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Observations on Antagonism in the Arbuscular Mycorrhizal Systems

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Abstract: ***The majority of land plants are colonised by arbuscular mycorrhizal fungi, however ,members of few families notably Cruciferae, Chenopodiaceae, Caryophyllaceae and Polygonaceae, so called non-host are not colonized by these fungi. Previous studies have shown that the growth and development of non-host species were severely inhibited when grown in the presence of active AMF mycelia. There is therefore a need to understand the mechanistic bases of adverse effects of AMF mycelia upon seedlings of non-host species.***

In this experiment young roots of non-hosts Spergula arvensis and Arabis hirsuta, and host Centaurium erythraea were exposed to mycorrhizal and non-mycorrhizal extracts. The results of this experiment showed that mycorrhizal extracts significantly reduced the growth of radicles and root hairs development of non-host species whereas had no effect on radicles and root hairs development of host species. These results indicate that toxicity effects rather the nutritional factors are the drivers of the negative responses of non-host species to the presence of the AMF.

Keywords: mycorrhizal fungi, host and non-host plants, Plantago lanceolata , Glomus mossae , mycelium.

I. INTRODUCTION

Whereas the vast majority of land plants responded positively to the presence of arbuscular mycorrhizal fungi (AMF), antagonistic reaction between these fungi and some plant species has been observed. Some studies have been reported that the develpment of seedlings of a number of species representative of typically non-mycorrhizal plants such as C. hirsuta and Arenaria serpyllifolia was strongly inhibited when thier roots were exposed to an actively grown AM mycelium[1]. These effects were observed immediately after germination and appeared to rise without invasion of the root tissue by the fungi. It was suggested that the antagonism was sufficiently severe to explain the exclusion of these typically ruderal plant species from closed communities of plants that are compatible with arbuscular mycorrhizal fungi. In view of the ecological significance of these observations, it is clearly important to consider them on physio-chemical basis. Because the negative impacts of the presence of AM mycelia were seen rapidly after germination and in the absence of tissue penetration it was hypothesized that they were as a result of substances released from the living hyphae of the fungus In order to test this hypothesis, an experiment was carried out to examine the possibility that antagonism was attributable to water soluble chemical toxin produced by the fungus.

II. MATERIAL AND METHODS

The experiment was designed to investigate the basis of inhibition of non –host species caused by the presence of AM fungal mycelium

Plants of Plantago lanceolata were raised in mycorrhizal (M) or non-mycorrhizal (NM) condition in trays of sterilized sand. In order to produce mycorrhizal plants (M), inoculum of the AMF Glomus mossae was added to half of the trays in two thin layers each separated by a layer of sterilized sand .Trays containing sterilized sand without inoculum were used to produce non-mycorrhizal plants (NM). All trays kept moist by the addition of 1/5th strength Rorison's solution lacking phosphorus. Examination of the root systems after a growth period of 6 weeks revealed presence of AM mycorrhizal in the root systems of Plantago lanceolta whereas the same plants remained non-mycorrhizal in NM treatment. The plants were then transferred to chambers which were designed to enable separation of compartments containing roots from those into which AMF mycelium alone could grow(Fig.1). In each chamber there were three compartments A, B and C. In M treatments these were further designated A-MO (Outer mycorrhiza), B-MI (Inner mycorrhiza), and C-MO (Outer mycorrhiza). In NM treatments the designations A-NMO (Outer non- mycorrhiza), B-NMI (Inner non- mycorrhiza), and C-NMO (Outer non- mycorrhiza) were used. These chambers were incubated for 5 weeks during which the root systems of Plantago lanceolata M or NM could proliferate in the inner (B) compartments, and the AMF mycelium could grow into (A & C) outer compartments of the mycorrhizal treatments.

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After 5 weeks 200g sub-samples of sand were removed from each compartment of all of the experimental systems and processed to extract the residual solution surrounding the sand particles.

To each 200g of sand, 100ml of deionised water were added to form a paste. The paste was left for 1 hour to attain equilibrium before being placed on a Whatman No 1 Filter paper in a Buchner funnel. The sand solutions were then extracted under mild vacuum. The extracts were subsequently concentrated to about one third of their original volumes by freeze drying. Seedlings of two species of non-host species were employed as bioassay plants to investigate the impacts of sand extracts upon radicle development. These were non-hosts Spergula arvensis and Arabis hirsuta and host Centaurium erythraea was also assayed as a control.

Seeds of the three bioassay species were first incubated on moist filter paper to the stage of radicle emergence. At this time (time zero) seedling radicles were immersed in single Eppendorf tubes containing 0.2 ml of the extract from the particular treatment. The growth of the seedling roots was monitored over a period of 6 days. The root tips were also closely examined and photographed.

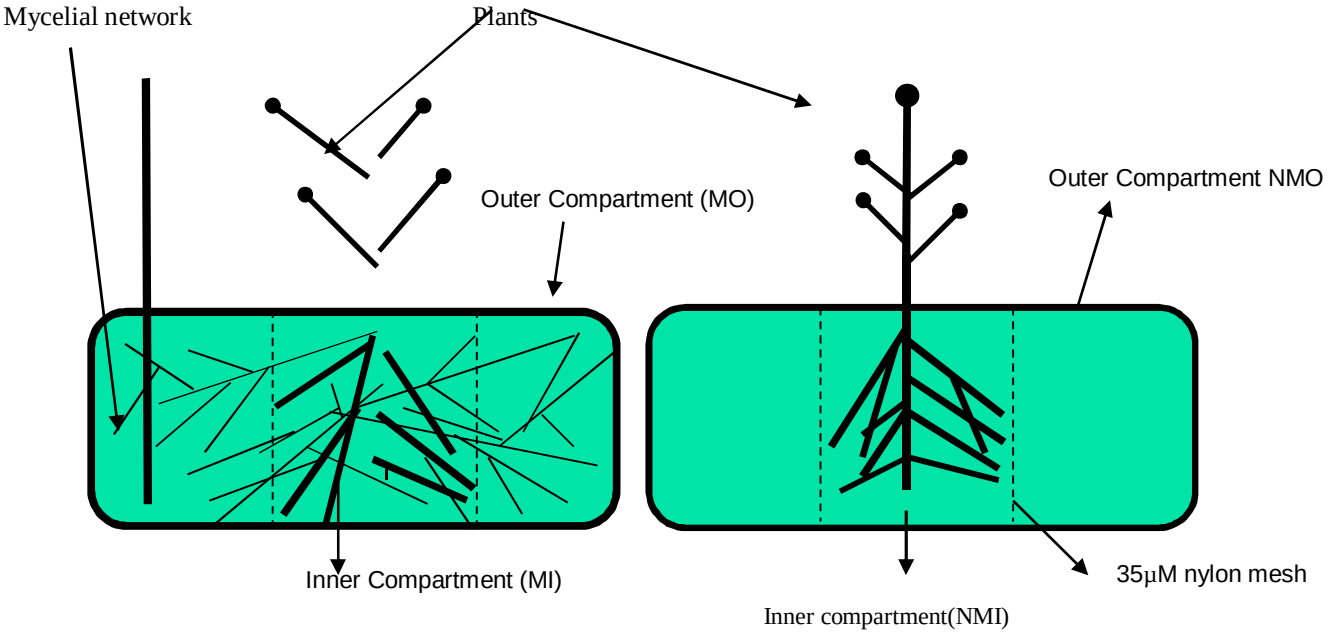

Figure :. Mycorrhizal Chamber (left) and Non-Mycorrhizal Chamber (right).

III. RESULTS

A. Non-host- Spergula arvensis (S. arvensis)

Growth and development of radicle

When seedlings of S. arvensis were exposed to M and NM extracts for 2, 4 and 6 days distinctive differences became progressively evident in their radicle lengths (Fig.2). In both MO and MI treatments, the lengths of radicles increased slowly with time but their growth was restricted relative to those in both NMO and NMI treatment (Fig.2). From day 4 of exposure, the radicle lengths were significantly greater in the NMI vs M treatment ($p<0.05$) and the NMO relative to the MO ($p<0.05$) treatment (Fig.2). These statistical differences were maintained at the subsequent harvest.

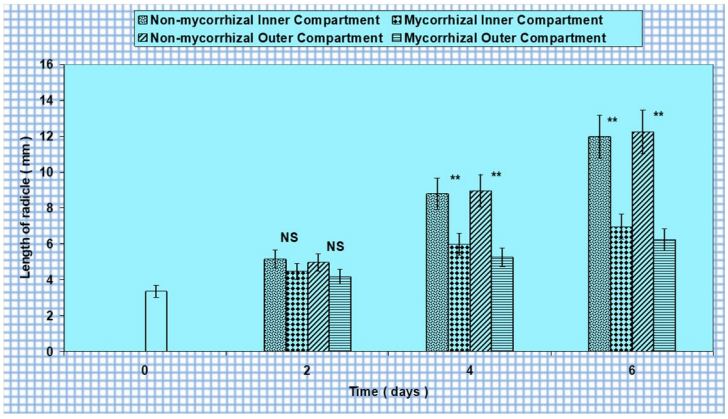

Figure 2: Length of radicle of seedlings of S. arvensis (** indicate significant differences at p< 0.05; NS- differences were not significant, p>0.05). Each value represents a mean of 6 replicates.

B. Production of Branches

No branches were produced in either of the M extracts (MO & MI) over the 2 and 4 days of exposure whereas branches appear at day 4 in both NMO and NMI extracts. By day 6, some branches were initiated in the M treatments. However the number of branches produced was significantly higher in both the NM extracts than in those with mycorrhiza (Fig.3).

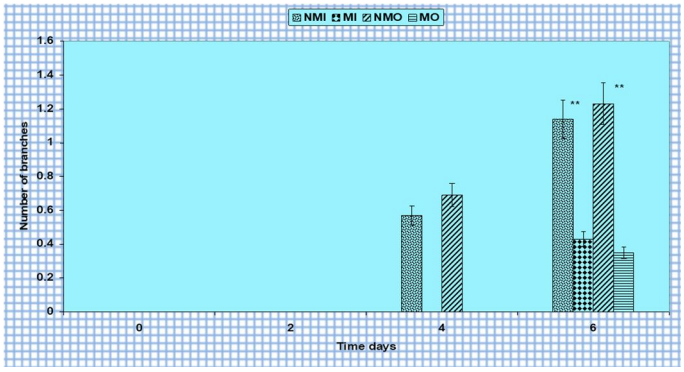

Figure 3: Number of branches on S. arvensis seedling radicles (** indicate significant differences at p< 0.05).

C. Production of Root Hairs

Analysis of the impacts of sand extracts upon production of root hairs revealed that while the number of root hairs increased progressively with time over the 6 day period in both NMI and NMO extracts, they increased only slightly in both MO and MI extracts .From day 4 of exposure, the number of root hairs was significantly higher in the NMI than in the MI treatment and in the NMO relative to the MO treatment. These statistical differences were maintained at the subsequent harvest (Fig.4).

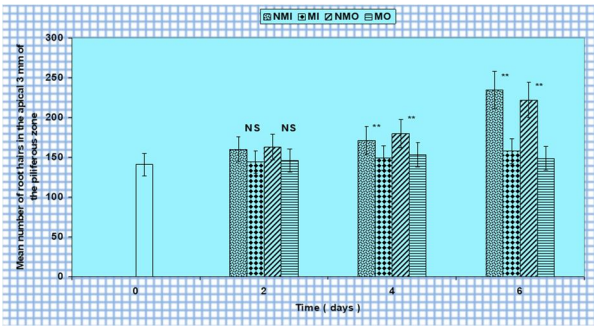

Figure 4: Mean number of root hairs of S. arvensis (** indicate a significant differences at p< 0.05; NS- differences were not significant, p>0.05).

D. Appearance Of Root Apices And Root Hairs

Distinctive differences were observed in the appearance of radicles of Spergula arvensis grown in M treatments and those grown in NM treatments (Figs 5,6 ,7 ,8). Swollen root hair tips and darkening of the apical meristems were observed in radicles exposed to M extracts whereas there were no such features in radicles exposed to NM extracts.

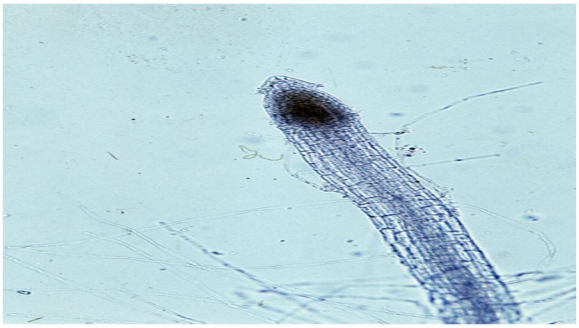

Figure 5: Light micrograph of part of radicle of S. arvensis collected from M extracts at the day 6 harvest, showing dark coloured apical region

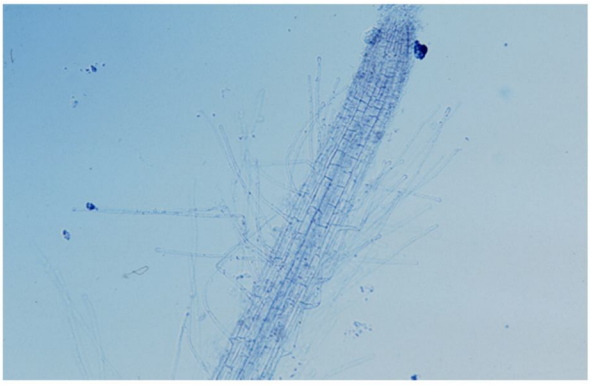

Figure 6: Light micrograph of part of radicle of S. arvensis collected from NM extracts at the day 6 harvest, showing transparent apical region

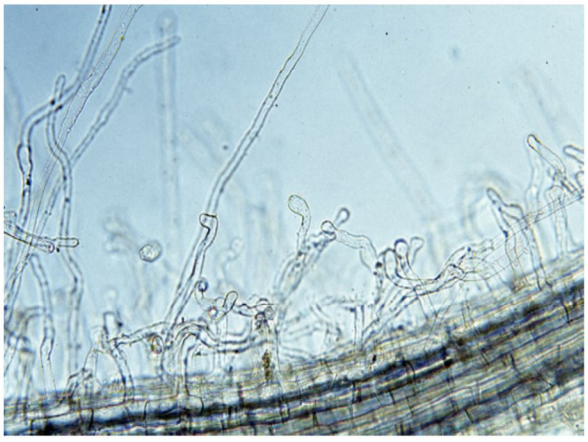

Figure 7: Light micrograph of root hairs of S. arvensis collected from M extracts at the day 6 harvest, showing swollen hair tips. 250X

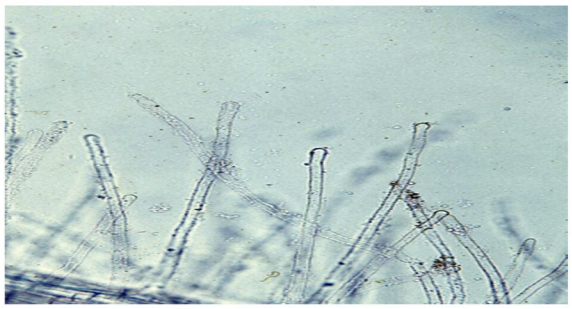

Figure 8 : Light micrograph of root hairs of S. arvensis collected from NM extracts at the day 6 harvest, showing absence of swellings. X250

E. Non-host- Arabis hirsute (A. hirsute)

Growth and development of radicle

Comparison of the impacts of sand extracts upon the growth and development of seedling radicles of A. hirsuta showed that while the lengths of the radicles increased progressively with time over the 6 day period in both NM extracts, they increased only slowly with time in extracts from both M compartments. At day 2 of exposure, the differences between the lengths of radicles grown in NM extract and those grown in M extract were not significant. By day 4, the lengths of NM radicles were significant greater in both NMO ($P< 0.05$) and NMI ($P< 0.05$) treatments than in either of the M treatments (Fig.9.). These statistical differences were maintained at the subsequent harvest (Fig.9)

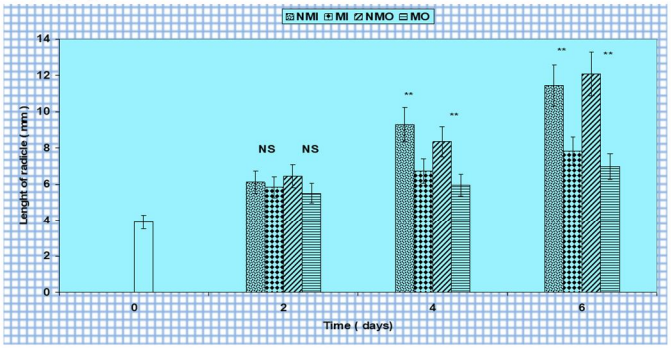

Figure 9: Lengths of radicles of seedlings of A. hirsuta (** indicate a significant differences at $p< 0.05$; NS- differences were not significant, $p>0.05$).

F. Production of Branches

No branches were produced in either of the NM or M extracts at day 2 harvest (Fig.10). Branches appeared at the day 4 stage in both NM and M extracts and at the subsequent harvest (Fig3.j). The numbers of branches were significantly greater in the NMI than in MI treatment and in the NMO than in MO treatment at day 4 and 6 harvests.

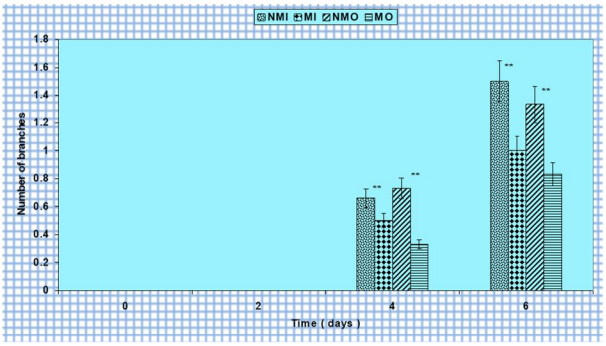

Figure 10: Number of branches A. hirsuta seedling radicle (** indicate significant differences at $p<0.05$).

G. Production of Root Hairs

There were no statistical significant differences in root hairs production between the NM and M extracts at 2 the day harvest (Fig.11). However, after 4 days of exposure to extracts, the number of root hairs was significantly higher in both NMO (P< 0.05) and NMI P< 0.05) treatments than in either of the M extracts (MO & MI). The statistical differences were maintained at the subsequent harvest (Fig.11).

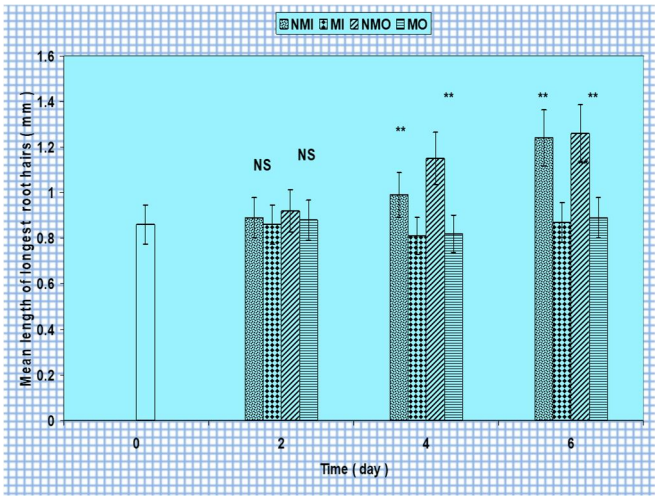

Figure 11: Mean number of root hairs of A. hirsuta (** indicate a significant differences at p< 0.05; NS- differences were not significant, p>0.05).

H. Appearance of root Apices and root Hair

There were distinctive differences in the appearances of radicles of seedlings of
A. hirsuta grown in M extracts relative to those grown in NM extracts (Figs.12,13). The most distinctive feature of radicles exposed to M extracts was the darkening of the apical meristems.

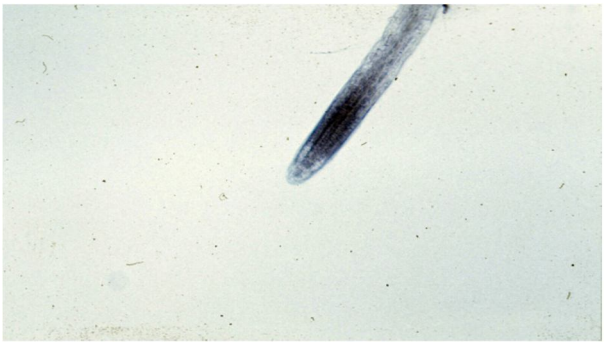

Figure 12: Light micrograph of part of radicle of A. hirsuta collected from M extracts at the day 6 harvest, showing dark coloured apical region and absence of root hairs.

Figure13:. Light micrograph of part of radicle of A. hirsuta from NM extracts at the day 6 harvest, showing light apical meriste.

I. (C. erythraea) Host species-Centaurium erythraea

Growth and development of radicle

When seedlings of C. erythraea were exposed to M and NM extracts for 2, 4 and 6 days, distinctive differences became progressively evident in their radicle lengths. From day 2, mycorrhizal extracts were seen to increase the growth and development of seedlings radicles compared with those grown in non-mycorrhizal extracts (Fig.14). There were no statistical significant differences in radicle lengths between M and NM extracts at day 2 and 4 harvests (Fig.14) By day 6 of the exposure, lengths of C. erythraea radicles were significantly higher in both MO ($P< 0.05$) and MI ($P< 0.05$) treatments than in either of NM treatments (NMO & NMI).

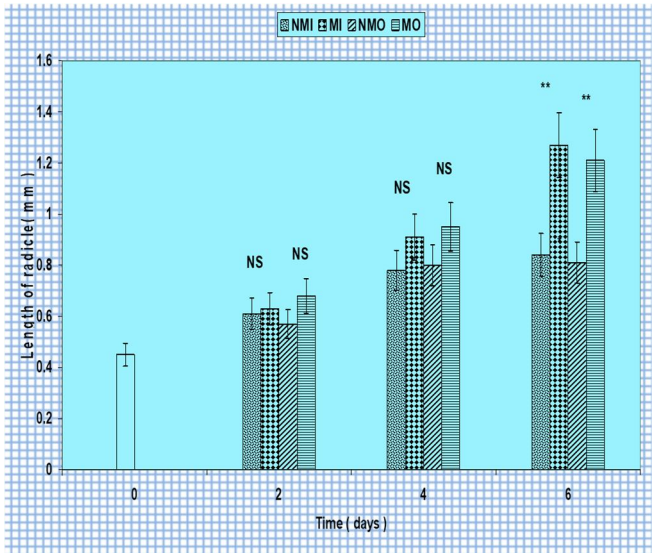

Figure14: Length of radicle of seedlings of C. erythraea (** indicate significant differences at $p< 0.05$; NS- differences were not significant, $p>0.05$). Each value represents 8 replicates.

J. Production of Root Hairs

Comparison of impacts of sand extracts upon production of root hairs showed that the number of root hairs was higher in both MO and MI treatments than in either of the NM extracts (Fig.15). No significant differences in the numbers of root hairs between MO and NMO treatments were found at any harvest (Fig.15). In the MI treatment, the number of root hairs was not significant at day 2 and 4 harvests. By day 6, there was significantly higher number of root hairs in MI relative to NMI treatment.

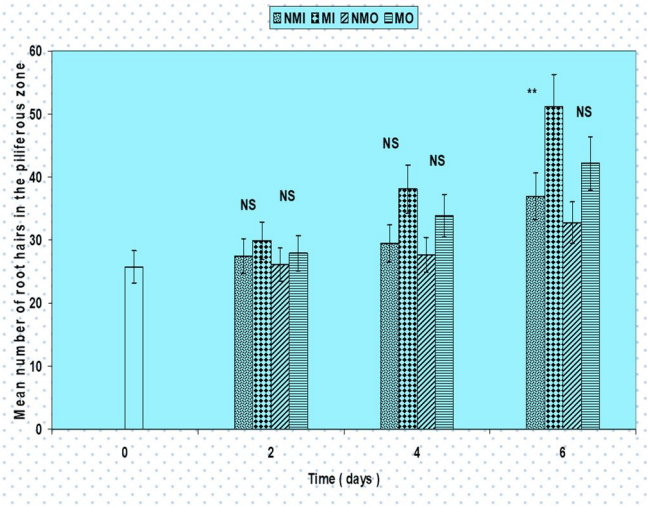

Figure15: Mean number of root hairs of C. erythraea (** indicate a significant differences at $p< 0.05$; NS- differences were not significant, $p>0.05$).

K. Appearance Of Root Apices And Root Hairs

No distinctive differences were observed in the appearance of radicles of C. erythraea grown in M treatments and those grown in NM treatments (Figs. 16,17). No swollen root hairs tips or darkening of the apical meristems were found in either of the M or NM treatments (Figs.16 ,17)..(

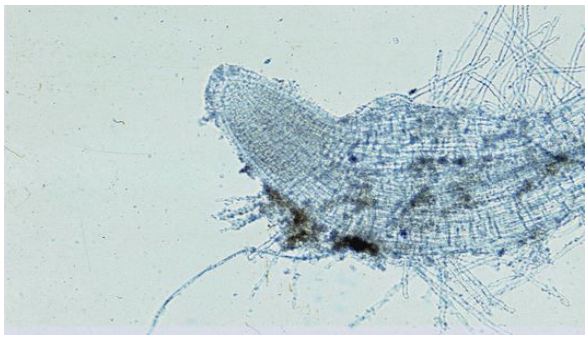

Figure16 : Light micrograph of part of radicle of C. erythraea collected from M extracts at the day 6 harvest, showing light apical region and absence of swellings. 100X

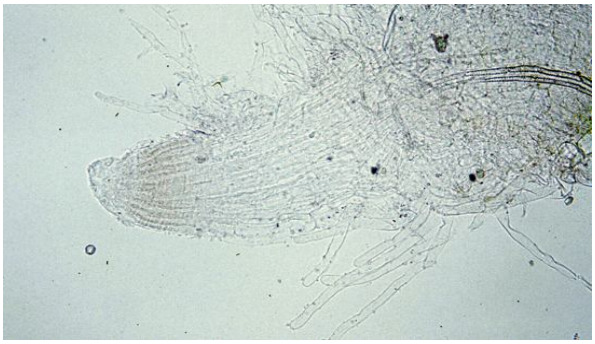

Figure 17: Light micrograph of part of radicle of C. erythraea collected from NM extracts at the day 6 harvest, showing transparent apical region and absence of swellings. 200X

IV. DISCUSSION

There has been a long history of debate about the causes of what Harper referred to as 'interference competition'[2]. This has led to a very extensive literature in which it is demonstrated repeatedly that the balance between species grown in mixture is changed by the addition of a particular nutrient, or of any of a number of other changes in the shared environment. The older of the experiments were reviewed by Rice[3]. None of the experiments described by Rice recognized the possibility that mycorrhizal fungi might be involved in the interactions. Experiments of the kind described by Francis and Read[1-4], and in this study, largely eliminate roots as being the potential source of any toxins both because roots are excluded from the bulk soil of the experimental systems and because roots of non-mycorrhizal donors fail to produce inhibitory effects. The demonstration here that active labile components can be extracted exclusively from AMF occupied compartments and that they are particularly potent inhibitors of root and root hair development in non-host plants is strongly indicative that the 'toxic substance' referred to by Fenner [5], was in fact the product of AMF mycelia. The analyses of the effects of aqueous extracts of AMF colonised soils confirm that labile antagonists are present and that these are potent inhibitors of extension growth of both roots and root hairs. In so far as inhibition of root extension, whether of the primary axis or the branches arising from it, restricts the differentiation of the piliferous zone these impacts upon the root must all be interactive. However, it is likely that interference with roots hair production, either directly or indirectly, is the key to the adverse effects of these toxins upon the plants. It is of interest to speculate on the possible nature and source of the 'toxic' component(s) found in aqueous extracts of AMF dominated soils. Until recently, few studies had contemplated the possibility that such mycelia left any physiological imprints other than P depletion, upon the soils through which they permeate. However, increasing awareness of the quantitative and qualitative importance of mycelial exudates and residues of AMF [6] . Jastrow, Miller and Lussenhop have changed perspectives on these matters[7]. Of particular interest in this connection is the release of compounds collectively referred to as 'glomalins'[8-9].

These, when exposed as residues by means of harsh hydrolytic extraction techniques (Wright et al., 1996) have been shown to be macromolecular glycoproteins. These are now thought to play key roles in soil aggregate formation[10-11]. Little is yet known of the biochemical characteristics of glomalin macromolecules or their precursors. However, it is increasingly recognised that there are both labile and non-labile forms of the glycoprotein, It is possible that by-products of these relatively labile components of the hyphal exudates are the sources of toxicity observed in the present study. Alternatively, since the whole hyphal surface of AMF is thought to be covered with glomalin-like material, it could be that the adverse effects seen, for example, as deformation of root-hairs, may arise from direct contact between these structures and coated hyphal surfaces ,the possibility also remains that the toxic agents are chemically quite distinct from glomalins

The appearance of the symptoms on non-hosts, most notably the browning of the meristems and distortion of the root hairs are indicative of toxicity rather than nutrient deficiency .

The most likely explanation for the observed effects is that the non-hosts are sensitive to factors, released by the AMF, which specially target those species with which the fungus is not compatible.

V. CONCLUSION

It be concluded that, the appearance of the symptoms on non-hosts, most notably the browning of the meristems and distortion of the root hairs are indicative of toxicity rather than nutrient deficien

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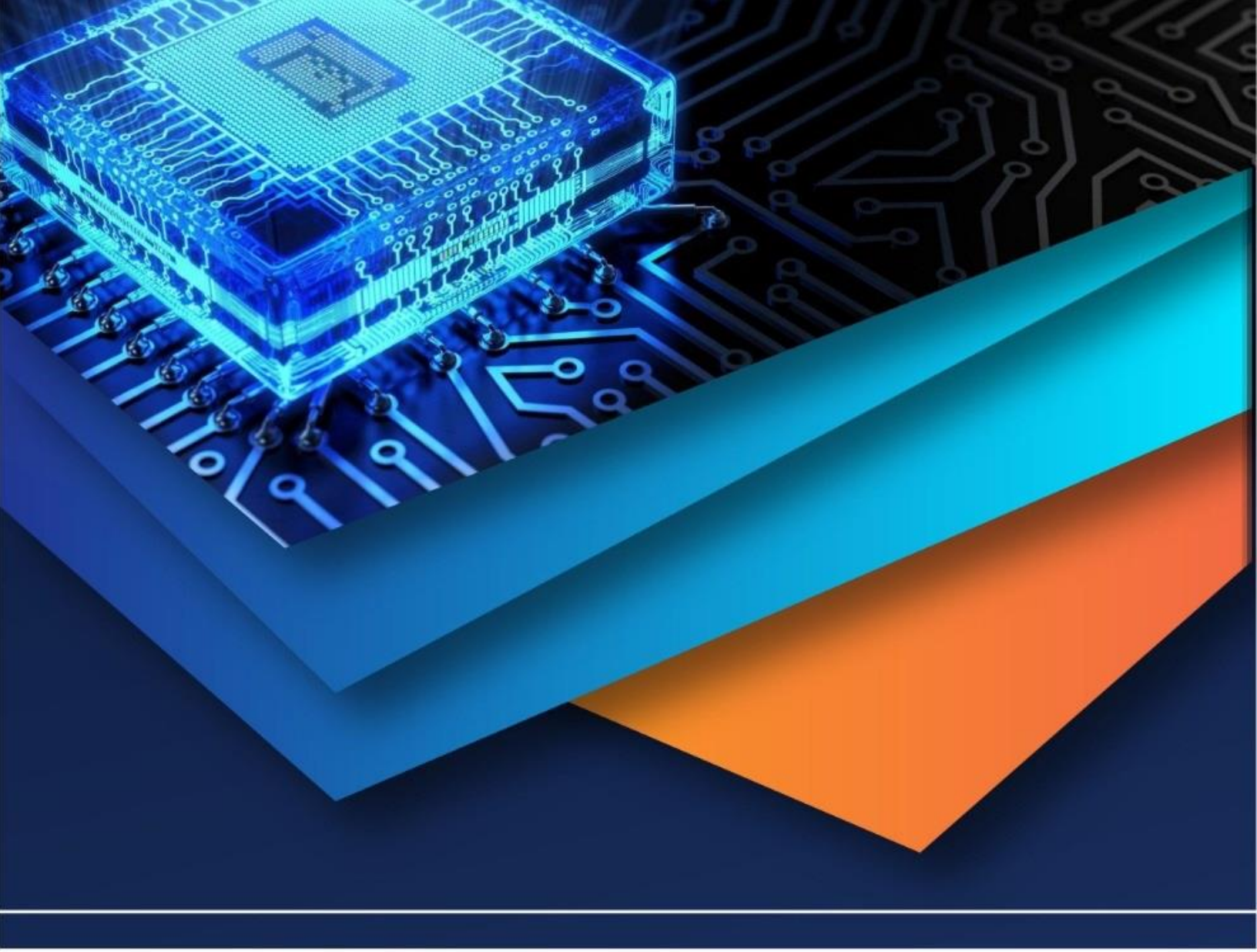

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