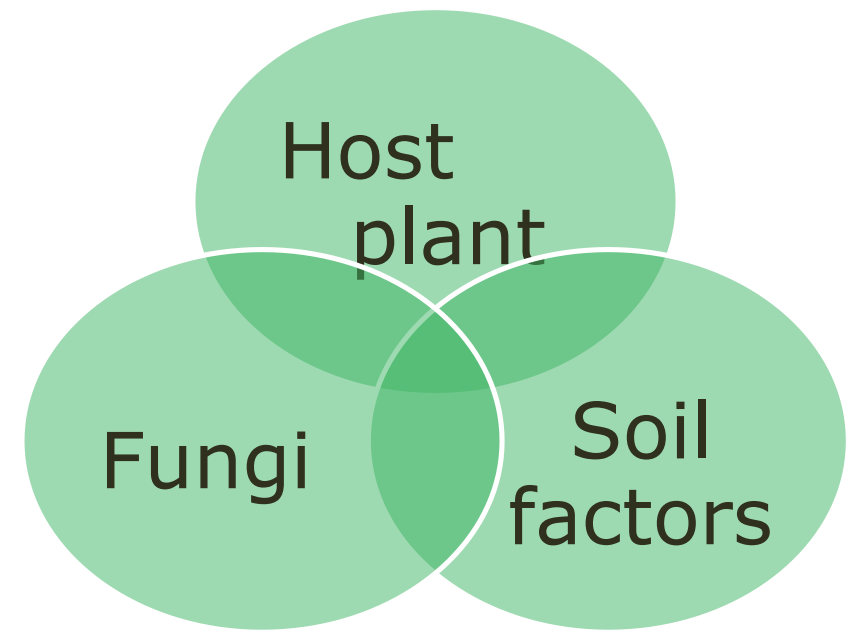


# **MYCORRHIZAL FERTILIZER**

PROSAINTA SAHA

- ❖ **Mutualistic association between plant roots and fungi**, both organisms benefit from the associations.
- ❖ **The fungus receives carbohydrates (sugars) and growth factors from the plant, which in turn receives many benefits, including increased nutrient absorption.**
- ❖ **In this association, the fungus takes over the role of the plant's root hairs and Acts as an extension of the root systems.**
- ❖ **Mycorrhizae** are highly evolved, mutualistic associations between soil fungi and plant roots. It is commonly known as **root fungi**.
- ❖ This association are members of the fungus kingdom (**Basidiomycetes, Ascomycetes and Zygomycetes**) and most vascular plants.
- ❖ Host plant receives mineral nutrients while the fungus photosynthetically derived carbon compounds from the plants.
- ❖ **In absence of host roots, the VAM fungi remain in soil as spores, which germinate near host roots and colonize there..**



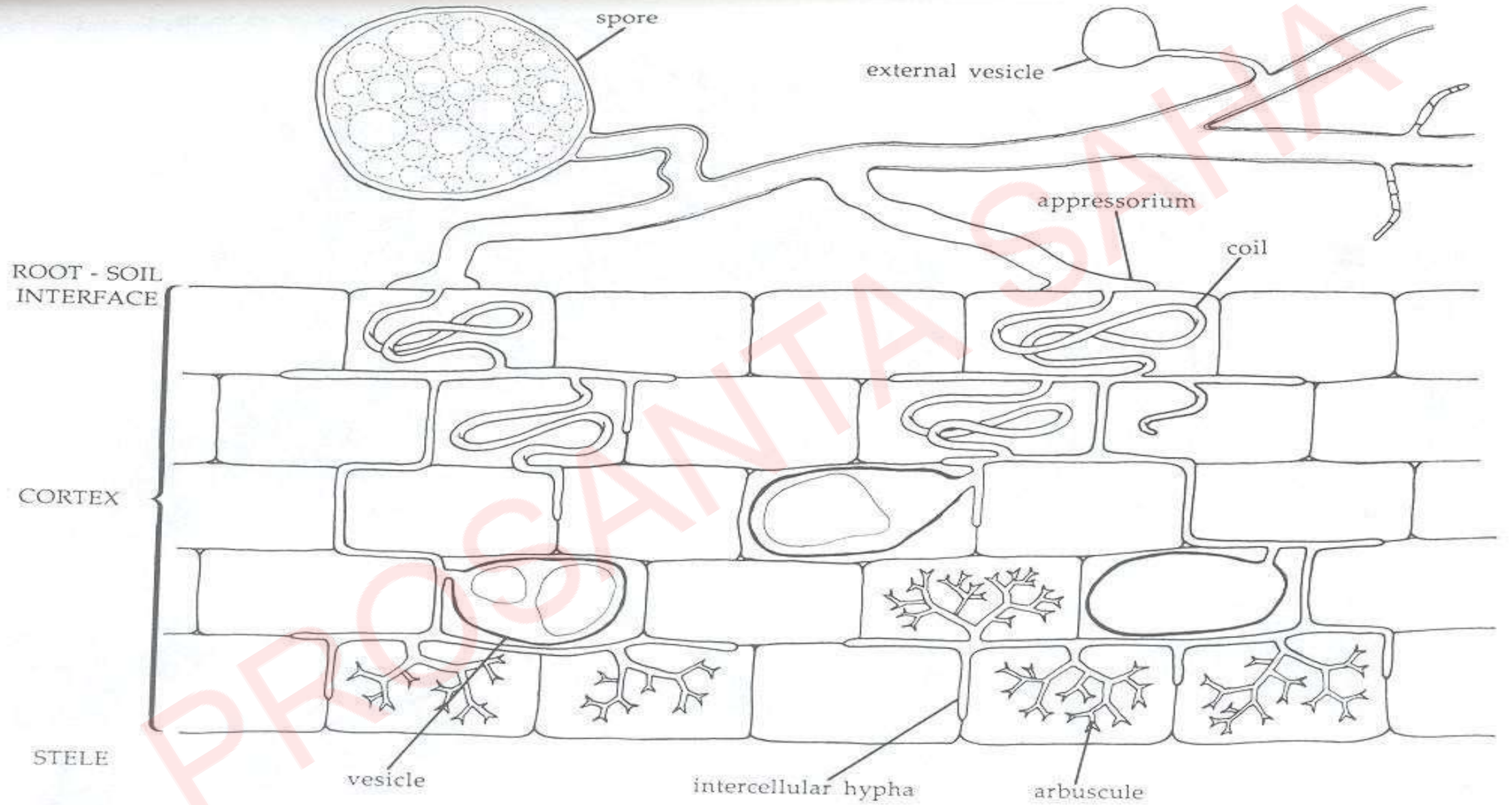
# Types of Associations

- ❖ Mycorrhizas are commonly divided into **ectomycorrhizas** (extracellular) and **endomycorrhizas** (Intracellular).
- ❖ The two types are differentiated by the fact that the hyphae of
  - **ectomycorrhizal fungi do not penetrate individual cells within the root**
  - **endomycorrhizal fungi penetrate the cell wall and invaginate the cell membrane.**
- ❖ Mycorrhizas are variable and have been further classified as **arbuscular (endomycorrhiza), ericoid (endo), arbutoid (ectomycorrhiza), monotropoid (ecto), and orchid (endo) mycorrhizas.**
- ❖ Arbuscular mycorrhizas, or AM (formerly known as **vesicular-arbuscular mycorrhizas, or VAM**), are mycorrhizas whose **hyphae enter into the plant cells**, producing structures that are either balloon-like (**vesicles**) or dichotomously branching invaginations (**arbuscules**).
- ❖ **Ectomycorrhizas, or EcM**, are typically formed between the roots of around **10% of plant families**, mostly woody plants including the birch, dipterocarp, eucalyptus, oak, pine families, and fungi belonging to the **Basidiomycota and Ascomycota**.
- ❖ Some EcM fungi, such as many **Leccinum** and **Suillus**, are symbiotic with **only one particular genus of plant**, while other fungi, such as the **Amanita**, are generalists that form mycorrhizas with many different plants.

- **Fungi exist as multi-nucleated round shaped resting spores.**
- **Fungi uses its triglyceride, glycogen reserves.**
- **Under suitable water, temp. conditions-  
Spores germinate & nuclei from the spore move into the extending mycelium.**
- **If host root absent- growth ceases (2-4 weeks)**

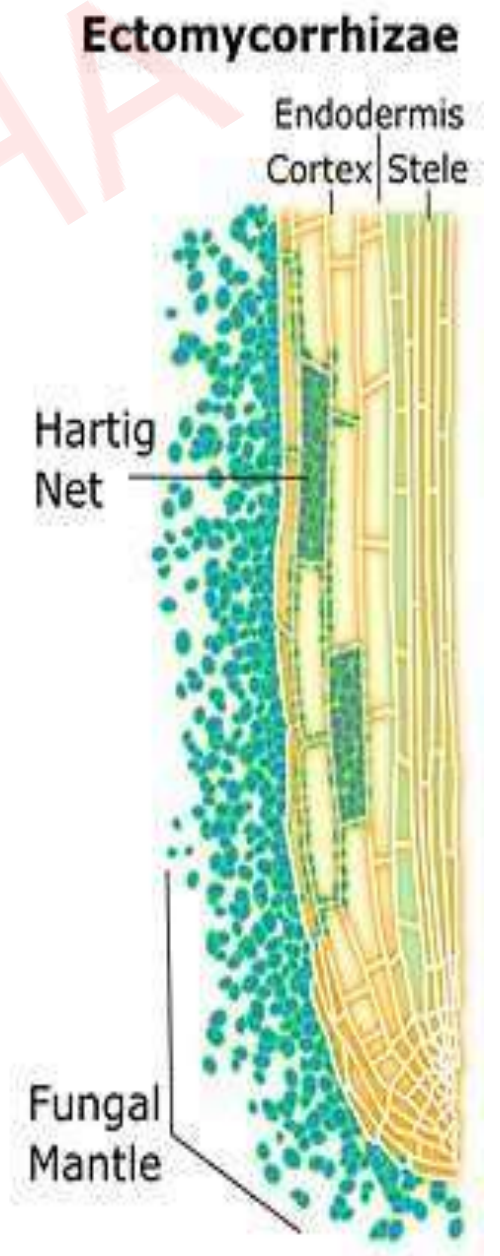
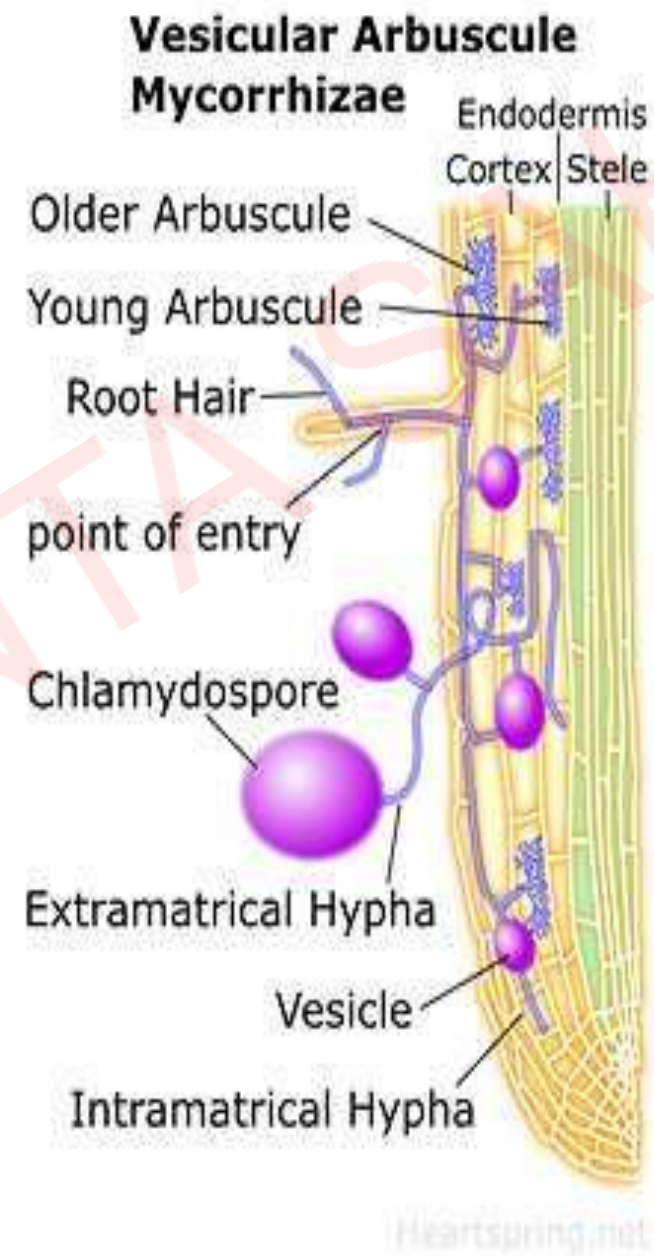
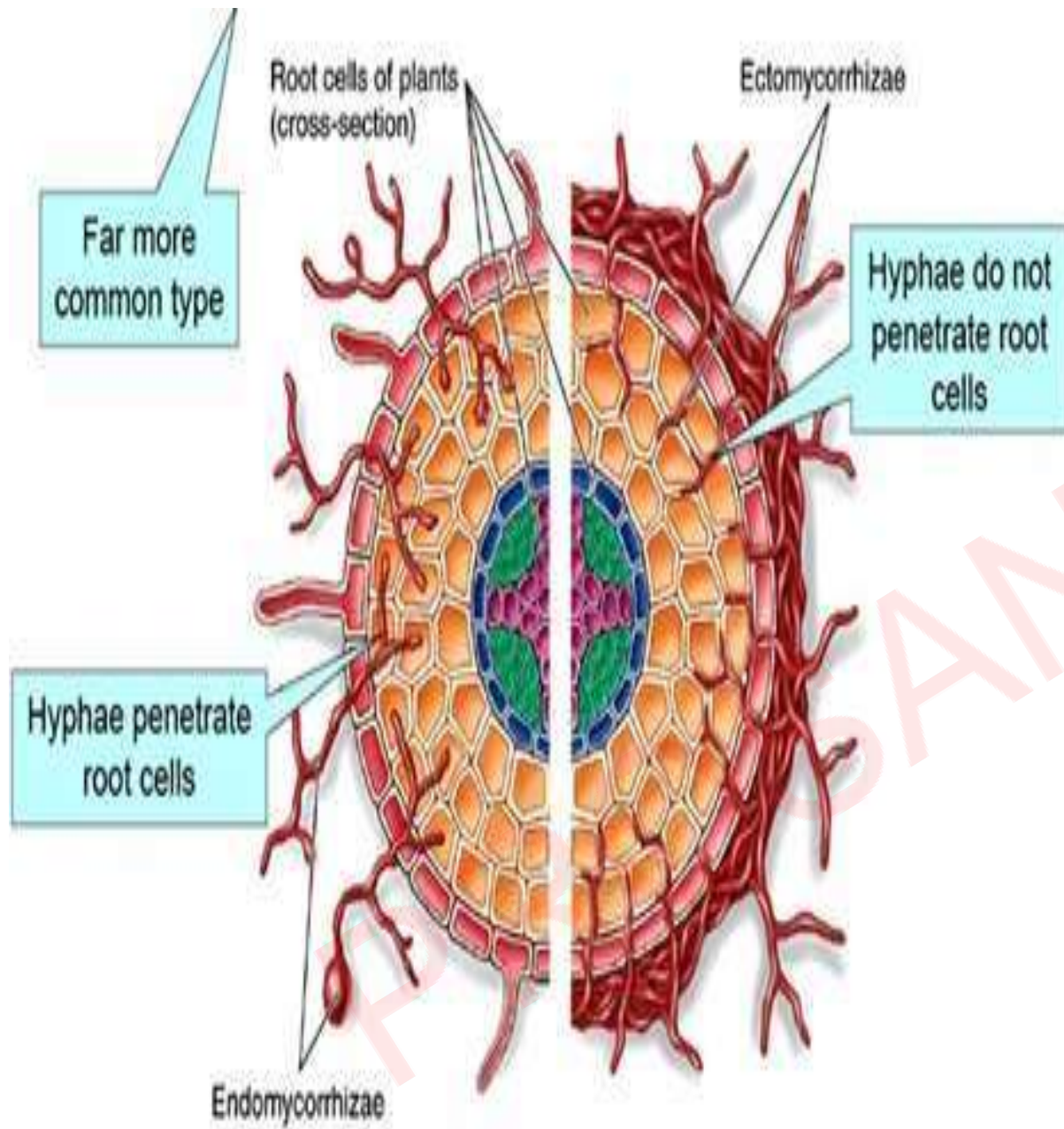
## **II. SYMBIOTIC STAGE**

- **Begins with the colonization of hyphae with compatible root.**
- **After attachment-**
  - i. Appresorium formed (fungus enters in cortex).**
  - i. Formation of specialized structures- inter- & intra- cellular hyphae, coils, arbuscules.**



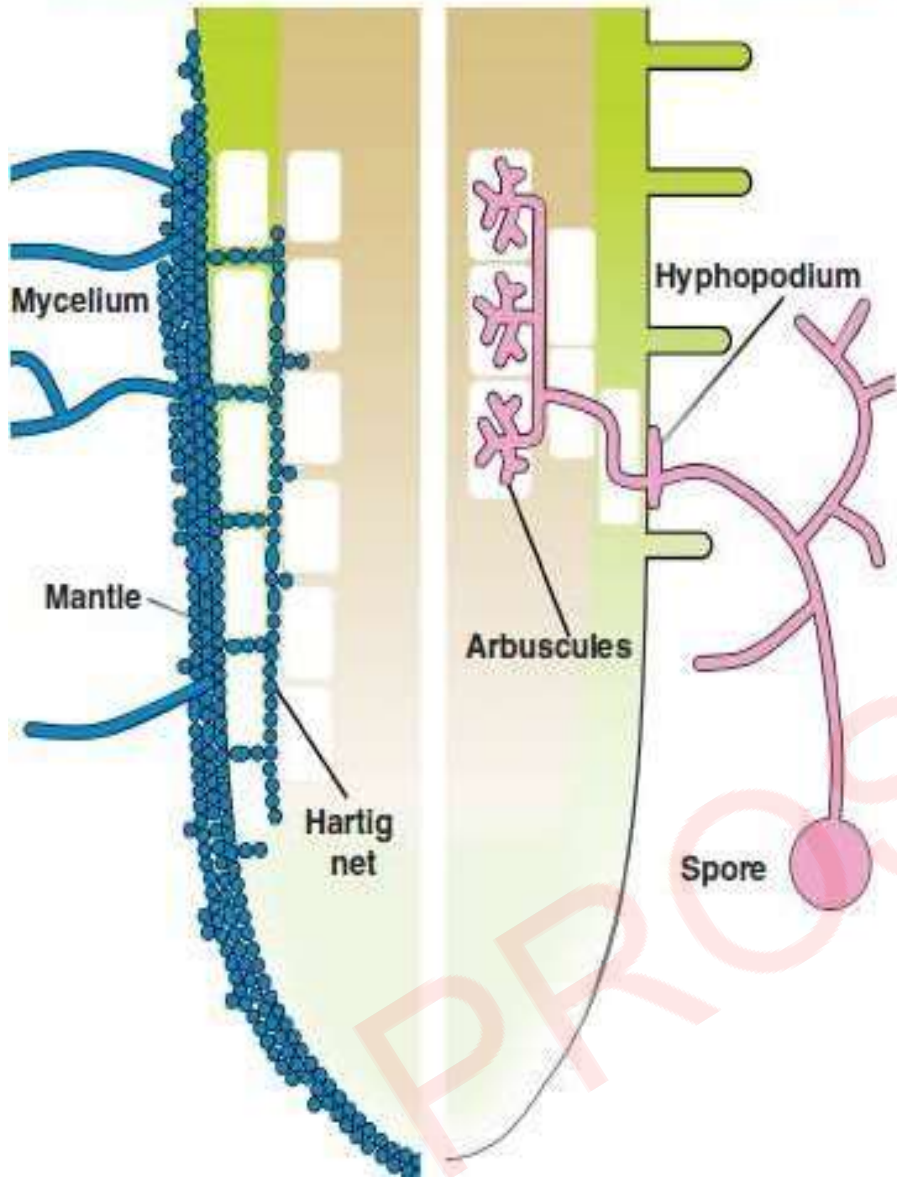
**Fig. 5.4** The relationship between the fungus and the host in vesicular-arbuscular mycorrhizal relationships. New arbuscules are formed and older ones become senescent continually during the relationship.





### Ectomycorrhiza

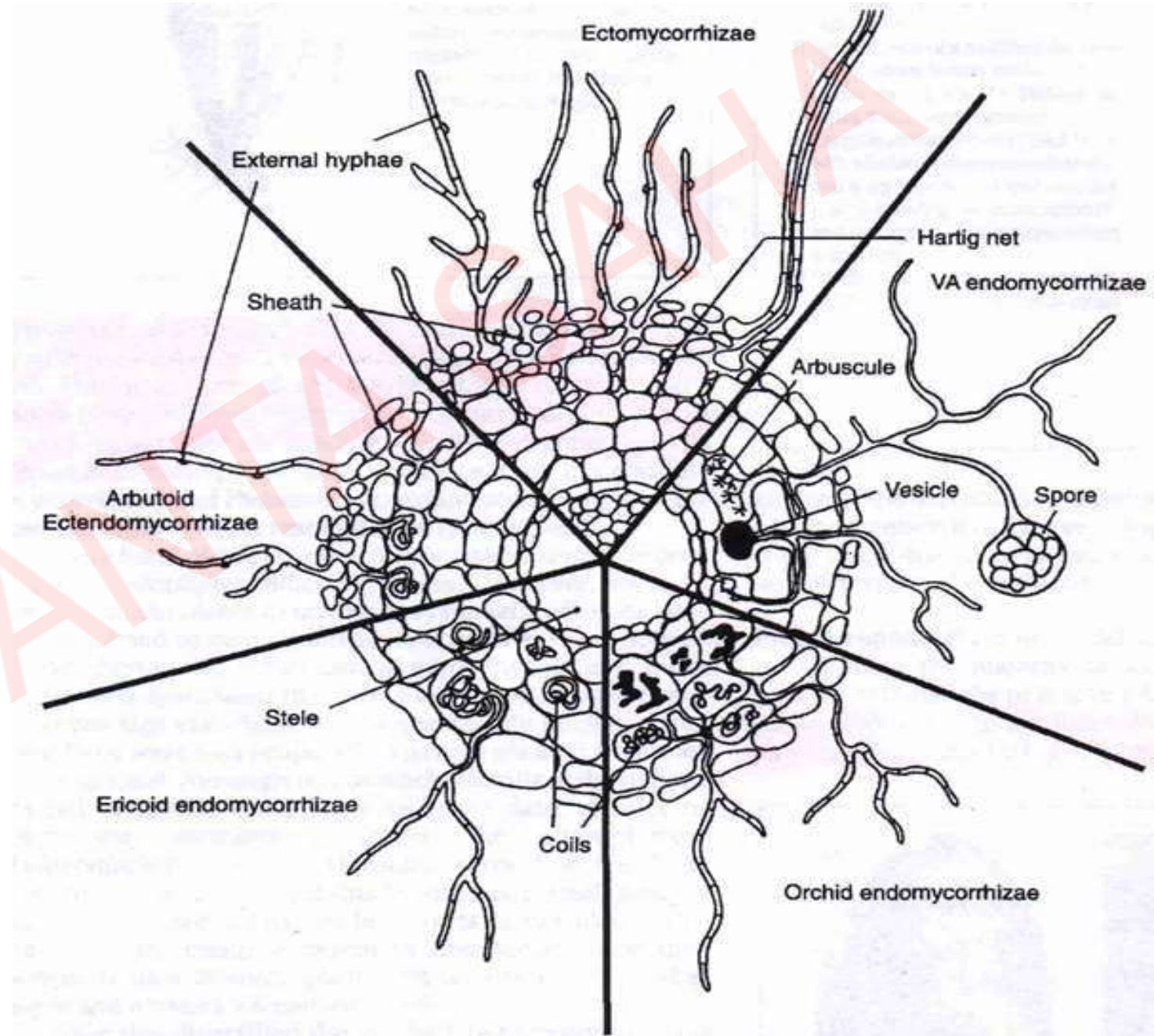
### Arbuscular mycorrhiza



Endomycorrhizae	Ectomycorrhizae
Generally fungi produce its typical structures, vesicles and arbuscules inside the root system.	Fungi produce majority of its structure outside the root system.
Commonly associated with agricultural, horticultural and tropical trees.	Commonly associated with trans temperate forest tree roots.
Have a loose network of hyphae in the soil and an extensive growth within the cortex cells of the plants.	Form a complete mantle or sheath over the surface of the rot and hyphae grows out into the soil.
Cannot be cultured on artificial media.	Can be cultured on artificial media.
Doesn't cause morphological changes in roots.	Cause morphological changes in roots.

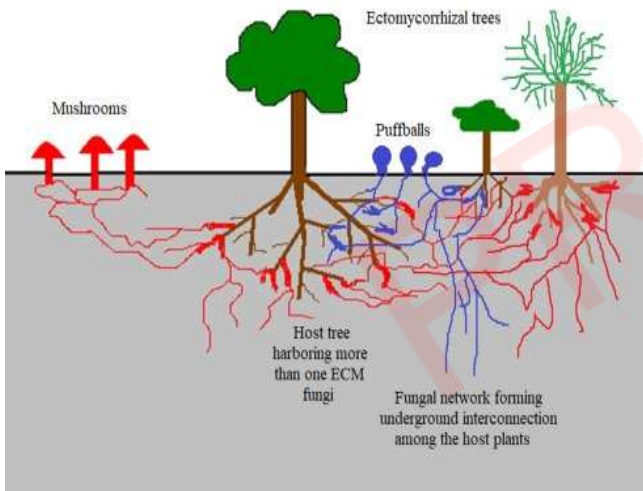
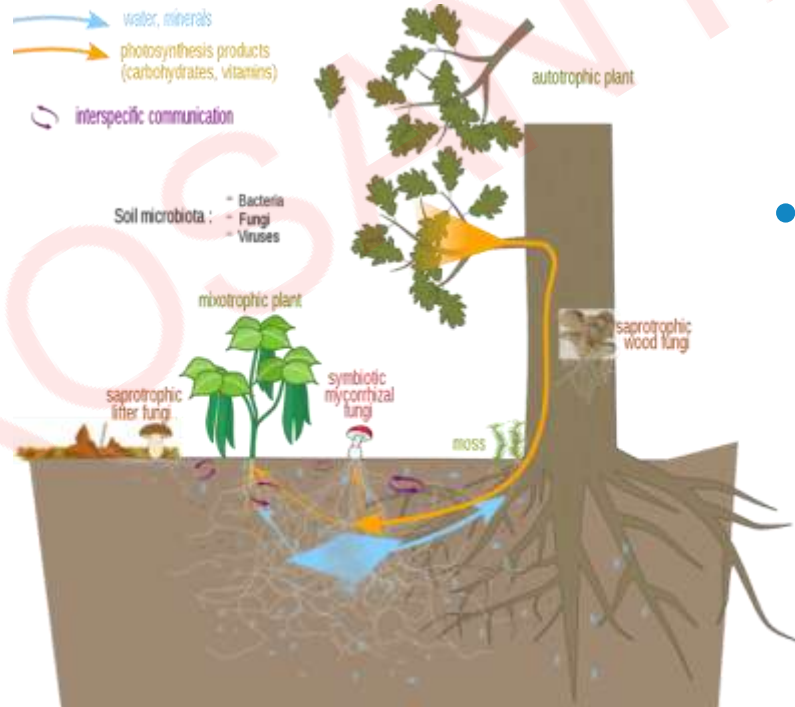
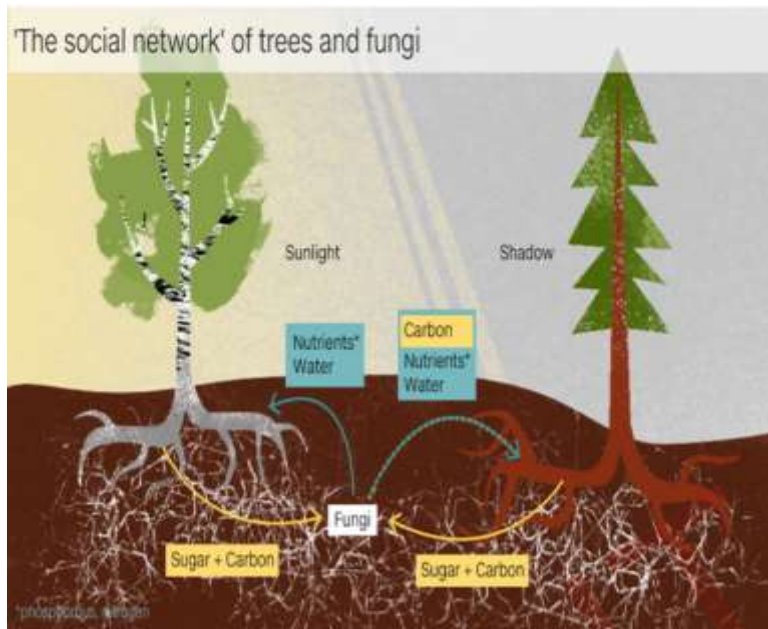
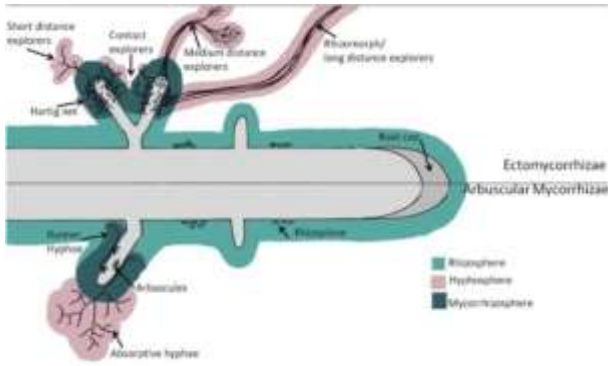


- Ectomycorrhiza (EcM)
- Endomycorrhiza (AM / VAM)
- Ectendomycorrhiza
- Monotropoid mycorrhiza
- Arbutoid mycorrhiza
- Orchid mycorrhiza
- Ericoid mycorrhiza





Mycorrhiza	Host range	Types of relationship
Ectomycorrhiza	Gymnosperms and Angiosperms	Sheath, intercellular hyphae
Endomycorrhiza (VAM)	All groups of plant kingdom	Coiled intracellular hyphae, vesicle and arbuscules present
Ectendomycorrhiza	Gymnosperms and Angiosperms	Sheath optional, inter and intracellular hyphae
Monotropoid mycorrhiza	Very restricted, Monotropaceae	Sheath, inter and coiled intracellular hyphae
Arbutoid mycorrhiza	Very restricted, Ericales	Sheath, inter and coiled intracellular hyphae
Orchid mycorrhiza,	Restricted, Orchidaceae	Only coiled intracellular hyphae
Ericoid mycorrhiza	Very restricted, Ericales	No sheath, no intercellular hyphae, long, coiled

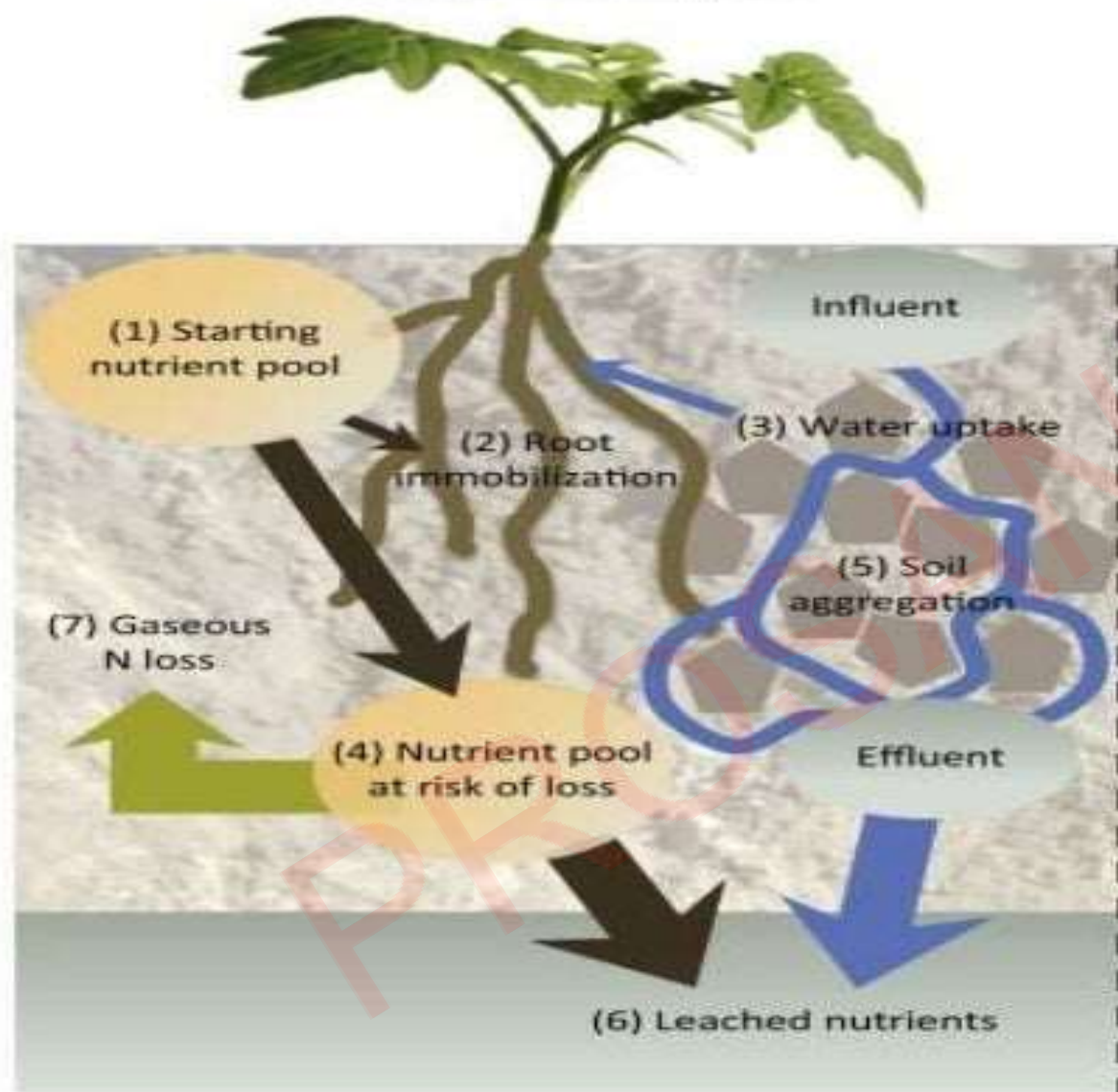


- **Wood wide web' (www)**—**Dr. Suzanne Simard** (1997). It is the **underground network of microbes that connects...** Millions of species of fungi and bacteria swap nutrients between soil and the roots of trees, forming a vast, interconnected **web** of organisms throughout the woods.
- Research has shown that beneath every forest and wood there is a complex underground web of roots, fungi and bacteria helping to connect trees and plants to one another.
- In the past few years, researchers have demonstrated that plants connected to the wood-wide web can **exchange more than just nutrients**. When broad bean plants come under attack by aphids, they release chemicals that not only repel their attackers, but also attract wasps that prey on the aphids.

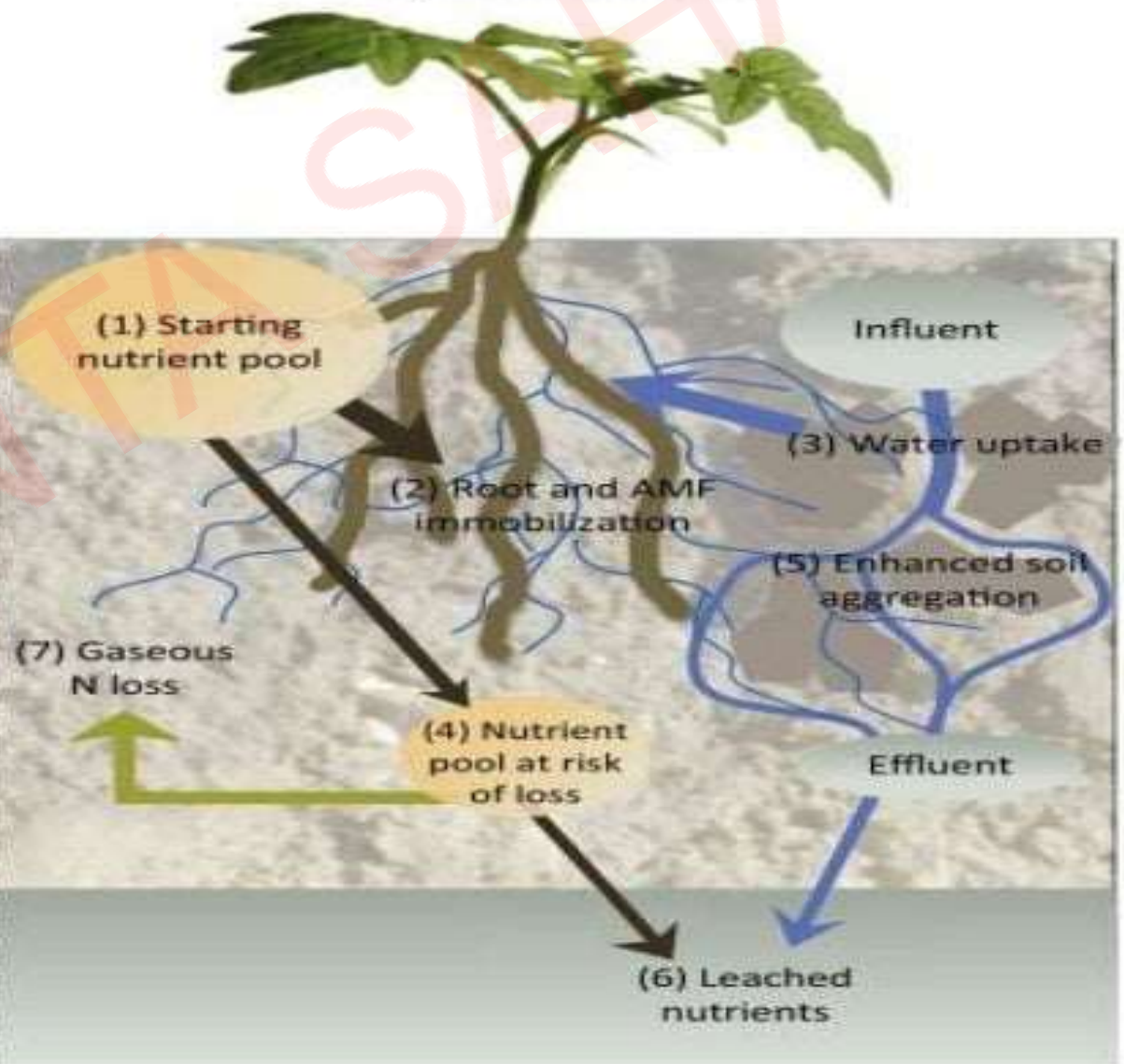




**Non-mycorrhizal plant**



**Mycorrhizal plant**





# ECTOMYCORRHIZA BIOFERTILIZER

- Inoculum production using **basidiospore, chopped sporocarp, sclerotia, fragmented mycorrhizal root, etc.**
- **MycoRhiz – Commercial formulation....Mycelial inoculum of *Pisolithus tinctorius*. In an vermiculite / peat substrate.**
- **For biofertilizer production , grow *Pisolithus tinctorius* in solid culture of MMN medium at 25deg C for 2-3 weeks and then transfer agar pieces to liquid MMN medium for mass culture.**
- **Solid substrate Fermentor: Steam sterilization of vermiculite, liquid MMN, vermiculite, carbohydrate and nitrogen source at 80deg C.....**
- **Cool and inoculate with *Pisolithus tinctorius*.**
- **MYCOBEADS..... Hydrogel beads of *Laccaria, Discolea, etc, EcM species***

The MMN medium presented the following composition (g.L<sup>-1</sup>): **glucose 10.0; malt extract 3.0; CaCl<sub>2</sub> 0.05; NaCl 0.025; (NH<sub>4</sub>)<sub>2</sub>PO<sub>4</sub> 0.25; KH<sub>2</sub>PO<sub>4</sub> 0.50; MgSO<sub>4</sub>.**

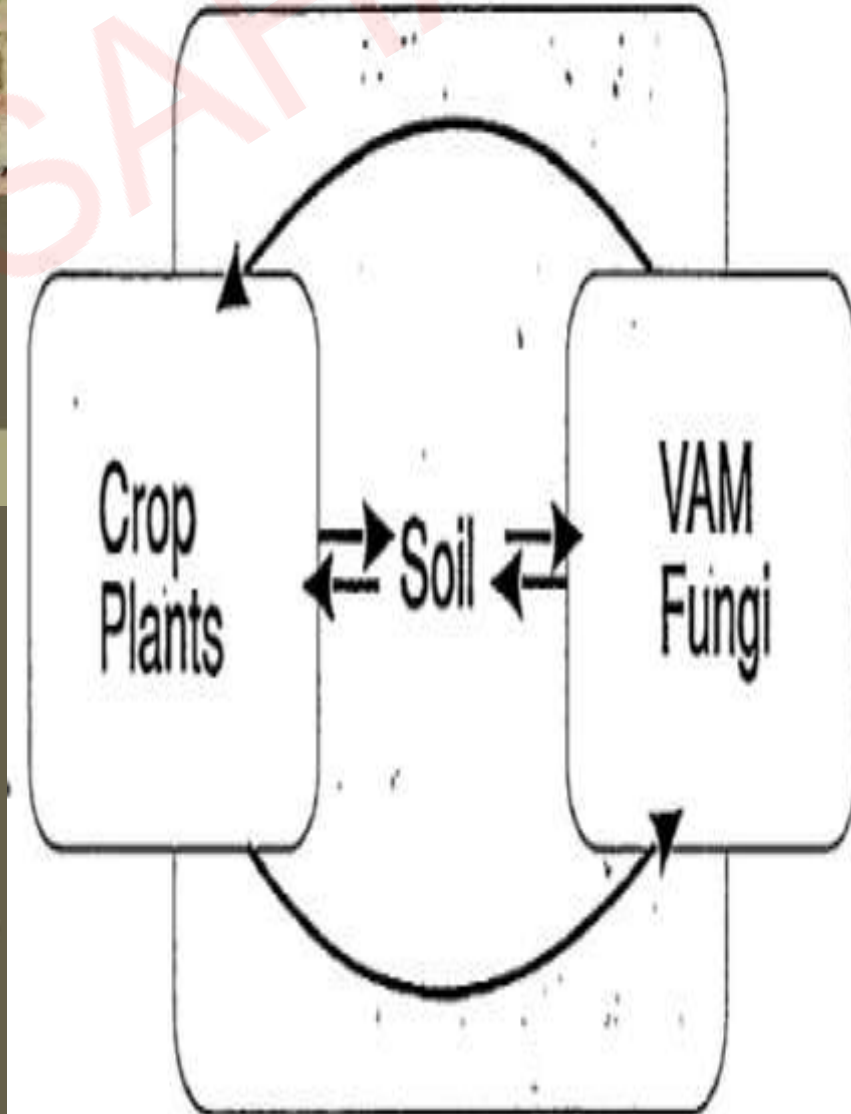
# Vesicular Arbuscular Mycorrhiza (VAM)

- ➔ The term mycorrhiza was taken from Greek language meaning '**fungus root**'. term was coined by Frank in 1885
- ➔ The mycorrhiza is a mutualistic association of fungal mycelia and plant roots.
- ➔ VAM is an endotrophic (live inside) mycorrhiza formed by aseptated phycomycetous fungi.
- ➔ VAM help in **nutrient transfer** mainly of phosphorus, zinc and sulfur.



- ➔ Mycorrhizae is the symbiotic association between plant roots and soil fungus of the 7 types of mycorrhizae,
- ➔ VAM plays a great role in inducing plant growth.
- ➔ VAM are symbiotic entophytic soil fungi, which colonize the roots of approximately 80% plants.
- ➔ The VAM hyphae also help in retaining moisture around the root zone of plants
- ➔ It increases the resistance to root borne or soil borne pathogens and Nematodes.

## Mycorrhizal Systems

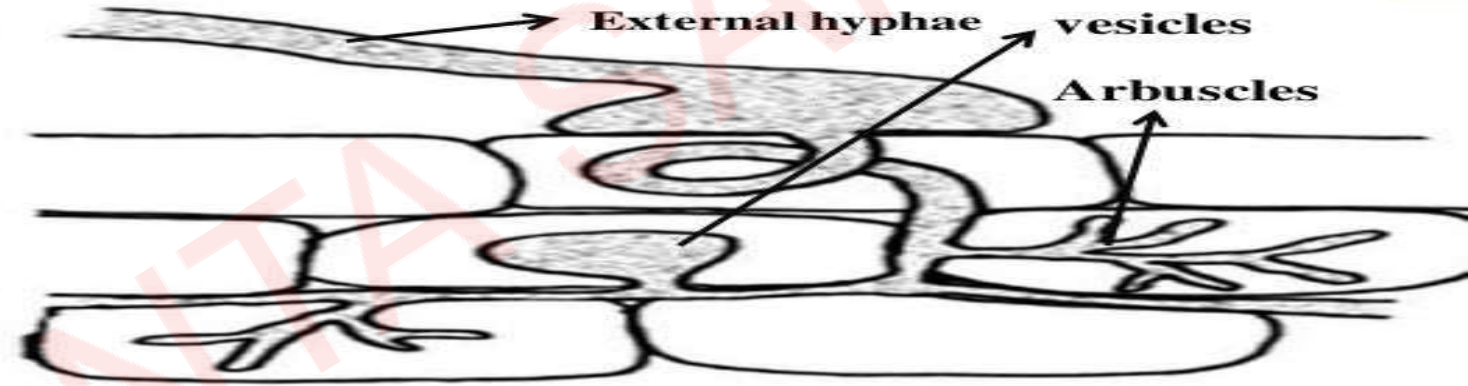




- ➔ They also **mobilize different nutrients** like Cu(copper), K(potassium), Al(aluminum), Mn(manganese), Fe (iron)and Mg (magnesium) from the soil to the plant roots.
- ➔ They posses vesicles (sac like structure) for storage of nutrients and arbuscular for funneling them into root system.

## Morphology

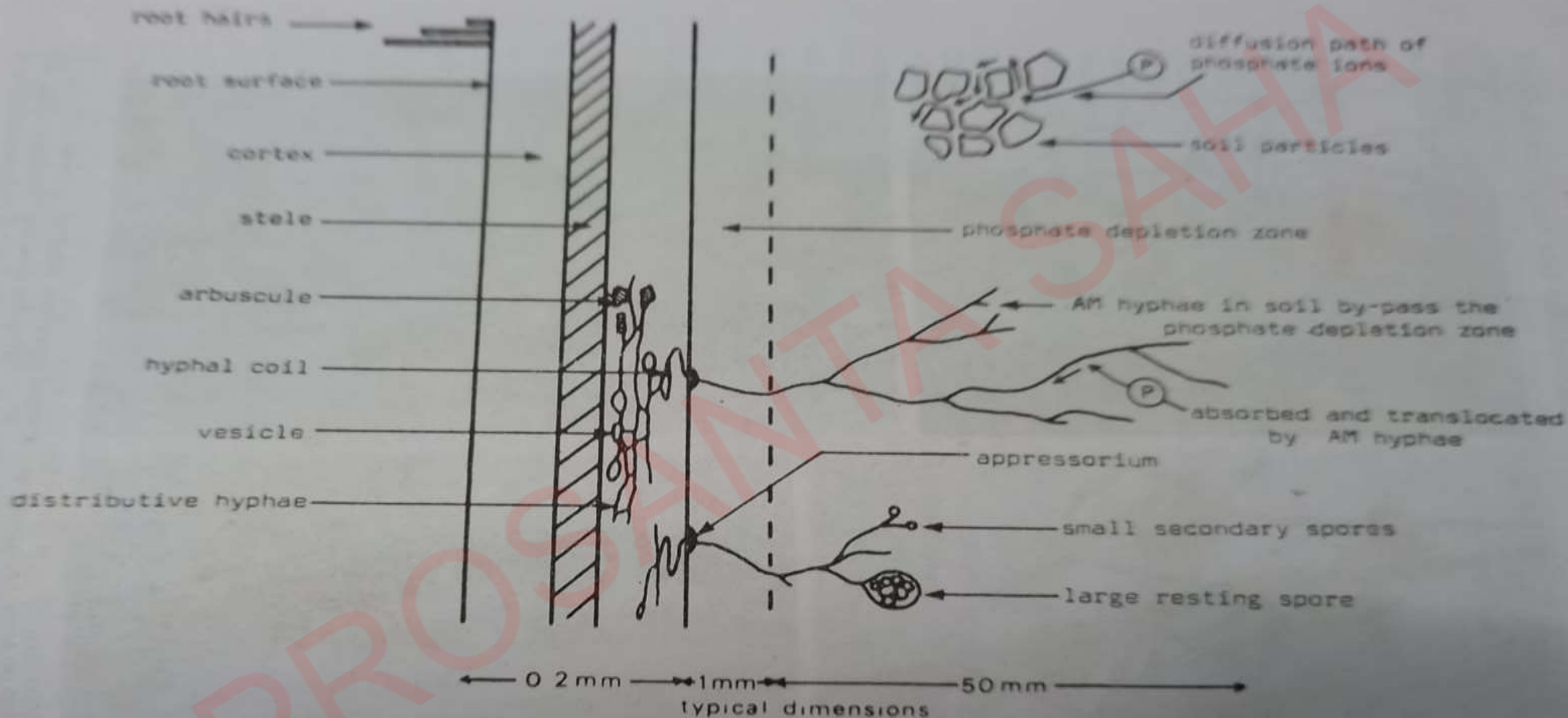
- ✓ External hyphae
- ✓ Arbuscles
- ✓ Vesicles



## Mechanism of Action

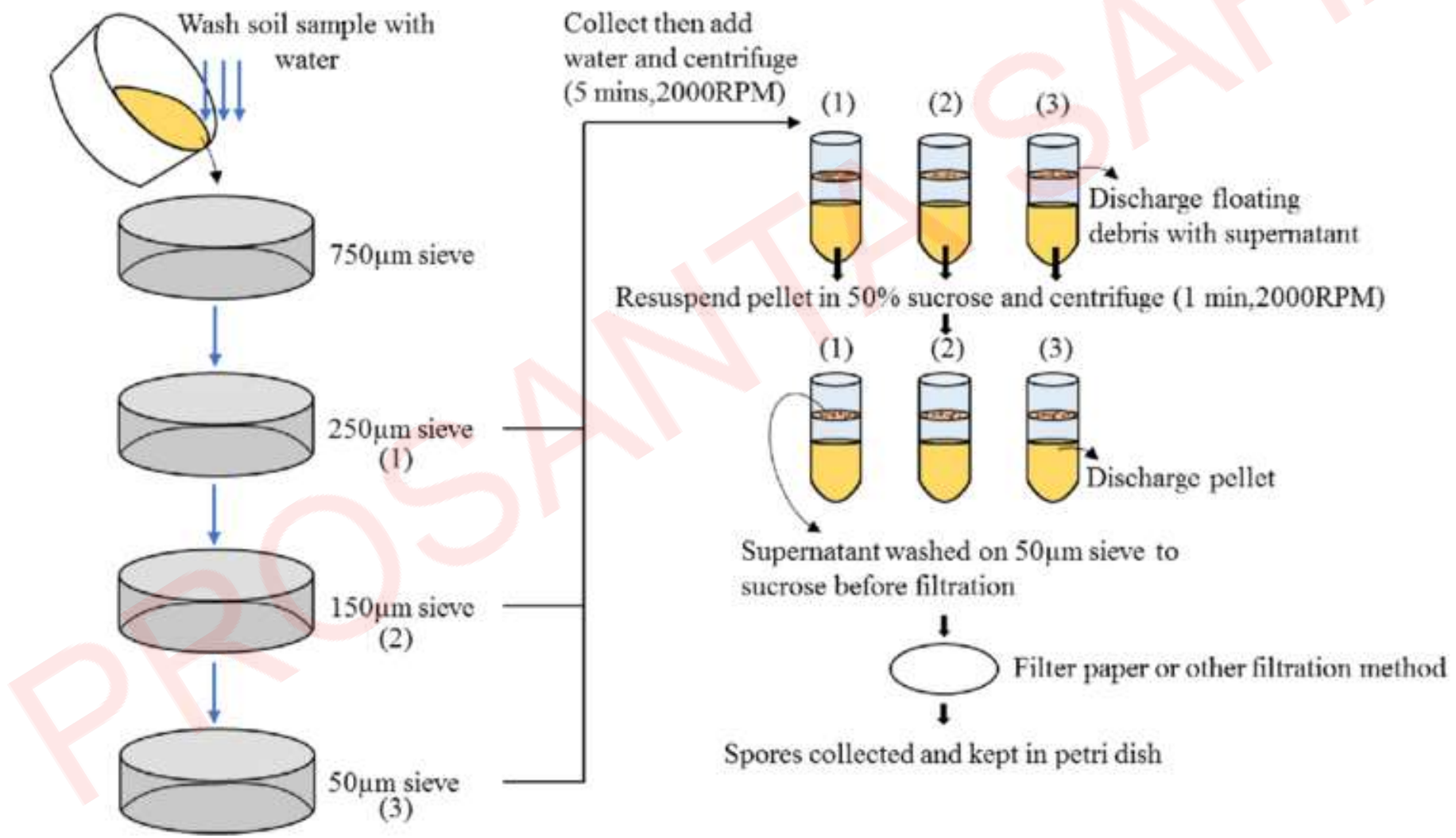
- ➔ The VAM forms an association with plant roots.
- ➔ It **penetrates in the root cortex** and spreads around the roots of the plant.
- ➔ As the name indicates, they posses sac like structure called **vesicules** which stores phosphorus as phospholipids.
- ➔ The other structure called arbuscule helps bringing the distant nutrients to the vesicules and root.

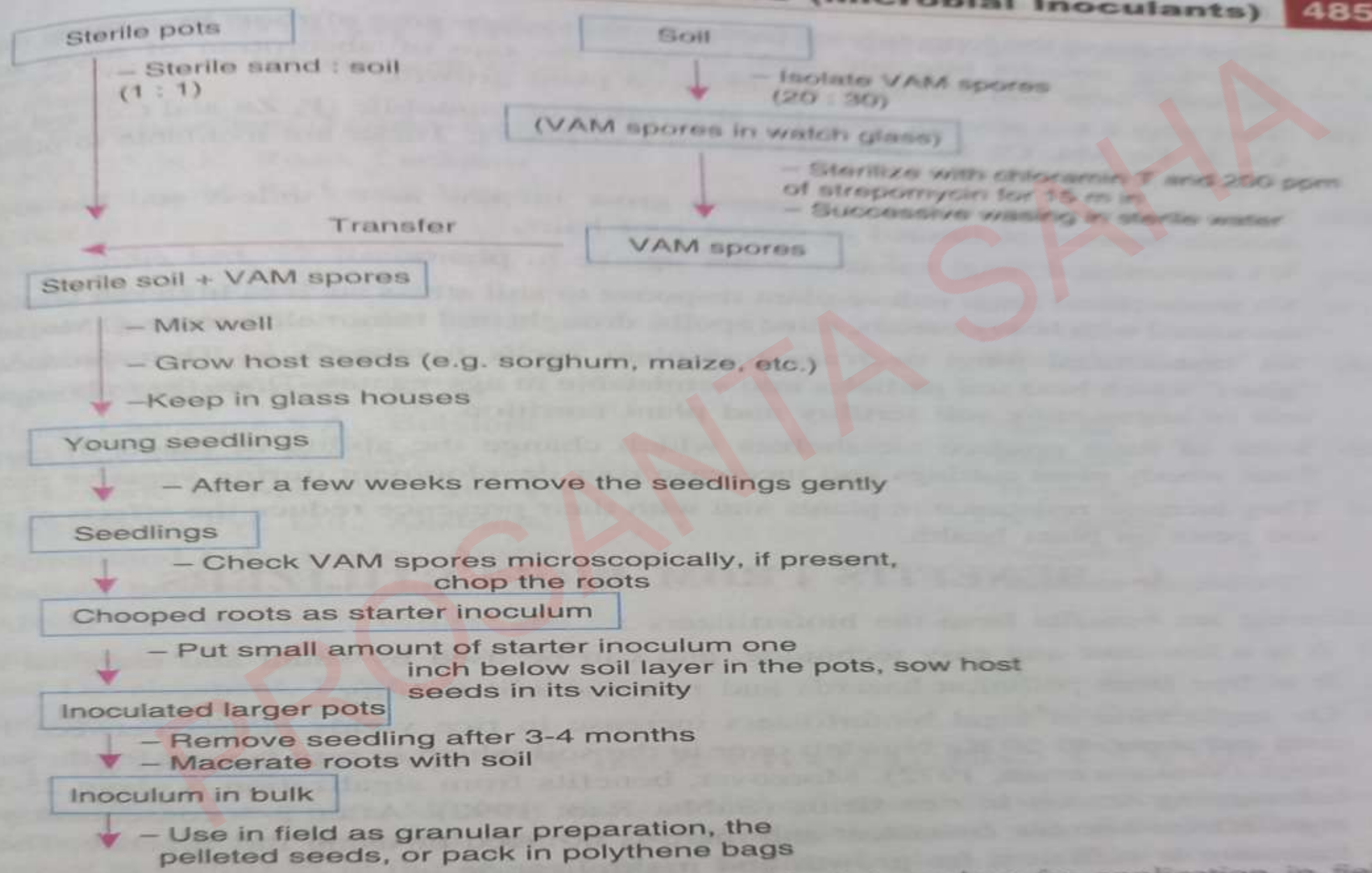




96 Diagram (not to scale) illustrating the major features of an arbuscular mycorrhiza and the chief mechanism whereby it is believed to enhance the uptake of phosphate from soil. The slow diffusion path of phosphate ions in soil, resulting from tortuous pathways and reversible adsorption to the soil particles, is short-circuited by direct transfer of phosphate to the root through the fungal hyphae which extend well beyond the root hair zone. P = primarily  $\text{H}_2\text{PO}_4^-$ . (From Hayman, 1982).

**Isolate spores of VAM from soil by Wet Sieving and Decantation technique and use as starter culture.**





20.7. Method of production of VAM (VA mycorrhizal) inoculum for application in fields.



## Mass production

VAM spores isolated



Spores mixed with sterilized soil



Soil filled in pots



Host plant transplanted in pots



Kept 3-4 months in green house



Soil in the pot along with roots of host plant is macerated



Dried till it attains 5% moisture



Dried soil inoculants used for field application

# a. *In vivo* culture

- AM fungi are **grown on roots of green house plants** and chopped mycorrhizal roots, often mixed with growth media containing **hyphae and spores, are used as source of inoculum.**
- **Soil could be replaced by inert substances such as vermiculite, perlite, sand or a mixture of these for crude inoculum production.**

## Method of production



1. Tank for mass multiplication of AM



2. Sprinkling of water in tank with vermiculite



3. Making of furrows to sow maize seeds

# Method of production



**4. Sowing the seeds in furrows**



**5. View of the maize sown AM pit**



**6. Vermiculite contained raised AM infected maize plants**



# Mass production of VAM



Mass production of VAM inside  
Bricklined tank



Mass production of VAM inside  
a cement tank

## **i) Solution culture**

- Involves **growing infected roots in aqueous medium enriched with mineral nutrients** required for the growth of the roots under controlled biotic and abiotic conditions.

## **ii) Aeroponic culture**

Involves **applying a fine mist of nutrient solutions to colonized roots for AM fungal inoculum** production.

## **iii) Root organ culture**

- Use of a **modified agar medium (MS rooting medium)/ liquid medium for creation of increased amount of roots from callus tissue and these roots are infected by AM spores** or by surface sterilized root bits obtained from mycorrhizal plant.

# Uses of VAM

- ➔ Enhances the feeding areas of the plant root as the hyphae spreads around the roots.
- ➔ Mobilizes the nutrients from distantance to root.
- ➔ Stores the nutrients (sp. phosphorus).
- ➔ Removes the toxic chemicals (example : phenolics) which otherwise hinder nutrient availability.
- ➔ Provide protection against other fungi and nematodes
- ➔ It increase growth rate in plants (*citrus, maize, wheat, etc.*)
- ➔ It reduces sensitivity of crop towards high level of salts and heavy metals



# Disadvantage of Mycorrhizae

- When the nutrient levels in the soil are so low and the fungus cannot extract extra nutrients and no benefit accrues to the plant in return for carbon transferred to fungus parasitism may be occur.
- Sometimes plant growth suppression has been attributed to mycorrhizal colonization, but usually this occurs only under unusual circumstances that affect plant photosynthesis (low light and cold temperatures) or high-phosphorus



## Plant Response to AM Fungal Inoculation

Soil phosphorus is a critical factor in plant response and responses are generally better under low phosphorus levels. Host genotypes and fungal strains seem to influence the response of plants to inoculation. Several investigators have reported response of plants to AM fungal inoculation. The results obtained with *Leucaena leucocephala* are illustrative where it was observed that growth enhancement was correlated with increases in AM fungal colonization of roots and uptake of phosphorus, copper and zinc (Fig. 97). Worldwide field experiments have provided evidence to show that under marginal p-deficient soils lacking in effective AM fungal endophytes, increase in yield of wheat, maize, barley, potatoes, white clover, red clover, lucerne, cowpea and other legumes are possible. Increased uptake of zinc has also been shown in AM fungus inoculated peach, maize, wheat and potato in zinc deficient soils. Other observations implicated in AM associations relate to increased uptake of sulphur and cadmium. Improved water absorption and tolerance of plants to water stress in citrus and avocado seedlings have also been noticed. There are also reports of increased levels of cytokinins and chlorophyll by AM fungus infected plants.

## Legume Arbuscular Mycorrhiza Interaction

Rhizobia and arbuscular mycorrhiza often interact synergistically resulting in better root nodulation, nutrient uptake and plant yield. In soils with low P content, this interaction is marked, especially with added phosphate. Such beneficial interactions have been shown in the following legumes: *Stylosanthes guyanensis*, *Centrosema pubescens*, *Medicago sativa*, *Phaseolus* sp., *Glycine max*, *Arachis hypogea*, *Vigna unguiculata*, *Pueraria* sp., *Trifolium repens* and *Trifolium subterraneum*.

The practical utility of this dual effect remains to be explored in spite of the inherent limitation that AM fungi are obligate symbionts and cannot be mass multiplied in pure culture by any known method.

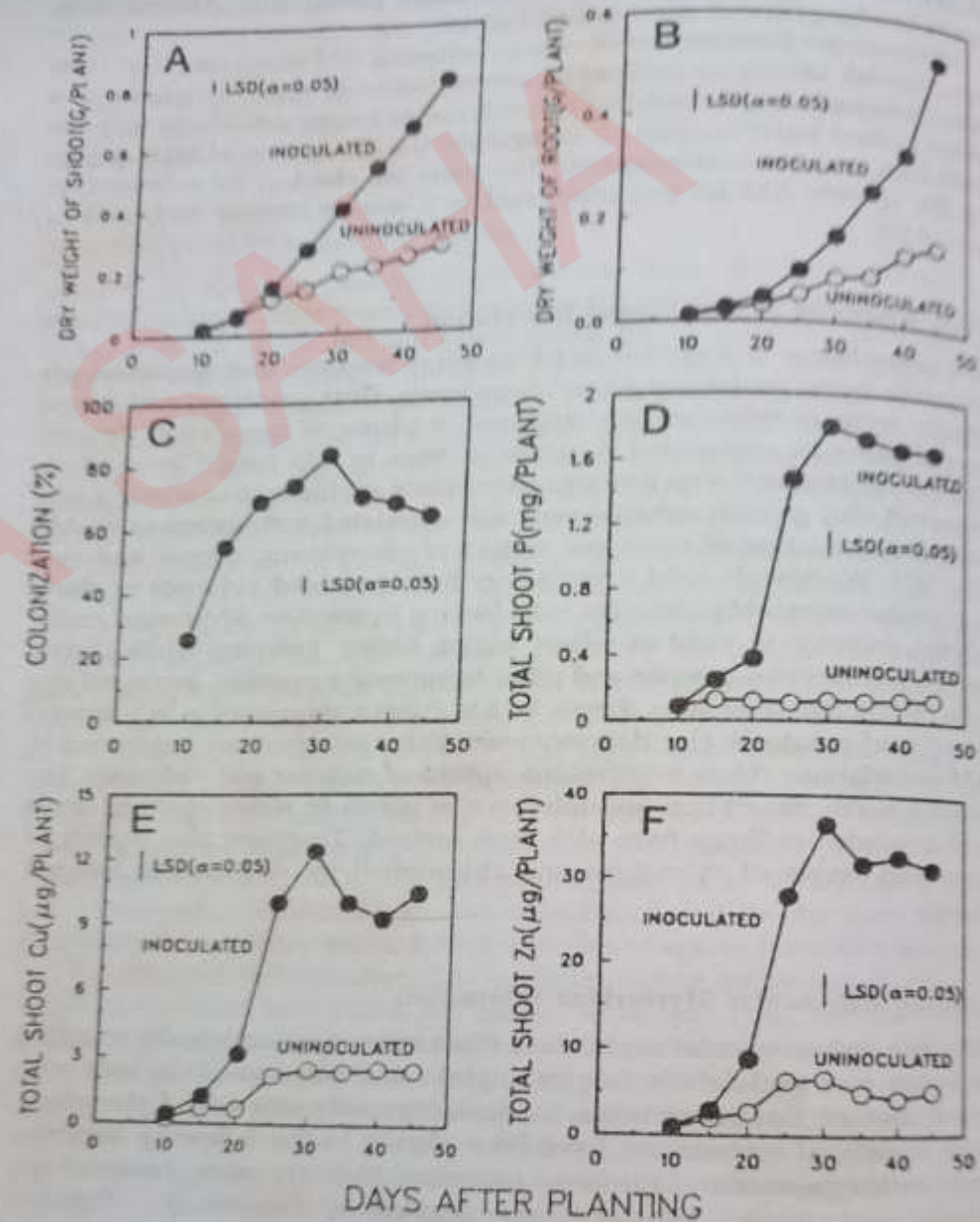


Fig. 97 Influence of soil infestation by *G. aggregatum* on (A) shoot dry weight, (B) root dry weight, (C) root colonization, (D) shoot phosphorus uptake, (E) shoot copper uptake, and (F) shoot zinc uptake of *L. leucocephala*. (Manjunath and Habte, 1988).



# Mycorrhiza Biofertilizer





**THANK YOU**

PROSANTA SAHA