Isolation of Mycorrhizal Spores and Nematodes in the Soils, of Some Arid Land Species in University of Maiduguri Campus, Borno, Nigeria

¹Yagana Tayib Alkali. ²Sule Baba Sarah. ³M. Bulama-Modu. ¹Department of Remedial Science, Ramat Polytechnic, Maiduguri, Nigeria. ^{2,3}Department of Biological Sciences, University of Maiduguri, Nigeria.

Abstract:- The study was to investigate the role of arbuscular mycorrhiza Fungi spore in soil and tree species create distinct biogeochemical signatures on soils. AM symbioses are usually beneficial to the host as it improves plant survival and performance, extra metrical mycelium the quantification of spores from the soil and extraction techniques. The protocols used for spore extraction are as follows wet-sieve and decant method which was developed by Gerdemann and Nicolson since in the nineteen sixties. Samples were collected from Plate of Mycorrhizal spores that are isolated. Extracting of nematodes serve two purposes and Picking nematodes is easiest with fine forceps and a rubber pipette, under a microscope at low magnification, in a Petri dish as indicated in the case of isolation of Mycorrhizal spores. It is transferred into slide with drop of glycerine and covered with cover slip. Mycorrhizal spores where isolated as shown of the plate.

I. INTRODUCTION

Mycorrhizae form a network of filaments that associate with plant roots and drawn nutrients from that the root system would not able to access otherwise. It also makes the plant less susceptible to soil-borne pathogens and to other environmental stresses such as drought and salinity. In return the plant provides carbohydrates and other nutrients to the fungi. They utilize these carbohydrates for their growth and to synthesize and excrete molecules like glomalin (glycoprotein). Spores: form as swellings on one or more subtending hypha in the soil in roots and extra metrical spores are commonly found in cultivated soils. M.R. Cartera and E.G. Gregorich (2008). Nematodes are tiny, round-bodied, unsegmented, worms. Most yards typically have billions of them in the soil, feeding on organic matter, bacteria, insects and plants. Nematodes are one of the most common of all animals, but because they usually can't be seen without a microscope, they aren't very well understood outside the scientific community of nematologists. Approximately 10% of all nematodes feed on plants, living around or in the roots. The most well known is the root knot nematode (Meloidogyne spp.), because of the distinctive galls it causes on infected roots, it's wide distribution, and the wide range of plants that it attacks (including most common vegetables, ornamentals, and fruit trees). There are thousands of nematodes. Not only are there more than 15,000 known species of roundworms, but there are many

thousands of individual nematodes in even a single handful of garden soil. Nematodes were once classified with a very large and heterogeneous cluster of animals grouped together on the basis of their overall worm-like appearance, simple structure of an internal body cavity called a **pseudocoelom**, and the lack of features such as cilia and a well-defined head that are found in most animals. (Wallace *et al.*, 1996.)

II. MECHANISM OF SYMBIOSIS

- 1- Spore formation
- 2- Root colonization
- 3- Exploration
- 4- Network development

Arbuscular Mycorrhizal Fungi spore in soil and tree species create distinct biogeochemical signatures on soils. AM symbioses are usually beneficial to the host as it improves plant survival and performance of extra metrical *mycelium*. The spore serve as progagules to initiate the new colonization of young roots growing in their vicinity, because AMF spores are large and easily discernible compared to those of other fungi and to determine abundance and diversity of AMF in an organic and conventionally system. AMF spore extraction techniques are obtained in the experiments. Plate showing different mycorrhizal spores that are isolated are seen below. The objective to quantity AMF spores from a given amount of soil, the techniques prove spore viability as shown in the plate. The objectives is preserved extracted spores for inoculation of subsequent growth experiments, spore viability is a criteria on technique(s). Spore extraction was use for the experiments. The protocols used for spore extraction are as follows;

- 1. Wet-sieve and decant method which was developed by Gerdemann and Nicolson since in the nineteen sixties.
- PROCEDURES FOR SAMPLE COLLECTION
- 1. An area of about $0.5m^2$ was cleared around host plant
- 2. The top soil (10 to 30cm) was cleared and moist root-soil (rhizosphere) is collected with as many fine roots as possible.

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- PROCEDURE FOR WET-SIEVE AND DECANT TECHNIQUE
- 1. 100g of root-rhizosphere soil mixture is placed into a 1000ml beaker
- 2. The root-rhizosphere soil is then mixed with tap water
- 3. Agitate soil mixture vigorously to free AMF spores from soil
- 4. Allow 10 seconds pause to enable heavier particles and organic material. The remaining soil-root-hypae-spore suspension is slowly poured through a set of sieves. $(1mm to 45 \mu m)$
- 5. The extracts are washed away from the bottom sieve to Petri dishes.
- 6. Using a dissecting microscope spores are picked by means of a fine forceps (furlan et al 1980)
- 2. Sucrose centrifugation method using 60% sucrose P/v
- PROCEDURE
- 1. Wet-sieve the soil sample and wash into a beaker
- 2. In a centrifuge tube, fill it with decanted sample about half way (5ml)
- 3. (gently inject 5ml 60% (p/v) sucrose at the bottom of each tube using a pipette with a pipette holder extension
- 4. There should be a clear interface between the water (above) and sugar (below)
- 5. Centrifuge the capped tubes at approx 3,000 rpm for 10 minutes
- 6. Remove the spores caught at the interface of the two layers with the pipette and holder extension
- 7. Pour content into a Petri dish and view under a stereomicroscope

Plate 1: Isolated Mycorrhizal spores.



- PROCEDURES FOR SAMPLE COLLECTION IN NEMATODES
- Sampling and extracting of nematodes serve two purposes;
- 1. Diagnosis a current problem
- 2. Predict a future problem

➤ Variation in space: horizontal and vertical

One of the biggest problems while sampling soil nematodes is their distribution, which is not at random, but clustered. Many physical, biological and agronomic factors may play a role in nematode distribution patterns. The objective of sampling nematode identification is the extraction method use. (Anonymos, 2020)

Baermann Funnel and Decanting method

Pour soil sample to filter or tissue paper, incubate for 48hr, and collect nematodes in a beaker. Sub sample. Literature: Baunacke 1992, Oostenbrink 1950

> Picking nematodes

Picking nematodes is easiest with fine forceps and a rubber pipette, under a microscope at low magnification, in a Petri dish as indicated in the case of isolation of Mycorrhizal spores. It is transferred into slide with drop of glycerine and covered with cover slip.

III. CONCLUSION

Arbuscular mycorrhizae fungi in enhancing the biomass and increasing the rate of tree growth. It has also demonstrated that mycorrhizal fungi is one of the main pathways by which most plants obtain nutrients and therefore critical for terrestrial ecosystem functioning. *The protocols used for spore extraction are as follows wet-sieve and decant method which was developed by Gerdemann and Nicolson since in the nineteen sixties. Samples were collected from Plate of Mycorrhizal spores that are isolated and* picking nematodes

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