Effect of fungi associated with foliar diseases of *Ficus capensis* on the proximate, anti-nutrient and mineral composition of leaves

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Abstract

The *Ficus capensis* Thunb leaves are affected by some foliar diseases, which could reduce the nutrient benefits from these leaves. This study aimed at isolation and characterization of fungal pathogens associated with the plant's foliar diseases, analysis of the proximate and phytochemical composition of healthy and diseased fruits and leaves. The effect of isolated fungal species on these proximate and phytochemical contents of leaves was also investigated. *Hendersonula* sp., *Didymeria conferta*, *Rhizopus stolonifer* and *Fusarium oxysporium* were isolated and characterized as fungi associated with the leaves. Proximate analysis revealed the presence of protein, carbohydrate, lipid, ash, fiber, and moisture. Mineral nutrient analysis revealed the presence of copper, zinc, lead, manganese, magnesium, potassium, sodium, calcium, while that of anti-nutrients revealed saponin, alkaloid, flavonoid, tannin and cyanogenic glycoside, both in the diseased and the healthy leaves and fruits of the plant. The values of the food nutrients and minerals in healthy leaves were significantly different from those of the infected fruits and leaves, with the value of anti-nutrients in infected leaves were higher than those in health ones. The anti-nutrients in the infected leaves were higher than those in the healthy leaves and fruits of the plants, while the proximate and mineral composition of the supposedly healthy leaves was greater than those of diseased leaves.

Key words: *Ficus capensis*, Fungi, Minerals, Non-nutrient, Proximate.

Introduction

Wild edible plants have recently gained popularity all over the world because they are good providers of proteins, minerals, vitamins, dietary fiber, carbohydrates, essential fatty acids, antioxidants, phenolic compounds, and secondary metabolites (Khan *et al.*, 2016). In addition, many wild plants also possess various biological properties and are very useful in the treatment of different ailments (Naqvi *et al.*, 2020; Javed *et al.*, 2021; Ferdosi *et al.*, 2022). Native populations from various parts of the world use the fruits in fresh or dried form because they are edible. To create fig preserves, they are utilized (Hankey, 2003). Since they feel it is the knowledge of their forefathers that also acknowledges different religious and historical backgrounds, and which mainstream medicine seems to disregard, many rural populations place a lot of faith in traditional remedies, especially the unexplainable elements (Adesina, 2014). Yet, for a safe use of herbs as medication, suitable formulation and dosage are required (Uzoekwe and Mohammed, 2015).

Bongoni *et al* (2013), reported that the most significant and useful phyto-nutrients comprise natural vitamins (such as vitamins A, B group, C, D, E, etc) and minerals (zinc, iron, calcium, copper, and

others). Leaves of *F. capensis* contain 3.4% protein, 1.05% lipid, 14.5% carbohydrate, 14.96% fibres 1.5% ash and 17.94% water (Cho *et al*., 2004). Bamikole (2008) reported that lipids in *F. capensis* contained a soluble non-oxidant, particularly, γ-tocopherol, which helps to control hypercholesterolemia. The carbohydrate content of *F. capensis* has been reported by Kislev *et al*. (2006), to be sufficient to support body energy, and that it contains a considerable favorable ratio of the soluble to the insoluble fraction of fiber, weighed against alternative typical dietary fiber sources like wheat bran, oat fiber and rice bran. Hankey (2003), observed that diet soluble fibers were substantially more effective than lower cholesterol. Dietary fibers have also been shown to slow down carbohydrate digestion and absorption, as well as post-natal blood glucose content in the liver and plasma level (Harrison, 2005). The results of the anti-nutrient screening; mineral and proximate analysis by Uzoekwe and Mohamed (2015), showed the presence of saponin, tannins, alkaloids, flavonoids, cardiac glycosides; calcium, magnesium, iron, zinc, copper, phosphorus, manganese, sodium, potassium, moisture, proteins, crude fat, ash, crude fiber and carbohydrates in the leaves of *F. capensis.* Achi *et al.* (2017) reported that the leaves of *F. capensis* revealed significant concentrations of flavonoids, terpenoids, tannins, and alkaloids, but only negligible amounts of steroids, glycosides, and saponins. Also, they noted that the plant's leaves included low levels of fat (1.83%) and fiber (4.77%), but significant levels of carbohydrates (73.77%), ash (6.65%), and protein (6.31%) as well as a moderate amount of moisture (6.67).

Fig rust is commonly caused by a variety of fungi which include *Physopella fic*i, *Certelium fici*, *Cylindrocladium scoparium* and *Cercospora fici* (Ludwig *et al.*, 1999). Palmer and Pitman (1974) reported that Rhizopus smut was the fungal disease of the fig tree. El-ghorah *et al.* (2007) reported that anthracnose caused by *Collitotrichum* sp. is a common disease that attacks all plant parts during the growing stage, and causes small black to yellow or brown spots on leaves. They stated also that as the disease progressed, these spots enlarge to affect the entire area. They also reported that the disease could cause canker on the stems and petiole, resulting in severe defoliation and root damage. In different regions of Nigeria, the leaves of *F. capensis* are utilized as a vegetable in soup and yam pottage as reported by Otitoju *et al*., (2014). In addition to its conventional uses, research has shown that it has anti-inflammatory (Daikwo *et al*., 2012), anti-diarrheal (Owolabi, 2013), antioxidant (Ramde-Tiendrebeogo *et al.*, 2012), pro-fertility (Njoku-oji *et al*., 2016), anti-sickling (Umeokoli *et al*., 2013), and immune-stimulating properties (Akomolafe *et al*., 2016).

Many fig species are grown for their fruits and leaves, which are used for medicinal purposes and for nourishment, especially, in Ebonyi State of Nigeria. The young leaves are used as food for animals, and are cooked and eaten as vegetables. Mature fruits are both eaten raw, and could also be used alongside the young leaves to prepare sauce for eating yam, while some brew them as tea. They are also used by local people for treating a number of ailments and health challenges like malaria, erectile dysfunction by men etc. According to WHO (2014), medicinal herbs make up a sizeable portion of the pharmaceutical market. They are frequently utilized to provide first-line and basic health services, both to people living in remote areas where they are the only health service available, and to people living in impoverished areas where they offer the only affordable remedy. These herbal remedies may work by acting as antimicrobial and anti-inflammatory agents, antioxidants, hormone stimulating enzymes, interfering with DNA replication, and acting as antibiotics through physical actions (Khan and Javaid, 2019; Ferdosi *et al.*, 2021; Javaid *et al.*, 2021, 2022). Though the proximate and phytochemical composition of the leaves, and some fungi implicated in the foliar diseases of *F. capensis* have been studied by some workers, no work has been done on the possible effect of the foliar fungal pathogens on proximate and phytochemical compositions of the leaves. The focus of this research was on the impact of the fungi linked to these foliar diseases on the phytochemical and proximate components of the foliage, which may impair the nutritional value of the leaves to people.

Materials and Methods

Collection of plant material

Both healthy and diseased leaves as well as fruits of *F. capensis* were collected from a farm behind the Faculty of Science Complex of Ebonyi State University, Abakaliki. The proximate and phyto-chemical studies were conducted in the Department of Plant Science and Biotechnology, Plant Anatomy and Physiology Laboratory, University of Port-Harcourt, Choba , Rivers State; while fungal isolation and characterization was done at the Department of Biology Laboratory, Alex Ekwueme Federal University, Ndufu-Alike Ebonyi State, Nigeria.

Fungal isolation and characterization

On a sterile work bench, 32.5 g potato dextrose agar (PDA) was weighed and dissolved with little distilled water in a 500-mL conical flask, before transferring into 1 L measuring cylinder, and made up to 1 L with distilled water. It was dispensed into two 500 mL conical flasks and covered with aluminium foil. Eighteen glass Petri dishes containing prepared media were wrapped with

aluminium foil. The media and the Petri-dishes were autoclaved at 103 kPa and 121 °C for 15 min. After autoclaving, 0.5 g chloramphenicol was aseptically added into each of the media in the conical flasks inside a laminar flow, , to inhibit bacterial growth. After this, these were allowed to dissolve and blend with the media for about 5 min. These were dispensed into Petri dishes that were clean, and allowed to solidify. Infected leaves of *F. cpensis* were washed under tap water and mopped dry using sterile cotton wool. A sterile blade was used to cut out 4 mm square portions of the infected leaf samples and sterilized in 1% sodium hypochlorite for 2 min, washed with sterilized distilled water and allowed to dry in the laminar flow. Sterile forceps were used to inoculate the cut infected leaves onto the solidified media at four equidistant points, and incubated for 48 h at a temperature of 25±2 ºC in an incubator. Fungi that grew on the media were sub-cultured to obtain their pure culture. The morphological and spore/conidial characteristics were used to identify the isolated fungi, using fungal identification manual of Barnnett and Hunter (2000).

Proximate analysis

Standard methods of AOAC (2010) with some modifications were used.

Phytochemical analysis

This was done following the method of Uzoekwe and Mohammed (2015), with some modifications.

Determination of nitrate content using Brucine method

One-gram sample of *F. capensis* fruit, and healthy and infected leaves independently, were placed in 50 mL of acetic acid (2.5%) in 100-mL conical flask, and filtered into three different 250 mL clean beakers. One milliliter of each was pipetted into three different test tubes, with the addition of 0.5 mL Brucin reagent into each of them. In the presence of NO_3^{-1} ion, concentrated sulfuric acid (0.1 N) was added in amounts of two drops to produce a yellowish shade. The color formed was absorbed at 400 nm on a Genesys 10 Spectrophotometer, Thermo Scientific. Water was used as a blank. The working standard for standard nitrate was 0.1 mg NO_3 mL^{-1}, which was obtained by dissolving 0.72 g of potassium nitrate in 1000 mL of distilled water, and distilling the mixture ten times. For every sample, the trial was repeated three times.

Determination of phosphate

Using ascorbic acid method, 1 g sample of *F. capensis* fruit, apparently healthy and infected leaves were extracted with 50 mLof 2.5% glacial acetic acid respectively, into three different 100 mL conical flasks. The extracts were filtered into three different 250 mL conical flasks, followed by the addition of 8.0 mL^{-1} of the combined reagent each of the flask containing the samples. Phosphate ion concentrations in the blank and standard samples ranged from 0.0001 to 0.0007 was prepared, and 0.8 mL of a mixture of reagent was introduced. .

At an 840 nm wavelength in distilled water, the bluish color that appeared within a 30 min period, was noticed and filtered. The filtrate was examined, using an atomic absorption spectrophotometer, for different metal ions. The digests were inhaled into the burner of the atomic absorption spectrometer, which was then calibrated using the reference solution for the particular metal being analyzed. The Atomic Absorption spectrophotometer's operation handbook was followed while making additional settings, which included giving the hollow cathode lamp enough time to stabilize.The experiment was replicated three times for each sample.

Determination of metal ions

One-gram sample of *F. capensis* fruit, and healthy and infected leaves were turned into ash independently, 3 h at a temperature of 630 ºC in a Muffle furnace. Each sample's ash was heated on an electrothermal heater hot plate, after being dissolved in 10 mL of strong hydrochloric acid. Using distilled water, the ash's result was diluted to a volume of 50 mL as can be seen in Table below. The outcome was anatomized by AAS at various wavelengths, for various essence ions. Air and acetylene gas inflow, a wavelength with a narrow tear range, and other settings were adjusted in accordance with guidelines for the used and controlled device. Before aspirating solution for the purpose of calibrating the apparatus, the hollow lamp cathode was given enough time to stabilize. The system was repeatedly cleaned with distilled water after calibrating the apparatus with a standard metal concentration, before each sample's solution was aspirated under the same experimental conditions used for the standard. Using the standard graph of that metal ion plotted, the concentration of that metal ion in the sample was extrapolated. The trial was replicated three times for each sample.

Determination of saponin

Twenty grams of *F. capensis* sample of fruit, apparently healthy and infected leaves were weighed and dispersed into three different 200 mL of 20% ethanol. The suspension was heated over a hot water bath at about 90 ºC. The mixture was then filtered, and the residue re-extracted with another 200 mL of 20% ethanol for each sample. The combined extracts were concentrated to 40 mL over water bath at about 90 °C. Transferring the concentrate into a 250 mL separator funnel, 20 mL of diethyl ether was added, and the mixture was violently shaken. While the ether layer was discarded, the aqueous layer was recovered. The purification procedure was repeated. *n*-Butanol (60

mL) was added, and the combined n-butanol and extracts were washed twice with 10 mL of 5% aqueous sodium chloride for each sample. In a water bath, the residual solution was warmed. The samples were dried in the oven to a consistent weight, following evaporation. Percentages were used to determine the saponin content. The trial was replicated three times for each sample.

W_1 = Weight of empty beaker
W_2 = Weight of beaker with dried sample
W_3 = Weight of the original sample

$$\text{Saponin content (\%)} = \frac{W_2 - W_1}{W_3}$$

This was done three times for each of the samples *viz.* fruits, and healthy and infected leaves.

Determination of tannin

Air dry sample (0.1 g) of *F capensis* fruit, and healthy and infected leaves were weighed into a 100-mL conical flask each. Fifty milliliters of water were added to each sample, and boiled gently on hot plate for 1 h. They were each filtered through Whatman filter paper No. 44 into a 50-mL volumetric flask, and diluted to desired volume when cooled.

Tannic acid standard, 0 to 3 mL was pipetted into 50 mL volumetric flask to give a standard range from 0 to 0.3 mg tannic acid. A suitable aliquot of the sample was pipetted into a 50 mL volumetric flask. From this point, the samples and standard were treated in the same way. Water was added until the flask was two-thirds full. Folin-Denis reagent (3 mL) was added, followed by addition of 10 mL Na_2CO_3 solution. It was diluted to volume, mixed and stood in water bath at 25 °C for 20 min, to measure the optical density at 760 nm or with a red filter using water as a reference. A calibration curve was prepared from the standard reading, and used to obtain trig tannic acid in the sample aliquot. Blank determinations were carried out and subtracted when necessary.

$$\text{Soluble tannic (\%)} = \frac{C \times \text{extract volume}}{10 \times \text{alipuist} \times \text{sample}} \times 100$$

This was replicated three times for each of the samples.

Determination of alkaloid

Sample (5 g) of fruit, and healthy and infected leaves each of *F. capensis* were weighed into three different 250 mL beakers each, with 200 mL^{-1} of 10% acetic acid in ethanol, covered, and left to stand for 4 h. The extracts were concentrated to one-fourth of the original volume after being filtered and concentrated on a water bath. Drop by drop, concentrated ammonium hydroxide was applied to the extracts until all of the precipitates had formed.

The resulting solutions were filtered after being rinsed with diluted ammonium hydroxide, and allowed to settle. The residues were dried and weighed.

W_1= Weight of empty beaker,
W_2 = Weight of filter paper and residues
W_3 = Weight of samples

$$\text{Alkaloid (\%)} = \frac{W_2 - W_1}{W_3} \times 100$$

Determination of flavonoid

F. capensis fruit, leaves that are appeared healthy, and infected leaves were all taken as dry samples and extracted several times using 100 mL of 80% aqueous methanol at room temperature. The resulting solutions were filtered using No. 42 Whatman filter paper (125 mm). After drying the filtrates over a water bath, and weighing them to a constant weight, the filtrates were placed into a crucible.

$$\text{Flavonoid (\%)} = \frac{W_2 - W_1}{W_3} \times 100$$

W_1 = Weight of empty beaker
W_2 = Weight of filter paper and residues
W_3 = Weight of samples

Determination of cyanogenic glycoside

The dried sample (5 g) of fruit, apparently healthy and infected leaves each of *F. capensis* were weighed into a Distillation flask, before 20 mL of distilled water was added to each, and the samples were left overnight, standing (24 h) for proper evaluation. The samples were distilled into 20 mL sodium hydroxide containing 0.5 g crystals. The distillates were titrated with 0.02 N silver nitrate in the presence of 0.2 mL 5% potassium iodide and 1 mL 6 N ammonia hydroxide solution to a permanent turbidity.

$$\text{Alkaloid (\%)} = \frac{\text{Titer value}}{\text{Volume of the digested distilate}} \times 100$$

Statistical analysis

The results were analyzed statistically for the data generated from the proximate, phytochemical and mineral contents, utilizing the social science statistical package SPSS. In order to evaluate if there was a significant difference between and within the values of the parameters extracted from the leaves and fruits, the means and standard deviation, as well as the analysis of variance (ANOVA) at $P \leq 0.05$ were used.

Results and Discussion

The fungi isolated from the infected leaves of *F. capensis* included *Fusarium oxysporum*, *Hendersonula* sp., *Rhizopus stolonifer* and *Didymeria conferta* (Fig. 2 A-D). These were different from *Physopella fici*, *Certelium fici*, *Cylindrocladium scoparium* and *Cercospora fici* associated with leaf rust of the plant, as reported by Ludwig *et al.* (1999) and Palmer and Pitman (1974).

They also reported that *Rhizopus* smut was the causal agent of the fungal disease of the fig tree. El-Ghorah *et al.* (2007), had reported that anthracnose of *Collitotrichum* sp, a common disease that attacked all plant parts during the growing stage, caused small black to yellow or brown spots on leaves, and that as the disease progressed, these spots enlarged to affect the entire area. Schipers (2000), reported that *Didymeria conferta* and *Rhizopus* attacked the plant in the field. By the above results, two new fungi-three new fungi *Hendersonula* sp., *D. conferta* and *F. oxysporum* have been added to the list of foliar fungi linked to the plant's foliar disease.

The results of the proximate composition of the apparently healthy and infected leaves, and the fruits of *F. capensis* are shown in Table 2. The results showed that all the parameters determined in the fruits were lower, when compared with the infected leaves and apparently healthy leaves, except that of the fibre content in the infected leaves. The lower values of the food nutrients -protein, carbohydrate, lipid, ash, fiber and moisture in infected than apparently healthy leaves could have been due to the fact that these must have been partially absorbed by the fungi throughout their metabolic process. According to Ibiam and Nwigwe (2013), fungi must have used the infected leaves' fiber, protein, and carbohydrate content during their derivative activities on them, to account for their relative decrease in the infected leaves. The parameters determined in the infected leaves were observed to be lower, when compared with the healthy leaves, though higher than those in the fruits.These results have also corroborated the report of (Uzoekwe and Mohamme, 2015; Okoroh *et al.*, 2019; Ezeigwe *et al.*, 2020), that the leaves and fruits of *F. capensis* contained these food nutrients.

Results of the mineral nutrients such as copper, zinc and lead were all below detectable limits (BDL), while those of manganese (2.05 ± 0.02 mg 100 g^{-1}) content was higher in infected leaves, when compared with apparent healthy leaves and fruits, as shown in Table 3. The calcium (79.66 ± 0.04 mg 100 g^{-1}) content was observed to be higher in infected leaves compared to the seemingly healthy leaves and fruits. The higher value of phosphorus (phosphate) in apparently healthy leaves could be due to its involvement in higher energy bonding as in the conversion of higher energy in ATP, as reported by Dzungaria *et al* (2007).Clancy *et al.* (1998), reported that high levels of potassium and magnesium reduced the susceptibility of plant to fungal infection, hence, the higher level of these minerals in apparently healthy leaves. These results have also corroborated the report of earlier workers who stated that the leaves and fruits of *F. capensis* contain these mineral nutrients (Uzoekwe and Mohamme, 2015; Okoroh *et al.*, 2019; Ezeigwe *et al.*, 2020).

The results of anti-nutrient content of fruit and leaves that appear in both healthy and diseased *F. capensis* leaves are shown in Table 4. High anti-nutrient contents of the fruits shows that the fruit has a high cleansing ability. This result corroborated the report of Okoroh *et al.* (2019), that the fruits contained alkaloids, saponins, flavonoids, glycosides, and tannins.It was observed that the ant-nutrient contents in the fruits were higher when compared with those in the apparently healthy and infected leaves.This could explain why they are rarely attacked by microorganisms.The ant-nutrient composition of the infected leaves was higher, compared to the leaves that appear to be in good health, except for tannin. This result corroborates the report of of previous studiesthat the leaves of *F. capensis* fruits contained alkaloids, saponins, flavonoids, glycosides, and tannins (Uzoekwe and Mohamme, 2015; Okoroh *et al.*, 2019; Ezeigwe *et al.*, 2020). The higher values of the secondary metabolites like saponin, alkaloid, flavonoid, tannin and cyanogenic glycoside in infected leaves more than the apparently healthy, showed that more of these metabolites must have been produced to counter the effects of the fungi. Ebana *et al.* (1991) reported that these metabolites work against the activities of pathogenic fungi.

Conclusion

From the results obtained, it is observed that three new fungi *Hendersonula* sp., *D. conferta* and *F. oxysporum* have been added to the list of fungi associated with leaf spot of *F. capensis*. There was relative evidence in the variations of proximate and phytochemical composition of the plant's ostensibly healthy and diseased leaves, as well as the fruits. People are advised to eat only the apparently healthy leaves and fruits of the plant, because of the high nutrients in them, but at the same time, avoiding the infected ones. The fruits could also be enjoyed fresh without boiling, when they are tender.

Table 1: Standard wavelength for determination of metal ions.

Metal ion	Wavelength (nm)
Lead	283.3
Copper	324.8
Zinc	213.8
Manganese	279.5
Calcium	422.7
Magnesium	285.2
Potassium	766.0
Sodium	589.0
Iron	510.0

Table 2: The proximate composition of the fruits, and the apparently healthy and infected leaves of *Ficus capensis*.

Food Nutrients	Healthy leaves (%)	Infected leaves (%)	Fruits (%)
Protein	5.69 ± 0.29	5.25 ± 0.25	3.83 ± 0.01
Carbohydrate	24.25 ± 0.96	5.30 ± 0.30	3.83 ± 0.01
Lipid	5.45 ± 0.45	2.15 ± 0.15	3.45 ± 0.02
Ash	5.60 ± 0.30	5.69 ± 0.01	1.07 ± 0.01
Fiber	26.96 ± 0.31	16.65 ± 0.20	31.38 ± 0.04
Moisture	60.80 ± 0.01	52.10 ± 0.14	51.60 ± 0.04
Vitamin C	25.95 ± 0.01	18.86 ± 0.03	21.23 ± 0.01

± standard deviation of means of 3 replicates.

Table 3: Mineral nutrient composition of apparently healthy leaves, infected leaves and fruit of *Ficus capensis.*

Mineral nutrients	Healthy leaves (mg 100 g^{-1})	Infected leaves (mg 100 g^{-1})	Fruits (mg 100 g^{-1})
Copper	BDL	BDL	BDL
Zinc	BDL	BDL	BDL
Lead	BDL	BDL	BDL
Manganese	0.55 ± 0.15	2.05 ± 0.02	BDL
Magnesium	62.70 ± 0.62	29.43 ± 0.13	12.10 ± 0.01
Calcium	23.88 ± 0.01	79.66 ± 0.04	24.50 ± 0.00
Sodium	16.68 ± 0.11	9.82 ± 0.60	7.23 ± 0.14
Potassium	14.60 ± 0.06	7.15 ± 0.15	6.14 ± 0.02
Phosphates	20.67 ± 0.02	39.80 ± 0.00	3.33 ± 0.05
Phosphorus	67.30 ± 0.00	12.79 ± 0.00	0.76 ± 0.25
Nitrate	39.71 ± 0.01	35.31 ± 0.05	39.71 ± 0.04
Nitrogen	0.08 ± 0.00	0.11 ± 0.01	0.08 ± 0.24

± standard deviation of means of 3 replicates; BDL = Below detectable limit

Table 4: The non-nutrient composition of apparently healthy and infected leaves and fruits of *Ficus capensis.*

Non-nutrient contents	Apparently healthy leaves (%)	Infected leaves (%)	Fruits (%)
Saponin	2.55±0.00	5.05±0.11	7.32±0.28
Alkaloid	6.40±0.20	8.00±0.70	10.74±0.24
Flavonoid	1.80±0.10	2.60±0.26	12.39±0.44
Tannin	2.56±0.50	0.40±0.46	3.00±0.05
Cyanogenic glycoside	50.00±0.27	60.00±1.01	73.20±0.26

± Standard deviation of 3 replicates.

Fig. 1: (**A**): Apparent healthy leaves of *Ficus capensis.* (**B**): Infected leaves of *Ficus capensis.*

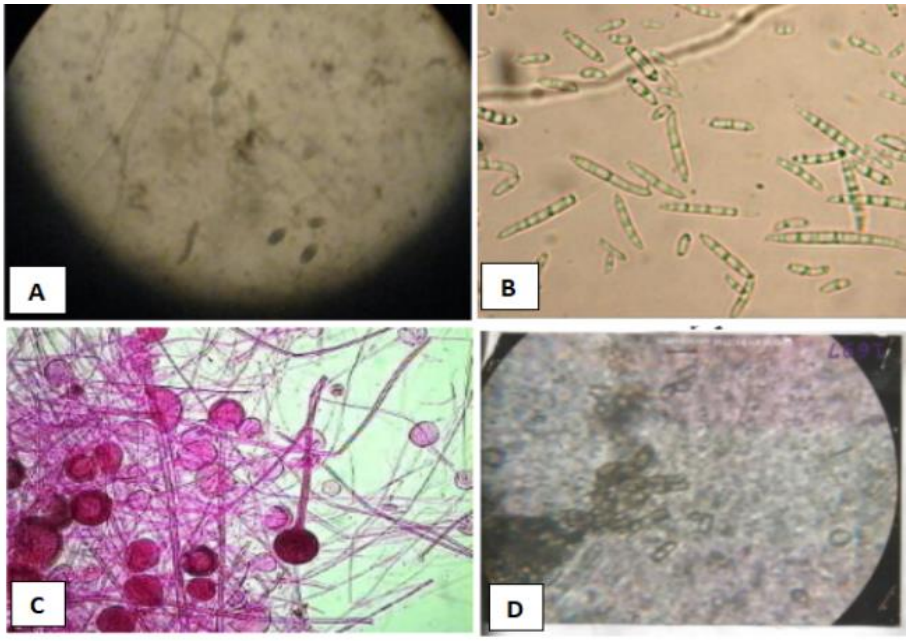

Fig. 2: (**A**): Reproductive structures of *Hendersonula* sp. x40, (**B**): Reproductive structures of *Fusarium oxysporium* x40, (**C**): Reproductive structures of *Rhizous stolonifer* x40 and (**D**): Reproductive structures of *Didymaria conferta* x40.

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