others (Lattanzio, Bianco, Crivelli, & Miccolis, 1983).

The composition of Bitter gourd fruits is being studied by the Metabolomics Facility of Universiteit Leiden. Analysis identified three metabolites from the class of triterpenoids (Xuedanoside H, Acutoside A, Karaviloside IX) that correlated (R=xxx, p<yyy) with improved glycemic control as induced by the cultivars "Palee" and "Good healthy". These research are still going on and under embargo.

Note that there is insufficient confirmation of the various bioactive components of the bitter gourd fruit.

#### Flavonoids

Flavonoids are polyphenolic secondary metabolites, that occur naturally in plants and can be found in almost all fruits and vegetables. Depending on the chemical structure, degree of oxidation and unsaturation of the linking chain (C3), flavonoids can be classified into several groups, such as anthocyanidins, chalcones, flavonols, flavanones, flavanonoes, flavan-3-ols, flavanonols, flavonos and isoflavonoids (Delage, 2015).

Bitter gourd contains these phenolic compounds, such as phenylpropanoids and flavonoids, and are known to have antioxidant activity. Normally this protects plantcells against biotic and abiotic stresses, including wounding, UV irradiation, exposure to pollutants and herbivores (Cuong, et al., 2018). Possibly, this could be the reason that the fruit has healthy benefits for the human body. These phytochemicals exhibit various health-promoting effects, such as reducing blood pressure and lowering possibilities of cancer and cardiovascular diseases (Cuong, et al., 2018; Tan, Parks, Stathopoulos, & Roach, 2014).

#### **Polypeptide-P**

An interesting component of bitter gourd is a hypoglycemic polypeptide, Polypeptide-P, which provides a sugar-lowering effect in persons with diabetes type I and type II. It is also seen as an analogue of insulin (Khanna P, 1981). The concentration of Polypeptide-P differs per bitter gourd variety and per season (Tian, et al., 2014). As a result, a choice can also be made for growing a certain variety, see also PARAGRAPH 10.2.

The gene sequence of the polypeptide isolated from seeds of *Momordica charantia* has been found, providing opportunities to analyse which bitter gourd varieties contain a high ratio of this polypeptide (Wang, et al., 2011). This fact is interesting for the grower as well as for the breeder.

## 10.2 Effect on strategy

The choice to grow on the basis of components and not on the amount of fruits influences several aspects of cultivation, such as cultivation strategy and choice of variety.

#### 10.2.1 Strategy of Het Nieuwe Telen

In addition to a high fruit yield, it is also interesting to steer cultivation towards a high yield of components. The principles of Het Nieuwe Telen (HNT) can also be applied to bitter gourd in the greenhouse. The assimilate balance, the balance between production and consumption of assimilates, is the most important player in this. By increasing the optimization of photosynthesis and making maximum use of the available light, the production of assimilates is increased. Planting less plants and leaf pruning also helps, see PARAGRAPH 6.2. In order to increase the transfer of the sugars produced to the fruits, it is important to keep the temperature high at a high light sum (Het Nieuwe Telen, z.d.).

A good balance between light sum and temperature can contribute to a constant product quality, with few fluctuations in content of metabolites. An example of such greenhouse settings are as follows:

- Heating temperature (minimum greenhouse temperature) day/night: 20/19 °C
- Ventilation temperature day/night: 25/24 °C

The temperature rises in the greenhouse when there is a lot of radiation, when the outside temperature is high or when lamps are present. When the ventilation and heating temperatures are closer to each other, this can create a more active climate where more moisture is discharged. However, this also costs more energy.

No follow-up research has been carried out into the influence of various climate settings on the amount of components.

#### 10.2.2 Breeding selection

The bitterness of the fruit is attributed to the secondary metabolites present such as alkaloids, momordicosides and momoricins (Behera, et al., 2010; Donya, et al., 2007). The more bitter the fruit, the more it contains the healthy metabolites. Flavour is important to the consumer, but the ingredients can also be extracted. It is important to choose a variety that has a very bitter taste, so that the yield of these ingredients is high.

The concentration of polypeptide-P varies by variety of bitter gourd and by season. Different harvest seasons have a significant effect on the level of polypeptide P. Fruits picked in September and October do not contain polypeptide-P, which also affects the harvest time of the fruit. There is also a relationship between the fruit colour and the polypeptide-P content. Fruits with a dark green colour have a lower polypeptide-P content than fruits with a light green or white colour, see APPENDIX 6 (Tian, et al., 2014).

# Literature

- Apeel Sciences. (2020). What is Apeel? Retrieved September 24, 2021, from https://blog.apeelsciences.com/what-is-apeel
- Bachok, M., Yusof, B., Ismail, A., & Hamid, A. (2014). Effectiveness of traditional Malaysian vegetables (ulam) in modulating blood glucose levels. *Asia Pac J Clin*, 369-76. Retrieved from https://pubmed.ncbi.nlm.nih.gov/25164446/
- Basch, E., Gabardi, S., & Ulbricht, C. (2003, Februari 15). Bitter melon (Momordica charantia): A review of efficacy and safety. *American Journal of Health-System Pharmacy, 60*(4), 356-359.
- Begum, S., Ahmed, M., Siddiqui, B. S., Khan, A., Saify, Z. S., & Arif, M. (1997). Triterpenes, a sterol and a monocyclic alcohol from Momordica charantia. *Phytochemistry*, 44(7), 1313-1320.
- Behera, T. K., Behera, S., Bharathi, L. K., John, K. J., Simon, P. W., & Staub, J. E. (2010). Bitter Gourd: Botany, Horticulture, Breeding. *Horticulture reviews.*, *37*, 101-141.
- Chen, Q., Chan, L. L., & Li, E. T. (2003). Bitter Melon (Momordica charantia) Reduces Adiposity, Lowers Serum Insulin and Normalizes Glucose Tolerance in Rats Fed a High Fat Diet. *The Journal of Nutrition*, 1088-1093.
- Cuong, D., Kwon, S.-J., Jeon, J., Park, Y., Park, J., & Park, S. (2018). Identification and Characterization of Phenylpropanoid Biosynthetic Genes and Their Accumulation in Bitter Melon (Momordica charantia). *Molecules*, 23(2), 469. doi:https://doi.org/10.3390/molecules23020469
- Delage, B. (2015, November). *Flavonoids*. Retrieved November 11, 2021, from Linus Pauling Institute, Oregon State University: https://lpi.oregonstate.edu/mic/dietaryfactors/phytochemicals/flavonoids
- Donya, A., Hettiarachchy, N., Liyanage, R., Lay, J. J., P., C., & Jalaluddin, M. (2007). Effects of processing methods on the proximate composition and momordicosides K and L content of bitter melon vegetable. *Journal of agricultural and food chemistry*, 55(14), 5827–5833. doi:https://doi.org/10.1021/jf070428i
- East West Seed Knowledge Transfer. (2019). How to plant and grow bitter gourd.
- East West Seed ROH Limited. (2019). CropWiki EWS.
- Ejelonu, O. C., Elekofehinti, O. O., & Adanlawo, I. G. (2017, Maart). Tithonia diversifolia saponin-blood lipid interaction and its influence on immune system of normal wistar rats. *Biomedicine & Pharmacotherapy*(87), 589-595. doi:https://doi.org/10.1016/j.biopha.2017.01.017
- Elekofehinti, O. O. (2015, Juni 1). Saponins: Anti-diabetic principles from medicinal plants A review. *Pathophysiology*, *22*(2), 95-103.
- Elekofehinti, O. O., Iwaloye , O., Olawale , F., & Ariyo, E. O. (2021). Saponins in Cancer Treatment: Current Progress and Future Prospects. *Pathophysiology*, 7(2), 250-272. doi:https://doi.org/10.3390/pathophysiology2802001
- Fan, J. M., Zhang, Q., Xu, J., Zhu, S., Ke, T., Gao, D. F., & Xu, Y. B. (2009). Inhibition on Hepatitis B virus in vitro of recombinant MAP30 from bitter melon. *Molecular biology reports*, 36(2), 381-388. doi:https://doi.org/10.1007/s11033-007-9191-2

- Fuangchan, A., Sonthisombat, P., Seubnukarn, T., Chanouan, R., Chotchaisuwat, P., Sirigulsatien, V., . . . Haines, S. T. (2011). Hypoglycemic effect of bitter melon compared with metformin in newly diagnosed type 2 diabetes patients. *Journal of ethnopharmacology*, 134(2), 422-428. doi:https://doi.org/10.1016/j.jep.2010.12.045
- Grover, J., & Yadav, S. (2004). Pharmacological actions and potential uses of Momordica charantia: a review. *Journal of Ethnopharmacology*, 123-131.
- Hertog, J. d. (2020, juli 23). Frank de Koning experimenteert met sopropo. Retrieved februari 03, 2022, from Biojournaal: https://www.biojournaal.nl/article/9237810/frank-de-koningexperimenteert-met-sopropo/
- Het Nieuwe Telen. (z.d.). *Module 4: Assimilatenbalans.* Retrieved from Kas als Energiebron: https://www.kasalsenergiebron.nl/content/docs/Het\_Nieuwe\_Telen/Cursusmap/04\_Module \_Assimilatenbalans.pdf
- HGVJ. (2021, September 14). *Sopropo kweken: van zaaien tot oogsten.* . Retrieved December 16, 2021, from https://hgvj.eu/sopropo-kweken-zaaien-oogsten/
- JinYanga, S., Choib, J., Park, S., Rheec, E., Young, W., Oh, K., . . . Park, C. (2015). Preventive effects of bitter melon (Momordica charantia) against insulin resistance and diabetes are associated with the inhibition of NF-κB and JNK pathways in high-fat-fed OLETF rats. *The Journal of Nutritional Biochemistry*, 234-240.
- Kaur, M., Deep, G., Jain, A. K., Raina, K., Agarwal, C., Wempe, M. F., & Agarwal, R. (2013). Bitter melon juice activates cellular energy sensor AMP-activated protein kinase causing apoptotic death of human pancreatic carcinoma cells. *Carcinogenesis*, 34(7), 1585-1592. doi:https://doi.org/10.1093/carcin/bgt081
- Khanna P, J. S. (1981, Nov-Dec). Hypoglycemic activity of polypeptide-p from a plant source. *J Nat Prod.*, 44(6), 648-655. doi:10.1021/np50018a002
- Krawinkel, M., Ludwig, C., Swai, M., Yang, R., Chun, K., & Habicht, S. (2018). Bitter gourd reduces elevated fasting plasma glucose levels in an intervention study among prediabetics in Tanzania. *J Ethnopharmacol, 216*, 1-7.
- Kumar, R., Khurana, A., & Sharma, A. K. (2014, Augustus). Role of plant hormones and their interplay in development and ripening of fleshy fruits. *Journal of Experimental Botany*, 65(16), 4561– 4575. Retrieved from https://doi.org/10.1093/jxb/eru277
- Lattanzio, V., Bianco, V., Crivelli, G., & Miccolis, V. (1983). Variability of Amino Acids, Protein, Vicine and Convicine in Vicia faba (L) Cultivars. *Journal of Food Science, 48*, 992-993. doi:https://doi.org/10.1111/j.1365-2621.1983.tb14950.x
- Lee-Huang, S., Huang, P., Chen, H., Huang, P., Bourinbaiar, A., Huang, H., & Kung, H. (1995, Augustus 16). Anti-HIV and anti-tumor activities of recombinant MAP30 from bitter melon. *Gene*, *161*(2), 151-6. doi:10.1016/0378-1119(95)00186-a
- Liu, J.-Q., Chen, J.-C., Wang, C.-F., & Qiu, M.-H. (2009). New Cucurbitane Triterpenoids and Steroidal Glycoside from Momordica charantia. *Molecules*, *14*, 4804-4813. doi:doi:10.3390/molecules14124804

- Liu, J.-Q., Chen, J.-C., Wang, C.-F., & Qiu, M.-H. (2010). One new cucurbitane triterpenoid from the fruits of Momordica charantia. *European Journal Chemistry*, 1(4), 294-296. doi:doi:10.5155/eurjchem.1.4.294-296.131
- Liva Harinantenaina, M. T. (2006). Momordica charantia Constituents and Antidiabetic Screening of the Isolated Major Compounds. *Chem. Pharm. Bull,* 54(7), 1017-1021.
- Lo, H.-Y., Ho, T.-Y., Lin, C., Li, C.-C., & Hsiang, C.-Y. (2013). Momordica charantia and Its Novel Polypeptide Regulate Glucose Homeostasis in Mice via Binding to Insulin Receptor. *Journal of Agricultural and Food Chemistry, 61*(10), 2461-2468.
- Lucas, E. A., Dumancas, G. G., Smith, B. J., Clarke, S. L., & Arjmandi, B. H. (2010). Health Benefits of Bitter Melon (Momordica charantia). In R. R. Watson, & V. Preedy, *Bioactive Foods in Promoting Health* (pp. 525-549). Academic Press. doi:https://doi.org/10.1016/B978-0-12-374628-3.00035-9
- Mahwish, Saeed, F., Sultan, M. T., Riaz, A., Ahmed, S., Bigiu, N., . . . Manea, R. (2021). Bitter Melon (Momordica charantia L.) Fruit Bioactives Charantin and Vicine Potential for Diabetes Prophylaxis and Treatment. *Plants, 10*(4), 730. doi:https://doi.org/10.3390/plants10040730
- Meewisse, S. (2021). Company Visit Bleiswijk. (R. Severens, Interviewer)
- Meewisse, S. (2021, November). Contact per mail and Feedback.
- Moses, T., Papadopoulou, K. K., & Osbourn, A. (2014). Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. *Critical reviews in biochemistry and molecular biology*, *49*(6), 439–462. doi:https://doi.org/10.310
- MSKCC. (2021, November 4). *Bitter Melon*. Retrieved November 15, 2021, from Memorial Sloan Ketting Cancer Center: https://www.mskcc.org/cancer-care/integrativemedicine/herbs/bitter-melon
- Parkash, A., Ng, T., & Tso, W. (2002). Purification and characterization of charantin, a napin-like ribosome-inactivating peptide from bitter gourd (Momordica charantia) seeds. *The Journal of Peptide Research, 59*, 197–202.
- Perez, J. L., Jayaprakasha, G. K., Crosby, K., & Patil, B. S. (2018). Evaluation of bitter melon (Momordica charantia) cultivars grown in Texas and levels of various phytonutrients. *Journal* of the Science of Food and Agriculture, 99(1), 379-390. doi:https://doi.org/10.1002/jsfa.9199
- Peter, E. L., Kasali, F. M., Deyno, S., Mtewa, A., Nagendrappa, P. B., Tolo, C. U., . . . Sesaazi, D. (2019). Momordica charantia L. lowers elevated glycaemia in type 2 diabetes mellitus patients: Systematic review and meta-analysis. *Journal of ethnopharmacology, 231*, 311-324. doi:https://doi.org/10.1016/j.jep.2018.10.033
- Plagron. (z.d.). *Het belang van gezonde wortels*. Retrieved November 18, 2021, from https://www.plagron.com/nl/grow-topics/het-belang-van-gezonde-wortels
- Raina, K., Kumar, D., & Agarwal, R. (2016, Oktober). Promise of bitter melon (Momordica charantia) bioactives in cancer prevention and therapy. *Seminars in Cancer Biology*, 40-41, 116-129.
   Retrieved from https://www.sciencedirect.com/science/article/pii/S1044579X16300281

- Ray, R., Raychoudhuri, A., Steele, R., & Nerurkar, P. (2010). Bitter melon (Momordica charantia) extract inhibits breast cancer cell proliferation by modulating cell cycle regulatory genes and promotes apoptosis. *Cancer Res, 70*(5), 1925-31. doi:10.1158/0008-54
- Remijn, N., Veenkamp, A., & Ruijssenaars (o.l.v.), A. (2022). *Onderzoeksverslag Bitter Gourd*. Dronten: Aeres Hogeschool.
- Rinki's Home Garden. (z.d.). How to Hand Pollinate Bitter Gourd / Karela Flowers. Youtube-video.
- Saeed, F., Afzaal, M., Niaz, B., Arshad, M. U., Tufail, T., Hussain, M. B., & Javed, A. (2018). Bitter melon (Momordica charantia): a natural healthy vegetable. *International journal of food properties, 21*(1), 1270-1290. doi:https://doi.org/10.1080/10942912.2018.1446023
- Schilperoord, R. (2021, Oktober 28). Company visit Mijnsheerenland. (D. Laanen, Interviewer)
- Severens, R. (2021). Onderzoek Bitter Groud Fotosynthese. Dronten.
- Tan, S., Parks, S., Stathopoulos, C., & Roach, P. (2014). Extraction of Flavonoids from Bitter Melon. *Food and Nutrition Sciences, 5*, 458-465. doi:10.4236/fns.2014.55054.
- Tian, M., Zeng, X. Q., Song, H. L., Hu, S. X., Wang, F. J., Zhao, J., & Hu, Z. B. (2014). Molecular diversity and hypoglycemic polypeptide-P content of Momordica charantia in different accessions and different seasons. *Journal of the Science of Food and Agriculture, 95*(6), 1328-1335.
- Toshihiro Akihisa, N. H. (2007). Cucurbitane-Type Triterpenoids from the Fruits of Momordica charantia and Their Cancer Chemopreventive Effects. *Journal of Natural Products, 70*, 1233-1239.
- van der Lugt, G. (2017, December). Nutrient Solutions for Greenhouse Crops. Yara. Retrieved from https://www.yara.nl/siteassets/toolbox/nutrient-solutions/nutrient-solutions-forgreenhouse-crops-dec2017.pdf/
- Wang, B.-L., Zhang, W.-j., Zhao, J., Wang, F.-J., Fan, L.-Q., Wu, Y.-x., & Hu, Z.-b. (2011). Gene cloning and expression of a novel hypoglycaemic peptide from Momordica charantia. *Journal of the Science of Food and Agriculture*, 91(13), 2443-2448. doi:https://doi.org/10.1002/jsfa.4485
- Wang, H., Kan, W., Cheng, T., Yu, S., Chang, L., & Chuu, J. (2014). Differential anti-diabetic effects and mechanism of action of charantin-rich extract of Taiwanese Momordica charantia between type 1 and type 2 diabetic mice. *Food Chem Toxicol., 69*, 347-56.
- Xiong, S., Yu, K., Liu, X., Yin, L., Kirschenbaum, A., Yao, S., . . . Levine, A. (2009). Ribosome-inactivating proteins isolated from dietary bitter melon induce apoptosis and inhibit histone deacetylase-1 selectively in premalignant and malignant prostate cancer cells. *Int J Cancer, 125*(4), 774-82. doi:10.1002/ijc.24325
- Zwet, R. v. (2021). Company Visit Almere. (I. v. Deijl, S. Dorst, A. v. Hoeflaken, T. M. Meijer, & D. Arendse, Interviewers)

# Appendix

	Pu	mice			Ston	e wool		0	rganic (	potting so	oil)
date	weight total	nr fruits	Avg. weight/ fruit	date	weight total	nr fruits	Avg. weight/ fruit	date	weight total	nr fruits	Avg. weight/ fruit
7-oct	123	1	123,0	7-oct	108	1	108,0	7-oct	269	1	269,0
14-oct	0	0	0,0	14-oct	482	2	241,0	14-oct	0	0	0,0
28-oct	1633	8	204,1	28-oct	768	5	153,6	28-oct	1334	7	190,6
4-nov	521	3	173,7	4-nov	769	5	153,8	4-nov	664	4	166,0
16-nov	706	4	176,5	16-nov	186	2	93,0	16-nov	230	1	230,0
24-nov	0	0	0,0	24-nov	148	2	74,0	24-nov	0	0	0,0
7-dec	0	0	0,0	7-dec	108	1	108,0	7-dec	128	1	128,0
15-dec	167	1	167,0	15-dec	387	3	129,0	15-dec	252	3	84,0
Total:	3150	17	185,3	Total:	2956	21	140,8	Total:	2877	17	114,1

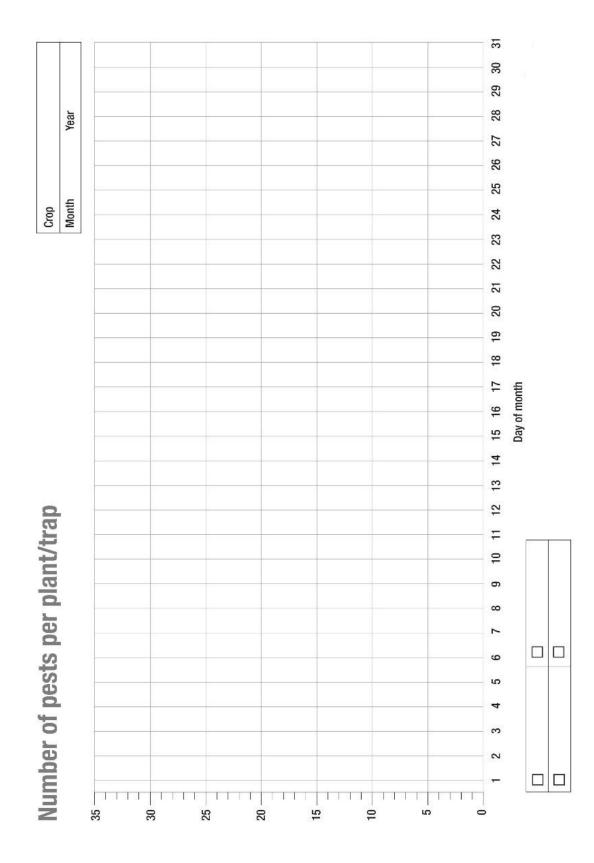
# Appendix 1: Research results substrate

# Appendix 2: Fertilizer schedule bell pepper

	Per 1000 L	Per 50 L
BAK A		
Calcium nitrate solid	59,4 KG	2,97 KG = 2970 G
Ammonium nitrate	5,6 L	0,28 L = 280 mL
Potassium nitrate	8,7 KG	0,44 KG = 440 G
Iron DTPA 6%	1400 G	70 G
Or Iron DTPA 3%	2800 G	140 G
ВАК В		
Potassium nitrate	46,9 KG	2,36 KG = 2360 G
Monopotassium phosphate	13,6 KG	0,68 KG = 680 G
Magnesium sulphate	24,6 KG	1,23 KG = 1230 G
Manganese(II)sulphate	135 G	6,75 G
Zinc sulphate 23%	115 G	5,75 G
Borax	240 G	12 G
Copper sulphate	19 G	0,95 G
Sodiummolybdate	12 G	0,6 G

Combined total of moles in recipe								
NO3	NH4	Р	К	S	Ca	Mg	Cl	H+
1200,138	99,654	99,960	650,400	99,876	275,022	99,876	0,000	0,000

Tank volume								1000	litres			
Concentration 100 x							х					
Total I	itres f	or nut	rients	olutio	n:						100000	
Concentrations crop (mmol/litre)												
NO3	NH4	Ρ	К	S	Са	Mg	Cl	H+	Equivale	nts	EC on basis of e	equivalents
12,00	1,00	1,00	6,50	1,00	2,75	1,00	0,00	0,00	29,9970	0064		1,49985032



# Appendix 3: Pests scouting record table (greenhouse)

## Appendix 4: Overview biological control

Bitter Gourd Pests					
CON .		2 3	3	4	
Red pumpkin beetle					
Raphidopalpa foveicollis	-	C			0 10
	5	6	7	8	
Serpentine leafminer					
Liriomyza trifolii	11	12			Fnemies
Melon fly Bactrocera cucurbitae					Biological
	3	13	2 3		
Mexican bean beetle					
Epilachna.sp			8		
Melon Aphida	14	15		9	16
Aphis gossypii Cucumber moth Diaphania inaica	17				

- 1. Pennsylvania leather wing beetle (*Chauliognathus pensylvanicus*)
- 2. Entomopathogenic nematodes
- 3. Celatoria cetosa
- 4. Braconid wasp
- 5. Chrysocharis Pentheus
- 6. Diglyphus isaea
- 7. Gronotoma micromopha
- 8. Lacewing
- 9. Lady beetle
- 10. Spider
- 11. Opius fletcheri
- 12. Ants
- 13. Braconid wasp (tachinid fly)
- 14. Aphidius colemani
- 15. Aphelinus spp.
- 16. Syrphid larva
- 17. Entomopathogenic fungi

# Appendix 5: IPM according to crop-stage bitter gourd in open soil cultivation

Stage	Management	Activity
Pre-sowing	Nutrients	<b>Apply farm yard manure (FYM):</b> 8-10 t/acre (20-25 t/hectare) and incorporate in the soil 2 to 3 weeks before sowing.
	Weeds	Field is kept weed free before sowing by ploughing.
	Soil borne fungus,	Cultural control:
	nematodes, resting	• Deep ploughing of fields during summer.
	stages of insects and weeds	• Soil solarization: Cover the beds with polythene sheet of (0.45 mm) thickness for three weeks before sowing for soil solarization which will help in reducing the soil borne pests.
		Biological control:
		<ul> <li>Apply neem cake at 100 kg/acre (250 kg per hectare) in soil at the time of last ploughing helps reducing nematodes, and soil dwelling pests.</li> <li>Apply <i>Trichoderma spp.</i> at 2.5 kg/acre (6 kg/hectare) along with FYM</li> </ul>
Seedling stage	Red pumpkin beetle	Cultural control:
		<ul> <li>Deep summer ploughing exposes the grubs and pupae.</li> </ul>
		<ul> <li>Biological control:</li> <li>Conserve predators such as Pennsylvania leather wing beetle (<i>Chauliognathus pensylvanicus</i>); larvae of which feed on pumpkin beetle larva.</li> <li>Conserve parasitoids such as: <i>Celatoria setosa</i> (grub)</li> </ul>
Applying <i>Trichodel</i> treatment and soil	· · · · · ·	atment and <i>Pseudomonas fluorescens</i> as seed, nursery
Vegetative stage	Nutrients	<ul> <li>Generally, cucurbit crops require 40: 32: 24 kg N: P: K/acre (100:80:60 kg N: P: K per hectare)</li> <li>Apply N in two splits first one (50%) at 25 days after sowing.</li> <li>Apply entire P and K at the time of sowing.</li> <li>Micro nutrient deficiency should be corrected by foliar spray of particular nutrient.</li> </ul>
	Weeds	• Regular hoeing and weeding should be done to keep the field weed free up to 30 days crop stage.

Fusarium wilt	<ul> <li>Cultural control:</li> <li>Use pathogen free seeds</li> <li>Remove and destroy the infected plants and plant debris</li> <li>Adopt crop rotation</li> <li>Avoid water stagnation and maintain proper drainage</li> <li>Use resistant varieties</li> </ul>
Serpentine leaf miner ( <i>Liriomyza brassicae</i> )	<ul> <li>Cultural control:</li> <li>Change in dates of sowing</li> <li>Growing castor, tomato or marigold as a trap crop.</li> <li>Use yellow sticky traps or cards at 10/acre (25 per hectare)</li> <li>Biological control:</li> <li>Conserve parasitoids such as <i>Tetrastichus ovularum</i> (egg), <i>Gronotoma micromorpha</i> (larval and pupal), <i>Diglyphus</i> sp (larval), <i>Opius phaseoli</i> (pupal), <i>Chrysocharis</i> sp, <i>Neochrysocharis formosa</i> (larval) etc.</li> <li>Conserve predators such as lacewings, lady beetles, spiders, fire ants etc.</li> </ul>
Aphids	<ul> <li>Cultural control: A seed mixture including self-sowing annual and perennial herbaceous flower species are viable options to grow within cucurbits or as field boundary crops to attract and increase beneficial insects and spiders for the control of sap-sucking insect pests.</li> <li>Biological control: <ul> <li>Conserve parasitoids such as Aphidius colemani, Diaeretiella spp., Aphelinus spp.</li> <li>Release larvae of green lacewing bug (Chrysoperla carnea) in first stage (25.000 per hectare)</li> <li>Conserve predators such as anthocorid bugs/pirate bugs (Orius spp.), mirid bugs, syrphid/hover flies, green lacewings (Mallada basalis and Chrysoperla carnea), predatory coccinellids (Stethorus punctillum, Coccinella septumpunctata, Menochilus sexmaculata, Hippodamia convergens), staphylinid beetle (Oligota spp.), predatory cecidomyiid fly and predatory gall midge (Aphidoletis aphidimyza, Feltiella minuta), earwigs, ground beetles, rove beetles, spiders, wasps etc.</li> </ul> </li> </ul>
Cercospora leaf spot	<ul> <li>Cultural control:</li> <li>Field sanitation</li> <li>Maintain good soil drainage and good aeration between vines.</li> </ul>

	Cucumber mosaic virus	<ul> <li>Cultural control:</li> <li>Raise 4 rows of barrier crops such as main or sorghum</li> <li>Avoid planting tomatoes next to cucurbits, spinach, or other vegetables and flowers susceptible to these diseases.</li> <li>Control of aphids (<i>A. gossypii</i>) will help reduce the likelihood of cucumber mosaic.</li> </ul>
	rowdery mildew	<ul> <li>Plant resistant varieties</li> <li>Increasing air movement inside the canopy</li> </ul>
	Downy mildew	<ul> <li>Cultural control:</li> <li>Trellising cucumbers</li> <li>Avoiding overhead irrigation or irrigating only in the late morning hours will limit the amount of time that leaves are wet.</li> <li>Control alternate weed hosts (wild cucumber, golden creeper and volunteer cucumbers) in neighbouring fence rows and field edges</li> </ul>
Generative stage	Nutrients	<ul> <li>Apply the second dose (50%) at 45 days after sowing</li> <li>Micronutrient deficiency should be corrected by foliar spray of particular micronutrient.</li> </ul>
	Weeds	• Left over weeds should be removed from the field to avoid further spread of weed seeds.
	Fruit fly	<ul> <li>Cultural control:</li> <li>Early maturing varieties are less affected than later ones.</li> <li>Changing of sowing dates.</li> <li>Collection and destruction of infested fruits</li> <li>Slight raking of soil during fruiting time and after the harvest to expose pupae from the soil.</li> <li>Use methyl eugenol (0.1%) based trap</li> <li>Biological control:</li> <li>Conserve parasitoids such as Opius fletcheri (pupal)</li> <li>Spray NSKE 5%</li> </ul>

# Appendix 6: Content polypeptide-p per accession

\*N represents no polypeptide-P. Values are given based on folds of EASTWEST using image J software (Tian, et al., 2014).

Accessions	Fruit colour	Harvesting time	Content*
DMW	White	2012-06-27	3.773
		2012-09-28	N
ZHGGF	Light green	2012-06-27	3.803
		2012-09-28	Ν
EASTWEST	Dark green	2012-06-27	1
TGCB	Light green	2012-07-10	8.959
		2012-10-09	N
GDQP	Green	2012-07-10	3.752
DAIDIA	Dark green	2012-07-10	1.159
		2012-09-28	Ν
НҮХС	Light green	2012-07-10	3.803
PN	White	2012-07-10	4.800
FLYH	Glossy green	2012-07-10	4.492
LSHCHB	Greenish white	2012-07-10	3.485
JGLSHDB	White	2012-07-10	3.863
		2012-10-09	Ν
YSHLB	Light green	2012-07-10	3.861
QP	Glossy green	2012-07-10	1.431
		2012-09-28	Ν
CHBWF1	White	2012-07-20	3.625
		2012-10-09	Ν
LM	Green	2012-07-20	1.249
CHL	Glossy green	2012-07-20	1.552
HLYL	Glossy green	2012-07-20	1.715
DDCL	Glossy green	2012-07-20	3.885
H&V	Green	2012-07-20	3.194
		2012-09-28	Ν