4 Mycorrhizal Inoculated Peat and Peat-Free Substrates in Potted Herbs

4.1 Introduction

Peat use in horticulture is problematic. It's a slow renewable resource with serious climate change implications when produced (Barkham 1993) . Peat is used in horticulture as it is an excellent growing substrate, however there is difficulty in replicating peat from other materials currently available (Robertson 1993). Issues arrise in not only replicating the chemical and physical attributes of peat, but also the economics of creating a like-for-like growing media with conventionally available material. The result of these elements is relatively limited amount of reliable, cost effective alternatives available [Alexander, Stuart, and Bragg (2017). The development of alternative peat substrates is continuously and cautiously progressing as new materials and processes evolve. There is no product that can currently reflect the reliability and range of crop compatibility of peat. *Coriandrum sativum* and *Ocimum basilicum* are commercially significant crops, grown globally for its leafy material and seed products (Parthasarathy and Zachariah 2008). The container grown herb sector in the United Kingdom is a huge market, with *C. sativum* and *O. basilicum* being a significant portion of the overall production. Although changes occuring through government advisory notices and awareness raised through environmental activism and lobbying, the use of peat remains a significant factor in container grown crop production and horticulture as a whole. The current alternatives have a reputation as being unreliable for large scale use due to the lack of homogeneity, issues in adapting irrigation requirements, quality control and changes in nutrient requirements.

This study aims explore the possibility of improving peat-free alternatives with microbial amendments, specifically Arbuscular mycorrhizal fungi (AMF). Research in this field has already yielded significant results regarding improved crop health and quality; increased oil content accumulation and changes in volatile composition as a treatment effect of AMF presence (Kapoor, Giri, and Mukerji 2002; Ravi, Prakash, and Bhat 2007), increased crop quality, growth and germination (Palencia, Martinez, and Weiland 2013; Gutowski 2015).

This study was designed to determine the differences, if any, between substrate types and

AMF inoculation. Observation of crop quality, biomass and rate of colonisation of AMF were recorded at different harvest points over the crops development. By understanding at which point the AMF begins to colonise the crop, efforts can be made to enhance this process at the earliest possible stage in order to convey the largest benefits to the crop in question. These results may also allow for adaptation of conditions in order to benefit both mycorrhiza and crop in a uniform and conjunctive manner.

4.2 Materials and Methods

The materials and methods used here are documented in the Materials and Methods section. Commercial inoculum was placed into pots with each peat and peat-free growing medias. Each pot was seeded with respective croppings (Basil, Coriander) and then placed into identical growing conditions for between 28-32days, at which point one or more crop would reach 16cm, harvesting would commence. The average height for commercial croppings to be produced to is 16cm. Harvesting and assessments would take the same day, along with sampling for further assessment later on.

Organisms

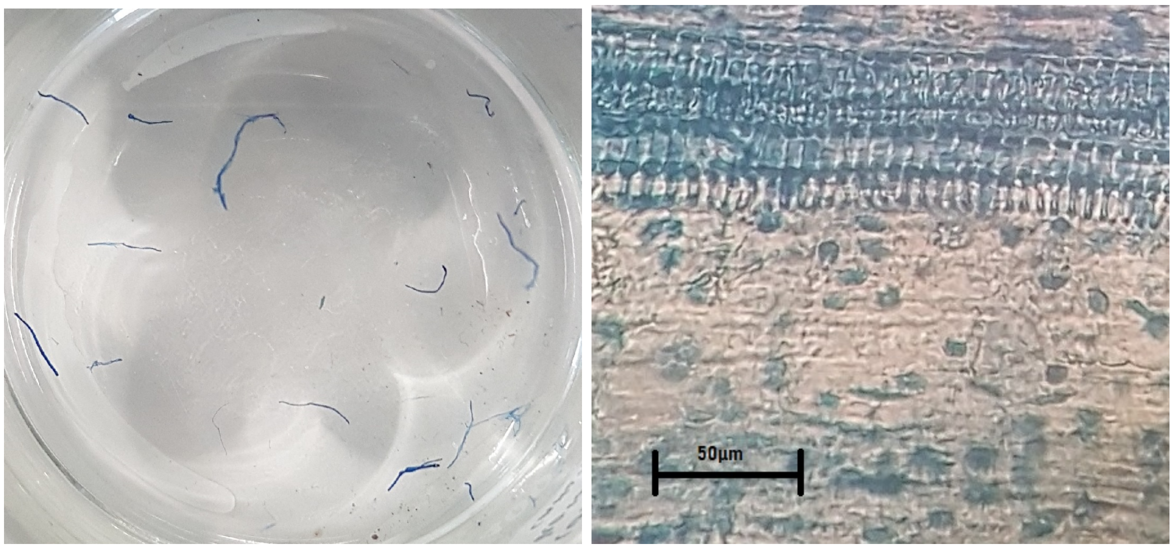
The Mycorrhiza was delivered through a commercial grade, granular product. It is a combination of six species of fungi that have demonstrated positive mychorrizal symbiosis with a variety of hosts (Strawberry (Palencia, Martinez, and Weiland 2013), Radish (Gutowski 2015), Tomato (Al-Karaki, Hammad, and Rusan 2001) to name some). The inoculum was added to the substrate pre-seeding. Seeds were then added on top and then covered with additional substrate (Coriander) or surface scattered and then covered with black-out plastic to promote germination (Basil).

Growing Media

The use of two growing media were employed for these studies. A third; peat-reduced (80% peat) was eliminated from potted trials entirely. The growing media used was Peat, a substrate sourced from a commercial nursery, manufactured for the purpose of growing potted herbs. The second media used was Peat-Free. This was created on-site from several ingredients using a cement mixer.

Mycorrhizal Colonisation

Root staining was achieved following methods laid out by Phillips and Hayman (1970) and further improved upon. Colonisation was assessed as a % of root length (RLC, root length colonisation) by observing methodologies established by McGonigle et al. (1990). An example of staining roots *in-situ* and free-floating in a solution can be seen in Figure 15.



(a) (b)

Figure 15: Example of roots cut and stained in an aqeous solution prior to mounting, and a potentially colonised root

4.2.1 DNA isolation and Amplification

DNA extraction was performed on harvested roots, suspended in 95% ethanol. Homoginised root material was used as the extraction basis. A postive control was made from crushed innoculum. After PCR with both species specific and universal primers, samples were analysed for DNA via spectography. No DNA was present.

4.2.2 Analysis

A variety of trials were collated here to produce are broader picture of the results. Treatments from trials consisting of either control values and the addition of mycorrhiza formed the basis for this study. Trials including and/or sharing Bacterial treatments were excluded entirely as a precaution against contamination.

One way ANOVA between various phytometric measurements and AMF treatments formed the basis of analysis for this study. Linear modelling was also employed to gather correlation co-coefficients between

4.3 Results

Overall, Peat based substrates were more successful in achieving higher yields and growth rates (height) for Basil croppings. Height was further increased by the addition of AMF inoculum. The addition of AMF increased yields, height, leaf diameter and RLC rates for Peat-Free grown Coriander only.

4.3.1 Height

During crop growth, AMF treated Peat and Peat-Free growing medias for both Coriander and Basil have increased height compared to none-AMF treated pots. This pattern is most prominent in Basil croppings for Peat based growing media with an increase of 1.67cm at 32days growth compared to controls.

Table 5: One way Anova between AMF and Control treatments for each Crop and Substrate over the growing period.

Crop	Substrate	Day	F-value	P
Basil	Peat	7	8.634	0.0045 **
-	-	14	28.69	9.44e-07 ***
-	-	21	16.89	0.000102 ***
-	-	28	14.4	0.000516 ***
-	-	32	45.69	2.79e-10 ***
-	Peat-Free	7	6.797	0.0112 *
-	-	14	2.406	0.125
-	-	21	0.374	0.543
-	-	28	0.005	0.942
-	-	32	7.133	0.00837 **
Coriander	Peat	7	23.9	1.28e-05 ***
-	-	14	19.17	2.74e-05 ***
-	-	21	1.357	0.248
-	-	28	14.48	0.000239 ***
-	Peat-Free	7	23.38	1.53e-05 ***
-	-	14	11.67	0.000875 ***
-	-	21	11.13	0.00129 **
-	-	28	7.692	0.00656 **

The addition of AMF inoculum appears to significantly increase height in treated pots over the growing period (see Table 5 and Table 6). The only treatment to not be consistently benefiting from the addition of AMF was Peat-Free Basil at 14, 21 and 28 days growth (see Figure 16). An example of ready to harvest Coriander croppings can be seen in Figure 17.

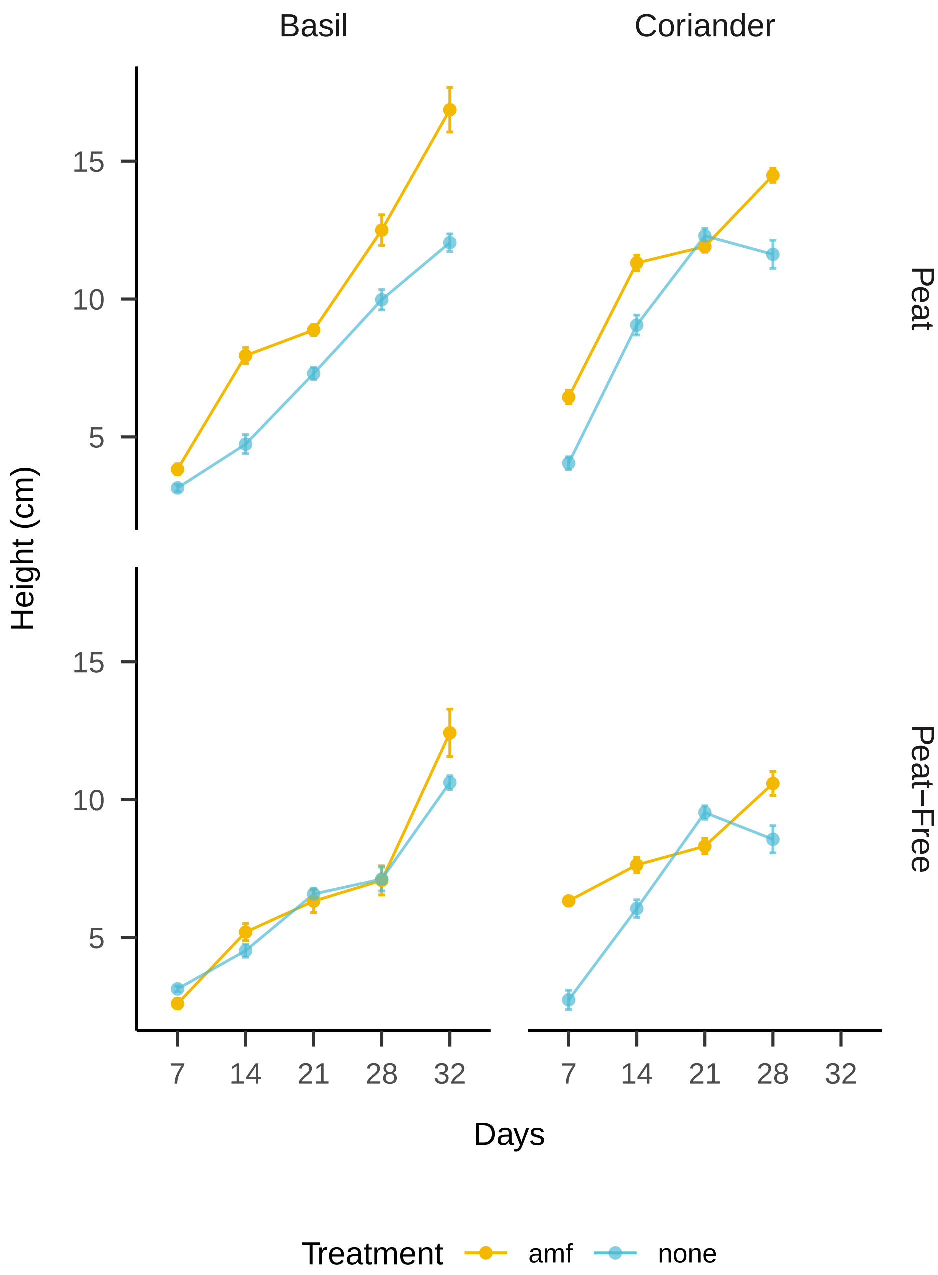


Figure 16: Height graph for Basil and Coriander with peat and peat-free substrates, treated with Mycorrhiza. Both Basil and Coriander in each Peat and Peat-Free substrates demonstrate increased crop height at harvest (28/32days). A reduction in height occurs for none-amf treated croppings for Coriander at harvest (28days).

Table 6: Crop height for both Coriander and Basil over the growing period (up to ca. 32days)

Coriander control	Peat	Peat-Free	p
7days	6.33 (0.50)	6.11 (0.60)	0.406
14days	10.83 (0.41	10.5 (1.22)	0.541
21days	11.33 (1.53)	13 (1)	0.189
28days	12.67 (0.58)	13 (1)	0.643
Coriander AMF	Peat + AMF	Peat-Free + AMF	p
7days	6.44 (0.73)	6.33 (0.50)	0.71
14days	10.83 (0.75)	10.67 (1.03)	0.756
21days	11.33 (1.53)	13.00 (1.73)	0.279
28days	12.33 (0.58)	13.67 (0.58)	0.047
Basil control	Peat	Peat-Free	p
7days	3.85 (0.74)	2.81 (0.74)	<0.001
14days	7.52 (1.25)	5.38 (1.19)	<0.001
21days	8.55 (1.35)	6.31 (1.26)	<0.001
28days	10.73 (1.90)	7.13 (1.82)	<0.001
32days	14.69 (2.52)	9.15 (3.25)	<0.001
Basil AMF	Peat + AMF	Peat-Free + AMF	p
7days	4.03 (0.58)	2.72 (0.72)	<0.001
14days	7.63 (1.09)	5.37 (1.22)	<0.001
21days	8.57 (1.18)	6.29 (1.54)	<0.001
28days	11.70 (1.75)	7.44 (1.70)	<0.001
32days	16.36 (3.05)	10.26 (3.56)	<0.001

(a)

(b)

Figure 17: Growth of Coriander, close to harvestable height (16cm).

4.3.2 Yield

Both substrate and crops had a significant impact on fresh yield and dry yields ($p < 0.05$). The addition of AMF had no significant impact on fresh yield for Peat substrates in both Coriander and Basil (see Figure 19). Significant differences in yields were observed in Peat-

Free substrates. A difference of 8.37g increase in fresh yield was observed (224% yield increase) between the control in Peat-Free and the addition of AMF in Coriander croppings only.

Dry Yield Significant differences were also found using dry yield as a response vector against both substrate and crop ($p < 0.05$). The addition of mycorrhiza on dry yield was not significant for Basil (see Figure 18). However and increase of 181% dry yield for Peat-free substrates in Coriander was observed, replicating the pattern established in Fresh Yields. This increase in dry yield may be a result of increased nutrient/mineral availability (Collingwood, Crossman, and Navarro 1991; Martin et al. 2017; Anwar et al. 2005) in peat-free growing media due to multi-component (coir, wood fibre etc.) structure.

The influence of AMF innoculumn can be clearly seen in Figure 20, wherein a stronger relationship between dry/wet weight can be seen in AMF treated crops.

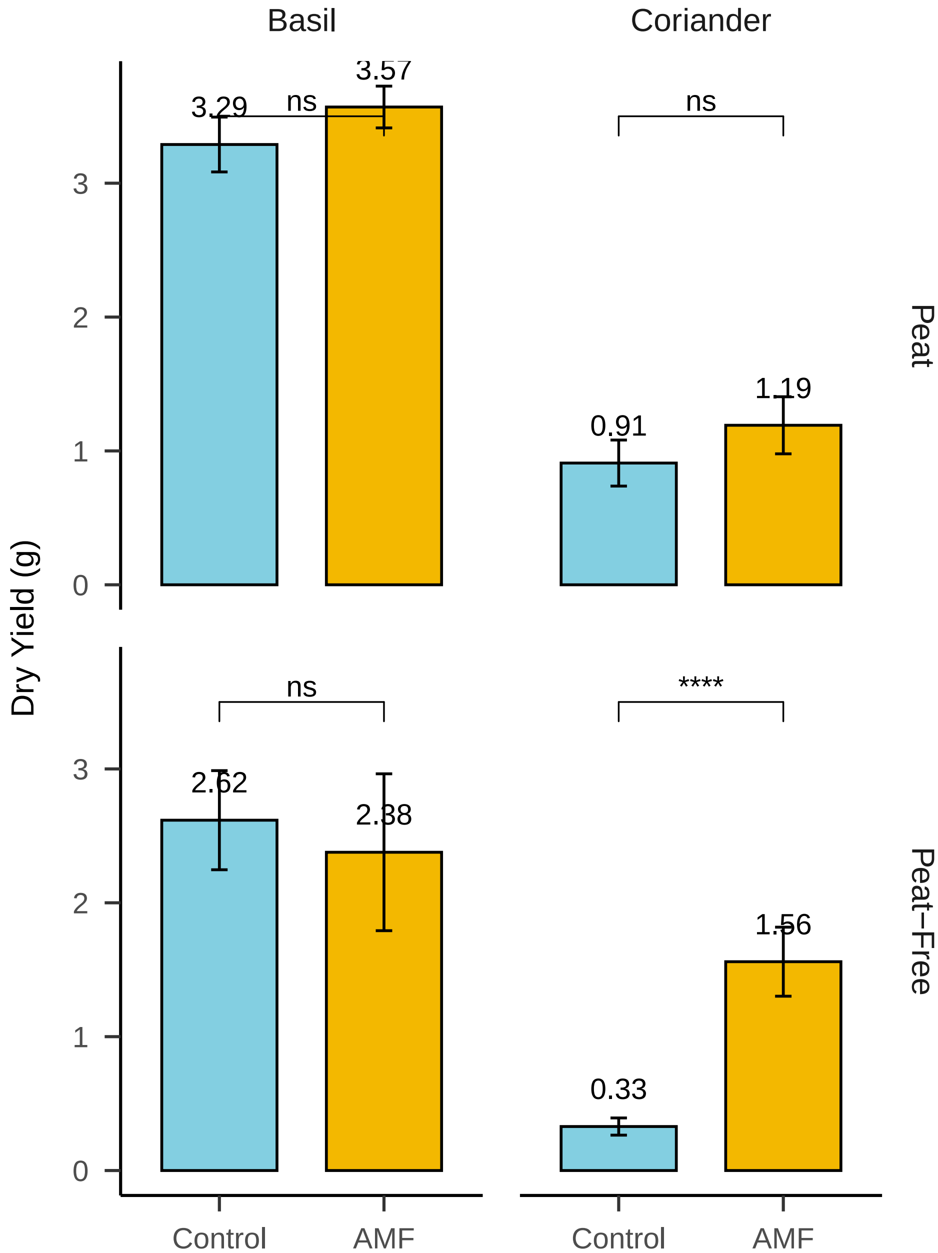


Figure 18: Dry yield from both crop types in Peat and Peat-Free growing media. The addition of AMF in Basil crops had no significant effect on dry yield. Coriander experienced increased dry yield when under the influence of AMF, but only in Peat-Free growing media. Large error bar occuring on Peat-Free, Basil AMF treatment indicates the variability of data, suggesting reduced homogenity.

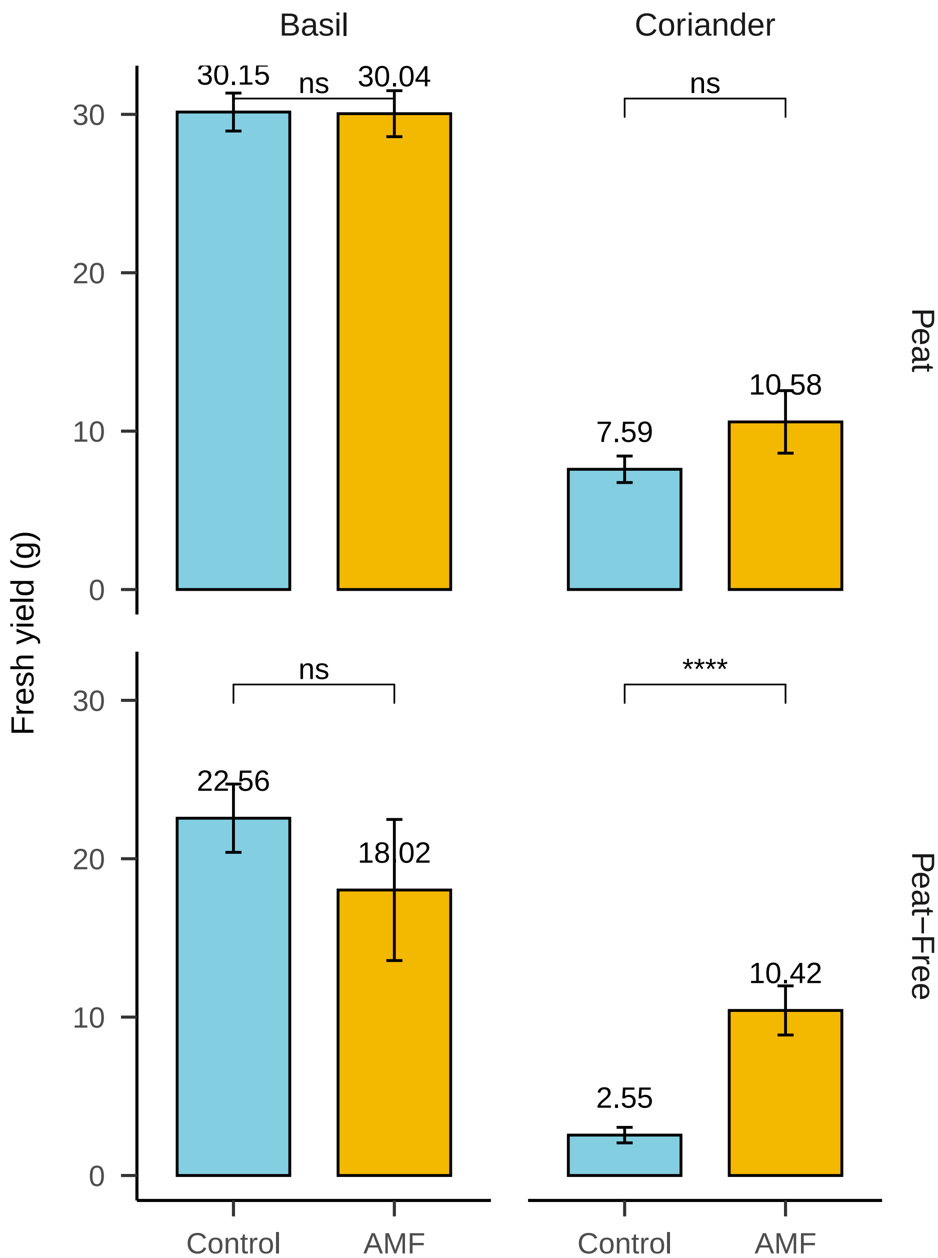


Figure 19: Fresh Yield for Basil and Coriander in Peat and Peat-Free growing media. The influence of AMF incolum on yeild only significantly effected one cropping; Peat-Free Coriander, which experienced an increase in yeild, replicating the pattern established in dry yield. Large error bar on Peat-Free, Basil AMF treatment appears similar to previous Dry Yield reporting and may suggest increased variability in recorded data.

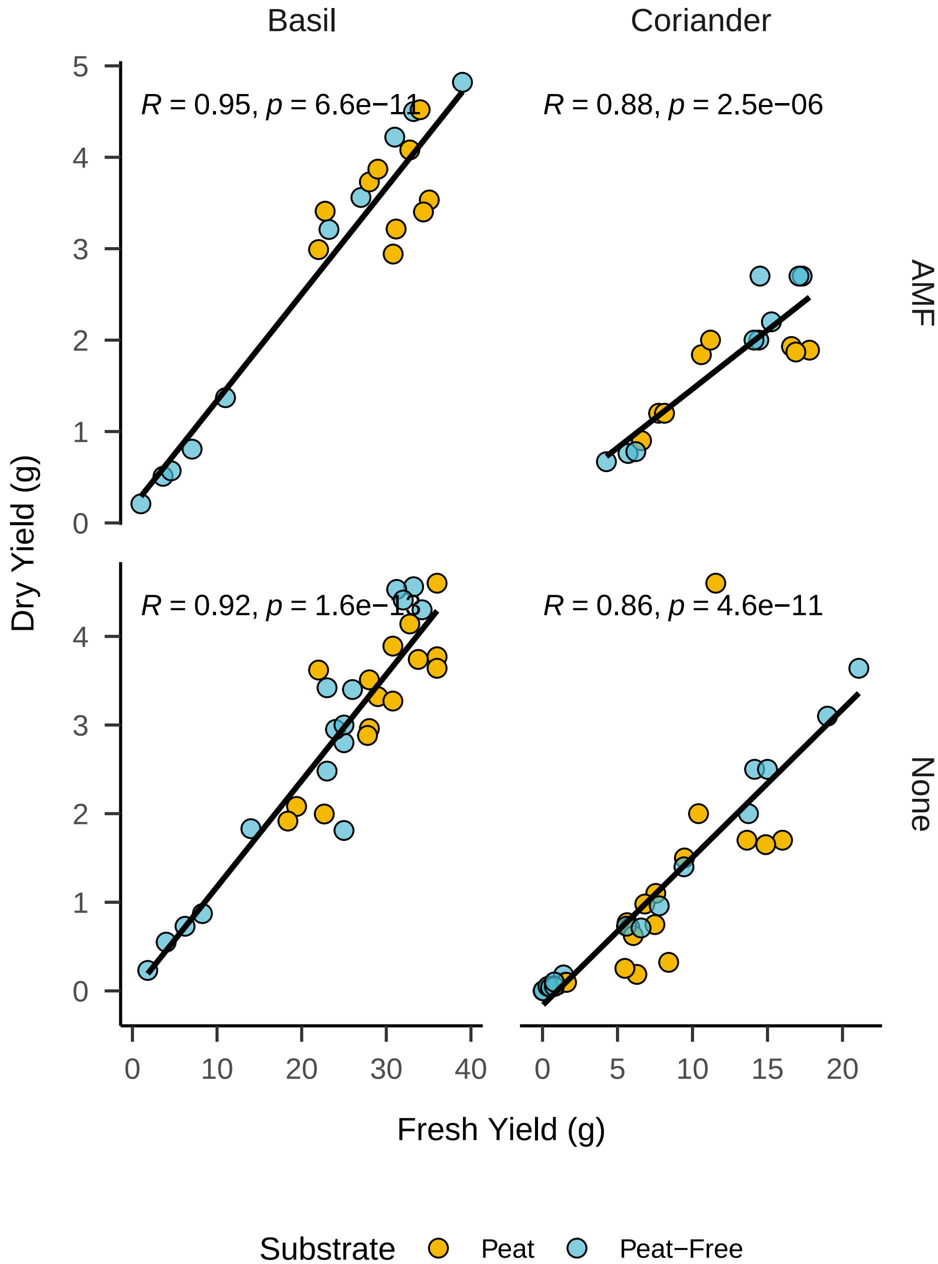


Figure 20: Linear model of Fresh Vs Dry Yield.A slightly increased correlation between yields can be observed in AMF treated pots. This relationship is however small, and not significant.

4.3.3 Leaf Diameter

Leaf diameter was significantly affected by growing media ($P<0.001$), with the exception AMF treated Basil crops. Crop type also influenced leaf-diameter with Coriander producing broader leafs during the trial periods. AMF had little impact on leaf diameter. Coriander in Peat-Free growing media inoculated with AMF had a significant increase in leaf diameter (2.55cm vs 2.21 cm for control) (see Figure 7). Leaf diameter is displayed in a violin plot (see Figure 21). A violin plot contains all the information of a box plot but with the addition of density distribution of the data projected on either side. This graph was chosen for displaying leaf diameter as the density distribution brings additional context for variablity in sizing for the leaves.

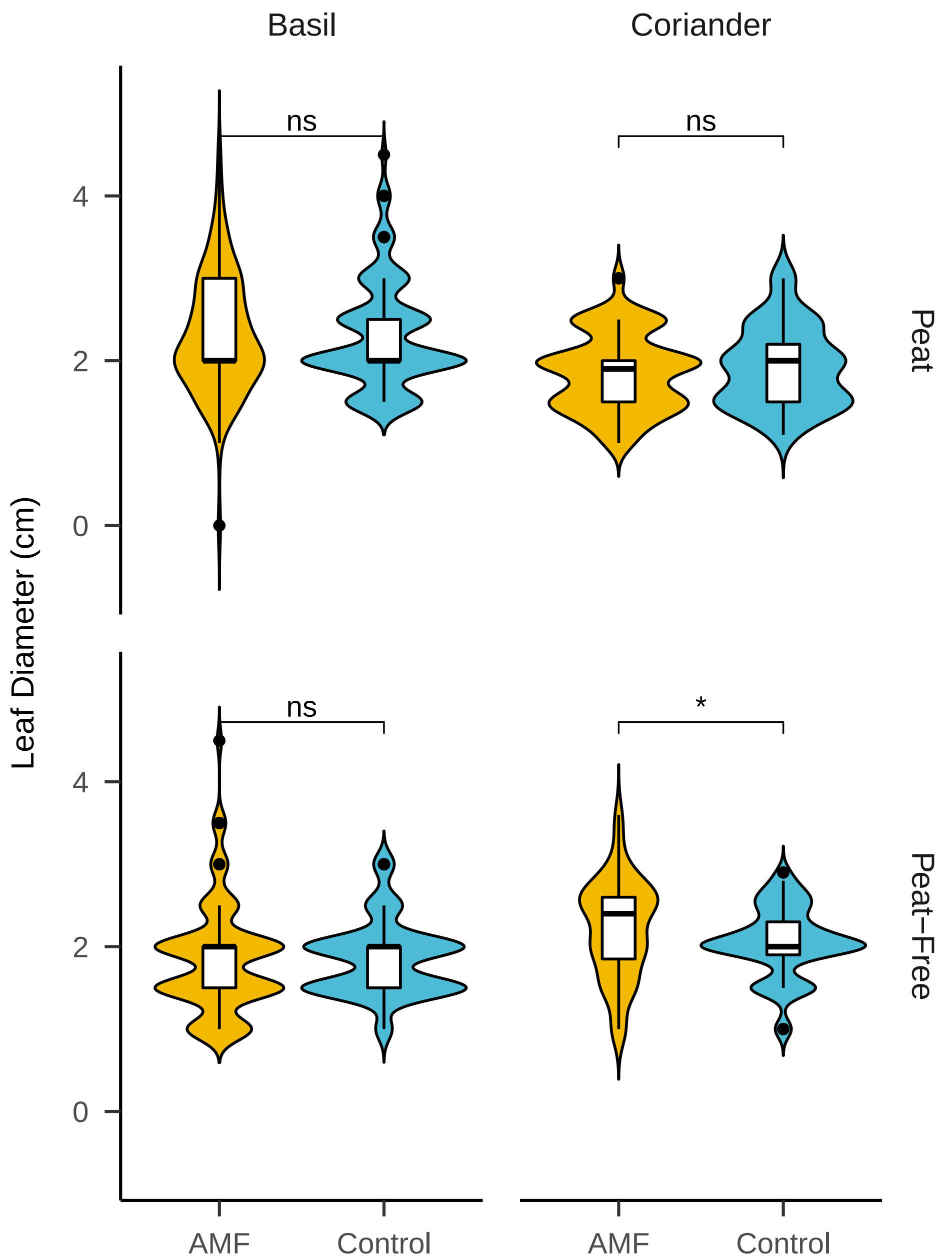


Figure 21: Leaf Diameter (LD) for both Basil and Coriander in Peat and Peat-Free growing media.The addition of AMF had a significant impact on LD for Peat-Free growing media in Coriander only.

4.3.4 Emergence

Crop Emergence was measured as a a simple count of emerged seedlings per pot for each treatment (see Figure 22). The addition of AMF on crop counts in the first week of emergence

Table 7: Mean Leaf Diameter Coriander and Basil and ANOVA results compared between substrate types.

Crop	Leaf Diameter	Peat	PF	p
Coriander	Control	1.64 (0.35)	2.21 (0.4)	<0.001
	AMF	1.58 (0.34)	2.55 (0.42)	<0.001
Basil	Control	2.10 (0.35)	1.65 (0.33)	<0.001
	AMF	1.90 (0.31)	1.55 (0.46)	0.007

had little to no effect on the rate/count of seedlings (see Figure 23). Substrate type however did influence crop emergence as seen in Figure 23, wherein Peat growing media had higher crop counts per pot at the end of the assessment period (5days).

(a) Coriander (b) Basil

Figure 22: Early growth of Coriander (a) and Basil (b). First true leaves are apparent on Coriander croppings. Typically after first true leaf emergence in Coriander, emergence assessments stopped. This period was to ensure both germinating pods per seed were allowed time for emergence.

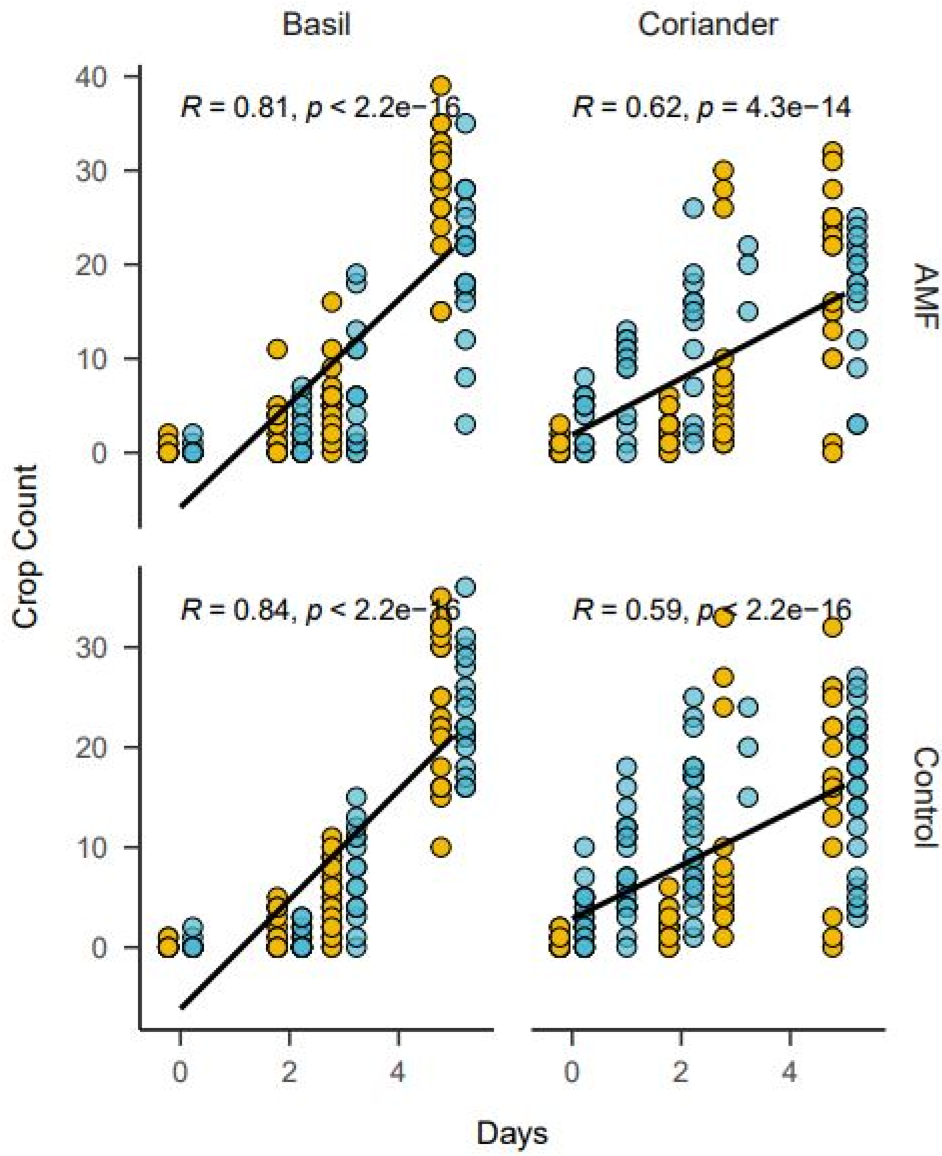
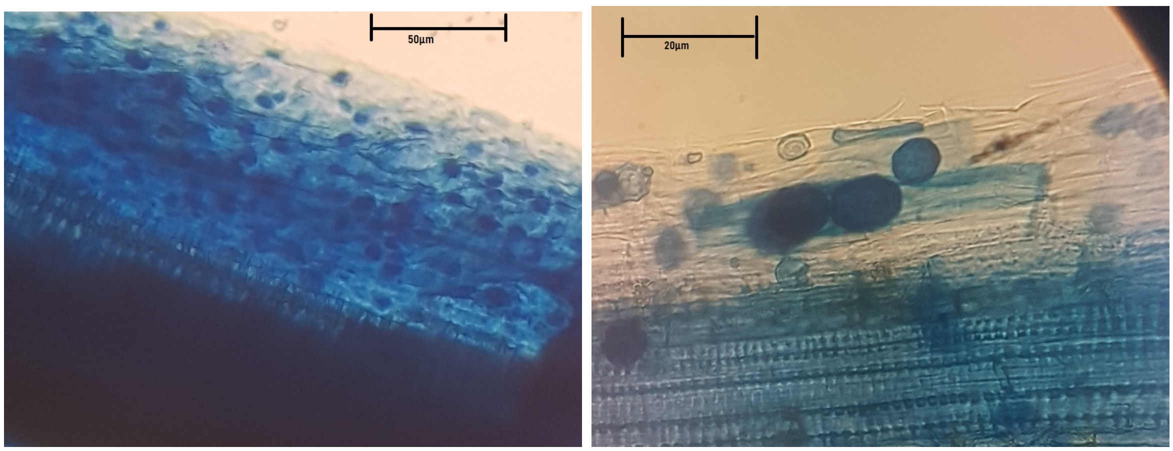


Figure 23: Emergence rate (crop count) correlated against days grown. The addition of Mycorrhiza had little to no significant effect on crop emergence.

4.3.5 Mycorrhizal Colonisation

Once staining was completed, roots were laid out on slides, mounted with PGEV and assessed at 200x magnification. Although typically AMF should demonstrate one of three structural features (Hyphae, arbuscules or Vesicles). Only arbuscules were observed using bright field microscopy (see Figure 24a). Initial colonisation of roots is weak after 7 and 14 days (see Tables 8 and 9). At 28 Days however, significant colonisation is apparent in both treated and untreated pots. Every effort was made to reduce any cross contamination for treatments, however colonisation was seen via staining in control pots, to a significantly lesser extent.

RLC overall in Peat based substrates was significantly less than that of Peat-Free substrates ($P<0.05$).The highest colonisation occurring at 28days in Coriander at a mean of 30.13% compared to 17.43% RLC for Peat AMF treated pots.



(a) Arbuscles (b) Unknown staining

Figure 24: Arbuscles apparent in the root structure (a), some unknown stained objects (b); possibly liquid retention.These pictures demonstrate the difficulty in disseminating mycorrhizal structures from possible contaminants.

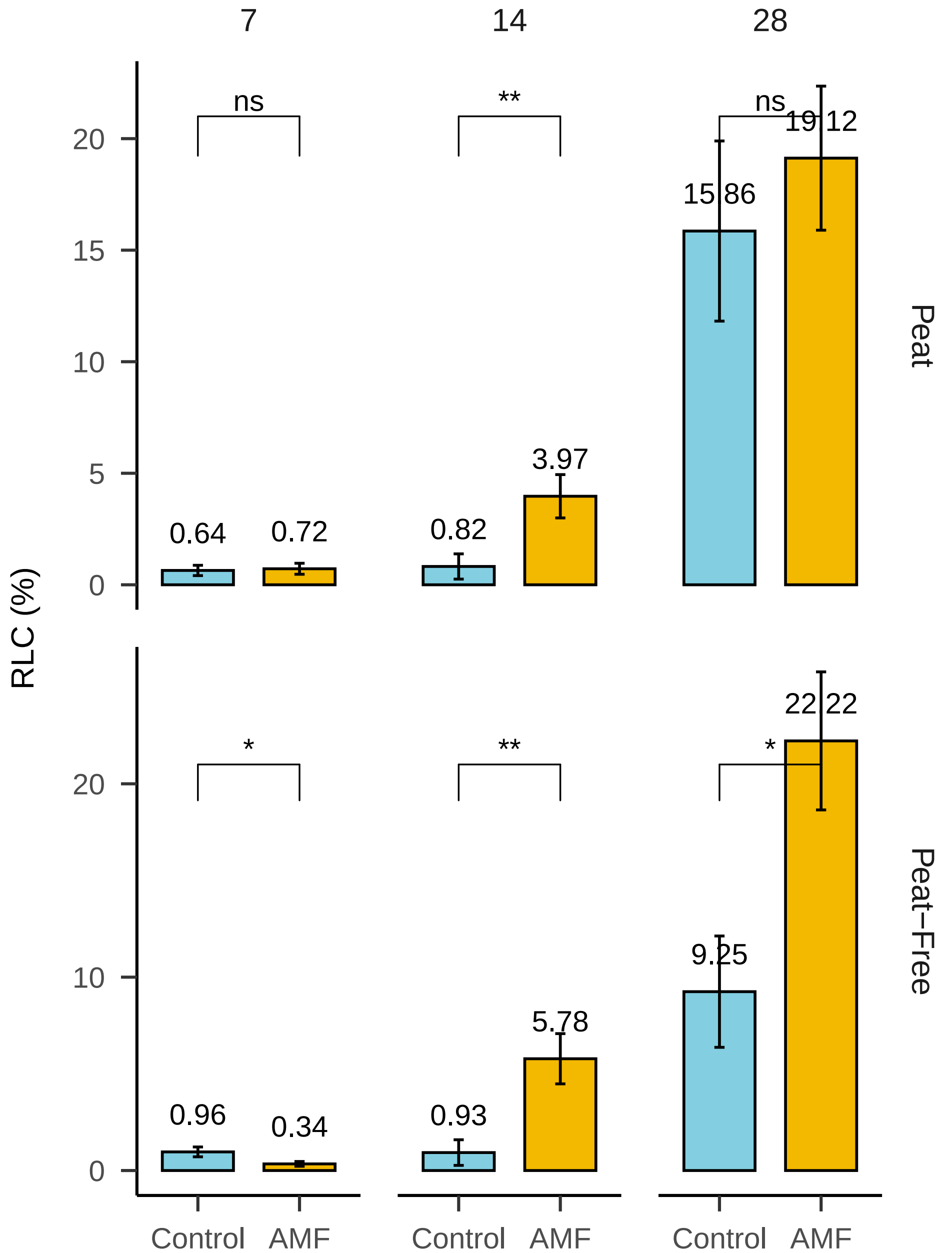


Figure 25: Root length colonisation in Basil croppings at 7, 14 and 28 days. Peat-Free crops treated with AMF demonstrated significantly higher levels of colonisation across each of the sample periods.

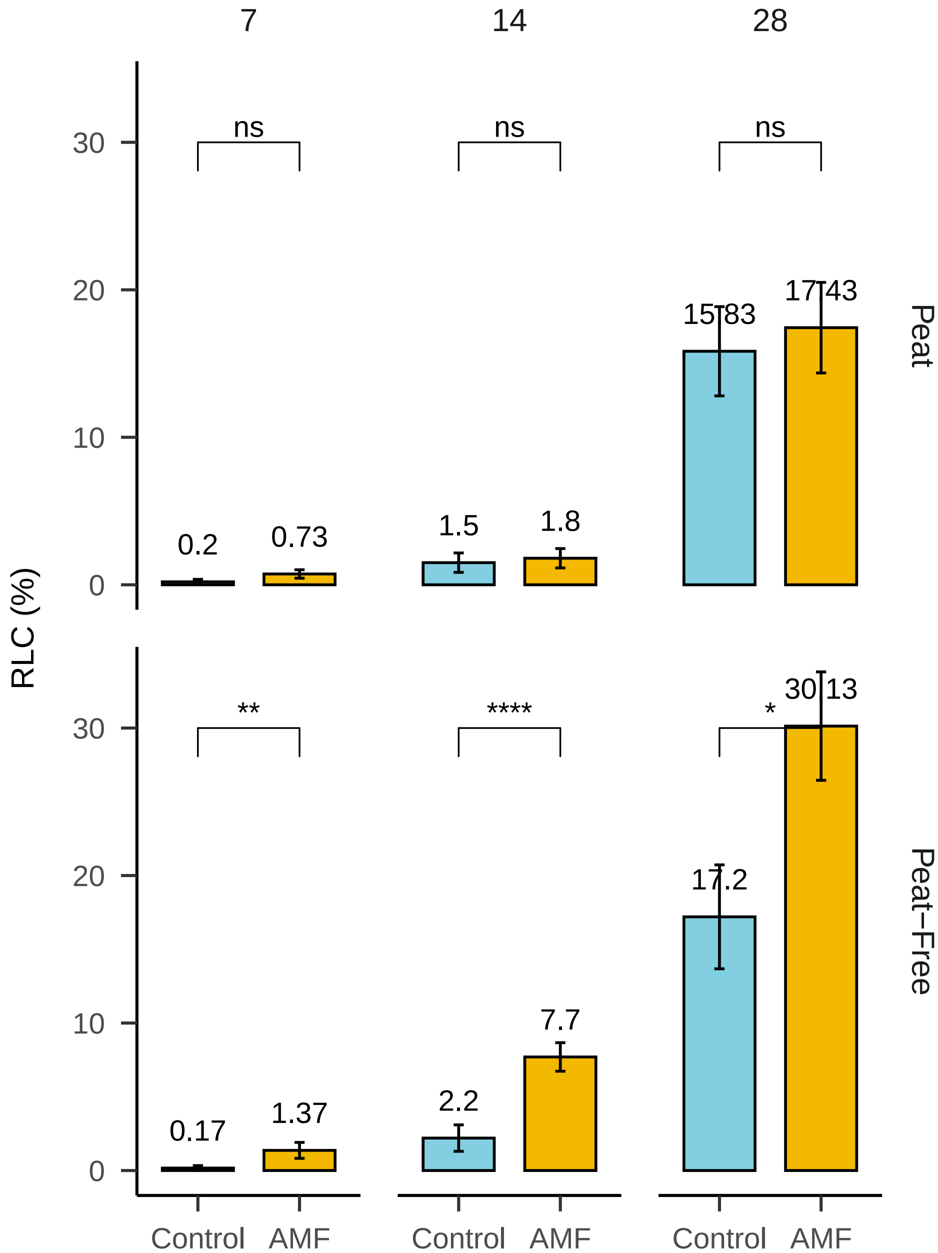


Figure 26: Root length colonisation for Coriander over 7, 14 and 28 days. Peat-Free growing media demonstrated significantly higher levels of colonisation in Coriander roots for AMF treated pots for each sampling period. Peat based croppings demonstrated no signifcant change in colonisation between control and AMF treated pots.

Table 8: Coriander RLC%

Days	Crop	Substrate	Treatment	RLC % mean
7	Coriander	Peat	Control	0.20 (0.92)
7			AMF	0.73 (1.57)
7		Peat-Free	Control	0.17 (0.91)
7			AMF	1.37 (2.97)
14	Coriander	Peat	Control	1.50 (3.61)
14			AMF	1.80 (3.60)
14		Peat-Free	Control	2.20 (4.91)
14			AMF	7.70 (5.30)
28	Coriander	Peat	Control	15.83 (16.56)
28			AMF	17.43 (16.82)
28		Peat-Free	Control	17.20 (19.30)
28			AMF	30.13 (20.14)

Table 9: Basil RLC

Days	Crop	Substrate	Treatment	RLC % mean
7	Basil	Peat	Control	0.64 (1.22)
7			AMF	0.72 (1.40)
7		Peat-Free	Control	0.96 (1.35)
7			AMF	0.34 (0.70)
14	Basil	Peat	Control	0.82 (2.99)
14			AMF	3.97 (5.49)
14		Peat-Free	Control	0.93 (3.51)
14			AMF	5.78 (7.35)
28	Basil	Peat	Control	15.86 (21.37)
28			AMF	19.12 (18.27)
28		Peat-Free	Control	9.25 (15.23)
28			AMF	22.22 (20.20)

4.3.6 Discussion

The addition of Mycorrhizal inoculum demonstrates increased crop growth in both Peat and Peat-Free substrates. None-AMF treated crops demonstrated levels of colonisation to a lesser extent than that of treated crops. This reduction in colonisation has a distinct impact on crop growth in Coriander as demonstrated by decreased crop height, leaf diameter and yield. This decrease in crop development was more apparent in Coriander grown in Peat growing media.

Increased levels of crop emergence were not seen with the addition of AMF, contrary to other research (Gutowski 2015; Fusconi 2014). This maybe due to positive growing conditions found in the glasshouse environment. Consequently negating any potential benefits of the presence of mycorrhiza seen in less favourable conditions.

RLC% is significantly increased at the later stage of growth (28/32days). This high level of colonisation and therefore ‘activity’ of AMF, and the potential benefits occurs at the end of the growth cycle for potted herbs. It may therefore be more advantageous to utlise nutrient resource and favorable conditions to increase colonisation at earlier stages in the life cycle in order to maximise the potential benefits of AMF.

It is apparent that increasing crop growth in potted Coriander and Basil grown in Peat-Free substrate is possible by the addition of Mycorrhizal Fungi. The addition of AMF may present however an additional cost for growers in commercial horticulture. This may impact the economic viability of utlising AMF for the improvment of Peat-Free substrates.