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ROOT COLONIZATION AND SPORE ABUNDANCE OF AM FUNGI IN COCONUT PALM WITH LOW-INPUT COCONUT CROPPING SYSTEMS OF KASARAGOD DISTRICT

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Abstract: *The coconut ranks second in gross values among agriculture crops in Kerala. Five rhizosphere soil and root samples from basins of coconut based multi species cropping system of coconut AMF spore count varied in different coconut cropping systems. The spore count was ranges from 122 to 167.3 spores per 100 gram soil. AM fungal species isolated from coconut cropping systems are belongs the genus namely Gigaspora, Glomus and Sclerocystis. The Arum-type mycorrhizal colonization was observed in coconut cropping system. Frequency of root colonization was found to be significant positive correlation with mycorrhizal spore count and also significant correlation with soil beneficial microflora.*

Key words: *Coconut, AM fungi, Glomus, Arum-type.*

Introduction

The coconut (*Cocosnucifera*L.) is the most extensively grown and used nut in the world and the most important palm. India is the largest producer of coconut in the world and the crop plays a significant role in the agrarian economy of some states. Coconut ranks second in gross values among agriculture crops in Kerala. Unfortunately, the coconut industry has recently faced with a major problem in yield reduction. One of the reasons attributed is depletion of soil fertility and inadequate nutrition. Mycorrhizas form a critical link between the aboveground plant and the soil by influencing plant nutrient cycling and soil structure (Korb et al., 2003) and make a large direct contribution to soil fertility and quality through soil organic matter (Rillig et al., 2001). The arbuscularmycorrhizal fungi (AMF) are beneficial soil micro-organisms used as bio-fertilizers to improve seedling vigour in plant nurseries as well as in fields to restore the soil fertility and thereby to improve crop growth. From this point of view the centre of attention of this study was the status of AMF spore count and root colonization in coconut palm in coconut cropping system also to enumerate the plant beneficialmicroorganisms in the rhizosphere.

Materials and methods

Study area

Soil samples from coconut and coconut based multi species cropping system were collected from Neerchal, Soorambail, Badiyadka, Vidyagiri and Thalanagaraof Kasaragod District during the month of October 2016.

Sample collection

Field sites selected for the study was strictly maintained under organic management practices. The soil sample and undamaged feeder root lets were collected at a lateral distance of one meter from the bole of the coconut palm and depth of up to 25 cm. The soil samples were stored in polyethylene bags.Three replications were maintained for each cropping system. The soil samples were analyzed for AM fungal colonization. Before processing, the soil samples were sieved (2 mm mesh size) and root segments were collected from each sample.Root samples were washed with running tap water, cut into small pieces (ca. 1 cm) and stained with 0.05 % trypan blue using the method described by

Kormanik and McGraw (1982). The stained root samples were observed under compound microscope for the presence of AM fungi. The characteristic features of endomycorrhizae were recorded as vesicles, hyphal coiling and arbuscules. When anyone of these was found on a sample, colonization was recorded as positive and calculated as:

$$\text{Percantageof root colonization} = \left(\frac{\text{number of mycorrhzal root segmentsobserved}}{\text{Total number of root segmentsobserve}}\right) X100$$

The collected air dried soil sample (100 gm) was directly used to estimate the AM fungal spores. Wet sieving and decanting method (Gerdemann and Nicolson, 1963) was followed to isolate the arbuscularmycorrhizal spores. Further, the intact and crushed spores were mounted on PVLG and examined under a compound microscope. Spores were identified to genus level based on spore colour, size, surface ornamentation and wall structure with reference to the identification manual of Schenk and Perez (1990).

Enumeration of beneficial microbes

Mycorhizospheric soil samples were analyzed for the enumeration of general (aerobic heterotrophic bacteria, filamentous actinomycetes and fungi) and function specific (free-living nitrogen fixers, phosphate solubilizers and fluorescent pseudomonads) microorganisms. Population was estimated by plate count method. Soil samples were serially diluted and pour plated on Nutrient agar, Rose Bengal Agar, Kenknight & Munaier's Medium, Kings B agar, Pikovskaya's agar and Jensons agar media for the determination of bacteria, fungus, actinomycetes, fluorescent pseudomonades, and phosphate solubilizers and nitrogen fixers.

Soil edaphic factors

Soil samples were analysed for different physico chemical parameters namely; soil pH, moister and organic carbon (Walke and Black, 1934).

Statistical Analysis

Pearsons's correlation analysis was used to determine relationship between AMF spore count, root colonization, diversity indices and soil physiochemical factors using SPSS Base 20.0 (SPSS, Cary, N.C.). One way ANOVA was used to test for the spore count, root colonization diversity indices and soil physiochemical factors in coconut using SPSS Base 20.0 (SPSS, Cary, N.C.).

Results and discussion

Five rhizosphere soil and root samples from basins of coconut based multi species cropping system of coconut farmers plot of Neerchal, Soorambail, Badiyadka, Vidyagiri and Thalanagaraof Kasaragod District during the month of October 2016.The soil and root samples were analysed for AM fungal spore count, AM fungal species diversity, root colonization, enumeration of soil beneficial microorganisms and physico-chemical characteristics.

Soil physicochemical characteristics

The cropping system studied had laterite soil. The results of the soil analysis are given in the Table 1. The soil in coconut cropping system is neutral slightly acid to neutral in reaction in reaction. The pH values ranged from 6.2 to 6.9 (average 6.5). The organic carbon content in the coconut cropping systems ranged from 1.1 to 1.9 % with an average of 1.5%. The soil moisture content was varied from 17.4 to 30.7 % with an average of 23.8. %.

Table 1: Physio-chemical properties of soil samples in coconut cropping systemsKasaragod District

Sampling area	pH	Organic carbon (%)	moisture %
Neerchal	6.3±0.1c	1.2±0.03b	17.4±1.1c
Soorambail	6.6±0.1a	1.9±0.03a	29.1±1.1a
Badiyadka	6.2±0.S1c	1.2±0.03b	18.5±1.1c
Vidyagiri	6.9±0.1a	1.9±0.03a	30.7±1.1a
Thalanagara	6.6±0.1b	1.1±0.03c	23.2±1.1b

AM root colonization and spore count

A high level of AM fungi spores were recorded from all the rhizosphere soil samples. Total spore count of AMF varied in different cropping systems. The spore count was highest in coconut cropping in systems Vidyagiri (167.3 spores per 100 gram soil) and lowest in Soorambail (122 spores per 100gram soil). The data was presented in the Table2. Johnson et al., (1991) reported that high spore count is an indication of a soil's mycorrhizalinoculum potential.There are many factors that could affect spore density in a given host rhizosphere. Rajeshkumaret al., 2015 reported that the AMF spore varied significantly in coconut cropping systems. AM fungal species isolated from coconut cropping systems are belongs the genus namely *Gigaspora, Glomus and Sclerocystis*. All of them were found in the all soil sample collected from rhizosphere soil of coconut cropping systems.

Percentage of root colonization

All the root segment observed were colonized for AM fungi. Mycorrhizal association was observed in coconut palm in coconut cropping systems of Kasaragod district. Microscopic observation of the root samples showed the presence of extensive hyphal, vesicular and arbuscular stages of AM fungal colonization and in some samples presence of spores was also seen within the roots. The mycorrhizal infection restricted to the epidermis and did not penetrate in to endodermis. Vesicles globose to elongate present in both intercellular and intracellular layer of cortical cells. The observation of presence of intercellular linear hyphae, arbuscules and vesicles indicated the mycorrhizal association in coconut roots to be of Arum-type as reported by Muthukumar and Prakash (2009).

The colonization of AM fungi in the roots however differed from location to location. The percentage colonization of AM fungi in roots of coconut in different cropping systems was ranged from 76.5 % (Soorambail) to 97.0 % (Vidyagiri),which were significantly different. The data was summarized in the table 2. It is evident from data that all the coconut cropping system had root colonization above 75%. This study confirmed widespread occurrence of AMF in association with coconut cropping systems at different locations though there was considerable variation in the root colonization and spore count in the rhizosphere soil (Rajeshkumaret al., 2015; Ambiliet al., 2012).

Table 2: Root colonization and spore count of AM fungi in coconut cropping system

Sampling area	Root colonization percentage	Spore count per 100 gram soil
Neerchal	92.2±0.9b	142.8±6.6
Soorambail	76.5±0.9d	122.0±6.6b
Badiyadka	80.4±0.9c	133.5±6.6b
Vidyagiri	97.0±0.9a	167.3±6.6a
Thalanagara	95.2±0.9a	129.1±6.6b

Pearson,s correlation of mycorrhizal spore load with root colonization and soil edaphic factors is given in the Table 3. Frequency of root colonization was found to be significant positive correlation with mycorrhizal spore count and Fluorescent pseudomonas in coconut cropping systems and week positive correlation with soil pH, bacteria, actinomycetes, nitrogen fixers and P solubilizers. Our study revealed that, increased spore count shows increased colonization. However, some researchers observed that no significant correlation between AM colonization and spore count (Li et al., 2007).

Table 3: Pearsons correlation analysis of mycorrhizal spore count, root colonization, edaphic factors and soil beneficial microbial populations

	Spore count	Organic carbon	Moisture content	pH	bacteria	fungus	Actinomycetes	Fluorescent pseudomonas	Nitrogen fixers	P-Solubilizers
Root colonization	0.56*	-0.27	0.01	0.40	0.39	-0.14	0.49	0.57*	0.49	0.16
Spore count	1	0.29	0.23	0.38	0.51*	-0.05	0.56*	0.36	0.45	0.09

*. Correlation is significant at the 0.05 level (2-tailed).

Generally, AMF colonization is influenced by spore availability (Muthukumar et al., 2003). The irregular spatial distribution of AMF spores and the complex structure of the underground root component should be considered as most important factors affecting AMF spore density that could contribute to variable rates of AMF colonization among plants (Zhao et al., 2001). But root colonization showed week negative correlation with organic carbon. The spore count also showed significant positively correlated with soil bacteria and Actinomycetes and week positive correlation with soil pH, organic carbon, Moisture content, Fluorescent pseudomonas, Nitrogen fixers and P-Solubilizers. But spore count showed negative correlation with soil fungi. Previous works have reportedthe presence of different beneficial groups of bacteria such as plantgrowth promoting rhizobacteria, phosphate – solubilizing bacteria (Toro et al., 1996) and nitrogen fixing bacteria (Smith and Goodman 1999) associated with therhizosphere of different plants colonized by AMF (SeciliaandBagyaraj 1987). In our studies root colonization and spore count showed positive correlation with soil beneficial bacteria and actinomycetes population. The previous report by Ambili et al., (2012) in coconut cropping system a significant correlation could not be drawn for thebeneficial microflora and AMF population.

Conclusion

The symbiotic association between AM fungi and the roots of plants is widespread in the natural environment. The present study confirmed wide spread occurrence of AMF in coconut cropping systems of Kasaragod districts. The spore abundance was a good indicator of soils AMF inoculum potential. AM fungal species isolated from coconut cropping systems are belongs the genus namely *Gigaspora, Glomus and Sclerocystis*. In coconut cropping system a significant correlation observed between AMF and beneficial microorganisms. Further research should be targeted towards understanding functional ecology of AM fungi in coconut cropping systems.

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