

Occurrence of Vesicular-Arbuscular Mycorrhizal (VAM) Fungi and Their Effect on Plant Growth in Endangered Vegetations

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Abstract A survey for vesicular-arbuscular mycorrhizae (VAM) occurrence was undertaken in three endangered vegetation sites in the area of Kudankulam atomic power station. Fifteen VAM fungal species were isolated from the root-zone soils of fourteen different plant species. There was a significant correlation observed between the number of spores and of percentage root colonization as exemplified by *Phyllanthus niruri* and *Paspalum vaginatum* (450, 95%; 60, 25%). Although VAM species are not known to be strictly site specific, the fact that *Acaulospora elegans* was observed only in site 1, *Glomus pulvinatum* in site 2 only, and *Gl. intraradices* in site 3 only, showed site-specificity in this study. To confirm the infection efficiency, two host plant species in the sites, *P. niruri* and *Eclipta alba*, were selected and inoculated in field with three selected VAM fungal spores. *Gl. fasciculatum* was found to be the most efficient VAM species in percentage root colonization, number of VAM spores, and dry matter content. When the nutrients in roots of *P. niruri* and *E. alba* were analyzed, there was higher uptake of K (4.2 and 3.4 times, respectively) and Ca (5.3 and 4.9 times, respectively), the analogues for ¹³⁷Cs and ⁹⁰Sr, respectively. From the results, it might be concluded that VAM association helps the plants survive in a disturbed ecosystem and enhances uptake and cycling of radionuclides from the ecosystem.

Key words: Endangered vegetations, occurrence of VAM fungi, *Glomus fasciculatum*, uptake of radionuclides

In recent days in India, due to eutrophication, siltation, land filling, construction of houses, construction of atomic

power houses, and conversion of paddy fields into other crops, the entire vegetation of an area is going under threat. In fact, Kudankulam, an area located near the distal southern tip of penninsular India, is going to face such threats. The construction of an atomic power station as well as construction of buildings and land filling undergoing in this area lead to some endangered vegetations, including some medicinal plants.

To protect vegetations growing under such disturbed conditions, application of vesicular-arbuscular mycorrhizal (VAM) fungi may play important roles [12]. A special type of root resulting from a mutualistic symbiotic association between a plant and a fungus is called mycorrhizae. A diverse group of soil fungi spanning the zygomycetes, ascomycetes, and basidiomycetes form mycorrhizae. These mycorrhizal fungi are ubiquitous and are found in diverse habitats. The mycorrhizal association is beneficial to the plants by its help in nutrient absorption, decrease in disease susceptibility, and increase in tolerance to adverse environmental conditions and biomass productivity. Infection by mycorrhizae (VAM) can enhance the plant to take up a vast range of trace and minor elements [14] as well as calcium and potassium [6].

Some plants have a high rate of radionuclide uptake. The growth of such plants may have potential as part of a future strategy to rehabilitate contaminated soil after radionuclide deposition. Both cesium, ¹³⁷Cs, and strontium, ⁹⁰Sr, being analogues of potassium and calcium, are highly bioactive and are readily taken up by components of the soil biota, particularly if they are limited by these nutrients. Although its atomic radius is slightly greater than potassium, the ¹³⁷Cs, a monovalent alkali metal cation, behaves quite similarly to potassium in the soil/plant system. The ⁹⁰Sr, a divalent cation, behaves similarly to calcium. The ¹³⁷Cs and ⁹⁰Sr are the products of nuclear fission reactions, therefore,

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regularly released into the environment as a result of weapons testing, nuclear power production, and nuclear fuel reprocessing [11].

Plants absorb ^{137}Cs and ^{90}Sr less efficiently than their nutrient analogues potassium and calcium, respectively [34]. As explained by White and Bradley [32], both ^{137}Cs and ^{90}Sr are taken up from the soil solution by plant root as K^+ and Ca^{2+} , as they are similar to those cations in chemical properties. Previous study [32] explained that K^+ , the analogue of ^{137}Cs , moves into root by a symplastic pathway, and Ca^{2+} , the analogue of ^{90}Sr , moves into root by an apoplastic pathway. Since VAM association is beneficial for the uptake of nutrients and the plants to survive even from a disturbed soil after radionuclide deposition [1, 9], a few study sites were selected in the constructed power house area of Kudankulam and this work was carried out to understand the occurrence of VAM fungal association and its influence on the survival of endangered vegetation sites.

In the case of accidental release of radioactivity, there are chances for the vegetations to face the effect of atomic radiations in Kudankulam area. Even though the effect of VAM fungi on plant growth is understood, the effect of VAM fungi on the vegetations facing atomic radiation was not studied yet. This work may be the first in this aspect.

Soil Characteristics of the Sites

Three different vegetation sites were selected from Kudankulam, Tamil Nadu, India and the surrounding area (Fig. 1). Site 1 is located within the campus of the newly constructed atomic power station, Kudankulam. Site 2 is located within the campus of the newly constructed staff quarters, Kudankulam. Site 3 is a private land located outside and opposite to the power house constructions, Kudankulam.

All three sites of the Kudankulam area were enumerated to have no base line information. The soils from the root-zone area of the vegetations were analyzed for the occurrence of VAM spores. The root samples were fixed in FAA [6 ml of formalin (40% formaldehyde), 1 ml of glacial acetic acid, 20 ml of ethanol (96%), and 40 ml of distilled water] immediately after collection [22] and

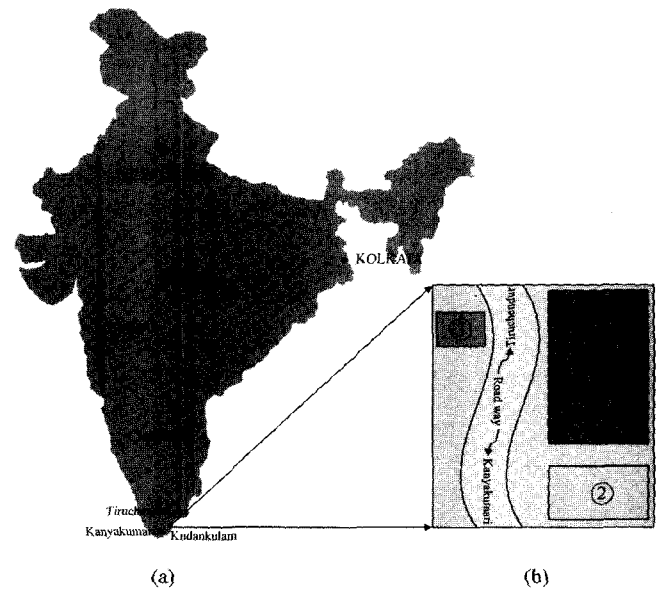


Fig. 1. Location of the sampling sites.

(a) Location of Kudankulam in India; (b) an enlarged view of the sites. Numbers represent the sampling sites: 1, atomic power station; 2, staff quarters; 3, a private land.

analyzed for the incidence of VAM structures in the root tissues. Plants of mycorrhizal and non-mycorrhizal families were analyzed for the presence of VAM structures and spores. In addition, physicochemical characteristics of the collected root-zone soils were also analyzed by following the standard methods [26].

Soils of all three sites were acidic (pH 5.7–6.8). The soils had black clay soil in site 1 and 2, and sandy loam soil type in site 3. The acidic nature and the variations among soil type in Indian soils have already been reported [3, 4, 23, 29]. Total N, available P, and available K of the soil ranged from 76.8 to 95.2, 25.4 to 53.1, and 250 to 350 kg/acre, respectively (Table 1). The organic matter ranged from 3.5 to 6.5. Earlier report [29] on the fertility of Indian soil revealed that N, P, and K levels in the soil were low, ranging 77.8 to 96.8, 24.2 to 52.1, and 244 to 603 kg/acre, respectively, thus the soil could only act as a low-fertility soil.

Table 1. Physicochemical characteristics of soils at three different sites of the Kudankulam atomic power station area.

Site ^a	Soil type	pH	Organic matter	N	P	K
				(kg/acre ^b)		
Site 1	Black clay	6.8±1.4	4.6±0.4	76.8±10.8	40.4±12.2	250±14.8
Site 2	Black clay	6.6±1.2	3.5±1.0	95.2±11.2	25.4±11.0	290±12.5
Site 3	Sandy loam	5.7±1.0	6.5±1.2	90.0±17.5	53.1±10.4	350±10.8

^aThe site 1, located within the campus of the newly constructed atomic power station, Kudankulam; the site 2, located within the campus of the newly constructed staff quarters, Kudankulam; the site 3, a private land opposite to the power station constructions, Kudankulam.

^bValues are mean of 10 replicates with 2% standard error.

Isolation and Identification of VAM Spores and VAM Spore Inoculation Studies in Some Experimental Plants

VAM spores were isolated and identified, and the dominant spores found in each site were recorded. The dominant spores were prepared for inoculum and were field inoculated into the experimental plants of mycorrhizal and non-mycorrhizal families. For the isolation of VAM spores, wet-sieving and decanting techniques [10] were followed. For the estimation of VAM fungal colonization, the Phillips and Hayman method [22] was followed. The identification of VAM spores was done by the synoptic keys of Raman and Mohankumar [24], and Schenck and Perez [27]. For inoculation, 1 g of soil with 250–500 spores was used. For the studies in the mycorrhizal family, the host plant *Eclipta alba* was selected, whereas the host plant *Phyllanthus niruri* was selected for the non-mycorrhizal family. Experimental plants were inoculated with different types of VAM fungi and harvested 75 days after inoculation for determination of the percentage root colonization of VAM fungi, number of VAM spores, dry matter content, and also the nutrients K [25] and Ca [16] level in root samples.

A total of 15 different VAM fungal species were detected in the root-zone soils. Among them, three species

belonged to the genus *Acaulospora*, another three species belonged to *Sclerocystis*, one species belonged to *Gigaspora*, and the remaining eight species to *Glomus* (Table 2). Fourteen commonly occurring herbaceous plants which belonged to 6 different families of angiosperms were selected from the sites and screened for VAM in the roots as well as for VAM structures in root-zone soils (Table 2). All 14 plant species were positive for VAM colonization of the roots, although the VAM colonization of the roots varied. VAM structures such as sporocarps and spores belonged to different VAM fungal species. The number of VAM fungal spores present in the root-zone soils ranged from 60–450, and the percentage root colonization varied from 25 to 95% (Table 2).

Among the 14 plants, only 3 plants were common for all three sites, whereas the remaining 11 plants were present only at specific sites. In particular, *Acalypha indica*, *Euphorbia hirta*, *P. niruri*, *Cyanodon dactylon*, and *Paspalidium punctatum* were present only at a single site. Furthermore, 8 plants which belonged to non-mycorrhizal families and 6 plants which belonged to mycorrhizal families were detected with mycorrhizal incidence in their roots (Table 2). All 14 plant species recorded in the three sites showed

Table 2. Percent root colonization, and number of VAM spores and VAM species associated in the root-zone soils of three sites of the Kudankulam area.

Serial No.	Family and plant species	Sites ^a	Percent root colonization	VAM spore number/100 g of soil	VAM fungal species associated ^b
I. NON-MYCORRHIZAL FAMILIES					
AIZOACEAE					
1.	<i>Gisekia pharnaceoides</i>	1, 2, 3	75	150	LCRD, LFSC, LMCC, ASCB, SSNS
AMARAN TACEAE					
2.	<i>Amaranthus spinosus</i>	1, 2, 3	45	90	LFSC, LMCC, SCVS, SRBF
3.	<i>Digera muricata</i>	1, 2	55	100	ABRT, ASCB, LFSC
4.	<i>Gomphrena globosa</i>	1, 2, 3	80	165	LFLV, LFSC, SSNS
EUPHORBIACEAE					
5.	<i>Acalypha indica</i>	1	80	240	ABRT, AELG, LFSC, LGSP
6.	<i>Euphorbia hirta</i>	2	90	350	GGGT, LGSP, LMCC, LPVN
7.	<i>Euphorbia rosea</i>	1, 3	60	165	LFSC, LMSS, GGGT
8.	<i>Phyllanthus maderaspatensis</i>	1, 2	70	198	ABRT, LFSC
9.	<i>Phyllanthus niruri</i>	3	95	450	LFSC, LINR, SSNS
II. MYCORRHIZAL FAMILIES					
ASTERACEAE					
10.	<i>Eclipta alba</i>	2, 3	60	140	LFSC, LGSP, SSNS
COMMELINACEAE					
11.	<i>Commelina bengalensi</i>	1, 2	50	120	LCRD, LFSC, LMSS
POACEAE					
12.	<i>Cyanodon dactylon</i>	1	45	100	LFSC, LGSP
13.	<i>Paspalidium punctatum</i>	2	30	80	LFSC, LGSP, SRBF
14.	<i>Paspalum vaginatum</i>	1, 3	25	60	LFSC, LMSS, SSNS

^aSites are the same as in Table 1.

^bAbbreviations: ABRT, *Acaulospora bireticulata*; AELG, *Acaulospora elegans*; ASCB, *Acaulospora scrobiculata*; GGGT, *Gigaspora gigantea*; LCRD, *Glomus claroideum*; LFSC, *Glomus fasciculatum*; LFLV, *Glomus fulvum*; LGSP, *Glomus geosporum*; LINR, *Glomus intraradices*; LMCC, *Glomus macrocarpum*; LMSS, *Glomus mosseae*; LPVN, *Glomus pulvinatum*; SCVS, *Sclerocystis clavispora*; SRBF, *Sclerocystis rubiformis*; SSNS, *Sclerocystis sinuosa*.

mycorrhizal incidence. This result agreed with the widespread occurrence of mycorrhizae reported in natural ecosystems [2]. Non-mycorrhizal families, Amarantaceae, Aizoaceae and Euphorbiaceae, and their members of eight plants were revealed to be mycorrhizal in this study, in agreement with the report of Vijayalakshmi and Rao [31]. Mycorrhizal and non-mycorrhizal plant species together in different sites examined were reported previously [13].

Among the plant species examined, *Gisekia pharnaceoides* in sites 1, 2, and 3 were associated with 5 VAM species, maximum number. Among the investigated plant species, variations in the percentage root colonization and species composition of associated VAM fungi were observed. The percentage of VAM colonization was the highest in medicinal plants *P. niruri* (95%) and *E. hirta* (90%). The lowest percentage of VAM colonization was recorded in *Paspalum vaginatum* (25%). There was a significant correlation between the number of spores and of percentage root colonization, as exemplified by *P. niruri* and *P. vaginatum* (450, 95%; 60, 25%) (Table 2). A similar correlation between spore numbers and percentage root colonization was also reported previously [15].

Although belonging to non-mycorrhizal and mycorrhizal families, all 14 plant species recorded in the three sites showed mycorrhizal association, showing that, even in the disturbed environment, the help of VAM association supports the plant species to survive. Reports already revealed that most of the vascular plants require mycorrhizae (VAM) to survive in the unfavorable environments [29, 30]. The occurrence of VAM fungi has been reported in a few medicinal plants. The occurrence of VAM fungi of the medicinal plants in this study agreed with Mukerji and Ardey [21] for *Eclipta alba*, and Selvaraj [29] for *Zingiber officinale*.

Status of Identified VAM Fungal Spores

At the site 1, three, one, six, and another three species of *Acaulospora*, *Gigaspora*, *Glomus*, and *Sclerocystis*, respectively, were recorded. At the site 2, two, one, seven, and three species of *Acaulospora*, *Gigaspora*, *Glomus*, and *Sclerocystis*, respectively, were recorded. At the site 3, one, another one, seven, and three species of *Acaulospora*, *Gigaspora*, *Glomus*, and *Sclerocystis*, respectively, were recorded (Table 3). The number of spores in the root-zone soils of the three sites varied significantly, in agreement with the earlier findings of Reddy and Manoharachary [23]. These could be due to the differences in the various environmental and edaphic factors of the sites, as reported previously [5, 15].

Among the 15 isolated VAM species, 11 species including *A. scrobiculata*, *Gig. gigantea*, *Gl. claroideum*, *Gl. fasciculatum*, *Gl. fulvum*, *Gl. geosporum*, *Gl. macrocarpum*, *Gl. mosseae*, *Scl. clavisporea*, *Scl. rubiformis*, and *Scl. sinuosa* were encountered in all the three sites. *A. bireticulata* was found

Table 3. Presence of VAM fungal species in the soils of three sites.

VAM fungal species ^a	Site		
	Site 1	Site 2	Site 3
ABRT	+	+	-
AELG	+	-	-
ASCB	+	+	+
GGGT	+	+	+
LCRD	+	+	+
LFSC	+	+	+
LFLV	+	+	+
LGSP	+	+	+
LINR	-	-	+
LMCC	+	+	+
LMSS	+	+	+
LPVN	-	+	-
SCVS	+	+	+
SRBF	+	+	+
SSNS	+	+	+

^aAbbreviations used as in Table 2.

only in two sites. The remaining 3 species including *A. elegans*, *Gl. pulvinatum*, and *Gl. intraradices* were found only in one particular site (Table 3). This investigation revealed the occurrence of both host specific and non-host specific association of VAM fungi. Among the investigated fourteen host plant species, *E. hirta* and *P. niruri* showed host specific association of the fungi *Gl. pulvinatum* and *Gl. intraradices*, as reported previously [7, 28]. Although VAM species are not known to be strictly site specific [20], the fact that *A. elegans* was observed only in the site 1, *Gl. pulvinatum* only in the site 2 and *Gl. intraradices* only in the site 3 showed site-specificity in this study.

Effect of VAM Spore Inoculations in the Experimental Plants

The plants of *P. niruri* in the non-mycorrhizal family and *E. alba* in the mycorrhizal family were field inoculated with selected dominant VAM fungal spores and analyzed for percentage root colonization, spore numbers, and dry matter content. It was observed that both the two plant species inoculated with *Gl. fasciculatum* gave higher level of percentage root colonization (90 and 92%), higher number of VAM spores (440 and 528), higher dry matter content (950 and 1,650 mg/plant), and higher level of potassium (48.12 and 45.42 mg/kg dry wt) as well as calcium (64.34 and 72.37 µg/g dry wt), followed by those inoculated with *Gl. geosporum* and *Scl. sinuosa* (Table 4).

The two experimental host plants *P. niruri* and *E. alba* inoculated with selected VAM spores revealed that *Gl. fasciculatum* was the most efficient VAM fungi with maximum VAM infection in the root, and was more beneficial for the plant growth. It was noticed that in spite of growth

Table 4. Percent root colonization, number of VAM spores, dry matter content, and nutrient level in the root of *Phyllanthus niruri* and *Eclipta alba* plant species, harvested 75 days after inoculation with different VAM species.

Treatment	Percent root colonization	VAM spores/100 g of soil	Dry matter content (mg/plant)	Nutrient level in root (dry wt)	
				K (mg/kg)	Ca (μ g/g)
<i>Phyllanthus niruri</i>					
Control	0	0	250 ^a ±12.5	11.56 ^a ±2.15	12.14 ^a ±2.80
<i>Glomus fasciculatum</i>	90±1.5	440±1.4	950 ^a ±13.4	8.12 ^a ±5.17	64.34 ^a ±6.12
<i>Glomus geosporum</i>	86±1.4	342±1.2	880 ^a ±12.4	2.16 ^a ±4.48	63.48 ^a ±5.57
<i>Sclerocystis sinuosa</i>	63±1.4	186±0.8	720±11.3	39.25±4.25	55.19±4.78
<i>Eclipta alba</i>					
Control	0	0	540 ^a ±12.4	3.50 ^a ±2.59	14.65 ^a ±3.22
<i>Glomus fasciculatum</i>	92±1.8	528±1.6	1650 ^a ±13.8	45.42 ^a ±5.43	72.37 ^a ±6.74
<i>Glomus geosporum</i>	80±1.5	370±1.4	1420 ^a ±11.8	39.96 ^a ±4.82	67.48 ^a ±5.94
<i>Sclerocystis sinuosa</i>	70±1.5	210±0.9	980±10.8	35.23±4.53	62.87±5.14

^aDifferences in the values are statistically significant ($p=0.5$) using F-test.

disturbance, VAM-inoculated plants were efficient in tolerating the endangered ecosystem. Similar results have been reported earlier [17, 19]. The two experimental host plants showed more uptake of K^+ and Ca^{2+} in the root of VAM inoculated plants, revealing the influence of VAM fungi for the plants to take up more nutrients like potassium and calcium from the soil area. The earlier reports revealed that mycorrhization raised the K^+ level in root [1, 9]. Since Ca^{2+} plays a role in cell wall integrity like K^+ , the requirement of Ca^{2+} for the root cell is more important, therefore, the VAM inoculation induces more Ca^{2+} absorption from the soil and translocates to the root and increases the level of Ca^{2+} in the root [30].

The earlier findings [6, 8, 14, 17] showed evidence for the involvement of VAM and the soil factors to support the vegetations in the radionuclide deposited soils. Although the effect of radionuclides was not carried out in this study, the chances are for more involvement of VAM fungi and the soil factors to prevent the activity of the radionuclides in the sites, if accidental release of radioactivity happens in the future. In higher mineral soils where Cation Exchange Capacity (CEC) is clay based, radionuclides will be held by charge only. Clays can, however, hold radionuclides, particularly cesium, with great strength. This is because the cesium becomes trapped within the clay lattice, preventing subsequent ion exchange with the soil solution.

Uptake and cycling of ^{137}Cs by *Calluna vulgaris*, for example, continued for 20 years after deposition on moorland soils after the Chernobyl nuclear disaster [8]. Even vegetation types that are not so responsive to potassium will still require it as a macronutrient, therefore, it will take up significant levels of radionuclide analogues. For example, ^{137}Cs was present in grass pasture in N.E. Scotland at levels of 10 to 20 Bq kg^{-1} for four months after the Chernobyl nuclear disaster [18]. The uptake of ^{90}Sr , a tracer and analogue for calcium, is enhanced by VAM infection [6]. Even the mechanisms by which ^{137}Cs and ^{90}Sr are taken up

by plant roots are not completely understood. Previous studies [32, 33, 34] revealed that Cs^+ is absorbed as K^+ by the K^+ -uptake system of the root. This evidence showed that the K^+ -uptake system strongly suppresses Cs^+ -uptake and the K^+ is efficiently transported by an isolated high affinity K^+ -uptake transporter of root cells. Since ^{137}Cs and ^{90}Sr behave like potassium and calcium, respectively, the soil characters showed its activity to hold the radionuclides and prevent the radioactivity. Chances are greater for the VAM fungi to absorb and translocate K^+ of the radionuclides cesium and Ca^{2+} of strontium, like usual potassium and calcium absorption and translocation [1, 9]. Thus, through the help of VAM fungi and the soil's nature to hold the radionuclide to prevent the expression of radioactivity, the chances are greater for the vegetations to survive in the disturbed ecosystem in a better way. There is very strong circumstantial evidence, therefore, that VAM infection will enhance uptake and cycling of radionuclides, particularly ^{137}Cs and ^{90}Sr , if they are deposited on an ecosystem as a result of accidental release, and are helpful for the plant to survive in that disturbed ecosystem by using the radionuclides just like potassium and calcium, thereby enhancing the plant growth.

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