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Comparative Studies of the Mycoflora of Dry and Fresh Maize (*Zea mays*) Rhizosphere

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Abstract

Comparative studies of the mycoflora of dry and fresh maize rhizosphere was evaluated in the laboratory. Rhizosphere and soil sample were taken during rainy season from two different maize farms in Bori, Khana Local Government Area, Ogoni, Rivers State. The maize stumps were collected by the use of pre-sterilized hand trowel. Collected maize stump with rhizosphere soil was carefully placed in polythene bag and the open end tied. Stock solutions were prepared and diluted serially for plate count and fungal isolation using pour plate method. Sabouraud dextrose agar was the preferred media. Discrete colonies were sub-cultured for precise identification of fungi. Total fungal count and the isolation frequency of each identified genera were determined. Analysis of the samples revealed the presence of four mould genera *Aspergillus, Fusarium, Penicillium, Trichoderma* and yeast. The frequency of occurrence was *Aspergillus* 16%, *Fusarium 2*%, *Penicillium* 68%, *Trichoderma* 9% and Yeast 5% in dry maize rhizosphere. The frequency of occurrence in fresh maize rhizosphere was *Aspergillus* 27%, *Fusarium* 0.0%, *Penicillium* 14%, *Trichoderma 4*7% and Yeast 12%. The results showed that *Penicillium* population within the rhizosphere of dry maize plant was very high compared to that in fresh maize plant rhizosphere. Some of these fungi genera are known to be pathogenic to both plants and animals; such as ear rot in maize plant, hematologic disorder in humans, among others, hence it was recommended that farmers should perform soil analysis for fungi before cultivating maize for maximal production.

Keywords: maize, mycoflora, rhizosphere, soil, comparative

Introduction

Maize (*Zea mays*) is the second most abundantly produced cereal in the world exceeded only by rice. It is a tall annual plant belonging to the grass family and is one of the most diverse species of plants. It is monoecious with terminal male inflorescence and auxiliary female inflorescences (lobs). The flowers are unisex, incomplete and zygomorphic. They reproduce by both cross-pollination and self-pollination (Ramalingan, 2019).

Cultivation

Maize is best grown in warm, tropical and subtropical regions as it requires warm soils to develop optimally. One of the most important requirements for growing maize is a high quality soil which is deep, fertile and well draining with pH between 6.0 and 6.9. Maize plants are very heavy feeders and even the most fertile of soils may need to be supplemented with nutrients as the plants develop, particularly nitrogen. Maize also requires plenty of space as it grows and is pollinated by the wind. It should be planted where it will receive full sunlight for most of the day and with ample moisture (Roney, 2009). Typical maize plants develop 18 to 22 total leaves. Silk appears about 55days after emergence and mature in around 125 days after emergence (Willy, 2010).

The maize kernel is the reproductive seed of the maize plant therefore, its structure and composition exist for the purpose of reproduction rather than processing. The kernel is composed of four main part expressed as a dry weight percentage of the whole kernel, and they include: germ, endosperm (horny and floury), pericarp and tip. Each of these sections has distinct compositional features that are important for the maize kernel (Chodosh, 2021; Tulin & Askun, 2006).

Storage

It is easier to dry, store and transport maize and these have made it a major source of energy for animal food and a stable raw material for the production of starch and starch -based industrial products. Maize is also a primary food source of many areas of the world, including South America, Central America and Africa where it is converted directly into food products via grinding, alkali processing, boiling/cooking, or fermentation (Karl, 2012; Sreenivasa et al., 2011).

Uses (Benefits)

Many parts of the maize plant are used in industry and several types of maize are grown primarily for their industrial applications. Maize (corn) is used to produce ethanol for industrial and local use. Maize is rich in Vitamin C, an antioxidant that helps protect cells from damage and wards of diseases like cancer and heart disease. Yellow corn is a good source of the carotenoids, which are good for eye health and prevent the lens damage that leads to cataracts (Bressani, 2011).

Corn has a high source of fibre which keeps the digestive system healthy and can also improve cholesterol and blood sugar levels. Corn also provides a high amount of carbohydrate which is the body's main source of energy. Carbohydrate helps to fuel the brain, kidneys, heart muscles and central nervous system. Corn is gluten free, which means that it helps reduce chronic inflammation, boosts energy and promotes weight loss. It is also rich in Manganese which plays a role in bone formation, blood clotting, and reducing inflammation (Kataki et al., 2007).

Corn has high amounts of Vitamin B constituents, thiamine and niacin, which are good for facilitating growth. Thiamine helps the body improve nerve health and cognitive functions while niacin can prevent a series of problems like dementia and dermatitis. Corn is also known for having high amounts of folic acid and is therefore good for pregnant women. Since corn is rich in Vitamin E, which is a natural source of antioxidant, it protects the body from various illnesses, helping you grow without the hindrance of disease (Nuss et al., 2011). It can help with various digestive problems like constipation and haemorrhoids, and can also protect one from getting colon cancer. Fiber is also good for your bowel movement because it backs up your stool and facilitates its movement down the digestive tracts. As a result, it is also good for diarrhoea and irritable bowel syndrome (Osborne & Voogt, 2008).

The maize plant root receives between 30-60% of net photosynthesized carbon. Of this, an estimated 40-90% enters the soil as a wide variety of materials including alcohols, ethylene, sugars, amino and organic acids, vitamins, nucleotides, polysaccharide and enzymes (Taylor et al., 2007). These materials create a unique environment of soil microorganisms called the rhizosphere. The rhizosphere is the narrow zone or region of soil that is directly influenced by root secretions and associated soil microorganisms (Raven et al., 2005). The plant root surface, termed the rhizoplane, also provides a unique environment for microorganisms. As these gaseous, soluble and particulate materials move from the plant to the soil, rhizosphere and rhizoplane microorganisms increase in number and when these newly available substrates become available, their compositions and functions also change (Taylor et al., 2007). In addition, rhizosphere and rhizoplane micro-organisms create a soil microbial loop, thereby playing critical roles in organic matter synthesis and degradation. A wide range of microbes in the rhizosphere can promote plant growth. They include certain chemicals such as auxins, gibberellins, glycolipids and cytokinins. A critical process that occurs on the surface of the plants and particularly in the root zone is associating nitrogen fixation, in which nitrogen-fixing microorganisms are on the surface of the plant root (Berea et al., 2005).

A fungus is a member of a large group of eukaryotic organisms that include microorganisms such as yeast, molds, and much familiar mush rooms (Moore et al., 1998; Willey et al., 2008). Fungi grow best in dark, moist habitats where there is little danger of desiccation, but they are found wherever organic material is available (Willey et al., 2008). Therefore, they are important

economically at the root of plant (maize) responsible for the majority of plant diseases. For example in maize, fungi cause leaf blight (*Helminthosporium turcicum pass*), smut of maize (*Ustiligo maydis*), head smut of maize (*Sphacelotheca reliana*), Rust of maize (*Puccinia sorghi*) and brown spot of maize (*Physodermazeae-maydis shaw*).

Paszkowski (2006) reported that fungi are highly beneficial in agriculture, horticulture and forestry. Fungi activity in farm lands contributes to the growth of plants by about 70%. (Chandler, 2017) reported also that fungi are animal pathogens. Thus they help in controlling the population of pests. Some fungi do not infect plants and animals and an example is *Beauveliabassana* which is used as pesticide to control the spread of emerald ash borer (Deshpande, 1999). Fomina et al. (2017) reported that fungi are also used in agricultural research. Some species of fungi are used in the detection of certain elements such as Uranium and Arsenic in soil and in the production of enzymes.

Zhuo and Fan (2021) reported that certain fungi in particular white roof fungi can degrade insecticide, herbicide and heavy fuels and convert them into carbon dioxide, water and basic elements.

Materials and Methods

The following materials were required and used for adequate analysis in determining the mycoflora of dry harnessed maize and fresh maize stand: oven, incubator, petri dishes (20), test tubes (40), pipette (60), Sabouraud dextrose agar, chloramphenicol, dry and fresh maize stands, hand trowel, polythene bags, electronic balance, distilled water, spreader, 95% alcohol, lactic acid, light microscope, hand lens, fungal identification manual.

Sample Collection

The maize stumps were collected by the use of pre – sterilized hand trowel. The hand trowel was washed and wiped thoroughly with 95% alcohol after each use. Collected maize stump with rhizosphere soil was carefully placed in polythene bag and the open end tied.

Preparation of Media

Petri dishes, test tubes, pipettes, flasks and beakers were all washed and sterilized by placing them upside down in an oven at the temperature of 160^{o}c for 1hr. Sample preparation was done in accordance with the manufacturer's recommendation. With the aid of an electronic balance 16.25g of Sabouraud dextrose agar was weighed into 500ml conical flask and prepared for sterilization by addition of 250ml distilled water. The preparation was plugged with cotton wool and wrapped with aluminium foil before autoclaving for 121^{o}c for 15 minutes.

It was allowed to cool to some degree, lactic acid and a capsule of 250mg of chloramphenicol was dissolved in it to inhibit the growth of both gram positive and gram negative bacteria. Aseptically, the autoclaved Sabouraud dextrose agar was carefully poured in to 20 petri dishes (10 petri dishes each for dry and fresh maize samples), was allowed to set and then inverted to avoid condensed water settling back on the already set medium.

The samples from the rhizosphere of dry and fresh maize were prepared by weighing 1g of soil into test tubes and diluted with 9ml distilled water to form the stock. The stock was used to prepare aliquot of dilutions of 10^{-1}, 10^{-2}, and 10^{-3} to 10^{-10}. 1ml of various dilutions were transferred to the agar plates and incubated at ambient temperature for 5 days. Then these cultures were inverted and incubated at ambient temperature (30± 2^{o}c) for up to 120hrs.

Data Collection

Data collected were the average plate count for each sample of both dry and fresh maize rhizosphere, population (cfu/g), as well as their isolates. All data were subjected to analysis of variance and significant means were separated using LSD at 5% level of probability.

Results

Table 1 shows the cultural characteristics of fungal genera of rhizosphere on dry and fresh maize cultures. Each organism; A*spergillus, Fusarium, Trichoderma, Penicillium,* and yeast shows their different characteristics on its cultural plates. With the aid of the fungal identification manual, their various characteristics were reached and identified.

Table 1: Cultural characteristics of fungal genera of rhizosphere on dry and fresh maize

Organisms	**Cultural Characteristics**
Aspergillus	Colonies spread, rapidly, mycelium initially white then turning black.
Fusarium	Colonies spread, woolly, cottony and flat, colour varying.
Trichoderma	Colonies spread rapidly, forming a some-what thin mycelia layer with irregular shaped parches of verdigris green.
Penicillium	Colonies form a tough flat with surface slightly floccose or ropy, radially furrows (line in between), pale grey-green or greenish grey, reverse reddish, to purple.
Yeast	Colonies forming a flat creamy surface with thin mycelia layer and irregular shape.

The result of plate count for each sample is shown in table 2. It was observed that the total plate count in fresh maize was higher when compared to the dry maize which was 14.6×10^2 and 12.2×10^2 respectively. The result also shows that on fresh maize samples, the number on each plate count out numbered that of the dry maize samples except plate 3 and 7. Meanwhile, plate 4 on both fresh and dry are the least.

Table 2: Plate count for each sample (cfu/g)

Sample	**Fungal count on Fresh maize(cfu/g)**	**Fungal count on Dry maize (cfu/g)**
1	1.1×10^1	1.0×10^1
2	0.7×10^0	0.3×10^0
3	1.5×10^1	2.0×10^1
4	0.4×10^0	0.2×10^0
5	0.9×10^0	0.4×10^0
6	1.2×10^1	0.8×10^0
7	1.5×10^1	2.5×10^1
8	3.0×10^1	1.7×10^1
9	2.8×10^1	2.1×10^1
10	1.5×10^1	1.2×10^1
Total	$\mathbf{14.6}\times10^2$	$\mathbf{12.2}\times10^2$

Table 3 shows the populations of fungal genera on fresh maize culture. The result indicated that plate 8 has the highest isolates followed by plate 3 and the least were plate 2, 6 and 7 while plate 8 has the highest population (cfu/g) followed by plate 9 and 3, with the least was plate 4. Remarkably, the fungi *Fusarium* was not isolated from fresh maize rhizosphere.

Table 3: Population of fungal genera on fresh maize culture

Fresh sample	Isolates	Population (cfu/g)	Total
1	*Trichoderma*	8	11
	Penicillium	3	
2	Yeast	7	7
3	*Aspergillus*	3	
	Trichoderma	8	15
	Pencillium	4	
4	*Penicillium*	1	4
	Yeast	3	
5	*Trichoderma*	6	9
	Aspergillus	3	
6	*Trichoderma*	12	12
7	*Trichoderma*	15	15
8	*Trichoderma*	10	
	Aspergillus	8	30
	Yeast	7	
	Penicillium	5	
9	*Aspergillus*	20	28
	Pencillium	8	
10	*Aspergillus*	6	15
	Trichoderma	9	

Fungal genera on dry maize cultures were identified as shown in table4. As clearly shown, plate 8 has the highest isolates, while plate 7 has the highest population (cfu/g) followed by plates 9 and 3. The least plate is 4.

Table 4: Identified fungal genera on dry maize cultures

Dry sample	Isolates	Population (cfu/g)	Total
1	*Penicillium*	10	**10**
2	*Penicillium*	3	**3**
3	*Aspergillus*	12	**20**
	Penicillium	8	
4	*Trichoderma*	4	**2**
5	*Trichoderma*	8	**4**
6	*Penicillium*	2	**8**
7	Yeast	2	**25**
	Penicillium	23	
8	*Penicillium*	11	
	Yeast	4	**17**
	Fusarium	2	
9	*Trichoderma*	1	**21**
	Penicillium	20	
10	*Aspergillus*	8	**12**
	Trichoderma	4	

Table 5 shows the total population of each organism on all cultures of fresh and dry samples. The table further shows the comparative analysis of the total organisms. *Trichoderma* is the led organism on the fresh maize sample followed by *Aspergillus*, while on the dry maize sample, *Penicillium* is the most populated organism followed by *Aspergillus* as well. *Fusarium* was the least populated organism on both fresh and dry maize samples.

Table 5: Total population of each organism on all cultures of fresh and dry samples

Organisms	Samples	
	Fresh maize	**Dry maize**
Penicillium	21	83
Trichoderma	68	11
Aspergillus	40	20
Yeast	17	6
Fusarium	0	2
Total	**146**	**122**

Comparison of the total number of each organism in percentages on all cultures of dry and fresh sample can be seen on fig. 1, 2 and 3. Just as in table 5, *Penicillium* took the lead on dry maize sample while *Trichoderma* on fresh maize sample. *Fusarium* was also the least in percentages on both fresh and dry maize samples.

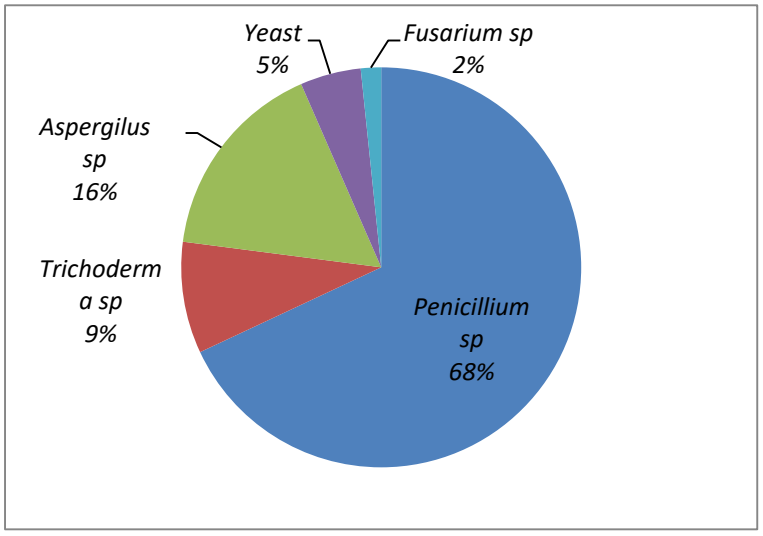

Fig .1: Isolation Frequency in Dry maize Rhizosphere

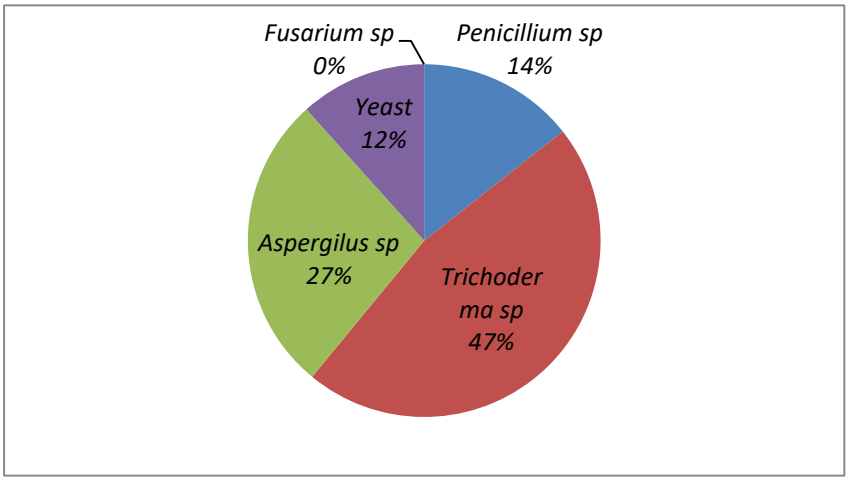

Fig.2: Isolation Frequency in Fresh maize Rhizosphere

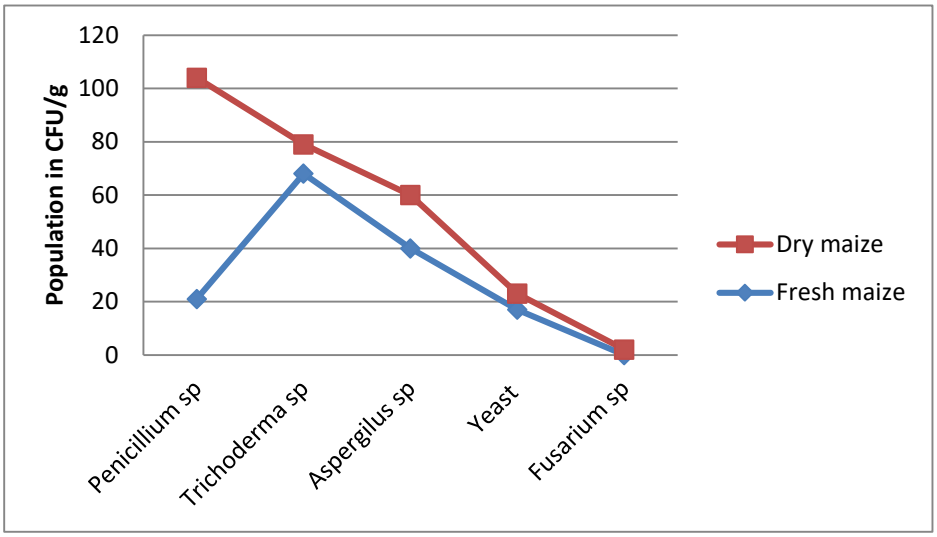

Fig.3: Isolation Frequencies of Mycoflora from Dry and Fresh Maize Rhizosphere

Discussion

Mycoflora of dry and fresh maize rhizosphere has been widely reported (Lim, 1969; Roney, 2009; Sreenivasa et al., 2011; Tulin & Askun, 2006). The findings of the present study is in agreement with this since about five different species of mycoflora were isolated from fresh maize and dry harvested stands as shown in table 1. Berea et al. (2005) also reported that a critical process that occur on the surface of the plants and particularly in the root zone is as a result of certain microorganism such as the fungi, the present study also attests to that fact because these mycoflora were isolated from the root zone which is the rhizosphere. Fungi grow best in dark, moist habitats where there is little danger of desiccation, but they are found wherever organic materials are available (Willey et al., 2008). The above findings are true and that can be attested to in tables2,3, 4 and 5. The total population of mycoflora as seen in fresh maize sample is higher when compared with the dry maize sample. Pandey (2009) shows that fungi are responsible for the majority of plant diseases, no wonder they are found at the rhizosphere as the present study shows.

Table 5 shows a total of 146 organisms as against 122 organisms, indicating that rhizosphere of maize when fresh is conducive in comparism to the rhizosphere of maize when it is dried. Fajard and Martinez (2008) reported that fungi at high concentrations act as chemical defense against competition with other microorganisms in species-rich environments, such as the rhizosphere. The finding of the present study agrees with that as seen in table 3. Previous studies involving the mycoflora of maize rhizosphere were associated with those that are found at the rhizosphere and not necessarily determining the comparison that exist between the population of mycoflora at dry harvested and fresh maize stands. The present study determines the comparative analysis between these developmental stages as well as comparing the mycoflora of dry harvested and fresh maize stands as shown in tables 1 and 4. It also enlists those mycoflora of dry and fresh maize rhizosphere. The present study also went further in comparing their percentages as shown in fig, 1, 2 and 3. From the statistical data, tables 4, and5, it was evidently clear that the population of *Trichoderma* was higher than other organisms, in fresh maize sample while on dry sample, *Penicillium* was the most populated organism. *Fusarium* was found only on dry maize sample.

Conclusion

Certain mycoflora are found at the rhizosphere of maize and they include: *Fusarium*, *Penicillium*, *Aspergilus*, *Trichoderma* and Yeast. From the study above, we can conclude that mycoflora of maize can be seen in their numbers especially at the root of fresh maize rhizosphere. Another positive effect is that it helps in recycling nutrients. They also affect maize in a negative way in that they cause both plant and animal diseases.

References

Berea, J. M., Pozo, M. J., & Azeon, R. C. (2005). Microbial co-operation in the rhizosphere. *Journal of Experiment Botany*, 56,1761-1778.

Bressani, R. (2011). Chemistry technology and nutritive value of maize tortillas. *Food Review International*, 6, 225-264.

Chandler, D. (2017). *Basic and applied research on entomopathogenic fungi microbial control of insect and mite pests*. Academic.

Chodosh, S. (2021). *The bizarre botany that makes corn a fruit, a grain and also a vegetable popular science*.

Desphande, M. V. (1999). Mycopesticide production by fermentation: Potential and challenge. *Critical Reviews in Microbiology, 25*(3), 229-243.

Fajard, A. & Martinez, J. L. (2008). Antibiotics as signals that trigger specific bacterial responses. *Current Opinion in Microbiology*, *11*(2), 161-167.

Fomina, M., Charnock, J. M, Millier, S, Alvarez, R., & Gadd, G. M. (2007). Fungal transportations of uranium oxides. *Environmental Microbiology, 9*(7), 1696-1710.

Karl, J. R. (2010). The maximum leaf number of the maize subspecies the maize. *The Maize Genetics Cooperation Newsletter*, *86*(4).

Kataki, P. K. & Babu, S. C. (2007). *Food systems for improved human nutrition linking agriculture, nutrition and productivity*. Haworth.

Lim, G. (1969). Some observation on soil and root surface mycoflora. *Plant Soil*. *31*, 143-148.

Moore, R., Clark, W. D., & Vodopich, D. S. (1998). *Botany (2nd ed.).* MacGraw-Hill.

Nuss, E. T. & Tanumihardjo, S. A. (2011). Quality protein maize for Africa: closing the protein inadequacy gap in vulnerable population. *Adv. Nutr 2*, 217-224.

Osborne, D. & Voogt, P. (2008). *The analysis of nutrients in foods*. Academic.

Pandey, B. P. (2008). *Plant pathology, pathogen and plant disease (11th ed.).* Chands.

Paszkowaszi U. (2006). Mutualism: The tin and tank of plant symbiosis. *Current Opinion in Plant Biology, 9*, 364-370

Ramalingam, S. T. (2009). *Modern biology (3rd ed.).* Africana

Raven, P. H., Evert, R. F., & Eichorm, E. S. (2005). *Biology of plants (7th ed.)*. Freeman, W. H.

Roney, J. (2009). The beginnings of maize agriculture. *Archeology Southwest*, *23*(1).

Sreeniva, M. T., Dass, R. S., ChairthRaj, A. P., & Janardhana, G. R. (2011). Mycological evaluation of maize grains produced in Karnataka (India) for the post harvest fungal contamination. *World App. Sci. J. 13*(4).

Taylor, D. J., Green, N. P. O., & Stout, G. W. (2007). *Biological science (7th ed.).* Cambridge. Tulin,

J. M. & Askun, D. I. (2006). Investigation of fungal species diversity of maize kernels. *J. Biol Sci.6*(2), 275-281.

Willey, J. M., Sherwood, L. M., & Woolwerton, C. J. (2008). *Prescott, Harley and Klein's microbiology (7th ed.).* McGrow-Hull

Willy, H. V. (2010). Growth and Production of maize: Traditional low-input cultivation. *Soils, Plant Growth and Crop Production*.

Zhuo, R. & Fan, F. (2021). A comprehensive insight into the application of white rot fungi and their lingo cellulolytic enzymes in the removal of organic pollutants. *Science of the Total Environmental*. *778*, 146132.