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MYCORRHIZAL AND ANTI BACTERIAL STUDIES OF MEDICINAL PLANTS OF RAWALPINDI AND ISLAMABAD

MUHAMMAD USMAN RAJA, NAJMA AYUB, MIR AJAB KHAN AND ATIF JAMAL

Department of Biological Sciences,
Quaid-i-Azam University, Islamabad, Pakistan.

Abstract

A study was carried out to investigate the vesicular- arbuscular mycorrhizal association with the medicinal plants of Rawalpindi and Islamabad. Mycorrhizal infection was studied in thirty-two medicinal plants. Slide length method was used to estimate vesicle, arbuscule and hyphal infection percentages. Number of spores per 50 gm of soil was counted by wet- sieving and decanting method. Soil pH and EC were measured. Soil was also analyzed for N, P, K, organic matter and $CaCO_3$ contents. Mycorrhizal infection was found in all the medicinal plants studied. The study of spores yielded two types namely *Glomus* sp. and *Gigaspora* sp. Antibacterial activity of root extracts of some of these plants for bacterial pathogens viz., *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* was also conducted. Most of the studied plants exhibited antibacterial activity. These findings can be used to grow AM inoculated medicinal plants, which will provide more raw material for the commercial production of drugs.

Introduction

Mycorrhiza is a kind of mutualistic symbiosis; the condition in which both symbionts are benefited from the association (Stackman & Harrar, 1957). There are numerous reports on the incidence of VA mycorrhizal infection in plants including grasses, vegetable crops and legumes. But reports on occurrence and morphological characteristic of VA mycorrhiza in medicinal plants are few (Burni *et al.*, 1994). With modernization of drugs and the study of chemical composition of various drug-producing plants, scientists have diverted their attention to the understanding of VA mycorrhiza and their occurrence in medicinal plants. Results indicated that VA mycorrhizas have a wide range of hosts among medicinal plants (Ueda *et al.*, 1992). Burni *et al.* (1995) listed some medicinal plants possessing mycorrhizae belonging to 16 different families, which have been reported by various workers.

Medicinal plants play a vital role in curing diseases for centuries, while mycorrhizal association improves the plant growth. The objective of this study was, therefore, to examine the natural mycorrhizal association with the medicinal plants of Rawalpindi and Islamabad and also to find the antibacterial activity of some of these plants.

Materials and methods

Soil pH was determined by glass electrode and Electric conductivity was determined by conductivity meter methods (Iwasri, 1992; Page *et al.*, 1982). Total nitrogen was measured by Kjeldahl method of Jackson (1962). Available phosphorus was determined by Molybdenum blue method (Allen *et al.*, 1974). Extractable potassium was measured by using flame photometer (Bower *et al.*, 1962). Organic matter was determined by methods of Nelson & Sommers (1982). Calcium carbonate percentage was measured by using acid base titration (Iwasri, 1992).

Plant roots of thirty-two plants with soil from the rhizosphere were sampled carefully from October 1999 to March 2000. For this purpose roots of the plants were dug carefully without injuring the fine rootlets. Approximately 1 kg of each soil sample along with the roots was stored at 2°C in the polythene bags. Collected samples of roots were carefully brought into the laboratory, washed gently under tap water to remove the attached soil particles. Fine root segments were then fixed in FAA (Kormanik *et al.*, 1980). Fixed roots pieces were washed with tap water to remove FAA, then these roots were cleared in 2.5 - 5% KOH solution and heated in a water bath at 90°C for 10-60 minutes (Koske & Gemma, 1989). The stained root samples were cut into pieces (1 cm). Ten pieces of each root sample were carefully placed on the slide and gently covered with cover slip. These segments were then examined under the microscope for the assessment of VA mycorrhizal infection. For the assessment, slide length method was used (Giovannetti & Mosse, 1980). Arbuscular mycorrhizal spores were extracted from the rhizosphere soil by wet-sieving and decanting method of Gerdemann & Nicolson (1963).

The root extraction was made with Soxhlet extractor (Kenneth, 1975). Diffusion method was followed for antibacterial study (Rios *et al.*, 1988). Microorganisms in this study were provided by Bacteriology laboratory of Public Health Division, National Institute of Health Islamabad.

Results and discussion

The main characteristics of the rhizospheres of studied plants are given in Table-1. Microscopic examination of roots showed that all plant roots studied were mycorrhizal (Table-2). So the present study supports the contention that most plants growing under natural conditions possess AM mycorrhizae in their roots (Gerdemann, 1968). Natalia *et al.* (1996) reported that mycorrhizae are key components of natural ecosystems because of their essential role in sustaining vegetation cover. This is well supported by present study that all of medicinal plants examined from Rawalpindi and Islamabad were infected with mycorrhizae, indicating high level of mycotrophy of existing vegetation within this degraded ecosystem, where lack of appropriate nutrient levels and water stress make it difficult for plants to survive.

Vesicular arbuscular mycorrhizal association was found in medicinal plants of Chenopodiaceae, Amaranthaceae, Euphorbiaceae, and Brassicaceae, which were reported less, infected with mycorrhiza (Gerdemann, 1975).

The results showed that arbuscule infection was low or even absent in some plants roots studied (Table-2). These results are in accordance with findings of Powell and Bagyarag (1986) who found that in older plants the situation of senescent arbuscules is more frequent than that of active arbuscules easily observed within young mycorrhizal roots. Moreover, due to their short span of life 4-15 days after which the arbuscules branches deteriorate and collapse, so it becomes difficult to locate the presence of arbuscule in roots (Paul & Clark, 1989).

The number of spores is low in the rhizospheres of studied plants. These findings suggest that this propagule is not main source of mycorrhizal innoculum in this ecosystem as suggested by McGee (1989) for semi arid site in Australia. The study yielded two types of spores namely *Glomus* sp. and *Gigaspora* sp. *Glomus* sp. spores were most common and abundant. The findings are also in consistent with

those of Bagyarag (1991) who recorded abundance of *Glomus* sp. spores in near neutral soils and their rarity in alkaline or acidic soils.

The root extract of the plants showed antibacterial activity (Table-3). The antibacterial activity of the plants is due to the presence of essential oils, terpenoids, flavonoids, phenolic acids etc. Several workers reported that AM infection enhance the production of these compounds (Ishii *et al.*, 1977; Bush *et al.*, 1997; Nagahashi, *et al.*, 1996). So it can be assumed that root extract taken from the plants infected with such kind of mycorrhizal fungi will enhance the ability to inhibit the growth of microorganisms.

In this study crude extracts of the plants showed antibacterial effects on the clinically isolated bacteria. If these extracts are purified and the compounds that possess antibacterial activity are isolated, a very effective drug could be prepared from these natural products. Vesicular arbuscular mycorrhizal inoculation will help them to grow in nutrient stress. Thus more raw materials will be available for the commercial production of drugs.

Table 1. Mean values of soil characteristics and components.

Soil parameter	Mean value
pH	7
Electric conductivity (dS/m)	0.3
Nitrogen (%)	0.02
Phosphorus (ppm)	2.5
Potassium (ppm)	125
Organic mater (%)	0.3
Calcium carbonate (%)	10

Table 2. Mean mycorrhizal, vesicle, arbuscule and hyphal infection percentages in roots and number of endophyte spores in the rhizosphere of medicinal plants of Rawalpindi and Islamabad.

S. No.	Plant Species	Vesicle Infection %	Arbuscule Infection %	Hyphal Infection %	No. of Spores
1.	*Achyranthes aspera*	2	0	5	10
2.	*Artemisia scoparia*	17	2	25	29
3.	*Bauhinia variegata*	8	0	16	20
4.	*Cannabis sativa*	2	0	6	15
5.	*Capsella bursa-pastoris*	2	0	5	5
6.	*Chenopodium album*	4	1	7	80
7.	*Cynodon dactylon*	18	6	30	80
8.	*Dicliptera roxburghiana*	7	2	13	26
9.	*Euphorbia helioscopia*	8	0	18	40
10.	*Euphorbia hirta*	9	2	15	25
11.	Fumaria indica	4	0	15	120
12.	Geranium wallichianum	4	0	13	100
13.	Lallemantia royleana	10	2	30	40
14.	Lantana camara	9	2	19	16
15.	Mallotus philippensis	5	2	11	20
16.	Micromeria biflora	7	3	20	21
17.	Oxalis corniculata	8	2	21	11
18.	Parthenium hysterophorus	2	1	10	20
19.	Plantago lanceolata	4	1	12	40
20.	Psoralea corylifolia	5	2	27	7
21.	Rumex dentatus	3	0	12	18
22.	Saussurea heteromalla	5	2	15	60
23.	Sida cordata	11	4	20	29
24.	Sisymbrium irio	3	2	11	10
25.	Solanum americanum	8	2	15	17
26.	Sonchus arvensis	9	1	25	33
27.	Tagetes minuta	4	1	25	3
28.	Trachyspermum ammi	5	2	18	20
29.	Trichodesma indicum	3	0	10	60
30.	Verbena officinalis	5	2	40	113
31.	Vetiveria zizanioides	10	4	38	30
32.	Viola canescens	2	2	32	10

Table 3. Antibacterial activity of root extracts of VA infected medicinal plants against three bacterial pathogens.

Sr. No.	Plant Species	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*
1.	**Artemisia scoperia**	-	+	+
2.	***Cannabis sativa***	-	-	-
3.	***Mallotus philippensis***	-	+	+
4.	***Oxalis corniculata***	+	+	-
5.	***Sisymbrium irio***	-	-	-
6.	***Trichodesma indicum***	+	-	+
7.	***Verbena officinalis***	+	+	+
8.	***Viola canescens***	-	+	+

+ = Zone of inhibition
- = Absence of zone of inhibition

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