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RESEARCH ARTICLE

Seasonal Fluctuation of AM fungi on two hydrocarbon yielding plant species of *Jatropha*

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Abstract

Seasonal fluctuation of AM fungal colonization and spore count on two hydrocarbon yielding plants revealed, that the soil type and physico-chemical features of the soil do not play a role in distribution of spores in selected 12 sites. However, the higher spore number was determined during summer in both *Jatropha curcas* L. and *Jatropha gossypifolia* L. and per cent root colonization was significantly higher in rainy season. The spore population was moderately low in rainy season in *Jatropa gossypifolia* L. and *Jatropha curcas* L. AM fungi belonging to genera *Glomus, Gigaspora, Acaulospora, Sclerocystis* and *Scutellospora* were isolated in Dharwad district soils. During the study period *Glomus* was dominated over other remaining four genera. The ecological and edaphic characteristics have been discussed with respect to spore distribution and root colonization.

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi form obligate symbiotic association with the roots and other underground parts of most of the terrestrial plants. They are more prevalent in phosphorus deficient soils. Arbuscular mycorrhizal fungi are capable of scavenging phosphorus from the soil into the root system mainly through exploration of soil by extra radial hyphae beyond the root hairs and the phosphorus depletion zone. These fungi have the potential to enhance plant growth and biomass is well documented (Smith and Read, 1997; Bagyaraj, 2006; Lakshman and Patil, 2004). Thus, AM fungi are also known to be a promising biofertilizers. The symbiotic association benefits plants by improving nutrients. Recent investigations revealed that mycorrhizal fungi enhance the growth and productivity of different crops (Mosse, 1991; Lakshman 1999).

In a number of developing countries the substitution of imported petroleum derivatives by domestically produced biomass-based engine fuels currently regarded as a promising strategy for eliminating energy shortages. Keeping all these things in mind, the present study was undertaken to evaluate the production of liquid bio fuels by the inoculation of AM fungi to the potential hydrocarbon yielding plants and utilizing them as a substitute for petroleum derivatives. Moreover, information on AM fungal seasonal diversity studies on hydrocarbon bearing plants is scanty. Therefore, in the present study on two hydrocarbon yielding plants *Jatropha curcas* L. and *Jatropha gossypifolia* L. have been undertaken.

Materials and methods

Root samples and the rhizospheric soil samples of *Jatropha curcas* L. and *Jatropha gossypifolia* L. were collected from 12 different places of Dharwad during the year 2012-2013. The plants were uprooted for the collection of root samples. Freshly collected root samples were washed in water, cleared with 10% of KOH,

acidified with 2% HCl and stained in 0.05% tryphan blue in lactophenol (Phillips and Hayman, 1970). AM fungal spores were isolated from soil by using wet sieving and decanting technique following the procedure of (Gerdemann and Nicolson, 1963). Matured seeds were collected from 12 year old plants. Seeds surface was sterilized in 2% sodium hypochlorite, subsequently 2-3 times washed in tap water and soaked in distilled water for 6 hrs and they were sown in 20 × 25 (breadth x length) cm diameter. Earthern pots were filled with 4kg of soil contain (3:1) sterile garden soil: sterile pure sand. The physico-chemical characteristics of the soil used for experimental pots are shown in (Table 1).

Table 1: Physico-chemical characters of the soil used for experimental pots.

Characteristics	Mean Value
pH	6.8
Sand (%)	73.54
Silt (%)	19.43
Clay (%)	05.86
O.M (%)	0.97
E.C. mmhos/cm^2	0.89
N mg/kg	8.36
P mg/kg	0.83
K mg/kg	32.07

Table 2: Association of AM fungi in two hydrocarbon yielding plants *Jatropha* sp. showing percent root colonization and spore number at twelve different places of Dharwad district.

Places	Soil type	Plant species	%AM colonization	Spore number/50g Soil
Dummwad	Sandy loam	JC	63.4	123
		JC	61.5	118
Kalghatagi	Brown	JC	72.7	306
		JG	91.4	113
Dastikoppa	Brown	JC	79.3	127
		JC	94.6	201
Gudageri	Red	JC	58.1	198
		JC	49.8	144
Narendra	Black cotton	JC	61.5	205
		JG	52.2	124
Sanshi	Red	JG	54.1	222
		JG	73.6	133
Kamdolli	Sandy loam	JC	61.2	456
		JC	78.5	179
Amminbhavi	Black cotton	JG	93.3	183
		JG	69.4	207
Ramapur	Black cotton	JG	72.5	99
		JC	67.3	286
Hindasageri	Brown	JC	76.4	139

		JC	59.4	272
Nuggikeri	Sandy loam	JC	63.2	312
		JG	60.2	210
Alnavar	Sandy loam	JC	83.2	115
		JC	75.1	309

JC – *Jatropha curcas* L ; JG – *Jatropha gossypifolia*

Table 3: Effect of different seasons on the occurrence of AM spores and percentage root colonization in the rhizosphere/non-rhizospheric soils of *Jatropha curcus* L. growing areas of Dharwad region at four different sites.

Season	AM spore population in 50 gm of Rhizospheric soil				Root colonization (%)			
	Sites1	Sites 2	Sites 3	Sites 4	Sites 1	Sites 2	Sites 3	Sites 4
Winter	191±2.3	134±5.2	177±3.1	140±0.0	66±1.1	47±3.1	56±7.1	58±3.3
Summer	395±1.0	330±3.1	380±2.0	343±1.1	54±2.2	33±4.4	45±3.4	48±2.1
Rainy	163±0.1	115±5.0	143±4.1	120±2.1	89±5.0	51±6.2	74±5.2	80±4.3

Each value is the mean of 8 samples ± standard error

Table 4: Effect of different seasons on the occurrence of AM spores and percentage root colonization in the rhizosphere/non-rhizospheric soils of *Jatropha gossypifolia* L. growing areas of Dharwad region at four different sites.

Season	AM spore population in 50 gm of Rhizospheric soil				Root colonization (%)			
	Sites1	Sites 2	Sites 3	Sites 4	Sites 1	Sites 2	Sites 3	Sites 4
Winter	180±2.1	148±2.6	163±2.4	154±0.2	60±2.0	50±4.2	63±8.1	51±4.8
Summer	346±1.4	320±4.8	359±3.5	343±2.6	58±2.4	39±5.9	40±4.6	42±1.6
Rainy	148±0.8	112±6.2	134±3.9	126±2.8	85±4.0	76±7.2	80±5.4	82±5.6

Each value is the mean of 8 samples ± standard error

Figure 1: Effect of different seasons on the occurrence of AM spores in the rhizosphere/non-rhizospheric soils of *Jatropha curcus* L. growing areas of Dharwad region at four different sites.

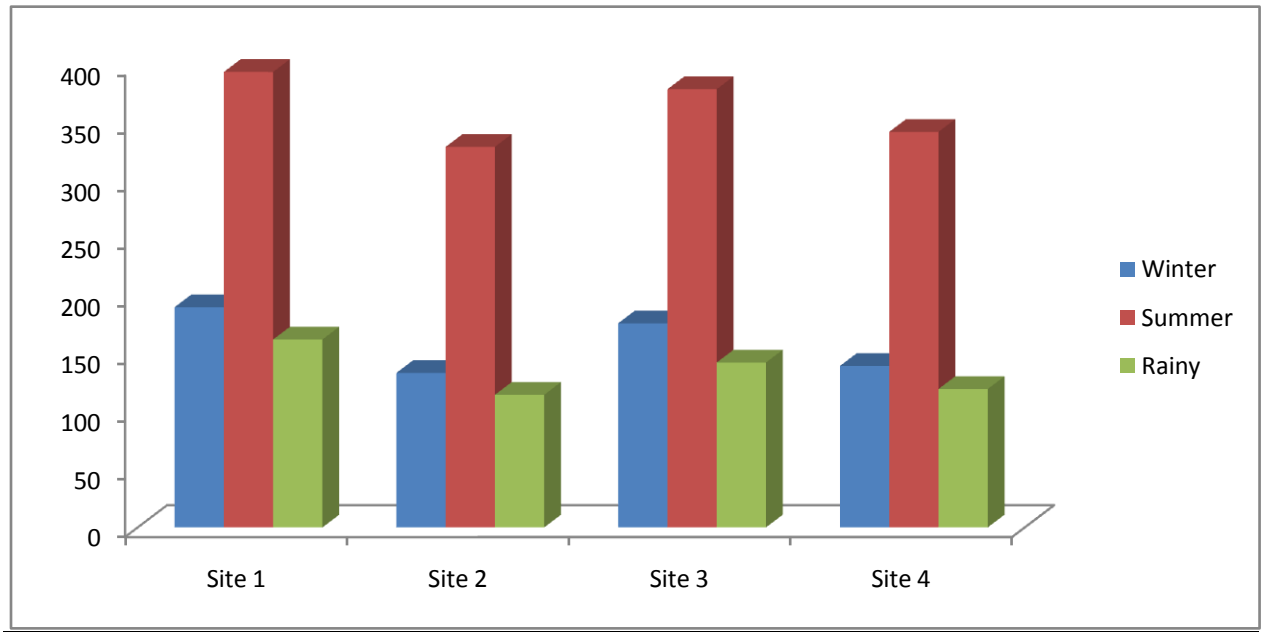

Figure 2: Effect of different seasons on the occurrence of AM spores in the rhizosphere/non-rhizopspheric soils of *Jatropha gossypifolia* L. growing areas of Dharwad region at four different sites.

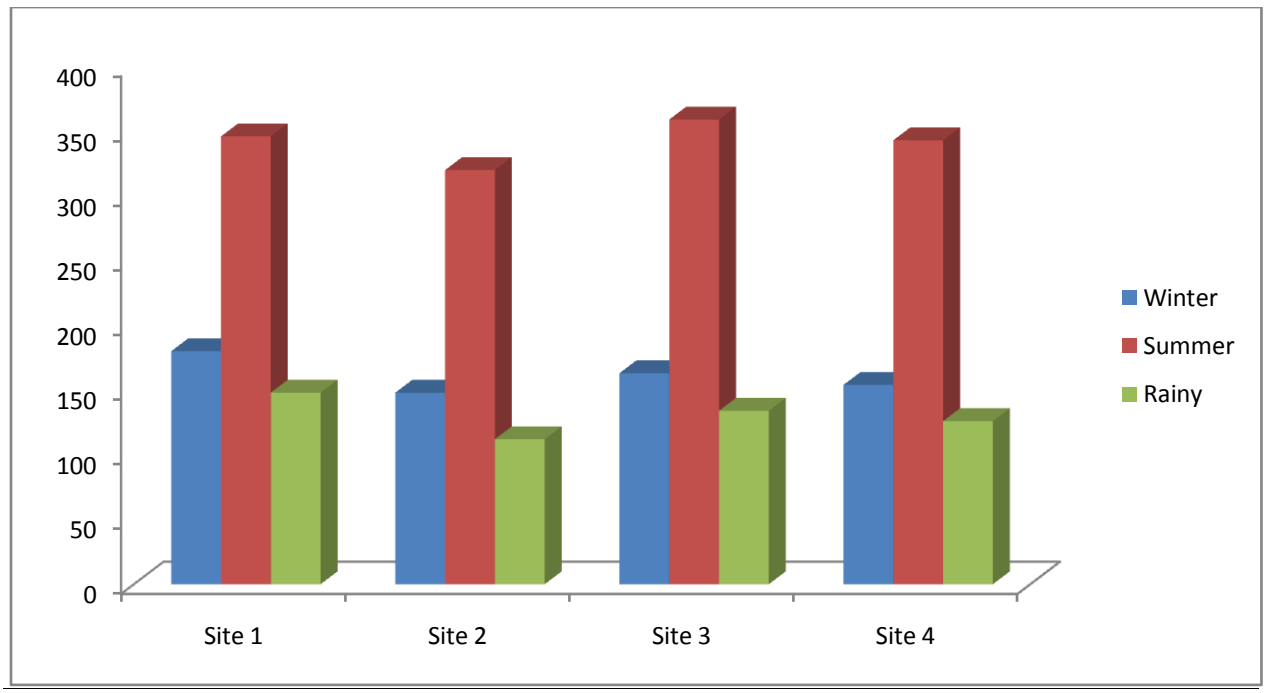

Figure 3: Effect of different seasons on the percent root colonization in *Jatropha curcas* L. growing areas of Dharwad region at four different sites.

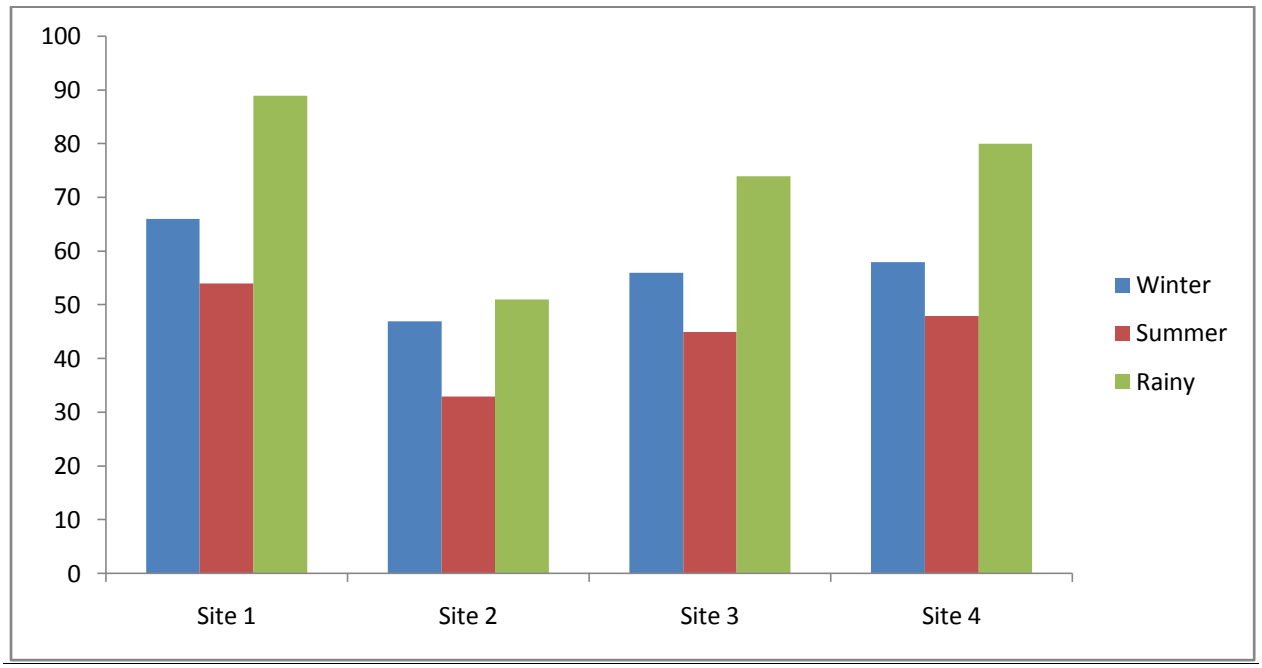

Figure 4: Effect of different seasons on the percent root colonization in *Jatropha gossypifolia* L. growing areas of Dharwad region at four different sites.

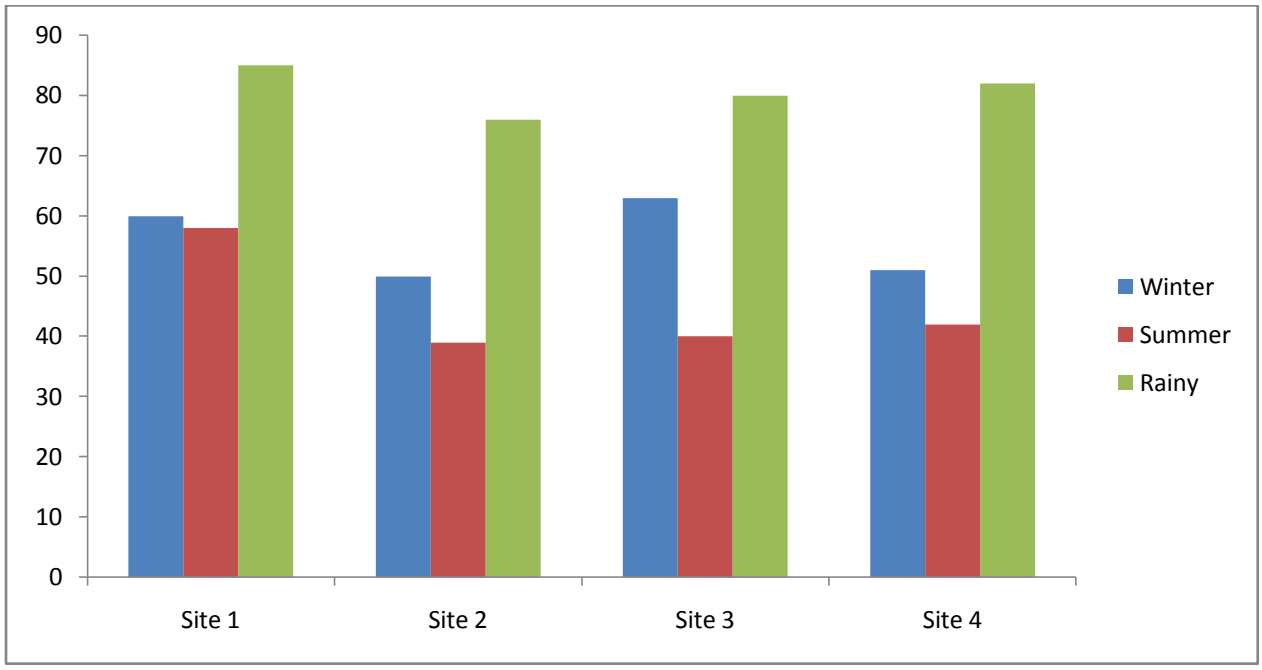

Table 5: Frequency and Distribution of AM fungi in rhizosphere soil samples of Dharwad district from 12 sites.

Fungus/places	1	2	3	4	5	6	7	8	9	10	11	12	frequency
Acaulospora scrobiculata Trappe	-	-	-	+	-	+	-	-	+	-	-	+	25
Gigaspora margarita Becker and Hall	+	+	+	-	+	-	+	+	-	-	+	-	58
Glomus aggregatum Schenck and Smith	+	+	+	+	+	+	+	+	-	+	-	-	75
Glomus botryoides Rothwell and Victor	-	+	+	-	+	-	-	-	+	-	-	+	42
Glomus deserticola Trappe, Bloss and Menge	+	+	-	-	+	-	+	-	+	-	+	-	50
Glomus geosporum (Nicol &Gerd)Walker	+	+	+	-	+	-	+	-	-	-	+	-	50
Glomus mosseae (Nicol & Gerd) Gerd and Trappe	+	+	+	+	-	-	+	-	+	-	+	+	66
Sclerocystis pachycaulis Wu and Chen	+	+	+	-	+	-	-	-	-	+	-	+	50
Sclerocystis pakistanica Iqbal and Bushra	+	-	+	-	+	-	-	+	-	+	+	-	50
Sclerocystis sinuosa) Gerd and Bakshi	+	-	+	-	-	-	-	+	+	-	+	-	42
Scutellospora calospora (Nicol &Gerd) Walker and Sanders	-	-	-	+	-	+	-	-	-	-	-	-	17

Note: + = Present, - = Absent

Results and discussion

The roots of *Jatropha curcas* L. and *Jatropha gossypifolia* L. showed the presence of hyphae, vesicles and arbuscules. Microscopic and anatomical observation of AM fungi colonized roots showed mycorrhizal components had clear arrangement with in outer cortex. The relative abundance of mycelium in roots based on root length and entry points and root hairs count. High percentage of mycorrhizal colonization was recorded in both the *Jatropha* species. Usually colonization was seen in second set of lateral roots from the top. Young terminal roots had significant colonization. However, no root colonization was recorded in meristematic zone of the roots. The penetration in inoculated plants occurred by the third and fourth day of inoculation. Appressoria formation was occasional but not frequent. In most cases, the penetration was direct, by rupturing the outer cell wall. The hyphae developed inter and intracellularly in cortical cells. Arbuscule formation was recorded on the third and sixth day penetration. The arbuscules lived for 5-16 days and then degenerated. Vesicle formation was noticed from the 10^{th} day onwards after penetration of the host cell. Extra matrical spores were observed on the 21st day after penetration irrespective of soil types (Table 1). The soil types do not influence in increase/decrease in percent root colonization and spore count in *Jatropha* species. However, the chemical composition especially higher concentration of P in soil, decreased the root colonization and spore count drastically. In case *J. curcas* spore number was higher, when plants growing in brown and sandy soil (83-94%). Similarly, in case of *J. gossypifolia* the plants growing in brown and black cotton soil showed (91-93%) root colonization. The number of spore count also varied, the brown soil of rhizosphere of *J. curcas* had higher spore count 306/50g. soil. The maximum number of spores (312/50 g. soil) were isolated in case of *J. gossypifolia* growing rhizosphere shown in (Table 1). Higher per cent of root colonization was observed during rainy seasons, which was followed by winter season but lower percent root colonization was noted down during summer in both the *Jatropha* species shown in (Table 3&4). Seasonal distribution of AM fungal spores also varied. The highest number of spores was isolated during summer; it is being followed by winter and rainy seasons respectively. This is being clearly shown the decreased number of spore population was lower during rainy seasons in both the *Jatropha* species shown in (Fig 1-2).

Dharwad district soils exhibited rich in AM fungi belonging to genera *Glomus Gigaspora, Acaulospora, Sclerocystis* and *Scutellospora* genera. *Glomus* was predominated other than the remaining four genera (Table 5). Similar observation was made by (Lakshman et al. 2003). The spore population was moderately low in rainy season and maximum during summer season in *Jatropa gossypifolia* L. and *Jatropha curcas* (figure 1&2). The results are in agreement with early workers observations (Hayman, 1970; Tapar and Khan, 1988; Airsang, 2007: Romana and Lakshman, 2013; Airsang and Lakshman, 2014)). Root colonization was found to be 58-93% in *Jatropha curcas* L. and 54-93% in *Jatropha gossypifolia* L. growing in natural soil. High colonization of roots of *Jatropha curcas* L.and *Jatropha gossypifolia* L. by AM fungi indicates that these species are mycorohizal dependent, interestingly for each plant species, the efficient of AM species were different. Soil amendments with fertile soil served as a source of inoculum for rapid development of fungi (Pagano et al. 2013), which in turn, efficiently nourished the plants with essential elements. Biological activities in soil also increased the absorbing capacity of roots.

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