RHIZOSPHERE FUNGI OF RED PEPPER {*Capsicum frutescens}*

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Abstract
Fungi present in the rhizosphere and rhizoplane of Capsicum frutescens at different stages of plant growth were studied. The experimental soil was loamy sand in texture. The pH of the rhizosphere soil in the field ranged from 6.4 to 6.7 and in the pots, it ranged from 6.3 to 6.8. The fungi isolated were Aspergillus niger, Rhizopus stolonifer, Aspergillus flavus, Aspergillus candidus, Penicillium expansum, Fusarium oxysporum, Fusarium poae, Penicillium citrinum, Mucor racemosus, Mucor mucedo, Verticillium lateritium, Fusarium avenaceum, Trichophyton mentagrophyte, Fusarium verticilliodes, Aspergillus ustus, Saccharomyces cerevisiae and Aspegillus fumigatus. The most frequently isolated fungi were Rhizopus stolonifer, Aspergillus nigers and Mucor mucedo. The highest fungal number occurred on the 30th day of plant growth for pots and field.

Key words : *Fungi , rhizosphere, rhizoplane, plant*
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Introduction

Soil is the topmost layer covering the earth crust. It is a living environment that supports extremely diverse communities of micro-and macro-organisms (Van -Elsas *et al*., 1997). Soil is one of the most dynamic sites of biological interaction in nature and it is the region in which many of the biochemical reactions concerned in the destruction of organic matter, weathering of rocks and nutrition of agricultural crops occur (Alexander, 1967).

The rhizosphere is the zone of the soil surrounding plant roots where biology and chemistry of the soil are influenced by the roots. The zone has no distinct edge ; it is an area of intense biological and chemical activity influenced by compounds exuded by the roots and by microorganisms feeding on the compounds (Rebecca, 2005). As plant roots grow through the soil, they release water soluble compounds such as amino acids, sugars and organic acids that supply food for the microorganisms. These compounds are broadly known as exudates. In turn, microorganisms provide nutrients to the plant, through their decomposition activities, thus creating a critical linkage between the plant roots and the soil (Davies, 1982). The plant root surface, termed the rhizoplane also provides a unique environment for microorganisms, as the exudates move from the plant roots to the soil. The rhizosphere and rhizoplane microorganisms not only increase in number when these new substances become available, but their composition and function also change. The exudates have several functions. They may function in the defence of rhizosphere and roots against pathogenic microorganism;, they may repel particular microbial species; they keep the soil around the roots moist; they provide nutrients for microorgarnisms;

they serve to change the chemical properties of the soil around the roots; they stabilize soil aggregates around the roots; and they inhibit the growth of competing plant species (Czames *et al.*, 2000).

Capsicum frutescens also known as red pepper or long cayenne is a short-lived evergreen shrub usually 1 to 1.5m in height and 0.5cm in basal stem diameter (Dutta, 1979; Bosland and Votava, 2000). It grows on soils of different types of texture and levels of fertility. . Moist, well drained soil with loose structure is best for rapid growth. The species is intolerant of shade, and fruits best in full sun. Soil pH of 4.3 to 9.7 is tolerated (Centre for New Crops and Plant Products, 2002). The plant flowers after 80 days of growth and fruits continuously as long as it lives (Centre for New Crops and Plant Products, 2002). However, fruiting starts between 90 to 120 days of planting. The flowers are insect pollinated (Bosland and Votava, 2000). Under continually favorable conditions, red pepper lives about 2 years. It grows rapidly during the first year, then much more slowly and finally dwindle and die (Centre for New Crops and Plant Products, 2002).

The microorganisms colonizing the rhizosphere could be both from the epiphytic microflora of the seed coat and soil microflora. The seed coat microflora of the seed also plays an important role as a protectant during the seedling stage (Bosland and Votava, 2000).

In Nigeria several workers have studied the rhizosphere microflora of some vegetable crops, cereals, oil seed crops, legumes and other plants (Mcdonald 1968, 1969; Giha, 1976; Odunfa and Oso, 1979; Oyeyiola,1992; Ayodele and Emretiyoma, 1999; Bayagbon,2001). Information is not available on the rhizosphere mycoflora of *Capsicum frutescens.* The aim of this study therefore, was to determine the fungal population and its diversity in the rhizosphere and rhizoplane of red pepper *(Capsicum frutescens)* at different stages of growth, both in the field and in the pots.

Materials and Methods

Procurement of seeds and raising of plants

The pepper (*C. frutescens*) seeds were obtained from National Horticultural Institute (NIHORT),Ibadan, Nigeria. Raising of the plants was done in two phases. The first phase was the nursery stage whereby the seeds were sown and allowed to grow into seedlings. The second phase was the post-nursery stage whereby the seedlings were transplanted onto the field and pots, and allowed to grow to maturity. The transplanting was done 45days after seed sowing. The field soil was properly turned and made into low ridges. The soil from the same field was put in the pots. The plants were raised in the Biological Garden of the University of Ilorin, Ilorin, Nigeria.

Determination of physicochemical characteristics of experimental soil, rhizosphere soil and non-rhizosphere soil

The water holding capacity, texture, pH, moisture content and organic matter content were determined from the experimental soil prior to sowing of the seeds. All these parameters, except water holding capacity and texture, were also determined from the rhizosphere soil and non-rhizosphere soil as the plant aged in the field and the pot. The water holding capacity and texture were determined using the methods of Pramer and Schmidt {1964}, Awolumate{1977}, and

Olaitan and Lombin {1984} while pH, moisture content and organic matter content were determined using the methods of Oyeyiola {2010}..

Collection of root, rhizosphere soil, and non-rhizosphere soil samples

Plant root and rhizosphere soil samples were collected by carefully uprooting a plant with the soil immediately around the roots using a sterile trowel. The soil immediately around the roots was collected in a sterile polythene bag as the rhizosphere soil. . Then the roots were cut off with a sterile scalpel at the base of the stem and collected in another sterile polythene bag. The non-rhizosphere soil sample was collected by using a sterile trowel to dig the soil from the surface down to a depth of about 20cm, and the soil sample was put in a sterile polythene bag. Each polythene bag was tied and taken to the laboratory for analysis.

Preparation of culture medium

The culture medium used was potato dextrose agar(PDA). Commercially produced dehydrated medium was used and the medium was reconstituted following the manufacturer's directions.

Isolation of fungi from the rhizoplane

The roots were cut into 1-mm pieces and one gram of root pieces were put in a sterile boiling tube. 9ml of sterile distilled water was then added. The boiling tube was shaken gently, giving 10^{-1} dilution. This dilution was serially diluted until 10^{-4} dilution was obtained. Isolation was made from 10^{-4} dilution using duplicate potato dextrose agar plates. The plates were incubated at 25^{0}C for 48-72hours and representative colonies were taken for subculturing until pure cultures were obtained.

Isolation of fungi from rhizosphere soil and non-rhizosphere soil samples

One gram of each soil sample was put in a sterile test tube and 9ml of sterile distilled water was added.The test tube was shaken gently, giving 10^{-1} dilution. This dilution was serially diluted until 10^{-4} dilution was obtained. The subsequent steps were as stated for the rhizoplane above.

Results And Discussion

Table 1: Physicochemical characteristics of the experimental soil, rhizosphere soils in the field and pots ,and non-rhizosphere soil

Sample /Characteristics	**Period of sampling (days)**							
Experimental Soil	0	15	30	45	60	75	90	105	120
pH	6.9	-	-	-	-	-	-	-	-
Water holding capacity (ml/g)	0.35	-	-	-	-	-	-	-	-
Moisture matter content (%)	15.5	-	-	-	-	-	-	-	-
Organic matter content (%)	3.45	-	-	-	-	-	-	-	-
Texture	Loamy sand	-	-	-	-	-	-	-	-
Rhizosphere soil in the field									
pH	-	6.4	6.3	6.3	6.7	6.4	6.5	6.6	6.7
Moisture content (%)	-	11.4	19.0	23.0	24.0	25.0	27.0	20.0	27.0
Organic Matter Content (%)	-	8.9	18.1	12.7	16.9	32.0	17.3	13.0	22.3
Rhizosphere soil in the pot s									
pH	-	6.4	6.3	6.3	6.8	6.6	6.9	6.6	6.7
Moisture content (%)	-	11.4	19.0	23.0	24.0	25.0	27.0	20.0	27.0
Organic Matter content (%)	-	8.9	18.1	12.7	16.9	32.0	17.3	13.0	22.3
Non -rhizosphere soil									
pH	-	6.8	6.6	6.2	6.2	6.5	6.6	6.4	6.4
Moisture content (%)	-	8.6	22.0	23.0	30.0	23.0	24.0	12.0	25.0
Organic Matter content (%)	-	4.1	17.8	17.8	12.0	7.6	3.8	12.0	17.5

Note: O day represents experimental soil prior to seed sowing
Data are means of three replicates.

The physicochemical characteristics of the experimental soil prior to seed sowing are shown in Table 1. The pH of the soil prior to seed sowing was 6.9 and it ranged from 6.3 to 6.7 in the rhizosphere soil for both field and pots. These pH values were favourable for the growth of the plant. Many soils require no management of soil acidity as their pH remains between 5.5 and 8.5 at which plants do well (Singer and Muns, 1996). Moreover the desirable microorganisms in the soil thrive best between pH 5.5 and 7.8 (Wrigley, 1981). The soil texture was loamy sand and the pH was near neutrality. The pH, moisture content and organic matter content were the same for the rhizosphere soils in the field and the pots, for the first 45 days because the seedlings were in the nursery stage. However after transplanting (from 60 to 120 days), variations occurred in these parameters in the rhizosphere soils. The organic matter contents were higher in the rhizosphere soils in the field and pots than the non-rhizosphere soil throughout the period of plant growth from the nursery to maturity. This would be due to the greater microbial activity and root activity in the rhizosphere soils than in the non-rhizosphere soil.

The fungi isolated throughout this study were *Aspergillus niger, Rhizospus stolonifer, Aspergillus flavus, Aspergillus candidus, Penicillium expansum, Fusarium oxygsporum, Fusarium poae,*

Penicillium citrinum, Mucor racemosus, Mucor mucedo, Verticillum lateritium, Fusarium avenaceum, Trichophyton mentagrophyte, Fusarium verticilloides, Aspergillus utsus, Saccharomyces cerevisiae and *Aspergillus fumigatus.* Most of these fungi have been isolated from the rhizosphere and rhizoplane of sorghum, wheat, *Amaranthus hybridus*, groundnut and okro by previous workers (Odunfa, 1979; Abdel-Hafez, 1982; Oyeyiola and Hussain, 1992; Oyeyiola, 2002; Dongmo and Oyeyiola, 2006; Oyeyiola, 2009). *F. verticilloides, F. oxysporum, V. lateritium* and S. *cerevisiae* were present in both the rhizosphere soil and rhizoplane in the field and pots, but they were absent from the non-rhizosphere soil. More fungi occurred qualitatively in the rhizosphere soil and rhizoplane than in the non-rhizosphere soil. This should be expected because microorganisms in the rhizosphere react to the many metabolites released by plant roots and as rule, a general increase in the microorganisms in the rhizosphere is always noted (Morgan *et al.*, 2005). As roots grow through the soil, the loss of organic material (as root exudates and dead root cells) provides the driving force for the development of active microbial population (Whipps, 2001). Similar increases in the number of fungi in the rhizosphere over the number in the non-rhizosphere have been reported by previous workers (Odunfa, 1979; Abdel-Rahim *et al.*, 1983). The must predominant fungus in the rhizosphere soil and rhizosplane was *M.mucedo.* This fungus occurred in the highest number of times. Predominance of fungi in the rhizosphere is a common phenomenon (Odunfa, 1979; Odunfa and Oso, 1979; Odunfa, 1980; Abdel-Hafez, 1982; Oyeyiola, 2009).

During the nursery stage, which occurred within the first 45days after seed sowing, the number of fungi on the rhizoplane was higher than the number of fungi in either the rhizosphere soil or the non-rhizosphere soil (Fig. 1). However after transplanting the seedlings, more fungi occurred in both the rhizosphere and rhizoplane in the field than in the pots (Figs. 1and 2). Also after transplanting the seedlings the highest number of fungi occurred in the rhizosphere soil at 90th day for the pots (Fig.1), but at 75^{th} day for the field (Fig.2). For the field, the highest rhizosphere effect occurred at 75th day after seed sowing, while for the pots, the highest rhizosphere effect occurred at 90th day after seed sowing (Fig. 3). The two days would be when the greatest numbers of fungi occurred in the rhizosphere soils as caused by availability of nutrients, especially root exudates. It would have taken many days (75 days and 90 days) for the effect of exudates on the rhizosphere fungi to reach its maximum because according to Funck-Jensen and Hockenhull (1984), the rate of exudation is influenced by the age of the root, environmental factors, cultural factors and the presence of microorganisms.

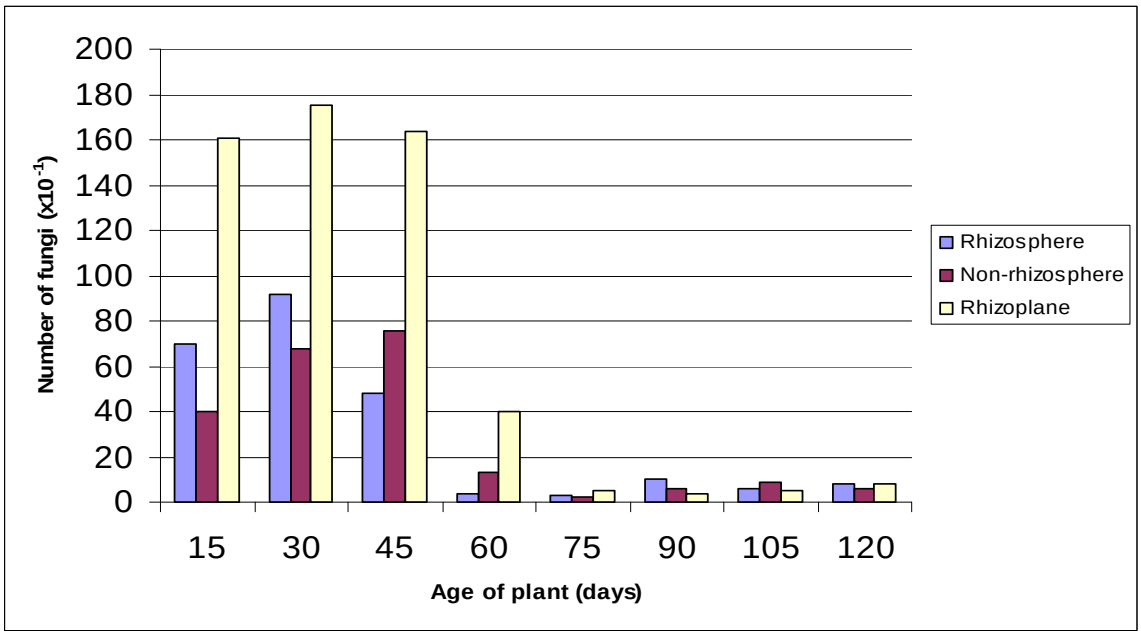

Figure 1: Number of fungi in the rhizoplane and rhizosphere soil of *Capsicum frutescens* in pots and the non-rhizosphere soil as the plant aged

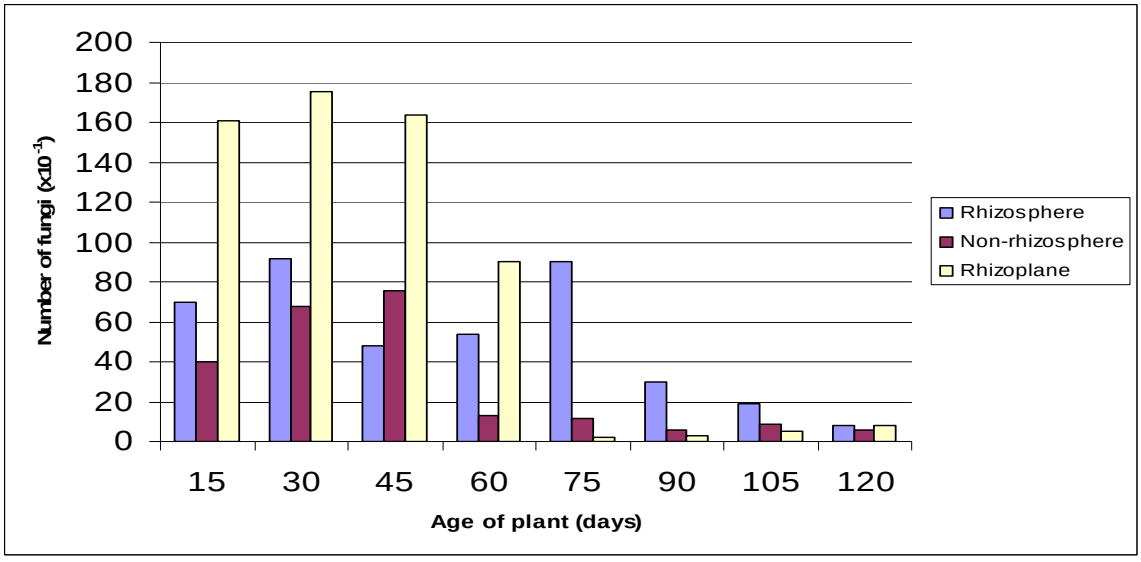

Figure 2: Number of fungi in the rhizoplane and rhizosphere soil of *Capsicum frutescens* on field and the non-rhizosphere soil as the plant aged.

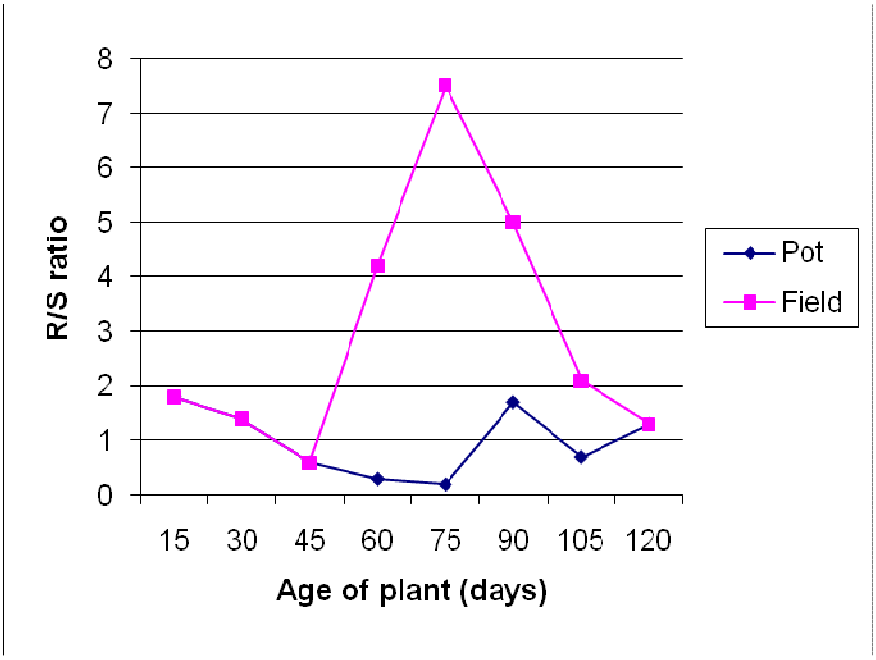

Figure 3: Rhizosphere effect (R/S ratio) of fungi isolated from the field and pots

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