**Jewel Journal of Scientific Research (JJSR) 3(1): 128 – 131, 2015**
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# Isolation and Identification of Fungi Associated with the Rhizosphere and Rhizoplane of Tobacco (*Nicotiana tabacum*).

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## Abstract
Fungi present in the rhizosphere and rhizoplane soils of tobacco (*Nicotiana tabacum*) were isolated and identified using standard methods. The fungi were *Aspergillus niger*, *A. terreus*, *A. oryzae* and *Schizo saccharomyces* pombe. However, *A. oryzae* was absent from the rhizoplane soil but present in the rhizosphere soil. The rhizosphere soil has average pH value of 5.8 while that of the rhizoplane soil was 6.2. The average percentage moisture content of the rhizosphere soil was 10.27% and higher than that of the rhizoplane soil (8.80%). The fungi isolated are not harmful or pathogenic to tobacco.

**Key words:** Pathogenic, standard method, soil

## Introduction
Tobacco (*Nicotiana tabacum*) is a robust annual little branched herbaceous plant within the genus *Nicotiana* of the Solanaceae family (Wikipedia, 2014). It grows up to 3metres high. The leaves are large and oval, with rounded or pointed ends (Purseglove, 1977). Both the leaves and stems are covered with hairs. The flowers are large trumpet – shaped and white – pinkish in colour (Wikipedia, 2014). The fruit is 2 – valved, ovoid capsule, 1.5 – 2.0 cm. long, almost completely covered by the calyx (Purseglove, 1977). The seeds are minute, oval to spherical, 0.5mm. long, finely reticulate light to dark brown in colour. The seeds contain several phytochemicals (Wikipedia, 2014). Tobacco is propagated by seed.

Dried tobacco leaves are mainly smoked in cigarettes, cigars, pipe tobacco, etc. They are also consumed as snuff, chewing tobacco and dipping tobacco (Wikipedia, 2014). Tobacco oils can be used in biofuels. In India, an extract from tobacco called solansole is used in several drug types (Anonymous, 2014). Tobacco plant is also medicinal as the leaf juice can be taken orally to induce vomiting and narcosis (Wikipedia, 2014). Ebola drug made from tobacco plant saved United States aid workers according to Langreth *et al.* (2014). Tobacco plant is also grown around houses and farms to ward – off snakes and termites (Wikipedia, 2014).

Rhizosphere is the area around a plant root that is inhabited by a unique population of microorganisms influenced by the plant exudates (McNear Jr., 2013). It is the most active portion of the soil in which biogeochemical processes influence a host of landscape and global scale processes (McNear Jr., 2013). Rhizoplane is the external surface of roots together with the closely adhering soil particles and debris (Singer and Donald, 2006). Soil microbes and macrobes compete for food and space in the rhizosphere.

The pH of the rhizosphere soil can influence the species mix and functions of microbes.

This study was meant to isolate and identify the fungi associated with the rhizosphere and rhizoplane soils of tobacco, an economic and medicinal plant of numerous uses, as well as to determine the moisture content and pH values of the soil samples. Previous studies have focused on the microorganisms associated with the rhizosphere and rhizoplane soils of other economic crops. This research will therefore bridge this gap.

**Materials and Methods**

**Description of the study area**: The study area is a tobacco farmland in Olopolo village, Ilorin – West Local Government Area of Kwara State, Nigeria.

**Collection of soil samples:** Soil samples from the rhizosphere and rhizoplane of two matured tobacco plants were collected using the methods of Oyeyiola *et al.* (2013).

**Physicochemical analyses of the soil samples:** The rhizosphere and rhizoplane soil samples were analysed for moisture content and pH using the methods of Warcup (1954) as well as Henry and Boyd (1988) respectively inside the Biology laboratory of University of Ilorin, Ilorin, Nigeria. Each analysis was done in duplicate and the average recorded.

**Preparation of Potato Dextrose Agar (PDA):** This was done according to the manufacturer's instructions (BIOMARK Laboratories, India).

**Isolation, purification, preservation and identification of fungi from the soil samples:** Fungi were isolated from the rhizosphere and rhizoplane soil samples on freshly prepared PDA plates incubated at 25± 2ºC using the spread plate techniques of Dubey and Maheshwari (2005); Pelczar *et al.* (2005) and John *et al.* (2010). The fungi isolated were sub – cultured on fresh PDA plates according to the methods of Fawole and Oso (2007) for purification. The purified fungal isolates were preserved on fresh PDA slants stored in a refridgerator set at 4 - 8ºC. The preserved fungal – isolates were identified based on their macroscopic and microscopic characteristics using the methods of Cambell and Stewart (1980).

**Results and Discussion**

The average percentage moisture content was 10.27 for the rhizosphere soil and 8.80 for the rhizoplane soil (Table 1). These are in line with the optimum requirement of tobacco plant for water as the plant does not tolerate water – logging (Purseglove, 1977). The average pH values of the rhizosphere and rhizoplane soils were 5.8 and 6.2 respectively (Table 1). These values show that the soil samples were slightly acidic and agrees with the values reported by Purseglove (1977) which are 5 – 6.5 as the pH requirement of tobacco plant.

**Table 1: The average percentage moisture content and pH values of the rhizosphere and rhizoplane soils of tobacco**

| Soil zone | Average moisture content (%) | Average pH value |
|---|---|---|
| Rhizosphere | 10.27 | 5.8 |
| Rhizoplane | 8.80 | 6.2 |

Four pure fungal isolates were obtained from the soil samples and they were identified as *Aspergillus niger, A. terreus, A. oryzae* and *Schizosaccharomyces pombe.* All the four fungal - isolates were present in the rhizosphere soil while all but *A. oryzae* were present in the rhizoplane soil (Table 2).

**Table 2: Distribution of fungi in the rhizosphere and rhizoplane soils of tobacco**

| Soil zone | Fungal - isolates | | | |
|---|---|---|---|---|
| | *Schizosaccharomyces pombe* | *Aspergillus niger* | *A. terreus* | *A. oryzae* |
| Rhizosphere | Present | Present | Present | Present |
| Rhizoplane | Present | Present | Present | Absent |

The presence of more species of fungi in the rhizosphere soil than the rhizoplane soil agrees with previous results by Oyeyiola *et al.* (2013) as well as Sule and Oyeyiola (2012). Ogbonna *et al.* (2013) isolated fifty – eight aquatic *Phycomycetes* and Ascomycetous fungi from *Artemisia annua* plantation soil samples in Jos, Plateau State of Nigeria. Of these, predominant ones included *Chaetomium bastrychodes, C. cancriodeum, Aspergillus candidas, A. niger, A. oryzae, Fusarium oxysporium, Penicillium chrysogenum, Botrytis cinerea* and *Curvularia lunata.* Danladi *et al.* (2013) also isolated and identified forty strains of bacteria belonging to 7 species from the rhizosphere soil of *Citrus lunata* and *Amaranthus cruentus* in Ruggar Liman and Kwalkwala farming suburbs of Sokoto State, Nigeria. Oyeyiola *et al.* (2013) in their study on the rhizosphere bacteria flora of okro (*Hibiscus esculentus*) in Ilorin isolated and identified *Bacilus subtilis, B. pumilus, Pseudomonas putida, P. fluorescens,* etc.

The results of this study showed the presence of more fungi species in the rhizosphere soil than the rhizoplane soil, and this agrees with previous findings by Sule and Oyeyiola (2012) as well as Oyeyiola *et al.* (2013). According to Kennedy (1998), the differing physical, chemical and biological properties of the rhizoplane and rhizosphere are responsible for increased number and activity of microorganisms in the rhizosphere micro – environment of plants.

**Conclusion**

The rhizosphere and rhizoplane soils of tobacco in Olopolo village, Ilorin West Local Government Area of Kwara State have species of the genus *Aspergillus* and *Saccharomyces pombe* in them. These microorganisms are not harmful or pathogenic to the host plant, tobacco. Rather, they add to the compactness of the soil, thereby enhancing the soil structure and profile.

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