



MICROBIAL STATUS OF *PARKIA BIGLOBOSA* GERMPLASM IN SOUTH WESTERN NIGERIA

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ABSTRACT: This study investigates the incidence of microbial pathogen on the germplasm of *Parkia biglobosa* in Wasangare, Saki West Local Government of Oyo State. The germplasm comprises of eight provenances from West Africa. These countries are Nigeria, Senegal, Benin Republic, Cameroun, Burkina Faso, Guinea, Mali and Ghana. The incidence of microbial pathogen could be a threat to the survival of this all important tree hence the need to evaluate the microbial flora of its bark, leaf and soil. Different bacteria genera which include the Entobacteriaceae, Bacilli and Streptococci species were isolated from the soil. Fungi genera isolated include: *Aspergillus* totalling 46.66% out of which are soil saprophytes while others such as *A.niger*, *A.flavus*, *A.fumigatus*, *A.tamari*, *A.terreus* and *Fusarium sp.* with 13.82% present are not only pathogenic but can be mycotoxigenic. *Botryopilodiasp.* have the highest occurrence among isolates from both *P.biglobosa* parts and infected twig parts which is a pointer for die back incidence on the plantation.

Keyword: *Pakia biglobosa*, soils, pathogen, mycotoxigenic and agroforestry

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1. INTRODUCTION

Pakia biglobosa belongs to the genus *Pakia*, family fabaceae, it consists of about 35 species but five other well recognized species besides African locust bean include *Parkia filicoidea*, *P. bicolor*, *P. roxburghii*, *P. biglandulosa* and *P. madagascariensis* (Keay, 1989). It occurs in the natural and semi-natural habitats such as savannah and woodlands, sometimes on rocky slopes, stony ridges and sandstone hills. It is able to withstand drought because of its deep taproot (Builders *et.al.*, 2014). Commonly known as African locust beans together with shea butter (*Vitellaria paradoxa*) is one of the main components of agro forestry parklands in West Africa (Shao, 2002). In West Africa the bark, roots, leaves, flowers, fruits and seeds are commonly used in traditional medicine to treat a wide diversity of complaints, both internally and externally, sometimes in combination with other medicinal plants. The bark is most important for medicinal uses, followed by the leaves. Medicinal applications include the treatment of

parasitic infections, circulatory system disorders, such as arterial hypertension, and disorders of the respiratory system, digestive system and skin. In veterinary medicine, a root decoction is used to treat coccidiosis in poultry. Green pods are crushed and added to rivers to kill fish. Geographically, *Parkia biglobosa* can be found in a belt stretching from the Atlantic coast in Senegal to southern Sudan and northern Uganda. The tree currently exists within a wide range of natural communities but is most abundant in anthropic communities – places where cultivation is semi-permanent. Annual production of seeds in northern Nigeria is estimated at around 200 000t. While the products of the tree are not common in international trade, they form an important part of local and regional trade in West Africa. The seeds are especially prized, and much trade occurs locally in the Sahel region where they are transferred between borders.

Seeds are fermented to make dawadawa: a black, strong – smelling tasty food rich in protein. The yellow starchy

pulp that surrounds the seed is an important food supplement rich in vitamin C and carbohydrates. The dried powder is often mixed with water to produce a drink called dozim. The food products collected from *P. biglobosa* are especially important due to the seasonality of fruit maturation and food availability (Shao, 2002 and Abbiw, 1990). Branches of *P. biglobosa* are sometimes lopped for firewood, attract bees and a popular tree among beekeepers. Pods and root are used as sponges and strings for musical instrument. Its wood is used for timber, pulps for alcohol, tannin or dyestuff, gum or resin, barks and pods contain piscicides which contain alkaloid parkin which is poisonous (Builder *et. al.*, 2014). The bark is used as mouthwash, cure toothache and other skin and gastrointestinal disease.

Parkia biglobosa is known with some insects, small rodents and livestock which may damage the seedlings but seedlings survive easily and the leaves are quickly replaced. Weevil and the pyraid moth have been observed on the fruits, moth eats both the pulp and seed, the leaves are attack by Lepidoptera families and timber is readily attack by termites, marine borers and fungi complexes. Today's forest management decisions integrate increasingly complex and changing social, economic, and ecological values. Foresters not only are concerned with the basic silvicultural goals of harvesting and regeneration but also seek to sustain long-term productivity of forest sites, protect non timber resources such as water and scenery, and maintain biodiversity. All this requires understanding of how forest ecosystems function. Today, forestry and forest sciences are shifting dramatically from basing decisions on how trees operate to a holistic integration of how all the parts of a forest work together, how they are linked and interdependent.

Fungi diseases are serious problem in forest regeneration and sometimes fungi can cause heavy mortality in nurseries. Fungi attack cause discoloration and considerably reduce the value of the wood. Non-timber forest products derived from *P. biglobosa* such as food, medicine, animal fodder, soil amendments, charcoal, and firewood (Chandrasekharan, 1993) enhance the need to protect the regeneration of the plant. The microbial evaluation of plantations is necessary to prevent pathogenic invasion which may lead to total loss of important tree species. Hence the study aim to carry out microbial evaluation of the soils, leaf, stem and bark of *P. biglobosa* for the presence of associated microbes.

2. MATERIALS AND METHOD

2.1 Isolation and identification of associated microbes on *P. biglobosa* soils and parts

Samples of plantation soils, leaves and bark of *P. biglobosa* were collected from eight provenances: Nigeria, Mali, Senegal, Burkina Faso, Guinea, Cameroun, Benin Republic and Ghana of *P. biglobosa* established at Wasengari, Saki, Oyo State, Nigeria. All samples were aseptically collected, stored in a sterile transparent Zip loc sample bags and isolation was made in the laboratory within 48 hours of collection. The plant parts in each provenance were sectioned into smaller pieces using a sterile scalpel, surface sterilized in 10% sodium hypochlorite and blotted dry in between sterile Whatman No 1 filter paper. Serial dilutions to 10^{-3} dilution factor of soil sample in each provenance was done to assay microbial flora in the soil. Potato dextrose agar (PDA), Nutrient agar (NA), and MacConkey agar were routinely prepared in the laboratory, autoclaved at 121°C for 15 minutes.

The media were allowed to cooled to about 45°C, dispensed into a sterile Petri dish and left to set on the bench. The plant part tissues and 1 ml of soil dilution (10^{-3}) were separately inoculated onto the media (Pitt *et. al.*, 1995), PDA was used for mycoflora assay and incubated at room temperature $28 \pm 2^\circ\text{C}$ for 5 – 7 days. NA and MacConkey agar were used for bacteria assay and incubated for 24 – 48 hours. Microbial colonies from the soil and plant parts in each medium were counted depending on when growth occurred, frequency of occurrence of colonies and morphological appearance were observed. Pure cultures of associated fungi were obtained by subculture on fresh PDA. Morphological appearances were used to identify the fungi with reference to identification keys by Kulwant *et al.*, 1991 and Raper and Fernnel, 1965. Bacteria colonies were subculture on fresh NA for Gram staining and microscopic examination and on MacConkey agar to observe for the presence of Gram negative, lactose and weak lactose fermenting bacteria genera present.

3. RESULT AND DISCUSSION

Many bacteria belonging to different genera were isolated from soils of *P. biglobosa*, the microbial load and occurrences shows that *P. biglobosa* from Senegal has the highest number (5.7×10^3 cfu/ml) of bacteria load and Ghana *P. biglobosa* recorded the least of 2.7×10^3 cfu/ml. Mali soil has 4.7×10^4 cfu/ml as the highest fungi colony while Burkina Faso has the least at 1.8×10^4 cfu/ml as presented in table 1.

Table 1: Microbial count of *P. biglobosa* soil across Provenances

Provenances	Fungi colony (cfu/ml) x10 ⁴	Bacteria colony (cfu/ml) x10 ⁴
Nigeria	1.9	35
Mali	2.4	29
Burkina faso	1.8	23
Senegal	4.7	57
Cameroun	1.9	44
Benin	3.0	46
Guinea	2.7	37
Ghana	2.1	27

Fungi from varied genera were isolated from *P. biglobosa* soils indicating a similar trend to that obtained with bacteria load whereby *P. biglobosa* from Senegal also have the highest number of fungi colony with 4.7 x10³ cfu/ml but Burkina Faso showed least fungi load of 1.8x10³ cfu/ml (Table 1).

Entobacteriaceae, *Bacilli* and *Streptococci* species were isolated from *P. biglobosa* soils in the provenances. *Bacillus* spp. such as *Bacillus thuringensis*, *B. mycoides* and even the waxy appearance of *B. cereus* were part of the isolates. Also are *Streptococci*, *Diplococci*, and *Staphylococci* cultured from the soils of *P. biglobosa* across the provenances as shown in Table 2.

Different fungal genera were isolated from *P. biglobosa*; *Aspergillus* genera dominating with total of 69.86% which includes *A. niger*, *A. versicolor*, *A. flavus*, *A. fumigatus*, *A. tamari*, *A. terreus* and another *Aspergillus* spp. which are either saprophytic pathogenic and could even be mycotoxigenic. Others are *Fusarium sp.* 27.71%, *Rhizopus sp.* 8.43%, *Botryopodia sp.* 1.02%, *Pythium sp.* 3.61%, *Trichoderma sp.* 1.20% and *Penicillium sp.* 1.20% were the fungi present in the soil.

The bark and leaves of *P. biglobosa* were assayed for fungi colony by direct plating on PDA as shown in table 4 below. The bark and leaves of *P. biglobosa* known for its high medicinal use is colonized by pathogenic fungi such as *Botryopodia sp.* 44.44% and 27.78% occurrence respectively along with other pathogens as presented in the table 4.

Some areas in the *P. biglobosa* plantation show obvious level of disease incidence as a result of stem or twig infection which lead to total loss of the plant leaving space within the plantation. Infected twig with dark smoky head samples were taken from Senegal, Guinea and Mali and were assayed in the laboratory for the associated organisms the result were as presented in table 5.

The need to assay infected twig was as a result of incidence of disease across the provenance which leave part of the plant devoid of any growth. Generally assessment was back with laboratory analysis as *Botryopodia sp.* isolated with the highest occurrence is known for dieback in cashew florescence and also result to root and stem rot in *P. biglobosa* with obvious symptom of smoky twig.

The assayed stem confirm *Botryopodia sp.* with 50% occurrence as the highest occurred pathogen follow by *Pestalotia*. All the four isolates are known to be pathogenic fungi while the high incidence of *Botryopodia sp.* can be a pointer of die back disease in *P. biglobosa* young tree.

4. CONCLUSION AND RECOMMENDATIONS

The present study investigates the microbial invasion of germplasm of *Parkia biglobosa* from different provenances in Wasangare, Saki West Local Government, Oyo State. Based on this study, the following conclusions are drawn:

1. The arrays of organisms isolated from this study suggest that, *Parkia biglobosa* could be attacked by various fungi and bacteria across the provenances investigated
2. *Parkia biglobosa* tree parts (leaves and stem) and soil can be affected by bacteria and fungi despite the antimicrobial potential of *P. biglobosa* as a plant which could lead to die back or total death of the tree, if it is exposed to pathogenic or opportunistic pathogenic invasion which could be through soil or directly on its part.
3. There is the need for constant evaluation and maintenance of the plantation to keep the plants and maintain its existence because of the usefulness as source of protein, timber and also medicinal capabilities.

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Table 2: Bacteria in *P. biglobosa* soil

Provenanace	<i>Enterobacter</i> spp.	<i>P. vulgaris</i>	<i>Salmonella</i> spp.	<i>S. mercesem</i>
Nigeria	+	+	-	-
Mali	+	+	+	-
Burkina faso	+	+	-	+
Senegal	+	+	+	-
Cameroun	+	+	+	-
Benin	+	-	-	-
Guinea	+	+	-	-
Ghana	+	-	-	-

Table 2 Cont'd: Bacteria in *P. biglobosa* soil

Provenanace	<i>K pneumoniae</i>	<i>Bacillus</i> spp.	<i>Streptococcus</i> spp.	<i>Staphylococcus</i> spp.	<i>Diplococcus</i> spp.
Nigeria	-	+	-	-	-
Mali	-	+	-	-	-
Burkina faso	+	-	-	-	-
Senegal	-	+	+	+	-
Cameroun	+	+	+	-	-
Benin	-	+	-	-	-
Guinea	-	-	-	-	+
Ghana	-	+	-	-	-

Table 3: Percentage of occurrence of fungi in *P. biglobosa* soil

Provenanace	<i>A. niger</i>	<i>A. versicolor</i>	<i>A. lilanus</i>	<i>A. flavus</i>	<i>A. fumigatus</i>	<i>A. tamaris</i>	<i>A. terreus</i>	<i>A. spp.</i>
Nigeria	+	+	+	-	-	-	-	+
Mali	+	+	-	-	-	+	-	+
Burkina faso	+	-	-	-	+	-	+	+
Senegal	+	+	-	-	-	+	+	+
Cameroun	-	-	-	-	-	-	-	-
Benin	+	-	+	+	+	-	-	+
Guinea	+	-	-	-	+	+	-	+
Ghana	-	-	-	-	+	-	+	+
% Occurence of organisms	12.04	7.23	6.02	1.20	9.64	10.84	6.02	16.87

Table 3 Cont'd: Percentage of occurrence of fungi in *P.biglobosa* soil

Provenanace	<i>Penicillium</i> spp.	<i>Fusarium</i> spp.	<i>Trichoderma</i> spp.	<i>Pythium</i> spp.	<i>Botyros</i> spp.	<i>Rhizopus</i> spp.	% occurrence of fungi across provenances
Nigeria	-	+	+	+	-	+	57.14
Mali	-	+	-	-	-	+	42.86
Burkina faso	-	+	-	+	-	-	42.86
Senegal	-	-	-	-	-	-	35.71
Cameroun	-	+	-	-	-	+	14.29
Benin	-	+	-	-	+	+	57.14
Guinea	+	+	-	-	-	+	50.00
Ghana	-	+	-	-	-	+	35.71
% Occurrence of organisms	1.20	27.71	1.20	3.61	1.02	8.43	

Table 4 : Fungi isolates from *P.biglobosa* bark and leaf

Provenanace	<i>Botryopilodia</i> spp.		<i>A. niger</i>		<i>A. flavus</i>		<i>A. fumigatus</i>		<i>A. spp</i>		<i>Fusarium</i> spp.	
	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf
Nigeria	+	+	-	-	-	-	-	-	-	-	+	-
Mali	+	+	-	-	-	-	-	-	+	-	-	+
Burkina faso	+	+	+	-	+	-	-	-	-	-	-	-
Senegal	-	-	-	-	-	-	-	+	-	-	-	+
Cameroun	+	+	-	-	-	-	-	-	-	-	-	+
Benin	+	+	-	-	-	-	-	-	-	-	-	-
Guinea	+	+	+	-	-	-	-	-	+	-	-	-
Ghana	+	+	-	-	-	-	-	-	-	-	-	+
% Occurrence	44.44	27.78	8.33	-	2.78	-	-	2.78	5.56	-	2.78	16.67

Table 4 Cont'd: Fungi isolates from *P.biglobosa* bark and leaf

Provenanace	<i>F. verticilliodes</i>		<i>Pestotatia</i> sp.		<i>Penicillium</i> sp.		<i>Trichoderma</i> spp		<i>Bipolaris</i> spp		Occurrence of fungi across Provenance	
	Bark	Leaf	Bark	leaf	Bark	Leaf	Bark	Leaf	Bark	leaf	Bark	Leaf
Nigeria	-	+	+	-	-	-	-	-	-	+	50	42.86
Mali	-	+	+	-	-	-	-	-	-	-	37.5	42.86
Burkina faso	-	-	+	-	-	+	-	-	-	+	50	50.00
Senegal	-	-	+	+	-	-	+	-	-	+	37.5	71.43
Cameroun	-	-	+	+	-	-	-	-	-	+	25	71.43
Benin	-	+	+	-	+	-	-	-	-	+	37.5	42.86
Guinea	-	+	-	-	+	-	-	-	-	-	50	28.57
Ghana	-	-	+	-	-	-	-	-	-	+	12.5	42.86
% Occurrence	-	13.8	22.2	8.3	5.56	2.78	2.78	-	-	22.		
		9	2	3						22		

Table 5 : Fungi isolates from infected twig/stem of *P.biglobosa*

Provenanace	<i>Botryopilodia</i> spp.	<i>Fusarium</i> spp.	<i>A .niger</i>	<i>Pestotatia</i> spp.
Mali	+	+	-	+
Senegal	+	+	+	-
Guinea	+	+	+	+
% Occurrence	50	18.5	12.5	31.25