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Research article

MYCORRHIZAE AT POLLUTED SITE OF WESTERN RAJASTHAN

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ABSTRACT: Pollution has become a worldwide environmental issue it has created many health problems which may destroy the living life on the planet. Many of the regions have been extremely affected with the pollutants from the industries and automobiles. These can be remediated with the help of plants as well and plants are helped with the Mycorrhizae. These Mycorrhizae help the plant to accumulate pollutants within them or lock in the soil so that it can't move to another place or their form is changed to another so that it cannot make harm to other plants. Plants are having association with the mycorrhizae in a compatible manner. Mycorrhizae increase the root absorption zone by increasing the rooting of the plant. This help the plant to absorb more and more pollutants from the soil to store in the plant itself remediating the soil free from pollutants.

Keywords: Mycorrhizae, Pollutants, Remediation.

INTRODUCTION

Pollution results destruction in the environmental status having many floes in it, it will cause harm to soil and its environment and cause harm to the habitat to that particular area. Now a day the problem of pollution has been increased due to CFC'S released in the atmosphere. This CFC'S has depleted the ozone layer in the atmosphere, and by this ozone depletion the atmosphere is affected very much, harmful rays can easily penetrate the earth atmosphere and in result of this the temperature of the earth has been increased so much. This caused harm to many living things like insects, animals, human being and plants. The harm caused to plants is more dangerous because it is the producer of the ecosystem it producers food and rest all of the living things relay on it weather direct or indirect. Likewise the ability of the plant to withstand in the adverse conditions is carried out with the help of mycorrhizae. Mycorrhizae are the symbiotic association with the plants which help the plant to increase its rhizospheric area of the plant roots so that to absorb more nutrients as well as some plants have ability to absorb pollutants in them. Mycorrhiza helps the plant to accumulate pollutant in them with this rooting modification. Mycorrhiza are symbiotic associations that form between the roots of most plant species and fungi. These symbioses are characterized by bi-directional movement of nutrients where carbon flows to the fungus and inorganic nutrients move to the plant, thereby providing a critical linkage between the plant root and soil. In infertile soils, nutrients taken up by the mycorrhizal fungi can lead to improved plant growth and production. As a result, mycorrhizal plants are often more competitive and better able to tolerate environmental stresses than are nonmycorrhizal plants.

Mycorrhizal associations vary widely in form and function. Ectomycorrhizal fungi are mostly basidiomycetes that grow between root cortical cells of many plant species, forming a Hartig net. Arbuscular mycorrhizal fungi belong to the order Glomales and form highly branched structures called arbuscules, within root cortical cells of many herbaceous and woody plant species. Plant responses to colonization by mycorrhizal fungi can range from dramatic growth promotion to growth depression. Factors affecting this response include the mycorrhizal dependency of the host crop, the nutrient status of the soil, and the inoculum potential of the mycorrhizal fungi. Management practices such as tillage, crop rotation, and fallowing may adversely affect populations of mycorrhizal fungi in the field. Where native inoculum potential is low or ineffective, inoculation strategies may be helpful. With the current state of technology, inoculation is most feasible for transplanted crops and in areas where soil disturbance has greatly reduced the native inoculum potential.

Types of Mycorrhizae

Ectomycorrhizae

The diagnostic feature of Ectomycorrhizae (EM) is the presence of hyphae between root cortical cells producing a netlike structure called the Hartig net**,** after Robert Hartig who is considered the father of forest biology. Many EM also have a sheath, or mantle, of fungal tissue that may completely cover the absorbing root (usually the fine feeder roots). The mantle can vary widely in thickness, colour, and texture depending on the particular plant-fungus combination. The mantle increases the surface area of absorbing roots and often affects fine-root morphology, resulting in root bifurcation and clustering. Contiguous with the mantle are hyphal strands that extend into the soil. Often the hyphal strands will aggregate to form rhizomorphs that may be visible to the unaided eye. The internal portion of rhizomorphs can differentiate into tube like structures specialized for long-distance transport of nutrients and water. Ectomycorrhizae are found on woody plants ranging from shrubs to forest trees. Many of the host plants belong to the families Pinaceae, Fagaceae, Betulaceae and Myrtaceae. Over 4,000 fungal species, belonging primarily to the Basidiomycotina, and fewer to the Ascomycotina, are known to form ectomycorrhizae. Many of these fungi produce mushrooms and puffballs on the forest floor. Some fungi have a narrow host range, such as *Boletus betulicola* on *Betula* spp., while others have very broad host range, such as *Pisolithus arhizus* (also called *P. tinctorius*) which forms ectomycorrhiza with more than 46 tree species belonging to at least eight genera).

Arbuscular Mycorrhizae

The diagnostic feature of arbuscular mycorrhizae (AM) is the development of a highly branched arbuscule within root cortical cells. The fungus initially grows between cortical cells, but soon penetrates the host cell wall and grows within the cell. The general term for all mycorrhizal types where the fungus grows within cortical cells is Endomycorrhiza. In this association neither the fungal cell wall nor the host cell membrane are breached. As the fungus grows, the host cell membrane invaginates and envelops the fungus, creating a new compartment where material of high molecular complexity is deposited. This apoplastic space prevents direct contact between the plant and fungus cytoplasm and allows for efficient transfer of nutrients between the symbionts. The arbuscules are relatively short lived, less than 15 days, and are often difficult to see in field-collected samples. Other structures produced by some AM fungi include vesicles, auxiliary cells, and asexual spores. Vesicles are thin-walled, lipid-filled structures that usually form in intercellular spaces. Their primary function is thought to be for storage; however, vesicles can also serve as reproductive propagules for the fungus. Auxiliary cells are formed in the soil and can be coiled or knobby. The function of these structures is unknown. Reproductive spores can be formed either in the root or more commonly in the soil. Spores produced by fungi forming AM associations are asexual, forming by the differentiation of vegetative hyphae. For some fungi (e.g., *Glomus intraradices*), vesicles in the root undergo secondary thickening, and a septum (cross wall) is laid down across the hyphal attachment leading to spore formation, but more often spores develop in the soil from hyphal swellings. The fungi that form AM are currently all classified in the order Glomales [1] The taxonomy is further divided into suborders based on the presence of: (i) vesicles in the root and formation of chlamydospores (thick wall, asexual spore) borne from subtending hyphae for the suborder Glomineae or (ii) absence of vesicles in the root and formation of auxiliary cells and azygospores (spores resembling a zygospore but developing asexually from a subtending hypha resulting in a distinct bulbous attachment) in the soil for the suborder Gigasporineae. The term Vesicular-Arbuscular Mycorrhiza (VAM) was originally applied to symbiotic associations formed by all fungi in the Glomales, but because a major suborder lacks the ability to form vesicles in roots, AM is now the preferred acronym. The order Glomales is further divided into families and genera according to the method of spore formation. The spores of AM fungi are very distinctive. They range in diameter from 10 mm for *Glomus tenue*to more than 1,000 mm for some *Scutellospora* spp. The spores can vary in colour from hyaline (clear) to black and in surface texture from smooth to highly ornamented. *Glomus* forms spores on the ends of hyphae, *Acaulospora* forms spores laterally from the neck of a swollen hyphal terminus, and *Entrophospora* forms spores within the neck of the hyphal terminus. The Gigasporineae are divided into two genera based upon the presence of inner membranous walls and a germination shield (wall structure from which the germ tube can arise) for *Scutellospora* or the absence of these structures for *Gigaspora*.

The AM type of symbiosis is very common as the fungi involved can colonize a vast taxonomic range of both herbaceous and woody plants, indicating a general lack of host specificity among this type. However, it is important to distinguish between *specificity,* innate ability to colonize, infectiveness, amount of colonization, and effectiveness, plant response to colonization. AM fungi differ widely in the level of colonization they produce in a root system and in their impact on nutrient uptake and plant growth. The heavy metal contamination found in polluted soil are Zn, Co, Ni, Cu, Sn,Sb, Pb, etc.

MATERIAL AND METHOD

For the estimation of mycorrhizae presence at the polluted site the collection of soil has been carried out from different areas of Jodhpur, Barmer and Pali. From Pali these sites have been considered for the collection of soil they are near the Bandi River, near cloth industry. While from Jodhpur the soil nearby extrude of the factory, same as from Barmer it has taken from.

SOIL SAMPLING

The soil is collected from the plants grown near by the polluted site. Its rhizospheric soil has been considered for the collection and estimation of the mycorrhizal spores. The area should dig up to 10-15cm below the soil surface so that soil having mycorrhizal spores can be collected. The soil should be moisten so that it can easily dig out.

SPORE ISOLATION

Spore from the soil was extracted by the wet-sieving and decanting method technique of [2]. Total spore number of Mycorrhizal fungi in the soil sample was estimated by the method given by [3] and spore densities were expressed as the no of spore per 100 g of soil. The identification of spore was based on spore size, colour, wall layer and hyphal attachment using the identification manual of [4] and the description provided by the international collection of vesicular and AMF.

SOIL TESTING

Soil samples were analysed for pH and electrical conductivity. The organic carbon was estimated by the method of [5]. Gravimetrical estimation by hydrometer for soil texture was analysed by the method given by [6]. By surveying the above four sites it has been found that these plants have been found abundantly in these places because soil's physiochemical properties allow plants to grow and survive there. Here the soil pH ranges from 5.52-8.60, and the organic carbon content is between 0.12% and 0.45%. By observing and examining the soil collected from the different places, these five genera of spores have been identified these are:-

1. *Acaulospora*
2. *Gigaspora*
3. *Glomus*
4. *Scutellospora*

Acaulospora & *Glomus* were the most abundant in all four soil samples. So here we can conclude that the various species of AMF varies with the different plant. The no. of spores isolated from the collected soil samples ranges from 80-150 spores per 100 g of soil, and there is found variation in the no. of spore population from the different sites (Figure 1)

ROOT COLONIZATION

In order to determine the root colonization percentage the roots are treated by the method given by [7], for rapid assay of Mycorrhizal association, while by the method given by [8] the infection percentage can be determined. By applying above method the colonization of the root sample varies between 40% to 60%. The stained roots show the presence of globose to subglobose or ellipsoid bodies vesicles or spores, dichotomously branched structure arbuscules & hyphae in all the sites.(Figure 2).

TRAP CULTURE

Pot cultures are called trap cultures have been shown to useful tool for the inducing sporulation of AMF from field soils in semi arid ecosystem in order to facilitate the detection of AMF species that are present in the rhizosphere & roots. To establish this trap culture the field soil was autoclaved and planted with surface sterilized seeds (by 0.1% of $HgCl_2$ solution for 2 min. And then washed with distilled water) of *Pennisitum typhoides*.

MASS PRODUCTION

The spores isolated from the soil samples are then collected and the spore of similar types having same genera is collected separately. These are then allowed to grow with the plant in the trap culture. These spores are either saw directly with the help of syringe or making synthetic seeds of these spores. After a plant attains successive height the soil of these pots again collected for the isolation of spores from the soil. These spores are now called as pure culture because the spore are alike and of same genera. In this way the pure cultures of different five spores can be obtained.

SOIL PARAMETERS

Soil samples were analysed for pH and electrical conductivity on 1: 2.5, soil: water suspension. Organic carbon was estimated by the method of [5] using 1 N potassium dichromate and back titrated with 0.5 N ferrous ammonium sulphate solution. Available phosphorus in soil was determined by extraction with 0.5 M sodium bicarbonate for 30 min [9]. Soil texture was estimated gravimetrically by hydrometer method [6].

RESULT AND DISCUSSION

In the table no.1 vesicles in a root segment is abundant in the plants found near industrial area in the balotra, while the minimum no. is found at industrial area Pali and RIICO Jodhpur. As compare to this spore population is maximum in the soil near Bandi river Pali, Balotra Industrial area and RIICO Jodhpur, there is not much variation in the spore population in all the areas from where soil is collected. Maximum No. of AM species is found near Bandi river Pali and Balotra industrial area, while minimum no. is found in the soils of Pali industrial area and Textile Industry Basni Jodhpur. Another parameter is degree of colonization is maximum in the samples of Pali Bandi river, while lesser in the RIICO Jodhpur. From the above data it can be concluded that the soil near Bandi river and Balotra has more no. of efficient mycorrhizae which help the plant to grow and flourish there and survive in the polluted soil there.

Fig.1: Spore colony

fig.2:Glomus spore

Fig. 3: Acaulospora

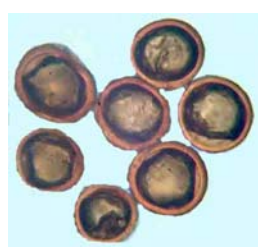

Fig.4: Group of Acaulospora

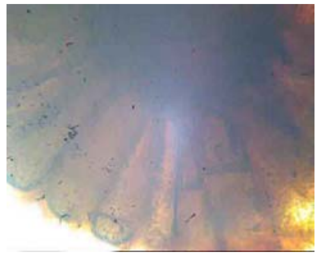

Fig.5: Sclerocystis

.TYPES OF ROOT COLONIZATION:

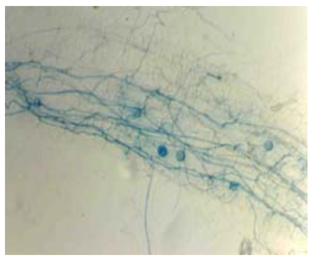

Fig.1: Roots with vesicles

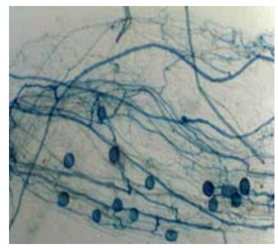

Fig.2: Roots with vesicles

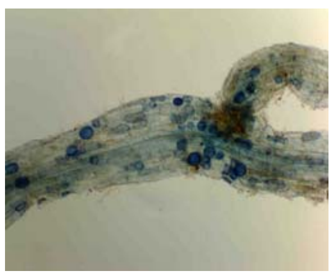

Fig.3: Roots with vesicles

Fig.4: Roots with vesicles

The table 2 reveals correlation analysis between AMF spore population and different edaphoclimatic factors. It is evident from the results that AMF spore populations were affected by soil pH, organic carbon and Olsen P content. A significant positive correlation with pH ($r = 0.85$, $p<0.01$) and organic carbon ($r = 0.68$, $p<0.05$) was recorded during present investigation.

In table 3 the pH of the Pali and Jodhpur is slightly acidic because of presence of heavy metals and other pollutants from the industrial waste. The pH, EC (dSm-1), OC(%), Olsen P (mgkg-1), Texture of Pali is the mean or average value which is calculated from different sites of Pali. Like wise same is calculated for Jodhpur and Barmer.

Table 1: Relationship between AMF spore population and different edaphic factors

Edaphic factors	AMF spore population
pH	0.88**
EC	0.53
OC	0.68*
Olsen P	-0.86**
Temp (max)	0.14
Temp (min)	-024
RH	0.08

*p<0.05; **p<0.01; n = 12

Table 2: Physicochemical characteristics of site soils for Desert Region

Physicochemical characteristics	District		
	Pali	Jodhpur	Barmer
pH	5.10	5.25	6.10
EC (dSm-1)	0.22±0.02	0.28±0.03	0.25±0.02
OC (%)	0.32±0.01	0.38±0.01	0.40±0.01
Olsen P (mgkg-1)	7.7±0.01	4.2±0.04	5.1±0.03
Texture	Sandy gravel	Coarse loam	Sandy gravel

± Standard error of mean

Table 3: Distribution of AMF species associated with Plants found in Polluted Region

AMF species	Pali	Jodhpur	Barmer
Acaulospora leavis Gerdmann & Trappe	+	+	+
Acaulospora morrawae Spain & Schenck	_	_	+
Acaulospora sporocarpia Berch	+	+	_
Gigaspora margarita Becker & Hall	_	+	_
Gigaspora gigantean Nicol & Gerd	+	++	_
Gigaspora rosea Nicol & Schenck	+	+++	++
Glomus aggregatum Schenck & Smith	+++	++	+
Glomus constrictum Trappe	+	+	_
Glomus desertocola Trappe Bloss & Menge	++	+++	+
Glomus mosseae Gerd. & Trappe	+	++	++
Scutellospora calospora Walker & sanders	+++	++	++
Scutellospora nigra Walker & senders	_	_	+

- = Absent, + = Low (< 20%), ++ = Moderate (20-50%), +++ = High (>50%),

Twelve species of AMF were identified in the rhizosphere soils collected from Pali,Jodhpur and Barmer field and successive pot cultures scattered over four genera viz., *Acaulospora*, *Gigaspora*, *Glomus*, and *Scutellospora*. *Glomus* species were most dominant and made up for more than 50% of the total isolates followed by *Acaulospora* (3 species), *Scutellospora* (2 species) and *Gigaspora* (3 species). It is evident that the occurrence of various species of AMF varied considerably with different plant species. . It is reported that genus Glomus to be the most common AMF genus distributed globally and it is also known to dominate in the tropical areas [10] as well as temperate region[11] of the World. Its dominance under various climatic conditions ranging from tropical [10] to high arctic region [12] has been reported earlier. Wide occurrence of genus *Glomus* in the present study as well as reports of several workers suggested that genus *Glomus* has a very wide ecological amplitude, that is responsible for its adaptability and survival in different habitats and vegetational composition.

CONCLUSION

The *Glomus sp.* is found abundantly in the regions of Jodhpur, Pali and Barmer. The study shows that the *Glomus sp*. have much more compatibility with the plants which are grown in the areas of polluted site, it help the plant to survive there and control the pollution by accumulating, absorbing, or degrading the pollutants within plant parts or near its vicinity. Furthermore studies can be done on the Mycorrhizal association with the plant at the polluted site in order to reduce the Heavy metal contamination from the soil and study the physiological, chemical and molecular modification or changes in plant as well as in Mycorrhizae also. This will help the future aspects of the environmental pollution to control and reduce it.

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REFRENCES

[1] Morton J. B. 2000. International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi. West Virginia University.

[2] Gerdemann, J.W. and Nicholson, T.H.: Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Transactions of the British Myological Society1963, 46: 235-244.

[3] Gaur, A. and Adholeya A.: Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Current Science 2004, 86(4): 528-533.

[4] Schenck, N.C. and Y. Perez. 1990. Manual for the identification of VA-mycorrhizal fungi. 3rd ed., Synergistic Publication, Gainesville, U.S.A.

[5] Walkley, A. and I.A. Black. 1934. An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. Soil Sci. 63:251-263.

[6] Jackson, M.L. 1958. Soil Chemical Analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ

[7] Phillips, J.M., Hayman, D.S.: Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc.1970, 55: 158–161.

[8] Biermann BJ, Linderman RJ (1981). Quantifying vesicular-arbuscular mycorrhizae: a proposed method toward standardization. New Phytol., 87: 63-67.

[9] Olsen, S.R., Sommers, L.E.: Phosphorus. In: Page, A.L. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. ASA, Madison1982, 403–430.

[10] Chaurasia, B., 2000. Ecological study of tropical forest trees with special reference to vesicular–arbuscular-mycorrhizal (VAM) association. Ph.D. Thesis. Dr. H. S. Gour University Sagar, M.P., India, 172 pp

[11] Vestberg, M., 1995. Occurrence of some Glomales in Finland. Mycorrhiza 5, 329–336.

[12] Dalpe, Y., Aiken, S.G., 1998. Arbuscular mycorrhizal fungi associated with Festuca species in the Canadian High Arctic. Can. J.Bot. 76, 1930–1938.