Effects of Micosat F granulate in *Ocimum basilicum (Basil)* and *Petroselinum crispum (Parsley)*

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Abstract

Micosat recently added a new product to the Dutch market: Micosat F, a mycorrhiza granulate for different kinds of crops. Micosat F has already been tested on some common crops like strawberries and it seems to have interesting positive benefits. Now Micosat would like to test the product on some more crops. Students of HAS University of applied sciences decided to take part in this by doing a research on the benefits of Micosat F granulate in the crops Ocimum basilicum (Basil) and Petroselinum crispum (Parsley) which are fast growing herbs. The experiment was conducted in the HAS climate chamber (Fytotron) supplemented with equal LED light- and climate settings. Two types of herbs (Ocimum basilicum (Basil) and Petroselinum crispum (Parsley) were cultivated. The research included 72 repetitions for each of the herbs, half of these were grown in treated substrates with Micosat F and another half on non-treated substrate. The crops were spaced after 19 days and harvested 26 days after sowing. The plant height, fresh weight and dry weight were measured. The results reveal that Micosat F granules in the treated substrate does have significant effect on the height of both herbs. Both herbs gives higher plants when growing in the substrate mixed with Micosat F. However, Micosat F does not have effect on fresh and dry weight of both herbs.

This study been realized with thanks to:

1. Introduction

About 95% of the world's plant species belong to families that are mycorrhizal, which means that mycorrhiza fungi have a fundamental importance on these plants (Trappe, 1987). One of these fundamental importance's is the well-known beneficial nutrition effect on the symbiosis which makes the plant roots able to absorb nutrients better (Figure 1) (Smith & Gianinazzi-Pearson, 1988). Mycorrhiza has also a positive influence on water absorption (Davies, Potter, & Linderman, 1992; Nelsen & Safir, 1982). AM fungi can reduce the level of root diseases (Bagyaraj, 1984; Caron, 1989; H.W., 1982), the roots will get a better environment because the development of soil aggregation (Sutton & Sheppard, 2011; Tisdall, 1994). Many studies have shown that, compared with non-mycorrhizal controls, there are positive significant differences between development of the root systems (Begon, 1988; Sanders, Tinker, Black, & Palmerley, 1977). It was found that root lengths, if they are classified by diameter class size, were longer because of the colonization by mycorrhizal fungi (Miller & Jastrow, 1990). Recently the company Micosat added a new product to the Dutch market named Micosat F which is developed by CCS Aosta in Italy (Micosat, 2014). Micosat F is granulate which contains the mycorrhizal fungi *Gloumus intraradices* (GB-67), *Gloumus mosseae* (GP-11) and *Gloumus viscosum* (GC-41). Except mycorrhizal fungi, Micosat F also contains other beneficial micro-organisms; *Agrobacterium radiobacter* (AR-39), *Bacillus subtilis* (BA-41), *Streptomyces spp.* (SB-14), *Streptomyces spp.* (ST-60), *Trichoderma harzianum* (TH-01), *Pichia pastoris* (PP-59) and *Pochonia chlamydosporia* (PC-50) (Micosat, 2014; van Klaren, 2014). The purpose of our study was to test the effect of Micosat F granulate in *Ocimum basilicum* (Basil) and *Petroselinum crispum* (Pasley) within a climate chamber. *Ocimum basilicum* and *Petroselinum* are fast growing herbs which can be harvested after 28 days when grown in a climate chamber under optimal circumstances (den Besten, 2014), while the common cultivation in glasshouses can be harvested after 36 days (Gipmans, 2014). Micosat claims to see visual differences in root system after 21 days (van Klaren, 2014). Based on literature and product information, the expectations are that Micosat F will develop a better root system than non-Micosat F controls, because of mycorrhizal fungi *Gloumus intraradices* (GB-67), *Gloumus mosseae* (GP-11) and *Gloumus viscosum* (GC-41). Another expectation is a higher plant height because of beneficial nutrition effect of the symbiosis and the positive effect of water absorption, which can mean that *Ocimum basilicum* and *Petroselinum* are able to absorb nutrients better because of the mycorrhizal fungi.

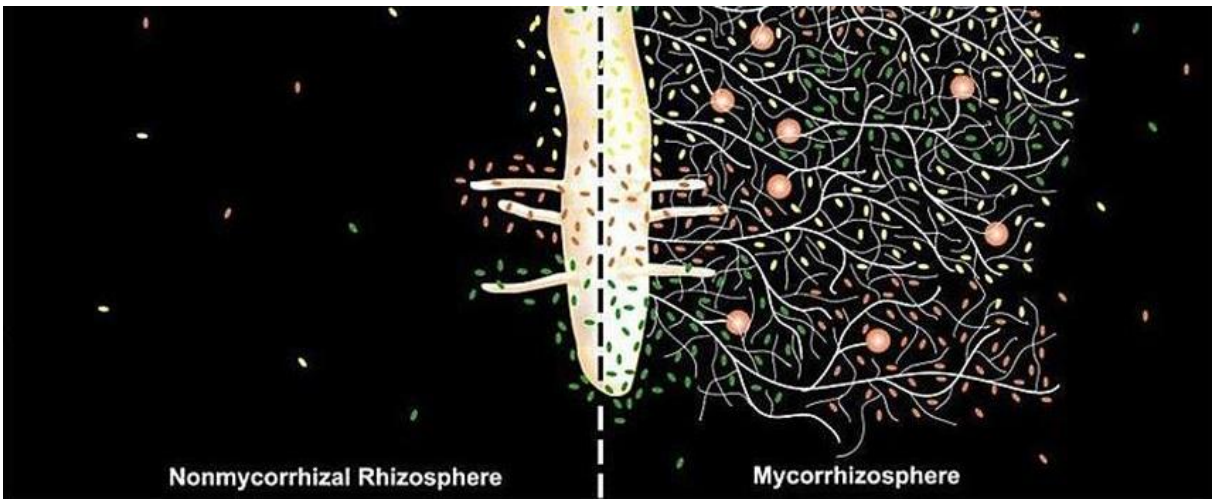

Figure 1: Difference between non-mycorrhizal rhizosphere and mycorhizosphere (Smith & Gianinazzi-Pearson, 1988)

2. Materials and methods

The research on the effects of the Micosat F granulates in *Ocimum basilicum* and *Petroselinum crispum* has been performed in a climate (HAS Fytotron nr. 8 and later moved to nr. 2) for in total 26 days under controlled conditions with LED-light. The experiment consisted two treatments (with and without Micosat F granulates mixed in the substrates) and two types of herbs (*O. basilicum* and *P. crispum*) were used. The number of repetitions were 72 pots for each variety (*O. basilicum* and *P. crispum*). Ach variety 36 pots were filled with substrate treated with the Micosat F granulate and another 36 pots were filled with substrate without this granulate. Twelve pots of each treatment were placed in one tray to make sure the treatments would not influence each other by the drainage or the watering system. Each tablet in the climate chamber contained 3 trays (36 pots per tablet).

The experiment was set up in 2014 on November 19th. The plants were sown in Ø 13 cm. pots containing each about 0,8 liter of substrate. For the treatment with the Micosat F granulate, 0,7 grams dose per pot was applied. The regular seed density of *Ocimum basilicum* and *Petroselinum crispum* differs. The *Ocimum basilicum* usually contains 40 seeds per pot, while *Petroselinum crispum* contains 50 seeds per pot (Gipmans, 2014). The used seeds in this experiment came from Gipmans Planten (a modern, family-owned plant nursery in Venlo who specializes in vegetables cultivation). Additionally, the company grows herbal plants for the international retail sector. A peat moss substrate (Euroveen) from BVB was used. The Micosat F granulate was supported by the company Micosat.

During the germination stage of the herbs a high humidity was needed. Light and CO_2 have less influence because of the absence of leaves. The climate in this stage was set on a humidity level of 85%, a temperature of 20 degrees Celsius and a CO_2 concentration of 600 ppm. The LED-light was set for 20 hours per day on about a maximum of 238 µmol/cm²/s light in a regular division between red (maximum of 112 µmol/cm²/s red, 59 µmol/cm²/s blue and 67 µmol/cm²/s far red) (den Besten, 2014). Measurements with the HortiSpec+ (HortiSpec K100723) and a usual PAR-meter (Li-Cor LI-250A) has shown no big differences of light level between treatments. The second of December (13 days after start of the research) most of the herbs were germinated. Therefore the climate settings were changed. The temperature was set on 21°C, combined with a humidity of 80% and 800 ppm as set point for CO_2. Also 10% more far red was added.

All pots both with and without Micosat F of each herb were placed on the same level tablet in order to minimize the difference in root temperature. The LED lights were installed underneath the tablet which influence the temperature on the tablet above. The upper tablets have a higher temperature than the lowest level tablets. During cultivation, watering was manually given by the school garden management equally on all plants. Sticky pads were used inside the climate chamber for pest control. The potted herbs were spaced out on day 19 after sowing. Harvesting was done on 26 days after sowing. Only 10 pots located in the middle of each tablet were harvested, which are a total of 10 repetitions each treatment per variety. All border pots were excluded in order to minimize the edge influences. The trial set up scheme and harvested pots can be seen in Figure 2 (Trial set up scheme and harvesting).

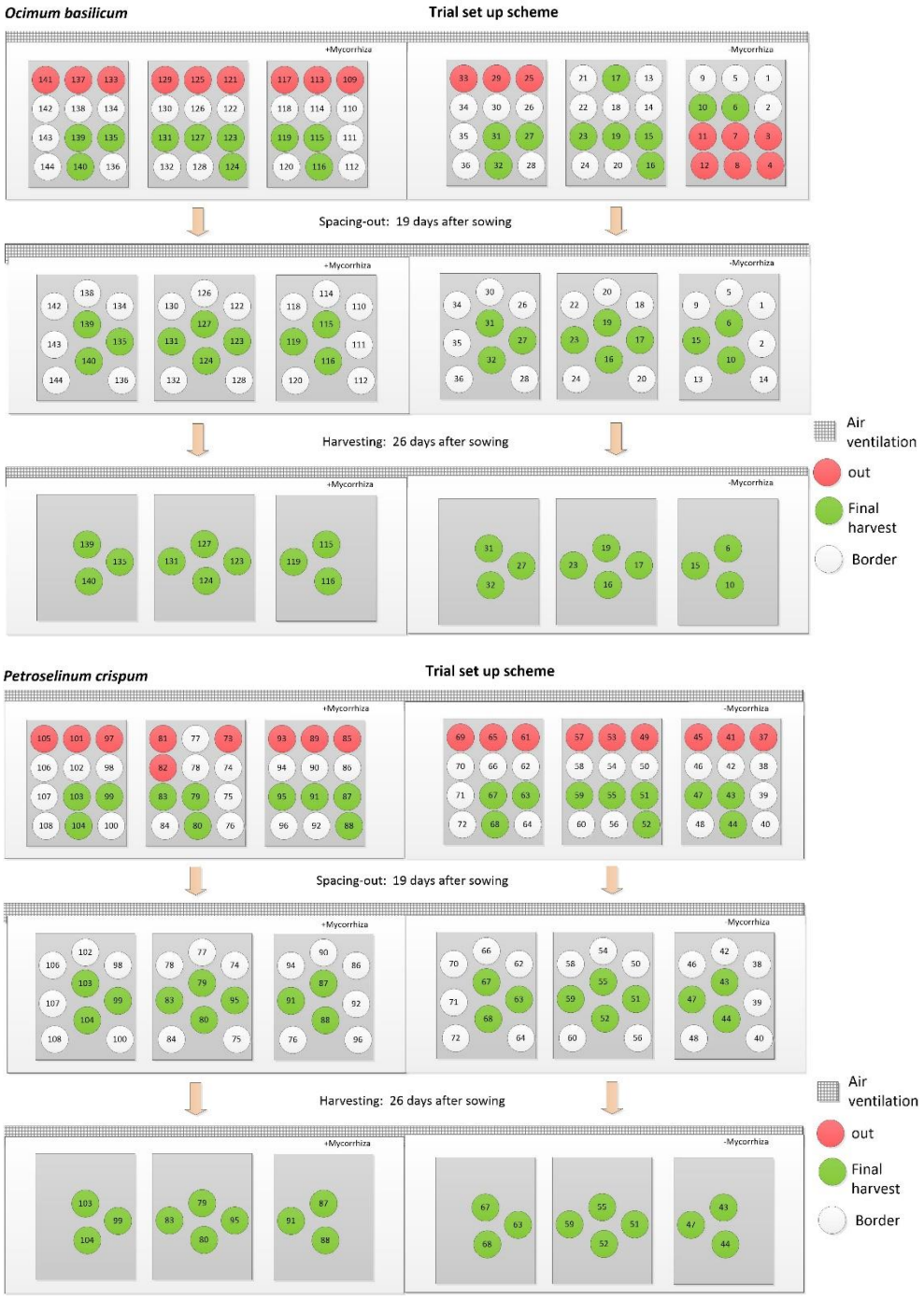

Figure 2: Trial set up scheme and harvesting

The plant height and fresh weight were measured per pot on the day of harvesting. The herbs in each pot were cut just above the substrate level in order to measure the fresh weight. After that fresh herbs from each pot were placed into the paper bag and dried in the oven at 80 degrees Celsius with 100% ventilation for 48 hours. Immediately after the bags of dried herbs were took out from the oven, the dry weight of each samples were measured.

The data were analysed with the statistical software Genstat (16th edition) using the Analysis of Variance method with a least significance level of 5%.

3. Results

This chapter presents the results of plant height, fresh weight and dry weight from two herbs *O. basilicum* and *P. crispum* cultivating in treated and non-treated substrates with the Micosat F granulate.

3.1 Plant height

The result shows that the mean height of *O. basilicum* is 22.27 cm. where *P. crispum* has a mean height of 21.48 cm. Each herb variety grown in substrate treated with the Micosat F granulate, gives higher plants than those who grow in substrates without Micosat F. *O. basilicum* gives 22.90 cm. height when growing in substrate with Micosat F and 21.65 cm. without Micosat F. Similarly, *P. crispum* gives 22.05 cm. height with Micosat F and 20.90 cm. height without Micosat F (Figure 3).

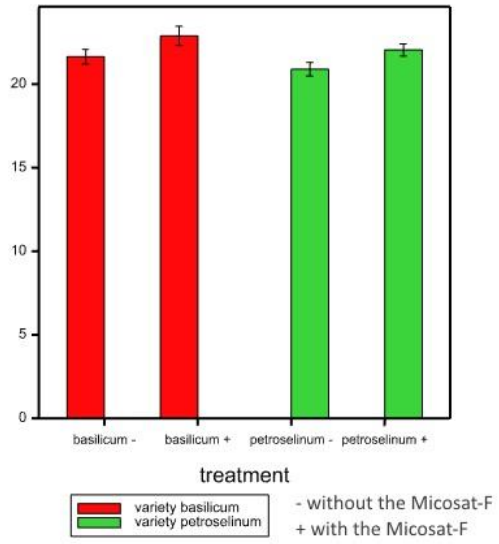

Figure 3: Mean height in cm. of *O. basilicum* and *P. crispum* between two treatments

Figure 4 and 5 shows potted herbs from both varieties on day 26 after sowing. In general *O. basilicum* plants look higher than *P. crispum* plants. Comparing both herbs, *O. basilicum* grown in substrate with Micosat F look taller and the ones without Micosat F look more compact in shape as whole pot.

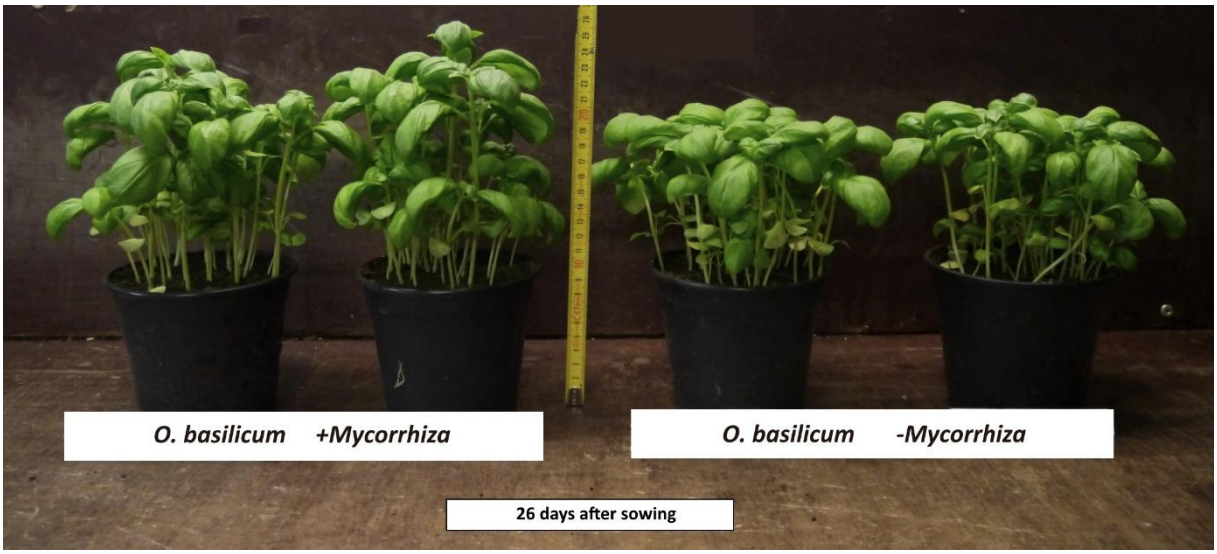

Figure 4: *O. basilicum* cultivates in substrate treated with and without with the Micosat F granulate on 26 days after sowing

Inversely of *O. basilicum*, *P. crispum* plants do not look much different between growing in substrate with and without Micosat F (Figure 4).

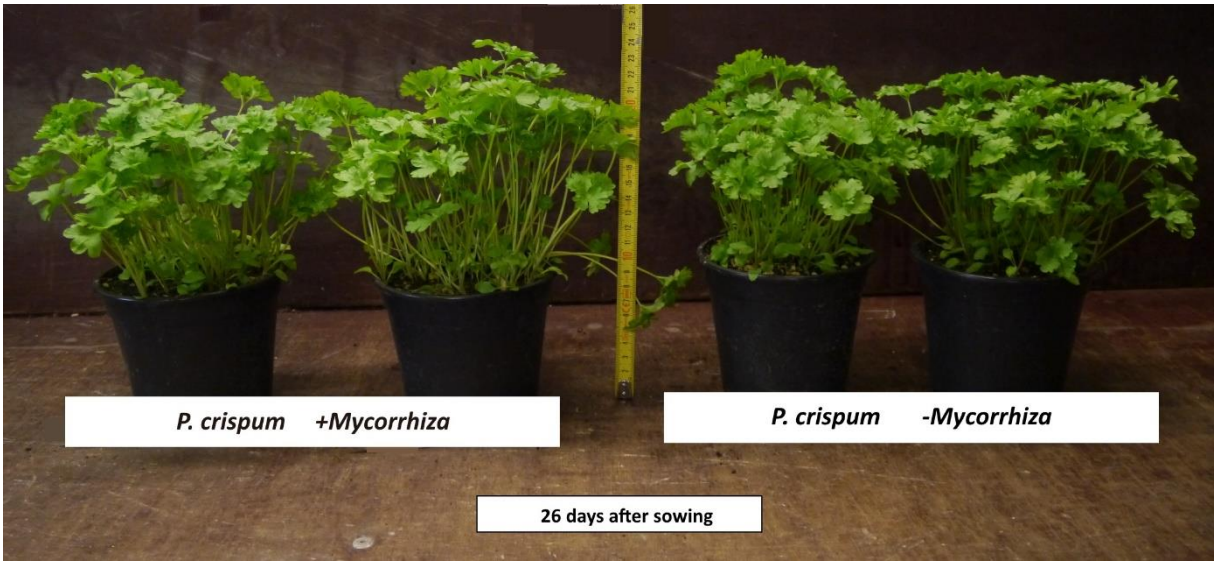

Figure 5: *P. crispum* cultivates in substrate treated with and without with the Micosat F granulate on 26 days after sowing

Table 1 the analysis of variance, height shows a significant difference (F-probability 0.012) regarding to herbs height between herbs that are grown in treated substrate with Micosat F and substrate without Micosat F.

Table 1: Analysis of variance, height (cm)

Variate: height (cm)					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Micosat F	1	14.400	14.400	7.05	0.012
Variety	1	6.400	6.400	3.13	0.085
Micosat F.variety	1	0.025	0.025	0.01	0.913
Residual	36	73.550	2.043		
Total	39	94.375			

The table of means of *O. basilicum* and *P. crispum* can be seen in Table 2 (LSD 0.917).

Table 2: Tables of means, height (cm) (LSD 0.917)

Variate: height (cm)		
Variety	***Ocimum basilicum***	***Petroselinum crispum***
Micosat F		
Without	21.65	20.90
With	22.90	22.05

3.2 Fresh weight

Regarding fresh weight, *O. basilicum* plants gives a mean of 26.03 g. while *P. crispum* gives only 15.82 g. mean per pot. *O. basilicum* plants with treated and non-treated substrates has almost the same fresh weight (26.04 g.) in treated substrate and 26.04 g. in non-treated substrate. *P. crispum* gives a fresh weight of 16.55 g. in substrate with Micosat F and 15.09 g. in substrate without it (Figure 6).

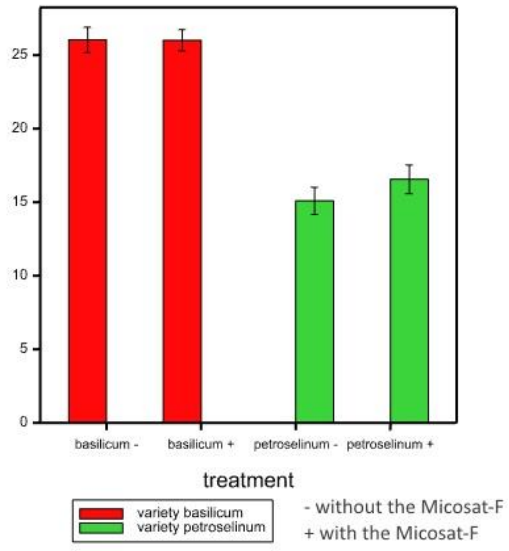

Figure 6: Mean fresh weight (g) of *O. basilicum* and *P. crispum* growing in substrates treated with and not-treated with the Micosat F granulate

Micosat F does not have a significant effect on the herb's fresh weight (F-probability 0.414) (Table 3).

Table 3: Analysis of variance, fresh weight (g)

Variate: fresh weight (g)					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Micosat F	1	5.198	5.198	0.68	0.415
Variety	1	1041.216	1041.216	136.10	<.001
Micosat F.variety	1	5.506	5.506	0.72	0.402
Residual	36	275.414	7.650		
Total	39	1327.334			

3.3 Dry weight

Mean dry weight (g) *O. basilicum* and *P. crispum* growing in substrates treated with and not-treated with the Micosat F granulate) shows the dry weight results of both herbs (Figure 7). *O. basilicum* has a mean dry weight of 2.23 g. per pot and *P. crispum* has a mean dry weight of 1.68 g. per pot. In substrate with Micosat F, *O. basilicum* has a dry weight of 2.21 g. per pot and 2.26 g. in a non-treated substrate. *P. crispum* gives a mean of 1.66 g. dry weight per pot in substrate with Micosat F and 1.70 g. in a non-treated substrate.

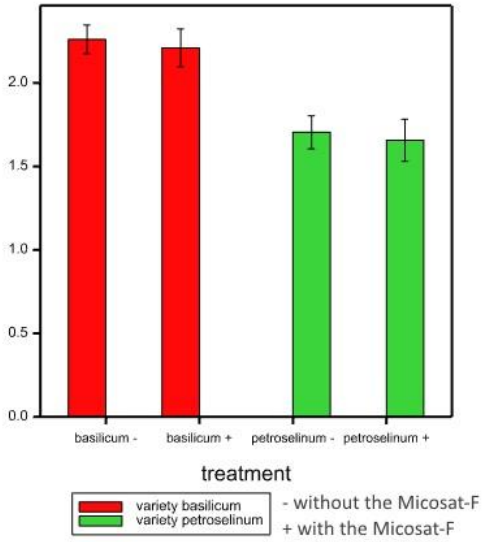

Figure 7: Mean dry weight (g) *O. basilicum* and *P. crispum* growing in substrates treated with and not-treated with the Micosat F granulate

The same as in fresh weight, species does effect the dry matter of herbs, but Micosat F does not have effect on dry weight (F-probability 0.647) (Table 4).

Table 4: Analysis of variance, dry weight (g)

Variate: dry weight (g)					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Micosat F	1	0.0245	0.0245	0.21	0.647
Variety	1	3.0747	3.0747	26.81	<.001
Micosat F.variety	1	0.0000	0.0000	0.00	0.989
Residual	36	4.1290	0.1147		
Total	39	7.2282			

4. Discussion

Micosat F does have effect on the plant height of *O. basilicum* and *P. crispum*. Herbs that were grown in the substrate treated with Micosat F granulates have a longer mean height than those without Micosat F, especially in *O. basilicum*. Basilicum plants have a more compact shape when growing without Micosat F treatment. However, in *P. crispum* both substrate treatments (with and without) do not give big different in plant shaped or size as whole pot visually. Micosat F does have a significant difference effect on herb height, but not on plant biomass. When the trial was moved from Fytotron nr. 8 to nr. 2 during cultivation, a little change in climate settings may affect herbs growth and developments.

Micosat F granulate was expected to have positive effect on herbs cultivation, especially better root system and expected visual differences in root system. However, the visual differences of herbs roots between growing in substrate with and without Micosat F cannot be obviously seen with bear eyes at the harvesting (Figure 8), the herb roots each variety between growing in substrate with and without Micosat F at the harvesting, 26 days after sowing.

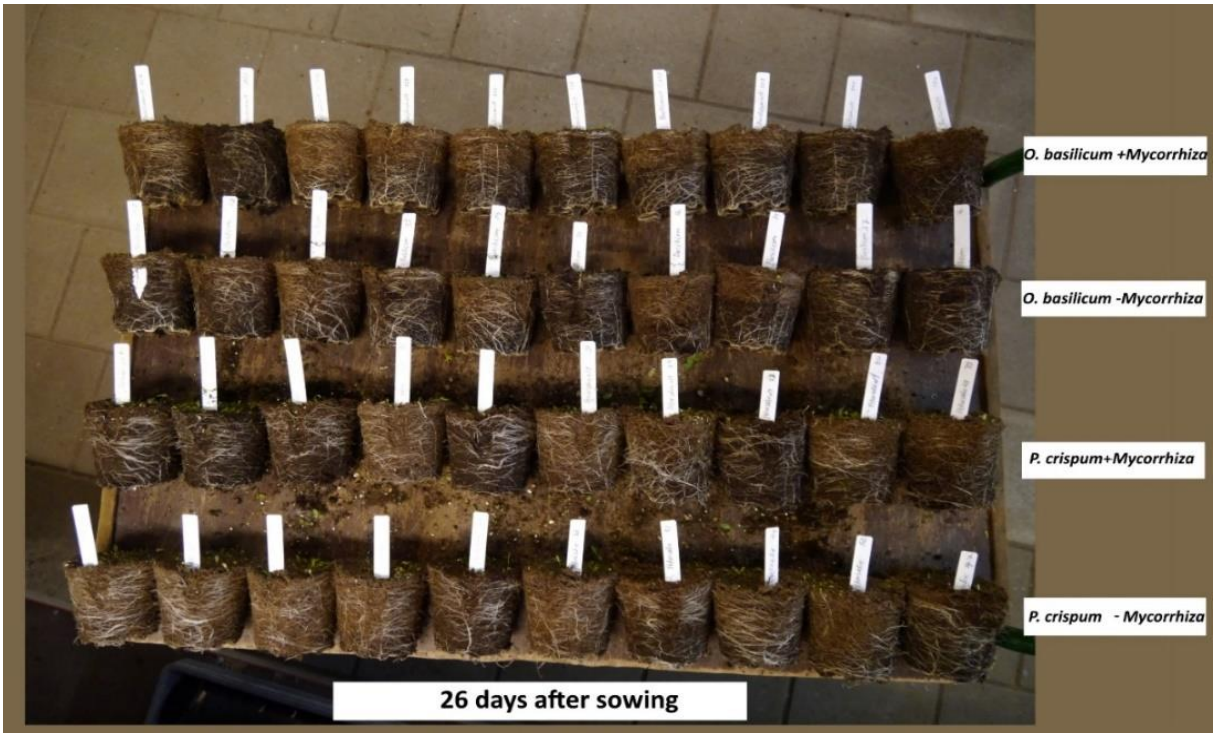

Figure 8: The herb roots each variety between growing in substrate with and without Micosat F at the harvesting, 26 days after sowing

In addition, a higher plant height was expected in this experiment because of the beneficial nutrition effect from mycorrhizal fungi in live symbiosis with roots and its positive effect on water absorption, which can mean that *O. basilicum* and *P. crispum* are able to absorb nutrients better. The result corresponds with the expectation; both herbs show longer plants when growing in substrate treated with Micosat F.

According to J. van Klaren, Mycorrhiza should work better than at a higher EC (Appendix C). However, EC is not measured in this research. The unexpected result is that the longer herbs height of both varieties give lower dry weight in comparison to the shorter herbs. There is no explanation on this. Micosat F did not improve biomass production (dry weight), only gave taller plants.

5. Conclusion and Recommendations

The cultivation of potted herbs *O. basilicum* and *P. crispum* using a substrate treated (and not treated) with so called "Micosat F" granulates (including Mycorrhiza fungi) in the HAS climate chamber shows some those results;

Micosat F has a positive effect on plant height in both herbs because the length of the plants where taller than non-Mycorrhiza treatment. The herbs that are grown in substrate mixed with Micosat F are higher than those who grow in non-treated substrate. In general *O. basilicum* is longer than *P. crispum.* In contrast, after these herbs were cut and dried, there is no significant difference in fresh- and dry weight between treatments. Micosat F does not have effect on fresh and dry weight of both herbs.

Whether Micosat F will benefit potted herbs grower, depends on the cultivation strategy and product specifications they want to achieve. If the goal is to gain height potted herbs then Micosat F could be interesting.

Since this experiment was conducted under well-controlled climate conditions with LED light supplements in the climate chamber, the result of Micosat F effect on potted herb cultivation might be differ from growing herbs in the nursery's greenhouse. It would be recommended to conduct the experiment under the same commercial potted herbs such as in the greenhouse for further study.

6. Acknowledgements

1) Gipmans Planten, a family owned nursery in Venlo who supplies the herb seeds for sowing, showing the nursery on the visit during the starting phase of this project. In addition, Gipmans gives a lot of useful information regarding herbs cultivation for setting up the experiment.
2) J. van Klaren, contact person from Micosat who supplies the Micosat F granulates to test in our trial. Moreover, he paid a visit on 17th December 2014 to look at our trial and gave us extra information about Mycorrhizal granulates work.
3) HAS school greenhouse staff by taking care of the crops, also during the holiday period and for all cooperation regarding the trial set-up.

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Appendix A: The measured data from harvesting

Table 5: Measured data from harvesting

Date: 16th of December 2014

Variety	treatment*	sample nr.	pot nr.	height (cm)	fresh weight (g)	dry weight (g)
basilicum	a	1	27	22,5	25,75	2,01
basilicum	a	2	32	23,0	24,79	2,03
basilicum	a	3	31	21,0	24,74	2,17
basilicum	a	4	23	23,5	30,39	2,74
basilicum	a	5	19	22,0	27,60	2,71
basilicum	a	6	10	20,5	28,24	2,31
basilicum	a	7	17	19,5	26,08	2,15
basilicum	a	8	15	21,5	24,11	2,26
basilicum	a	9	6	23,0	28,12	2,27
basilicum	a	10	16	20,0	20,54	1,95
basilicum	b	1	127	24,0	27,87	2,55
basilicum	b	2	139	20,5	27,27	1,64
basilicum	b	3	131	21,0	23,70	2,46
basilicum	b	4	115	25,0	29,96	2,81
basilicum	b	5	116	25,5	27,43	2,18
basilicum	b	6	135	21,0	22,30	1,82
basilicum	b	7	123	23,0	26,10	2,39
basilicum	b	8	119	21,5	25,43	2,21
basilicum	b	9	140	23,0	23,92	1,90
basilicum	b	10	124	24,5	26,17	2,13
petroselinum	c	1	67	22,5	17,34	2,16
petroselinum	c	2	55	23,0	20,58	2,12
petroselinum	c	3	43	19,5	12,40	1,65
petroselinum	c	4	63	19,5	13,22	1,60
petroselinum	c	5	59	21,0	15,58	1,48
petroselinum	c	6	68	21,0	16,91	1,82
petroselinum	c	7	51	21,0	13,94	1,44
petroselinum	c	8	44	21,0	16,03	2,02
petroselinum	c	9	52	19,0	10,15	1,24
petroselinum	c	10	47	21,5	14,75	1,51
petroselinum	d	1	103	23,0	15,76	1,49
petroselinum	d	2	99	20,0	11,78	1,09
petroselinum	d	3	104	22,5	14,91	1,38
petroselinum	d	4	88	21,5	18,46	2,01
petroselinum	d	5	95	21,0	13,84	1,24
petroselinum	d	6	91	23,0	20,89	2,13
petroselinum	d	7	87	23,5	19,51	2,02
petroselinum	d	8	83	21,0	13,19	1,28
petroselinum	d	9	80	23,0	19,12	2,07
petroselinum	d	10	79	22,0	18,07	1,85

** Small letters represent difference of treatments; a- Ocimum basilicum without Micosat F, b- Ocimum basilicum with Micosat F, c- Petroselinum crispum without Micosat F, d- Petroselinum crispum with Micosat F.*

Appendix B: GenStat output

Analysis of variance: Height (cm)

Variate: height_cm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
mycorrhiza	1	14.400	14.400	7.05	0.012
variety	1	6.400	6.400	3.13	0.085
mycorrhiza.variety	1	0.025	0.025	0.01	0.913
Residual	36	73.550	2.043		
Total	39	94.375			

Tables of means

Variate: height_cm

Grand mean 21.88

mycorrhiza	without Micosat F	with Micosat F
	21.27	22.48

variety	basilicum	petroselinum
	22.27	21.48

mycorrhiza	variety	basilicum	petroselinum
without Micosat F		21.65	20.90
with Micosat F		22.90	22.05

Standard errors of differences of means

Table	mycorrhiza	variety	mycorrhiza variety
rep.	20	20	10
d.f.	36	36	36
s.e.d.	0.452	0.452	0.639

Least significant differences of means (5% level)

Table	mycorrhiza	variety	mycorrhiza variety
rep.	20	20	10
d.f.	36	36	36
l.s.d.	0.917	0.917	1.296

Analysis of variance: Fresh weight (g)

Variate: fresh_weight__g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
mycorrhiza	1	5.198	5.198	0.68	0.415
variety	1	1041.216	1041.216	136.10	<.001
mycorrhiza.variety	1	5.506	5.506	0.72	0.402
Residual	36	275.414	7.650		
Total	39	1327.334			

Tables of means

Variate: fresh_weight__g

Grand mean 20.92

mycorrhiza	without Micosat F	with Micosat F
	20.56	21.28

variety	basilicum	petroselinum
	26.03	15.82

mycorrhiza	variety	basilicum	petroselinum
without Micosat F		26.04	15.09
with Micosat F		26.02	16.55

Standard errors of differences of means

Table	mycorrhiza	variety	mycorrhiza variety
rep.	20	20	10
d.f.	36	36	36
s.e.d.	0.875	0.875	1.237

Least significant differences of means (5% level)

Table	mycorrhiza	variety	mycorrhiza variety
rep.	20	20	10
d.f.	36	36	36
l.s.d.	1.774	1.774	2.5

Analysis of variance: Dry weight (g)

Variate: dry_weight_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
mycorrhiza	1	0.0245	0.0245	0.21	0.647
variety	1	3.0747	3.0747	26.81	<.001
mycorrhiza.variety	1	0.0000	0.0000	0.00	0.989
Residual	36	4.1290	0.1147		
Total	39	7.2282			

Tables of means

Variate: dry_weight_g

Grand mean 1.957

mycorrhiza	without Micosat F	with Micosat F
	1.982	1.933

variety	basilicum	petroselinum
	2.234	1.680

mycorrhiza	variety	basilicum	petroselinum
without Micosat F		2.260	1.704
with Micosat F		2.209	1.656

Standard errors of differences of means

Table	mycorrhiza	variety	mycorrhiza variety
rep.	20	20	10
d.f.	36	36	36
s.e.d.	0.1071	0.1071	0.1515

Least significant differences of means (5% level)

Table	mycorrhiza	variety	mycorrhiza variety
rep.	20	20	10
d.f.	36	36	36
l.s.d.	0.2172	0.2172	0.3072

Appendix C: Notes of visit Micosat at HAS

Visitor: J. van Klaren, contact person from Micosat

Taking notes: R. van der Spek

Date: 17th December 2014

It takes about 10 till 14 days before a population (colony) of Mycorrhiza is formed. Only than the fungi start entering the plant roots. During the formation of the colony, Mycorrhiza is not stable and can easily be killed. After forming a colony, Mycorrhiza cannot be killed that easy anymore. Mycorrhiza normally is dosed on the moment of planting or sowing. Mycorrhiza can survive low temperatures. It gets in dormancy than and becomes active when the temperature increases. Mycorrhiza also can survive in higher temperatures. However, when the temperature passes the limit of 45°C plants will die and the symbioses stops. For Mycorrhiza, a temperature of 20-25 °C is optimal.

Mycorrhiza takes sugars from the plants. Because of a good balance between sugars and nutrients, plant growth will not decrease by Mycorrhiza. Root differences in our experiment are due to Mycorrhiza.

An important benefit of Mycorrhiza is the high fertilizer efficiency it gives. A lower EC can be given by using Mycorrhiza. A normal dose of Micosat F (new) is 500-700 grams per m^3. In the open field, a reduction of 33% on fertilization can be reached. Also the loss of fertilizer to the drain can be minimized by a better uptake of nutrients due by Mycorrhiza.

Mycorrhiza can be killed by fungicides like “Amistar” and “Paraat”. On the other hand, one of the ideas of Mycorrhiza is to get rid of chemicals in cultivations.

Micosat offers also some more products for plant health. They provide for example products which prevent against fungi diseases on leaves and roots. Some of their products also can be solved in water. However, the Micosat F granulate cannot. After solving in water, Mycorrhiza cannot multiply itself and will not be able to start a symbioses with roots.