Academic Research Journal of Agricultural Science and Research
Vol. 9(3), pp. 104-111, June 2021
DOI: 10.14662/ARJASR2021.110

ISSN: 2360-7874
http://www.academicresearchjournals.org/ARJASR/Index.htm

Full Length Research

Fungal foliar diseases of Balanite *(Balanites aegyptiaca* L.*)* in Sokoto State, Nigeria

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Accepted 3 June 2021

A survey on fungal diseases affecting the leaves of *Balanites aegyptiaca* was conducted in three Agricultural zones of Sokoto State, Purposive sampling procedure was used to identify three location, each location was divided into three replicate from which a diseased leaf samples were collected with the objective of identifying the disease and organisms responsible for the disease. The samples were taken to mycology laboratory of the Department of Biological Sciences for identification of the pathogens responsible for the diseases. *Aspergillus* leaf rust and *Aspergillus* leaf spot caused by *Aspergillus species* and Mucor leaf rust caused by *Mucor spp*. were the diseases identified. Highest incidence and severity of Aspergillus leaf rust disease were visually assessed and recorded in Tambuwal Zone, followed by Sokoto Zone; this was attributed to space arrangement of the tree stands, while location 3 has a less visually incidence and severity. This was also attributed to the closeness of tree stands. Further research should be carried out to evolve the control measures of the diseases identified in the study area, and also management of other non-pathogenic that could be responsible for the disease leaves of *Balanites aegyptiaca*

Key words: - *Balanites aegyptiaca*, Fungi, Pathogens, Aspergillus, Leaf rust and Diseases

Cite this article as: Nasiru A. M., Alhassan K. (2021). Fungal Foliar Diseases of Balanites (*Balanites Aegyptiaca L*.) in Sokoto State, Nigeria. Acad. Res. J. Agri. Sci. Res. 9(3): 104-111

INTRODUCTION

Balanites aegyptiaca belongs to the family *Zygophyllaceae* which composed of about 25 genera and 240 species and predominant in tropical, sub-tropical and warm temperate, often in drier areas including Africa (Chapagain and Wiesmon, 2006). This tree reaches 10m (33ft) in the height with a generally narrow form, the branches are thorny, it produces several forms of inflorescence bearing yellow green bisexual flowers which exude nectar, and the dark green compound leaves are made up of two leaflets which are variable in size and shape. The yellow single seeded fruit is edible but bitter (Ndoge and Gassama, 2004).

B. aegyptiaca had been used over thousands of years (Von Maydell, 1986). The flowers are greenish white fragment with 5-6mm in diameter. Flowering and fruiting occurs during October Nix *et al.,* (2007*)*, seeds are pendulous and ex-albuminous the leaves are alternate, two foliate, petioles are 3-6mm long, leaflets are elliptic and have broadly pointed petioles up to 5mm long. The kernel cakes aqueous leaf extract and saponins was reported to possess anti-bacterial activity and potential larvicidal activity respectively (Nadro *et al.,* 2014).

Balanites aegytiaca are usually consumed by both urban and rural dweller especially during the dry season.

Balanites aegyptiaca like other trees species in the savanna is being endangered most importantly by disease incidence, other are activities of man to source for fire wood, medicine and other potentialities. *B. aegyptiaca* is one of

the most common trees, but neglected wild plant species of the dry land of Africa. However a number of diseases were reported to attack *B. aegyptiaca* causing loss in yield and quality of the tree or its part which range from the loss of chlorophyll from the tissue of leaves (yellowing), necrosis of the die-back tissue (canker), lesion on the fruits which are some of the diseases affecting *B.aegyptiaca.* Despite the vast values of *B. aegytiaca* in terms of medicinal uses, nutritional and economical importance, there is inadequate information of the disease affecting the tree with the respect to the pathogens that are responsible for the diseases Nix *et al.,* (2007*).*

The study is therefore aimed to determine the incident and severity of the diseases affecting *Balanites aegytiaca* and to identify the pathogens that are responsible for the diseases.

MATERIALS AND METHODS

Study Area

The research was conducted in three Agricultural zones of Sokoto State (Sokoto Zone, Isa Zone and Tambuwal Zone), Nigeria. Sokoto state is located in the Sudan Savannah zone in the extreme north western part of Nigeria between latitude 11.6^0 N and 13.9^0 N and longitude 3.7^0 E and 6.9^0 E (Mamman *et al*., 2000). Sokoto state share common border with Niger republic to the North West, Zamfara state to the south and Kebbi to the south west.

Sokoto state has an annual average temperature of 28.3^0 C (82.9^0 F) Sokoto is on the whole, a very hot area. However, maximum daytime temperature is for most of the year generally less than 40^0 C (104.0^0 F) and dryness makes the heat bearable. The warmest months are February to April when daytime temperature can exceed 45^0 C (113. 0^0 F). the rainy season is from June to October during which showers are daily occurrence (Nix *et al.* 2007*).* From the late October to February, during the cold season, the climate is dominated by the harmattan wind blowing Sahara dust over the land. The dust dims the sunlight where by lowering temperatures, significantly and also leading to the inconvenience of dust everywhere in houses (Ndoge and Gassama, 2004).).

Sokoto is located in the Sudan savannah from where grass cover is more or less inter-speare by short tree and shrubs. The soil is predominantly ferruginous tropical type, texturally sandy and P^H of the soil ranges between 6 and7. Some common trees species found include (Bello, 2002).

Sampling Procedure and Collection of Samples

A reconnaissance survey of the selected location was done and each Zone was divided into replicates in three different locations which were labeled as follows:

1.	Location A	Sokoto Agricultural Zone:	Kware, Sokoto South and Wamakko
2.	Location B	Tambuwal Agricultural Zone:	Yabo, Tureta and Tambuwal
3.	Location C	Isa Agricultural Zone:	Isa, Goronyo and Rabah

Trees were selected randomly in each location for the study; the selected trees were examined for the presence of the disease. The specimen of the disease leaves were collected and place into the sterilized polythene bag and were taken to the mycology laboratory of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto for identification.

Identification of the Disease causing organisms

Glassware Petri dishes were washed and cleaned and wrapped with aluminum foil paper for sterilization in the hot air oven. The oven was set at 160^0C for one hour.

Media preparation: the media was prepared in the conical flask; Potato Dextrose Agar (PDA) and streptomycin were measured by the use of electric weighing balance. According to the manufacturer instruction i.e 39g of PDA was dissolved in 1000ml of distilled water; 1kg of streptomycin was added to inhibit the growth of bacteria, the conical flask was then closed with cotton wool and wrapped with aluminum foil paper.

The mixture was heated using thermo plate and dissolved completely and was autoclaved at 121^0c for 15 minutes. It was allowed to cool, after which it was poured into the petri-dishes aseptically and allowed to solidify.

Inoculation: little portion of the sample was cut and placed at the center of the media in the Petri dish by the use of needle.

Sub culturing: fungal mycelium of the isolated organisms was sub-cultured on fresh Potato Dextrose Agar (PDA) in order to obtain pure culture. The pure culture were used to identify the fungal organisms by the used of microscopic.

Assessment of Incidence and Severity of Leaf spots and Rusts of *Balanites aegyptiaca*

Disease incidence is the number of plant unit infected, expressed as a percentage of the total number of units assessed as follows;

$$\text{Disease incidence (DI)} = \frac{\text{Number of infected plant units}}{\text{Total No. (Healthy and infected) of units assessed}} \times 100$$

$$\text{Disease severity (\%)} = \frac{\text{Sum of all leaves disease rating}}{\text{Total Number of rating X maximum grade}} \times 100$$

Disease severity were obtained using 0-10adapted from Horsfall and Barratte (1945)

1. 0% No symptom on the leaves
2. 1-10% Number of leaves diseased
3. 11-20% Number of leaves diseased
4. 21-30% Number of leaves diseased
5. 31-40% Number of leaves diseased
6. 41-50% Number of leaves diseased
7. 51-60% Number of leaves diseased
8. 61-70% Number of leaves diseased
9. 71-80% Number of leaves diseased
10. 81or more Number of leaves diseased

The data collected were for incidence and severity of the leaf disease, and mycilia growth after two weeks of inoculation.

RESULTS

The study discovered that the fungal diseases affecting the leaves of *Balanites aegyptiaca* in the study area include the following:-

I. **Leaf rust**; the Disease is caused by *Aspergillus tamaiikita.* It is characterized by colonies growing rapidly, dark brown. Rusts first appear as a yellowish – green fleck on the upper leaf surface. Almost simultaneously pustules appear as small raised bumps on the lower leaf surface.
II. **Leaf Spots**; the diseases is cause by *Aspergillus terreus Thom*. It is also characterized by brown, black, tan or reddish in color. Occasionally the necrotic areas have a red or purple border partial to complete defoliations may occur under favorable condition for the causal fungus.
III. **Mucor leaf rusts**; it is characterized by hyphen white or colour, varying from a few millimeters to some centimeters in height. Sporangiophores often branched, always ending in a many spore, sporangium without an apophysis. Sporangia varying in size, columellae well – developed, spores are variable in shape, smooth – walled or slightly ornamented.

Incidence and Severity of Fungal Foliar Disease

Aspergillus leaf rusts of *Balanites aegyptiaca*

The Incidence and Severity of *Balanites aegyptiaca* is presented in Table 1, with significant difference among the treatments, the result shows that, Zone B recorded the highest Disease Incidence (88.33%), followed by Sokoto Zone (Zone A) (72.72%), Zone C has the lowest incidence (59.47%).

The severity was significantly higher in Zone B which was recorded as (42.12%), followed by the Zone A (41.43%), and lastly Zone C (32.26%).

Table 1. Incidence and Severity of Aspergillus Leaf Rusts of *Balanites aegyptiaca.*

Zones	Incidence (%).	Severity (%)
Sokoto Agricultural Zone	72.72±11.16[ab]	41.4±3.03
Tambuwal Agricultural Zone	88.33±12.58[a]	42.12±9.48
Isa Agricultural Zone	59.47±8.61[b]	32.26±2.496
Standard Error	5.23	2.33
Significance	✳	ns

Means followed by the same letter (s) do not differ significantly according to Duncan's Multiple Range Test (DMRT) at 5% level of significance**.**

Aspergillus leaf spots of *Balanites aegyptiaca*

The Incidence and Severity of Aspergillus leaf spots of *Balanites aegyptiaca* is presented in Table 2 with significant difference among the treatments. The result shows Tambuwal Zone recorded the highest Disease Incidence (88.33%), followed by Sokoto Agricultural Zone (72.72%), and Isa Agricultural Zone (59.47%).

The Severity was significantly higher in Tambuwal Agricultural Zone (42.12%), followed by Sokoto Agricultural Zone (41.43%), and Isa Agricultural Zone (32.26%).

Table 2. Incidence and Severity of Aspergillus leaf spots of *Balanites aegyptiaca*

Location	Incidence (%).	Severity (%)
Sokoto Agricultural Zone	72.72±11.16[ab]	41.4±3.03
Tambuwal Agricultural Zone	88.33±12.58[a]	42.12±9.48
Isa Agricultural Zone	59.47±8.61[b]	32.26±2.496
Standard Error	5.23	2.33
Significance	✳	Ns

Means followed by the same letter (s) do not differ significantly according to Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Mucor Leaf Rusts of *Balanites aegyptiaca*

The Incidence and Severity of Mucor leaf rusts of *Balanites aegyptiaca* is presented in Table 3: with no significant difference between among the treatments. The result shows that Tambuwal Zone recorded the highest Diseases Incidence (78.33%), Sokoto Agricultural Zone (72.72%), and Isa Agricultural Zone (59.47%).

The Severity was also significantly higher in Sokoto Agricultural Zone (15.72%), followed by Tambuwal Agricultural Zone (13.83%), and Isa Agricultural Zone (11.33%) respectively.

Table 3. Incidence and Severity of Mucor Leaf Rusts of *Balanites aegyptiaca.*

Location	Incidence (%).	Severity (%)
Sokoto Agricultural Zone	72.72±11.16	15.72±5.12
Tambuwal Agricultural Zone	78.33±20.21	13.83±2.38
Isa Agricultural Zone	59.47±8.61	11.33±.37
Standard Error	4.96	1.14
Significance	Ns	Ns

Means followed by the same letter (s) do not differ significantly according to Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Morphological Features of Fungal Pathogens Affecting the Leaves of *Balanites aegyptiaca.*
Aspergillus tamarii kita

Colonies on Potato Dextrose Agar (PDA) at 25^0C were attaining a diameter of 4-5cm within 7days, mostly sporulating densely and initially forming a dense felt at yellow-brown conidiophores, which rapidly turn dark green-brown. Conidiophores stalk hyaline. Mostly conspicuous rough-walled

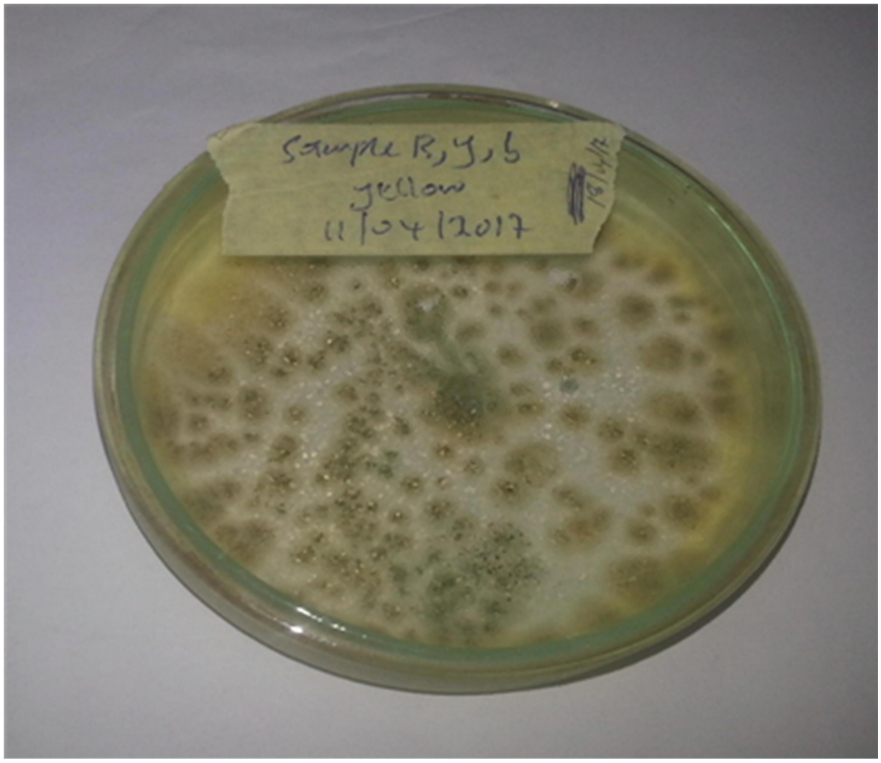

Plate 1: An isolate of *Aspergillus tamarii kita.*

Aspergillus terreus thom

Colonies on PDA at 25^0C attaining a diameters of 3.5 – 5.0cm within 7days, mostly consisting of a dense felt of yellow-brown conidiophores, becoming darker in age. Conidial heads compact. Columnar

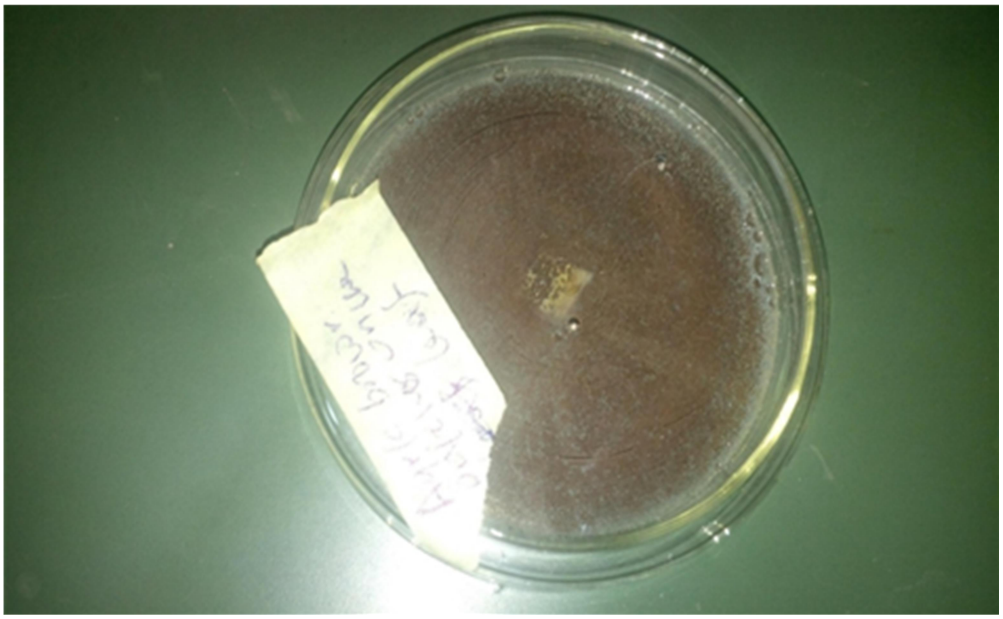

Plate 2. An isolate of *Aspergillus terreus Thom.*

DISCUSSION

Diseases in the Study Area

Aspergillus leaf rust is characterized by small (1.2mm) angular, pale bright yellow lesions (spots) on the upper surface of infected leaves. This rust is usually limited in size and shape by the finest vein lets and is often grouped in small irregular clusters of three or more spots. With age, the spots may often turn to a golden yellow and then brown as the affected tissue within the spots dies (Chothani and Vaghasiya, 2011).

Mild moist condition favours the development of rust disease. This agrees with the finding of (Mark *et al., 2006),* that rust is spread by windblown spores from infected plant to healthy plants. Spores are also spread by splashing of water, they need wet leaf surface to germinate and cause infection. Rusts disease is common in late summer and early rainfall, since free water on leaf surface is usually necessary for infection.

This rust is also responsible for leaf distortion and mosaic in mulberry plant, symptom on which include a numerous pin-head sized circular to oval brownish to black eruptive spots on the surface of the leaves as observed on the leaves of *Balanites aegyptiaca*. This agrees with the finding of (Chothani and Vaghasiya, 2011). That the leaf rust is caused by bacteria and fungi, over a long period of time.

Aspergillus leaf spot is characterized by small and irregular yellow, brown, dark-brown or black spots. The spots can expand and merge to cover the whole area. The color of the infected part darkens as it ages. The disease can also produce cankers on petioles and on stem that can cause severe defoliations and rotting of fruits and roots, infected fruit has small, water-soaked, sunken, circular spots that may increase in size up to 1.2cm in diameter. As it ages the centre of an older spot become blackish and emits gelatinous pink mosses. This agrees with the findings of Ghose *et al.,* (2010).That Leaf rust are small circular spots with light brown center surrounding by dark concentric rings spotting is most prevalent on lower older leaves, but new leaves are susceptible too. Spots that are closely together may combine to form one large spot. Heavily spotted leaves will quickly turn to yellow, wilt and fall due to like of water. Leaves suffer the most from this symptoms but stems, flower, and seed pod can all be affected *(*Shweta *et al.,* 2014).

Mucor leaf rust; the first symptoms of rust are tiny specks or spot on leaves that are red in color from orange to rusty brown, brownish-yellow, purple and red. Left untreated spots get bigger and turn into bumpy-looking pustules (Anon*,* 2015*).*this goes in line with the finding of Nix*,(*2007*)* that the spores are spread by splashing of water, they need a wet leaf surface to germinate and caused infection.

Mucor leaf rust pathogen that cause reddish brown streaks turning parallel to the leaf veins, which aggregate to form larger dark-brown to black compound streaks. This streak eventually form bursiform or elliptical lesions that coalesce, form a water soak border with a yellow hallo eventually merge to cause extensive leaf necrosis. The disease does not killed the plant immediately, but weaken them by decreasing the photosynthetic capacity of leaves, causing a reduction in the quality and quantity of fruits, reducing the premature ripening of fruit harvested from infected plants. This agrees with the finding of *(* Mark *et al.,* 2006*) that* the leaf rust formed yellow or orange leaf spots form on the leaf, powdery yellow orange spore are produce on the underside of the leaf *of B. aegyptiaca.*

Incidence and Severity of Fungal Diseases of *Balanites aegyptiaca*

The highest incidence and severity of Aspergillus leaf rust 88.33% and 42.12% respectively was recorded along location 1 possibly because of the trees are closed to each other, this agrees with the findings of Shweta *et al.,* (2014) that Aspergillus species which found to be the causative agent for Aspergillus leaf rust, and diseases thrive very well in hot and harsh weather condition, also Shweta *et al.,* (2014), stated that Aspergillus leaf rusts turn as the disease progresses, causing premature defoliation, moist condition also favours the development of rust diseases. Abu-al-Futuhlm (1983) added that the disease increase when cooler weather is paired with high humidity. With respect to Aspergillus leaf rust, the highest incidence and severity of 88.33% and 42.12% obtained in location 2 was attributed to the same factors that enhanced the growth and development of Aspergillus leaf rust in location 1. However low incidence of 56.47% and 42.12% respectively could be attributed to space arrangement of the stands in the location. While the least incidence and severity could be due to more or less closeness of tree stands.

The highest incidence and severity of mucor leaf rust 78.33% and 15.72% respectively was also recorded in location 2. However, low incidence of 59.47% and 11.33% respectively could be as a result of the temperature in the study area which might have influenced the activities of the fungal pathogens. This agrees with the findings of Mark *et al.,* (2006) that rust spread by windblown spores from infected plant to healthy plants. The temperature is also a major factor that plays an important role in the growth of fungi, the higher the temperature the faster the spoilage will occur in the leaves (John, 1969).

Morphological Feature of Disease Pathogens Responsible for the Leaf spots and Leaf rusts Disease of *Balanites aegyptiaca*

Aspergillus tamarii kita, can be described morphologically by colonies on Potato Dextrose Agar (PDA) attaining a diameter of 4-5cm within 7days, mostly sporulating densely and initially forming a dense felt of yellow-brown conidiophores, This rapidly turn dark green-brown conidiophores stalk hyaline, mostly conspicuous rough-walled. Vesicles globose, 25-50mm in diameter. Phialides borne directly on the vesicles or on metulae (mostly on large heads).10-15 x 4-8µm. metulae 7-10 x 4-6µmconidia globose to subglobose5-6(8) µm in diameter. Brownish yellow conspicuously ornamented with tubercles and warts. The outer and inner conidial wall visible Mark *et al.,* (2006).

Aspergillus terreus Thom; can be described morphologically by possession of yellow-brown conidiophores. Colonies on PDA at 25^0c attaining a diameter of 3.5 – 5.0cm within 7days. Mostly consisting of a dense felt of yellow brown conidiophores, becoming darker with age. Conidial heads compact columnar, mostly 150 – 500 x 30 – 50µm. conidiophores hyaline, smooth – walled. Viseclessubglobose, 10 – 20 µmin diameter phialides borne on metulae, 5 – 7 x 2.0 – 2.5µm. conidioglobose to ellipsoidal, 1.5 – 2.5µm. in diameter.Hyaline to slightly yellow, smooth. Dense felt of dark green conidiophores intermixed with aerial hyphae bearing conidiophores. This agrees with the finding of (Rodrigues *et al.,* 2007*)*, that *aspergillus* and mucor, morphological feature includes conidial and mycelia colour, colony diameter, colony reverse colour, production of exudates and soluble pigments, presence of sclerotia and cleistothecia. Micro morphological characterization is mainly dependent onseriation, shape and size of vesicle, conidia and morphology of cleistothecia and ascospores. However all this morphological features have to be determined under standardized laboratory conditions by trained mycologists, in order to obtain an accurate identification?

Mucor racemosus fre's; can be described morphologically by possession of white, becoming brownish gray with age 2 – 20(30) mm in height, consisting of tall and short. Sporangiophores branched (in a mixed sympodial and monopodial way), the short branches sometime recurved, with encrusted walls. Sporangia hyaline becoming brownish to gray with age, with up to 80 to 90µm in diameter but mostly 70µm in diameter with spinulose (small sporangia up to 20µm in diameter with persistent walls). Columellarobovoid, ellipsoidal cylindrical, slightly fosiform, usually with truncate base, up to 37 – 55µm, height brown with collar. It is also characterized with hyphae white or colour varying from few millimeter to some centimeters in height. Sporangiophores often branched, always ending in a many spores sporangium without apophysis. Sporangia varying in size, columellar well developed, spores are variable in shape, smooth-walled or slightly ornaments, this agrees with the findings of Nix *et al.,* (2007*).* Colonies are floccose mucor ranging in colour from white, cream, pale greyish to brown.sporangiophores are hyaline and mostly sympodially branches with long branches creet and shorter branches becoming circinate (recurved). Sporangia are spherical, varying from 20 – 80µm in diameter, with small sporangia often having a persistent sporangia wall, columellae are spherical to ellipsoidal and are up to 50 µm in diameter, sporangiophores are hyaline, smooth – walled.

CONCLUSION

It was concluded that, the fungal foliar disease affecting the leaves of *Balanites aegyptiaca were Aspergillus Species* of fungi are responsible for this infection. This research work revealed that the leaf rust and leaf spot are the available symptoms of the disease of *Balanites aegyptiaca* in Three Agricultural Zones of Sokoto State. Prompt removal all disease leaves, fruits, or entire plants to avoid spread of diseases. To reduce disease severity if pathogens are present, and to lessen dependency on labeled fungicides and nematicides, pesticides include Biological control agents that may be commercially available in the future, must be used. Therefore all the possible management practice should be put in place so as to enhance the productivity of the plant.

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