STUDIES ON THE DIVERSITY OF FUNGI ASSOCIATED WITH SPOT SYMPTOMS ONSELECTED CULTIVATED AND UNCULTIVATED PLANTS IN SAMARU, NIGERIA.

BY

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES. AHMADU BELLO UNIVERSITY, ZARIA. IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER DEGREE IN BOTANY.

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DECLARATION

I declare that the work in this project Thesis entitle;Studies on the diversity of fungi associated with spot symptoms onselected cultivated and uncultivated plants in Samaru , Nigeria. Has been carried out by me in the department of Biological science. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Halima IBRAHIM _____ Signature _____ date

CERTIFICATION

This thesis entitled STUDIES ON THE DIVERSITY OF FUNGI ASSOCIATED WITH SPOT SYMPTOMS ON SELECTED CULTIVATED AND UNCULTIVATED PLANTS IN SAMARU, NIGERIA by Halima IBRAHIM) meets the regulations governing the award of the degree of masters in botany of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This project is dedicated to my beloved parents Alhaji Ibrahim Yero and HajiyaKarimatu Ibrahim Yero.

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ABSTRACT

Spot disease is a descriptive term applied to a number of diseases affecting different parts of plants. Fungi associated with spot symptoms on cultivated plants (*Vignaunguiculata*L Walp, and *Telfairiaoccidentalis*) and uncultivated plants (*Chlorophytum blepherophyllum, Spigelia anthelmia, Ipomoea nil, Trianthema portulacastrum, Senna singuena, Combretum molle, Porphyrostemma chevalieri* and *Clematis hirsute*) were studied. Fungi were isolated by tissue plating and sticking method on Potato Dextrose Agar (PDA). Fungi isolated from cultivated plants include:*Macrophomina* sp., *Rhizoctonia solani, Sclerotium* sp., *Septoria* sp., *Cladosporium* sp., *Bispora* sp.*Mycogone* sp., *Curvularia* sp. and *Gleosporium* sp. Fungi isolated from spot symptoms on uncultivated plants include: *Macrophoma* sp., *Trichoderma* sp., *Nigrospora aerophila,Choanephora* sp., *Monocheatia* sp., *Rhizoctonia solani, Fusarium equisetum,Alternaria* sp., *Macrophomina* sp. and *Helicosporium* sp.The percentage occurrence of fungi isolated from cultivated plants shows that *Rhizoctonia* sp.have the highest followed by *Sclerotium* sp.and *Cladosporium* sp.Percentage occurrence of fungi isolated from uncultivated plants was determined with *Nigrospora aerophila* having the highest followed by *Rhizoctonia solani*.Principal Component Analysis was used to determine the ecological and epidemiological relationship between fungal isolates, cultivated and uncultivated plants, which enhances understanding of the ecology and epidemiology of fungi inducing spot disease. *Invivo*pathogenecity test of fungi isolates on cowpea shows no symptoms. *Invitro*pathogenecity test using leaf disk method was carried out on uncultivated plants. Symptoms similar to those observed in the field were obtained.

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CHAPTERONE

1.0 INTRODUCTION

Spot disease is a descriptive term applied to a number of diseases affecting different parts of plants, which are one of the most important limiting factors for cultivation of crops in tropical and subtropical areas (Reis and Boiteux, 2010). Spot diseases are induced by various pathogenic groups of fungi and bacteria (Burrows, 2013).

Fungi causing spot diseases include;species of*Septoria, Alternaria, Cladosporium, Rhizoctonia, Nigrospora,Cercospora,Phyllosticta,* and *Ascochyta*(Singh and Allen, 1979; Allen *et al.*,1996; Howard and David, 2007; Verma and Gupta, 2008). Spot diseases may cause yield loss of up to 70%, while *Septoria* and *Alternaria* spp.cause yield losses of 70% and 100% respectively (RPD, 1999).

Cowpea (*Vigna unguiculata)* L.*Walp*belonging to the family Fabaceae is an annual legume; it is commonly referred to as southern pea, black-eye pea and Crowder pea. The history of cowpea dates back to ancient West Africa cereal farming, five to six thousand years ago where it was closely associated with the cultivation of sorghum and pearl millet (Davis *et al.*, 1991).

Fungi Pod diseases of cowpea include; brown blotch caused by *Colletotrichumcapsici*and *C. truncatum*(Emechebe and Florin, 1997; Singh and Allen, 1979;Alabi, 1994), lambs tail pod rot induced by *Choanephoracurcubitarum*(Adegbite and Amusa, 2008; Singh and Allen, 1979), *Phomopsis* pod spot induced by*Phomopsislongicola (*Royand Ratnayake, 1997) and scab induced by *Sphaceloma*sp.(Singh and Allen, 1979; Allen *et al.*, 1996).

Telfairia occidentalis commonly called fluted pumpkin in English, ugu in Igbo, Ikoro in Yoruba and Umeke in Edo, belongs to the family Cucurbitaceae (Staff, 2011; Odiaka*eta.l,* 2008). It is found in forest zone of West Africa, most frequently Benin, Nigeria and Cameroon (Kayode and Kayode, 2011). It is cultivated in Nigeria for its edible leaves and seed (NwufoAtu, 1987; Badifu and Ogunsina, 1991). The leaf is a good source of organic acids, mineral salts, oil, vitamins, proteinsand carbohydrate and also possesses medicinal values (Gruben and Benton, 2004).

The major fungal diseases of *Telfairia occidentalis* are leaf spot caused by *Phomasorghina*(Nwufo and Atu, 1987) and anthracnose caused by *Colletotrichum*sp.(Udo*et al.,* 2008).

Uncultivated plants should be considered when endeavoring to manage and control plant pathogens of cultivated plants. They can significantly influence disease incidence. Weeds can interact with pathogen management in several ways, including provision of weed biological control, weeds can serve as alternative hosts for pathogens (Wisler and Norris, 2005; McWhorter, 1989; Quénéhervé*et al* 1995 and 1996).*Sphaceloma*sp. of cowpea has been reported on *Ipomoeainvolucrata*and *I. eriopcarpa (*Adebitan, 1998). Black *et al.*(1996a and b) found a wide range of weed species as potential hosts for *Diaporthephaseolorum*var. *caulivora*Kulik and *Rhizoctoniasolani*J.G. Kühn AG-1 in soybean fields in Lousiana.

1.1 Statementof Research Problem

In the 1970s, about 94% of the world cowpea was produced in Africa, the situation remains much the same today but the optimization of production which is still grossly at the subsistence level in this region continues to be hampered by insects, pest and diseases (Maude, 1996; Amadi and Oso, 1996).

Diseases, parasitic weeds, insect pests, drought and low soil fertility are the major constraints to cowpea production in Nigeria (Singh *et al*., 1997; Asante *et al.,* 2001; Emechebe and Lagoke, 2002; Fawole*et al*., 2006).

Cowpea seed yields average only 100 to 300 kg/ha, largely due to the effects of insects and diseases (Singh and Singh, 1990). High incidence of pest and diseases reduces *Telfairia occidentalis* yield significantly and affect its marketability (Tindal, 1975; Williams *et al.,* 1991).

Uncultivated plants apart from competing with crops for nutrients, moisture, space and light also serve as reservoir host of plants pathogenic fungi.*Ipomoea involucrata*and *I. eriopcarpa*have been found to be susceptible host of *Sphaceloma*sp which serves as reservoir host for the pathogen *(*Adebitan, 1998). In India *Colletotrichumcapsici* has been reported on *Chlorophytumborivilianum* (Abdul*et al.,* 2005). In Florida *Rvenelia* rust disease was reported on *Sennasurattensis*(Brown, 2013). The interaction between wild plants and cultivated crops has a relevance to the management of plant pathogenic fungi.

1.2Justification

Cowpea is an important major staple food crop in sub-Sahara Africa, especially in Nigeria. The seeds form a major source of plant proteins, vitamins to man, and feed for animals. The young leaves and immature pods are eaten as vegetables (Dugje*et al*., 2009). The grain is rich in protein up to 30% in some varieties. In addition, the grain contains micronutrients such as iron and zinc which are necessary for healthy living (Boukar*et al*., 2010).

*Telfiariaoccidentalis*is grown all over Nigeria and used as vegetable. It has been reported to have antibacterial activity (Oyewale and Abalaka, 2012).

Generally crop losses due to plant diseases have been estimated to be as high as $30 billion per year (Pimentel *et al*, 2000). Scab cause yield loss of upto100% (Emechebe, 1980; Mbong, *et al.*,2010).

Alternaria and *Septoria* spp.causes major losses due to spot diseases on a wide range of crops (Woundenberg*et al.,* 2013). The losses from the disease may approach 100% in warm, wet seasons where control measures are not practiced. Harvestable fruits number, size and quality are all reduced by these diseases (Report of Plant Disease,1999).

Even though uncultivated plants have constrainedthe cultivation of crops they also serve for medicinal purposes (Ahmed *et al.*,2014; Kew, 2012; Neyenje, 2012).

Spot symptoms were observed on cowpea pods, leaves of *T. occidentalis* and someuncultivated plants in Samaru. Therefore there is the need to find out the fungi associated with the symptoms observed. There is the need to study the relationship between fungi isolated from cultivated crops and uncultivated plants.

At the end the information obtained will enhance the understanding of diversity and epidemiology of fungi inducing spot on both cultivated and uncultivated plants in Samaru. The information can be used by plant breeders, Quarantine stations and as a potential for biological control for weeds.

1.2 Aim of the Study

To isolate and identify fungi associated withspot symptoms onboth cultivated and uncultivated plants.

1.3 Objectives of the Study

1. To observe different types of spot symptoms on cultivated and uncultivated plants.
2. To isolate and identify fungi associated with spot symptoms on cultivated and uncultivated plants.

1.4 Hypotheses

1. Different types of spot symptoms were not observed on cultivated and uncultivated plants.
2. Fungi are not associated with spot symptoms on cultivated and uncultivated plants.

CHAPTER TWO

2.0LITERATURE REVIEW

2.1Spot Diseases

Spot is a common descriptive term applied to a number of diseases affecting plants. The majority of spots are caused by fungi, but some are caused by bacteria. Some insects also cause damage that appears like a spot disease.Leaf spot may result in defoliation in some plants(Nix, 2014).

Fungi causing spot diseases on different plantsinclude; *Septoria, Alternaria, Cladosporium, Rhizoctonia, Nigrospora,Cercospora, Phyllosticta,* and *Ascochyta*spp.(Singh and Allen, 1979; Allen *et al.,*1996; Howard and David, 2007; Verma and Gupta, 2008). Also *Curvularia* and *Gleosporium* spp.were reported to cause leaf spot (Olson, 1978; Akram*et al.*, 2014).

2.2Symptoms ofSpot Diseases

Spots come in a wide variety of shapes, sizes, and colors. A leaf spot disease creates spots on foliage. The spots will vary in size and color depending on the plant, the

organism involved and the stage of the host plant development. Spots are most often brownish, but may be tan or black. Concentric rings or a dark margin around the spot may be present. Over time the spots may combine to enlarge and form blotches. Spots or blotches that are angular and located around the veins are generally referred to as anthracnose. (Stanley, 2014).

*Alternaria*spot on curcubits and cotton produces brown, grey or tan lesions (spots) on cotyledons, leaves and bracts varying from 1 to 10mm in diameter (Report of Plant Disease, 1989). The lesions appear first on older leaves as small circular spots which are light brown with light centers and form concentric dark rings as they enlarge thus the name "Target spot". Lesions form on lower leaf surface tends to be more diffuse. Fruit infections begin as sunken brown spots and may later develop a dark powdery appearance as the fungus sporulates (Kucharek, 2000; Watt, 2013).*Alternariaaternata*was reported on *Cyperusrotundus*and *Cyperusbrevifoius.*

Septoria spot produceslesions on wild blueberry and banana that are dark red, circular to irregular, 2-4mm in diameter, appearing similar on both leaf surfaces (Singh and Allen, 1979). Numerous small, water soaked spots first appear on the lower leaves, these spots soon become circular to angular with dark margins and grayish white centers often bearing one or more tiny black bodies called pycnidia, which are spore-bearing structures. Individual lesion is seldom more than 0.80cm in diameter and is usually quite numerous on an infected leaf (Report of Plant Disease, 1999; Ojiambo*et al.,* 2007; Udugama, 2002).Wild blueberry leaf spot caused by *Septoria* sp, symptoms appears as small water soaked blisters on the bottom side of the leaf. The infected areas develop diffuse red margins (Wild blue berry Fact Sheet, 2007).

*Cercospora*leaf spot produces circular to irregular cherry-red to reddish brown lesions up to 10mm diameter on cowpea (Singh and Allen, 1979). Individual leaf spots initially occur on older leaves and then progress to younger leaves are approximately 0.80cm in diameter with ash colored centers and purple to brown borders and circular to oval in shaped. It is distinguished from other leaf diseases by their smaller size and shape. As the disease progress heavily infected leaves initially turn yellow which may coalesce and form larger areas of dead tissue (Harveson, 2013).Grey leaf spot of maize caused by *Cercosporazea-maydis*create symptom that is small dark moist spots that are encircled by a thin yellow radiance which leads to discoloration (Ward *et al.,* 1999).

*Ascochyta*spots are irregularly circular with grey to brown centers surrounded by a yellow halo. Such lesions become zonate and under favorable conditions spread rapidly causing extensive blighting of leaves, pods and stems of cowpea (Singh and Allen, 1979). The lesions develop also on petioles stem nodes peduncles and pods (Allen *et al.,* 1996).Initial symptoms on the leaves are small circular reddish-brown spots which enlarge, becoming surrounded by irregular shaped water-soaked areas. Under humid conditions the lesions develop rapidly and coalesce (Singh and Allen, 1979; Allen *et al., 1996).* Basidiospore produces distinct small necrotic and circular spots which do not enlarge. Pods and seeds also bear lesions (Verma *et al.,* 2006; Gonzalez *et al.,* 2011).

Cladosporium sp. produces spot on eggplant, pepper and tomatowith irregular borders but when infection is severe these spots coalesced and kill large areas of the foliage. The upper surface of affected leaves turn olive green with more intense color near the center of lesions. Leaves eventually curl, wither, and may drop from the plant (Howard and

David, 2007). Spots appear purplish-brown on the upper sides of leaves, on the lower side, spots are chestnut brown. Infection is generally more pronounced at margins of leaves (Jauron, 2012).

Nigrospora leaf spot appear in the form of small (2-5mm), circular to irregular red colored spots on leaflets, covering major area of the leaf on turf grass and cotton. Occasionally, the spots are seen delineated by midrib. In advanced stages of the disease, some spots cracked at the center. Eventually leaves dry and the plant defoliates (Nelson, 1992; Verma and Gupta, 2008; Zheng*etal.*, 2012).

Black leaf spot caused by *Diplocarponrosae*attack rose plant. It can attack any plant with fleshy leaves and stem if the conditions are right. It appears as tiny black spots on leaves no bigger than a pin head. As the fungus develops, those black spots are ringed with yellow. Soon entire leave turn yellow and falls (Rhoades, 2014).

Banana leaf spot (black sigatoa caused by *Mycosphaerellafijiensisvar.difformis*have been reported to vary according to location which reflect an important distinction between disease epidemics in temperate and tropical regions (Chuang and Jeger, 1987; Udugama, 2002; Mcmullen and Adhikari,2009).

All palms are susceptible to at least one of the pathogens that cause leaf spots and blight. Symptoms reported on palms are usually round to oval in shape and vary in color from yellow to brown to black (Rothrock*et al.*, 1998).

*Phyllostictacitricapa*have been reported to cause citrus black spot on citrus. Symptoms on older leaves are small, round, sunken necrotic spots with grey centers bordered with dark brown margin; young lesions are small, reddish and slightly rose. Foliage lesions are most commonly seen in lemon (Citrus disease fact sheet, 2013).

Tabebuia tree leaf spot leads to symptoms that are brown or black spots and patches may be circular, with a water soaked or yellow-edge appearance (Jim, 2007).

2.3Economic Lossdue toSpot Diseases

A spot disease causes yield loss of up to 70% to100% respectively (RPD, 1999). *Alternaria* causes major losses on a wide range of crops (Woundenberg*et al.*, 2013). The losses from the disease may approach 100 % in warm, wet seasons if control measures are not practiced. Harvestable fruits number, size and quality are all reduced by this disease (Report on Plant Disease,1999; Neely and Nolte, 1989). Indi *et al.*(1986a and 1986b) reported yield losses to the tune of 10 to 25% and under severe conditions to cause 50% loss in seed yield of safflower.Yield losses of 60% have been attributed to natural infection (Eyal, 1972; Shaner and Finney, 1976). Yield losses are often accompanied by corresponding decrease in grain weight (Narvaez and Caldwell, 1957; Shaner and Finney, 1976). William, (2003) report 30% yield loss on blue berry in Georgia.

Losses due to *Cercospora*leaf spot disease on sugar beets can approach 40%, and are represented by both root tonnage and sugar percentage in roots. Beets with low sugar level do not store well and losses in storage result from increased storage decay. Profitable yields are additionally reduced due to greater levels of impurities in roots and increased sugar loss to molasses during processing (Harveson, 2013).

*Ascochyta*leaf blight yield losses in commercial pea fields were estimated from 10to 20% but in some trials losses were over 50% (Xue*et al.,* 1997; Xue and Warkentin, 2001;Boros and Wawer, 2007).

Based on the assessment of nursery-wide annual losses due to *Rhizoctonia*web blight, about 0.5% of the plants are killed and 3 to 8% are unsalable during part of the year and experience reduced growth the following year (Copes, 2005).

2.4 ControlofSpot Diseases

Spot diseases can be control by maintaining soil health, proper nutrition, pH and irrigation. The disease may be controlled by application of fungicides, but there is no guarantee that treatments will be effective. Preventive control before spots become present is recommended for field with previous infections with treatments beginning in May (Alexis, 2011).

Fungicides are generally not necessary for *Alternaria* leaf spot management during most years in the high plains (Schwartz and Gent, 2007;Waterworth, 2010). Murumkar*et al.*(2007) reported spray of 0.1%carbendazim was a superior treatment for management of *Altenaria*leaf spot of safflower.

Crop rotations can reduce the initial inoculums of fungal leaf spot pathogens. Residue management involving tillage to burry infected residue. Also the use of fungicides (Mcmullen and Adhikari, 2009; Burrow, 2013).Use of best high yielding varieties that are tolerant or resistant to *Septoria* leaf spot (Burrow, 2013). Foliar fungicides can be useful if significant leaf disease occurs early in the season and the flag leaf is to be protected (Burrow, 2013).

Cultivation and rotation can reduce level of inoculums; use of leaf spot-tolerant cultivars can significantly reduce disease severity and yield losses. Fungicidal sprays may be necessary in fields where inoculums have carried over from the previous years (Harveson, 2013).

Ascochyta blight can be controlled through crop rotation, destruction of infected trash and chemical seed treatments which can significantly reduce the amount of primary inoculums. Fungicides have been used successfully to control the disease (Bretag, 2001; 2006). Application of non-amended suspensions of *Aureobasidiumpullulans*conidia to post –harvest chickpea debris resulted in 37.9% fewer *Ascochyta*blight lesions (Dugan *et al.*,2009). The use of clean seed and cultural practices such as rotation are recommended (Singh and Allen, 1979). Also field sanitation and some intercropping system can decrease disease severity. Mulching with banana leaves and rotation of legumes with a cereal crop seem particularly promising. Seed dressings of benomyl combined with Dithane M45 or Mancozeb have been found effective in Uganda (Allen *et al.,* 1996).

The pathogen survives as sclerotia in soil and on crop debris. The use of clean seed and cultural methods, avoidance of dense plantings and sowing timed to avoid peak rainfall (Singh and Allen, 1979). Various fungicides can control *Rhizoctonia* web blight. Cultural practices such as mulching can be highly effective (Allen *et al.,* 1996; Gonzalez, 2011). Benson and Cartwright (1996) reported plant spacing as a cultural practices routinely recommended to reduce *Rhizoctonia* web blight in nurseries that grow ornamental shrubs.

No biological control strategies have been developed for *Cladosporium* leaf spot. Cultural control include; planting of high quality seed free from *Cladosporium*, hot water

treatment can reduce seed contamination, crop debris elimination and use of resistant varieties . Fungicides are also used (Howard and David, 2007).

Maintain adequate amounts of balance fertilizer. Avoid moisture stress by constant irrigation but avoid night watering. The use of fungicides designed to control other diseases such as Dollar spot at labeled curative rates may reduce the severity of *Nigrospora* blight (Nelson, 1992).

2.5Ecology ofFungalSpot Diseases

Alternaria spot disease is favored by either repeated heavy dews or extended periods of wet weather. The fungi over winters as dormant mycelium in diseased and partly decayed crop refuse, in weeds and possibly in the soil. The conidia can survive under warm, dry conditions for several months. Conidia produced on diseased plants or crop refuse may be blown by wind to long distances. The germinating spores penetrate susceptible tissue directly or through wounds and soon produce a new crop of conidia that are further spread by wind, splashing rain, tools or workers (Kucharek, 2000).At least 18hours of high relative humidity, producing leaf wetness is required before infection can occur. The periods of infection and the appearance of symptoms varies from 3 to 12days. Young plants less than a month old and plants that are bearingfruit and 70 to 75 days old appear to be more susceptible than plants 45 to 60 days of age (Report of Plant Disease, 1989).

Frequent showers heavy dew, temperature of 68^{o} to 85^{o}F (20 to 29^{o}C) and overcrowding of plants favour the spread and development of *Septoria* leaf spot(RPD, 1999). The fungi may be carried on or in the seed or may overwinter in the soil or crop debris. In addition it also infect and over winter on several species of weeds as well as crops. The spores are

spread by water splash, on tools and farm equipments and by handling of plants (RPD, 1999). The severity of the disease is closely related to rainfall frequency and cool weather conditions (Sanderson *et al.,* 1985).

*Cercospora*requires periods of high humidity or leaf wetness periods longer than 11 hours and warm temperature (>60^{o}F). Since leaf wetness is not routinely measured, relative humidity above 90% humidity can be used as a substitute. Very little infection will occur below 60^{o}F or during periods of less than 11 hours of leaf wetness. Greater spore germination and leaf infection generally occur when night temperatures exceed 60^{o}F and temperature is between 80^{o} to 90^{o}F (Harveson, 2013). Initial inoculum potential depends on the survival of the fungus spores and spore bearing structures (Psuedostromata) from the previous year's infected crop residue, weeds may also be a source of inoculums. Spores can be carried by wind or splashing water to infect adjacent leaves and plants. Life cycle may be complete within 10days (Harveson, 2013).

Ascochyta is seed borne and also survives on straw. Secondary spreads occurs through rain splash. Infection depends on high relative humidity and cool temperature (21-24^{o}C) (Allen *et al.,* 1996).

Rhizoctonia survives between seasons which depends partly on the presence of crop debris, sclerotia and mycelium either on debris or free in the soil, are the main sources of inoculums. Infection follows splash inoculation with a range of weed species serving as source of primary inoculums (Allen *et al.,* 1996).The disease appear due to prolonged periods of rainfall or high humidity above 50% and high night time temperature above 65^{o}F (18^{o}C). *Rhizoctonia solani* infestations can occur over a wide range of air temperature generally from 60-90^{o}F (15-32^{o}C) (Nelson, 1992). The pathogen require wet

leaf surface or relative humidity of 95% to 100% and temperature from 25-32^{o}C to infect plant tissue (Gross *et al.,* 1998).

Cladosporium leaf spot occur in field when humidity is high. Fungal spores germinate under high humidity (85% or greater) and cool to warm temperatures (40 to90^{o}F) but disease rarely occurs below 50^{o}F. Wind splashing irrigation water and rain workers, tools and insects readily disseminate spores. Contaminated seed can also initiate epidemics. The pathogen survives between crops in crop residues and in the soil as spores (conidia) or dormant resting structures (Sclerotia) (Howard and David, 2007).

Periods of warm humid weather accompanied by nighttime temperature between 70-75^{o}F (21-24^{o}C) and free water on the surface of the foliage may result in severe outbreaks of *Nigrospora* blight (Nelson, 1992).

CHAPTERTHREE

3.0MATERIALSANDMETHODS

3.1Study Area

Samaru is located in Kaduna state, Nigeria. Its geographical location lies on latitude $9^{o}45'0''$ North and longitude $8^{o}23'0''$ East.

3.2SampleCollection

Cowpea pods showing leafsymptoms were collected from a farmer's field in Samaru Zaria. Infected *T. occidentalis* was collected from a kitchen garden in Area F, staff quaters, Ahmadu Bello University, Zaria. Also diseased leaves of uncultivated plants (*Clematis hirsuta,Senna singuena, Combretum molle, Porphyrostemma chevalieri,Spigelia anthelmia, Trianthema portulacastrum, Chlorophytum blepherophyllum, Ipomoea nil),* surrounding the cowpea farm were collected in polythene bags. The collected plants were taken to herbarium Department of Biological Sciences, Ahmadu Bello University, Zaria, for identification.

3.3Preparation of Media

Commercially formulated Potato Dextrose Agar was prepared as described by the manufacturer's instructions (Oxoid, England) and used throughout the study, 40g of Potato Dextrose Agar was weighed and dissolve in 1 liter distilled water and melted in a water bath. The media was sterilized in an autoclave at 121^{o}C for 15 minutes.

3.4 Isolation of Fungi from Plants with Spot Symptoms

Isolation of fungi was done through two methods; Plating out of intact lesions and maceration method.

3.4.1 Isolation by plating of intact lesions

Lesions were cut off with surrounding living tissues from infected plants with sterile surgical blades. All lesions were surface sterilized in 0.1% mercury chloride (HgCl) for three minutes and rinsed three times with sterile distilled water. Lesions were plated on Petri dishes (four lesions per plate) containing Potato Dextrose Agar (PDA) amended with streptomycin sulphate (1.5g/l) to inhibit bacterial growth. Each sample was in duplicate .The Petri dishes were incubated at room temperature. Observation was made daily for seven days, for the presence of colonies of fungal pathogen. The fungi colonies were sub cultured on fresh PDA for subsequent identification.

3.4.2Isolation by maceration method

PDA amended with streptomycin sulphate (1.5g/l) was put in 9cm diameter Petri dishes. About 5 lesions were cut out with sterile surgical blades from each sample of infected

plants. The lesions were surface sterilized with 0.1% HgCl for three minutes and rinsed three times with sterile distilled water. The lesions were then ground in a sterilized porcelain mortar using sterilized pestle until a smooth paste was obtained. Two mililiter of sterile distilled water and 50mg of streptomycin sulphatewere added to the paste to make a suspension. A sterile syringe was used to apply two drops of the suspension on each agar plate. Using sterile, L-shaped glass-rod, the suspension was spread on the PDA surface under aseptic conditions. The plates were incubated at room temperature and observed for fungal growth as above.

3.5Identification of Isolated Fungi

Identification was carried out by both macroscopic and microscopic observations of pure cultures.Fungi were identified by macroscopic characters such as colony texture, color, and its cultural characteristics on PDA.

Fungiwere also identified by microscopic characters such as conidiophores morphology and shape. Confirmation of the fungal identification was done using identification key (Barnett and Hunter, 1999), they were also compared with already described isolates in the Department of Crop Protection diagnostic unit, Institute of Agricultural Research, ABU Zaria.Some of the isolates were sent to Commonwealth Agricultural Bureaux International (CABI), United Kingdom for confirmations.

Percentage occurrence of fungi isolated was determined using the formula below(Gonzalez, 1995).

Percentage occurrence= frequency of occurrence of fungi x100 Total number of fungi

3.6Pathogenecity Test

3.6.1 *Invivo*pathogenecity test

Isolates of fungi weretested on two improved cowpea varieties; SAMPEA 7, SAMPEA 8and a local variety kanannado

Isolates were sub cultured onto PDA, once sporulating abundantly, they were flooded with sterilized distilled water, macerated in a blender and the suspension passes through four- folded layers of cheese cloth inside a funnel. To obtain a conidial suspension, concentration was standardized to the appropriate conidia/ml using a haemocytometer for each fungus (Appendix I).

In the green house, two cowpea seeds were sown in sterilized soil in 18cm (diameter) Perforated black polyethene bags; four polythene bags per isolate, making eight plants for each variety per isolate.

The cowpea plants were inoculated two weeks after emergence by spraying with appropriate conidial suspension (Appendix I) of the different isolates. Shortly after inoculation, all seedlings were placed for 48hours under an enclosure made of a white polyethylene sheet to encourage humidity. The plants were observed daily for disease development (Mungo, 1996).

3.6.2 *Invitro*Pathogenecity Test using Leaf Disks

Leaf disk inoculation method was carried out on leaves of uncultivated plants found around cowpea farm. Cork borer was used to cut8 mm diameter diskfrom healthy leaves of uncultivated plants. Inoculation with fungal spores was carried out using a micropipette by depositing 25µldroplet of conidial suspension on the leaf disc centrewhich had been superficially wounded using sterile needle. To prepare conidial suspension, isolates were sub cultured on fresh PDA for two weeks, once sporulating abundantly, it was flooded with sterilize distilled water, macerated in a blender and the suspension pass through four- folded layers of cheese cloth inside a funnel, to obtain a conidial suspension , each fungus was standardized to the appropriate concentration using a haemocytometer (Appendix I).

The leaf disk were placed in petri- dishes under humid condition (moistened filter paper) and covered with foil paper to prevent light. They were incubated at 22^{o}C temperature in an incubator for sevendays before evaluation (Batta, 2003).

3.7 Statistical Analysis

Principal Component Analysis (PAST VERSION 2.17c) was used to determine the ecological and epidemiological relationship between fungal Isolates, cultivated and uncultivated plants.

CHAPTERFOUR

4.0 RESULTS

4.1 Types of Spot symptoms Observed on Cultivated Plants.

Fourteen different types of leaf spot symptoms were observed and studied on cowpea pods which are shown in Table 4.1and Plates 1 to 3.Circular Dark brown spot on cowpea pod that leads to isolation of *Rhizoctonia solani*. Spot with grey center brown margin on cowpea pod, *Sclerotium* sp. was isolated from it. Grey center dark brown margin spot on cowpea pod no fungus was isolated from this spot. Mixed infection: *Septoria* sp. was isolated from the white center brown margin and *Cladosporium* sp. was isolated from brown spots also at the sutures.Round white center brown margin spot on cowpea, no fungus was isolated from the spot. Oval white center brown margin on cowpea pod.*Rhizoctonia solani* was isolated. Raised circular dark brown lesions which lead to isolation of *Rhizoctonia solani*.Circular brown lesions on cowpea pod. *Bispora* sp. was isolated.Coalesced dark brown lesion,*Macrophomina* sp. was isolated.Raised grey center brown margin on cowpea pods, no fungus was isolated.Lesions along sutures having grey center dark brown margin,*Rhizoctonia solani* and*Mycogone* sp. were isolated. Lesions on

cowpea peduncles with grey centre brown margin,*Rhizoctonia solani, Sclerotium* sp. and *Fusarium equiseti*were isolated. Purplish brown lesions on cowpea pod, no fungus was isolated from it.Two different types of symptoms were observed on *T. occidentalis*. Grey spot that makes hole on leaves and water soaked white patch on stem as shown in Table 4.1 and Plate 4.

Table 4.1: Fungi Isolated from Different Spot Symptoms on Cultivated Plants

Description of Symptoms	**Name of Plant and Part used**	**Fungi Isolated**
Circular dark brown spot	Cowpea pod	*Rhizoctonia solani*
Grey center brown margin	Cowpea pod	*Sclerotium* sp.
Grey center dark brown margin	Cowpea pod	-
Mixed infection; Grey center brown margin. Brown coalesced lesion	Cowpea pod	 *Septoria*sp. *Cladosporium*sp.
Circular white center brown margin	Cowpea pod	-
Oval spot with white center and brown margin	Cowpea pod	*Rhizoctoniasolani*
Raised circular dark brown spot	Cowpea pod	*Rhizoctoniasolani*
Circular brown spot	Cowpea pod	*Bispora*sp.
Coalesced dark brown lesions	Cowpea pod	*Macrophomina*sp.
Raised spot with grey center brown margin	Cowpea pod	-
Lesions along sutures having grey center brown margin	Cowpea pod	*Rhizoctonia solani* *Mycogone* sp.

Brown lesions with grey center on peduncle	Cowpea peduncle	*Rhizoctoniasolani* *Sclerotium*sp. *Fusariumequiseti*
Purplish scattered lesions	Cowpea pod	-
Grey spot that makes holes on leaf.	*T. occidentalis* leaf	*Curvularia* sp. *Rhizoctonia solani*
Water soaked patches on stem.	*T. occidentalis* stem	*Gleosporium* sp. *Cladosporium* sp.

Key: - =No fungus was isolated









Plate 1: Cowpea pods showing different spots symptoms I. A= Circular Dark brown spots on cowpea pod. *Rhizoctonia solani* was isolated from this spot. B= Grey center brown margin lesions on cowpea pod. *Sclerotium* sp. was isolated from it.C: Grey center dark brown margin spots on cowpea pod. No fungus was isolated from these spots. D: mixed infection, Da= *Septoria*sp. was isolated from the white center brown margin Db=*Cladosporium* sp. was isolated from brown spots also at the sutures.



Plate 2:Cowpea pods showing different spots symptoms II. A=Round white center brown margin. No fungus was isolated from the spots. B: Oval white center brown margin spots on cowpea pod. *Rhizoctonia solani* was isolated.C: Raised circular dark brown lesions which lead to isolation of *Rhizoctonia solani*.D: Circular brown lesions on cowpea pod. *Bispora* sp. was isolated.





Plate 3:Cowpea pod and peduncle showing spot symptomsA; Coalesceddark brown lesion. *Macrophomina* sp. was isolated. B: Raised grey center brown margin on cowpea pods. No fungus was isolated.C: Lesions along sutures having grey center dark brown margin. *Rhizoctonia solani* and*Mycogone* sp. were isolated. D: Lesions on peduncles with grey centre brown margin. *Rhizoctonia solani*, *Sclerotium* sp. and *Fusarium equiseti* were isolated. E:Purplish brown lesions on cowpea pod, no fungus was isolated from it.



Plate 4: *T. occidentalis* A=Grey spot that makes holes on leaf. B= Water soaked patches on stem

4.2 Identification of Fungi Isolated from Different Types of Spots Symptoms on Cultivated plants

From cowpea, eight different fungi were isolate and four different fungi from *T. occidentalis. Fusariumequiseti*macroscopic character showupper surface white, reverse pink, microscopic character shows macroconidia with septation.*Sclerotium*sp. macroscopic character shows upper surface white with sclerotia, reverse white, microscopic character shows non septate hyphae with clamp connection.*Rhizoctoniasolani* macroscopic character showsupper surface grey with pink wave edge, reverse dark brown, microscopic character showsbranched hyphae with point of septation.*Septoria* sp. macroscopic character upper surface white, reverse white. Microscopic character shows conidia hyaline elongated with several cells.*Cladosporium* sp. macroscopic character shows upper surface white reverse black, microscopic character shows conidia ovoid dark two celled.*Mycogone*sp.macroscopic character shows upper surface grey, microscopic character branched conidiophores with globose conidia.*Bispora*sp.macroscopiccharactershows upper surface grey, reverse brown, microscopic character shows conidiophores dark and short.*Macrophomina* sp. macroscopic character shows upper surface grey, reverse black. Microscopic character shows pycnidia with hyphae.*Gleosporium*sp. macroscopic character shows upper surface pink, microscopic character shows hyaline ovoid conidia variable in length.*Curvularia*sp. macroscopic character shows upper surface black reverse black, microscopic character showsconidia with four cells with one enlarged cell and hyaline mycelia. These are shown in Table 4.2 and Plates 5 to 14.

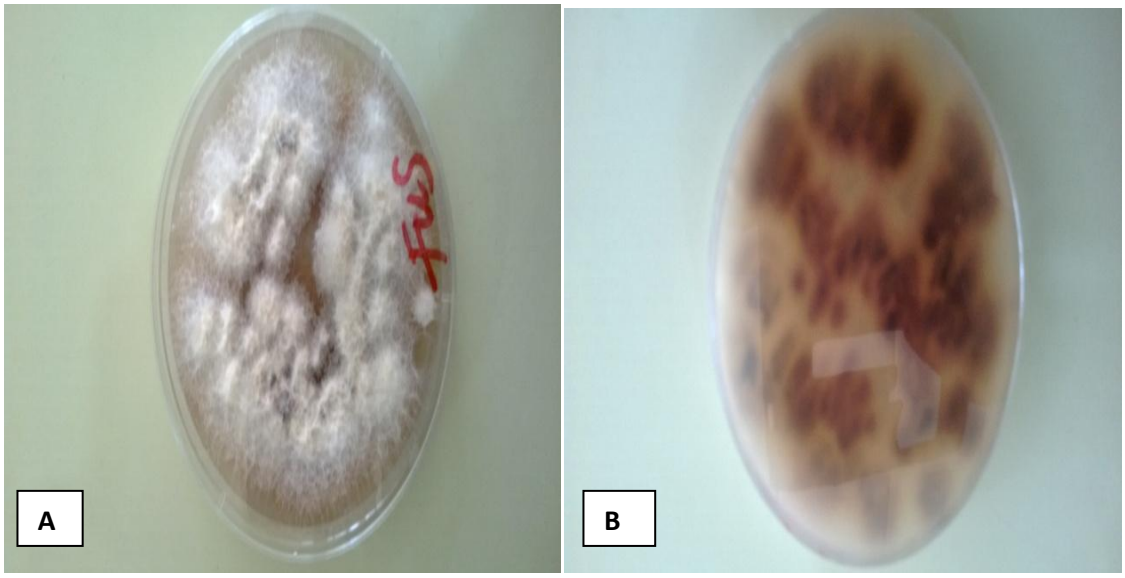
R. solani (Plate 7)and *Cladosporium* sp. (Plate 9) were isolated from both cowpea and *T. occidentalis. R. solani* was isolated from five different spot symptoms on cowpea. These were compared with already described species using keys and already established isolates at IAR, ABU, Zaria. To ascertain the identity of *Fusarium equiseti*and *R. solani* the isolates were sent toCommonwealth Agricultural Bureaux International (CABI), UK.

4.3 Percentage Occurrence of Fungi Isolated from Cultivated Crops

Percentage occurrence of fungi isolated from cultivated crops shows *R. solani* have the highest percentage occurrence of35.29%followed by *Sclerotium* sp.and *Cladosporium* sp.with 11.79%. *Macrophomina*,*Septoria*, *Gleosporium*, *Fusarium equiseti*, *Bispora*, *Mycogone*and *Curvularia* spp. have the lowest percentage occurrence of 5.88 %. These are shown in Table 4.3.

Table 4.2: Cultural and Microscopic Characters of Fungi Isolated from Cultivated Plants

Fungi	CultivatedCharacter	Microscopic character
Fusariumequiseti	Upper surface white cottony, Reverse pink.	Macroconidia with septation.
Sclerotium sp.	Upper surface white with sclerotia, Reverse white.	Non septate hyphae with clamp connection.
Rhizoctonia solani	Upper surface grey with pink wave edge, Reverse dark brown.	Branched hyphae with point of septation.
Septoria sp.	Upper surface white, Reverse white.	Conidia hyaline elongated with several cells.
Cladosporium sp.	Upper surface white cottony, Reverse black.	Conidia ovoid dark two celled, with dark conidiophores.
Mycogone sp.	Upper surface grey cottony, lower surface grey.	Branched conidiophores with globose conidia.
Bispora sp.	Upper surface grey. Reverse brown.	Conidiophores dark and short.
Macrophomina sp.	Upper surface grey, cottony, reverse black.	Pycnidia round shape
Gleosporium sp.	Upper surface powdery pink, reverse dark pink	Hyaline ovoid conidia variable in length.
Curvularia sp.	Upper surface black, reverse black.	Conidia with four cell and enlarged cell at the center of conidia, hyaline mycelia,



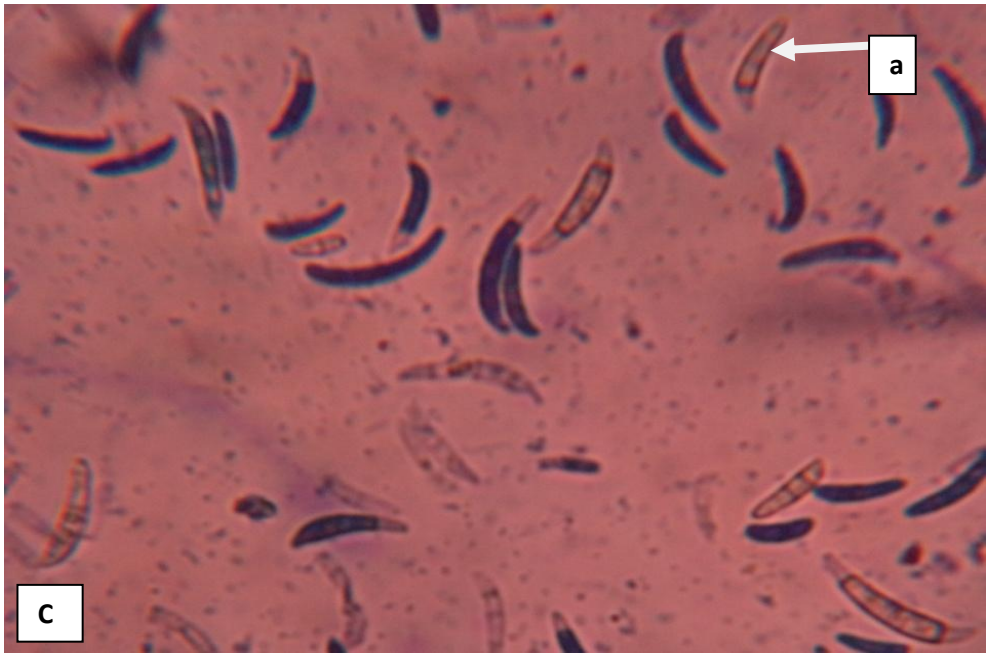


Plate 5: *Fusarium equisetum* Mg x40

A= Upper surface white Stain; Lactophenol cotton blue

B= Reverse pink,

Ca= Macroconidia with septation.

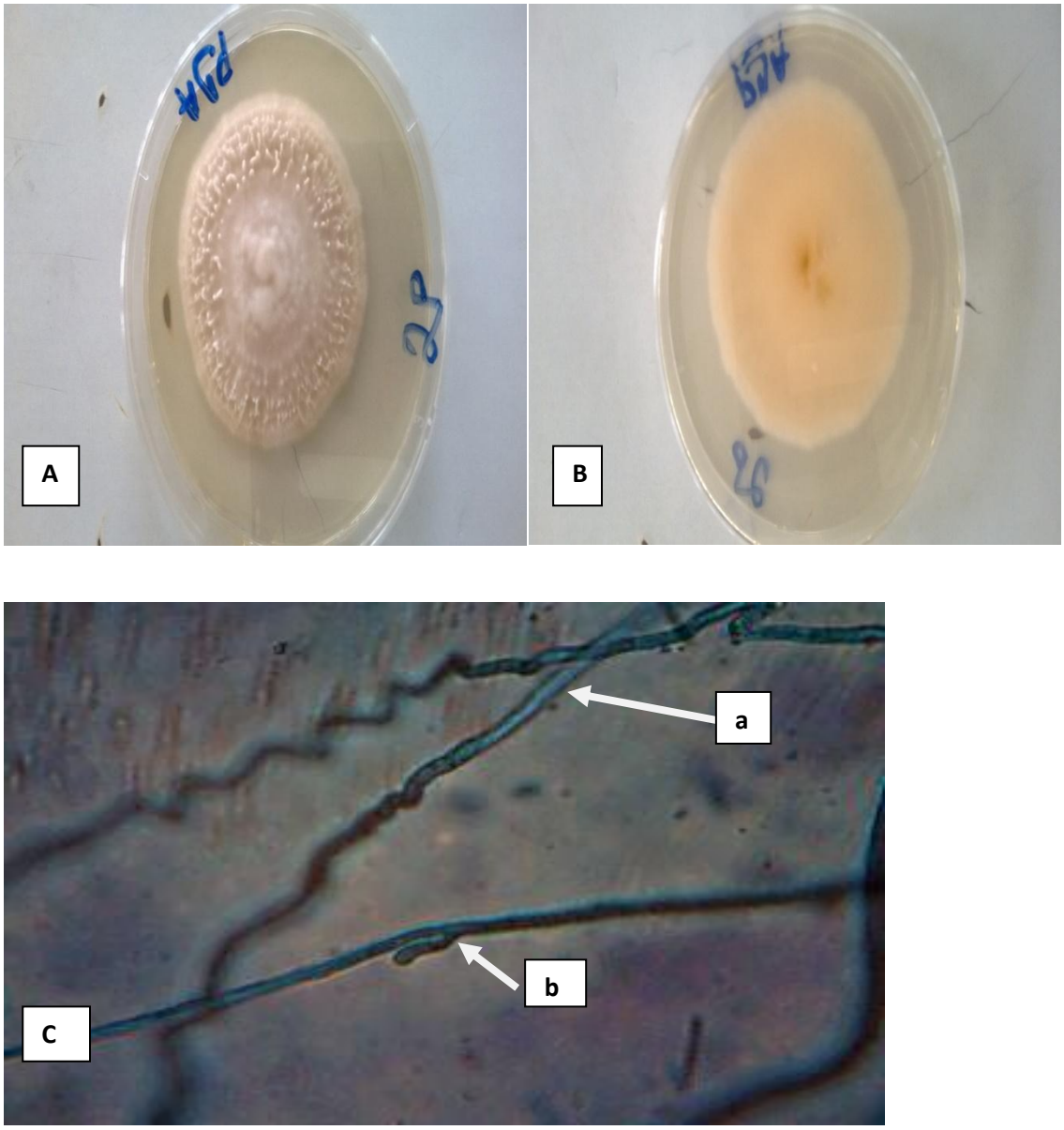


Plate 6: *Sclerotium*sp. Mg x40

A= Upper surface white with sclerotia, Stain; Lactophenol cotton blue

B= Reverse white,

Ca= Non septate hyphae, Cb= Clamp connection.

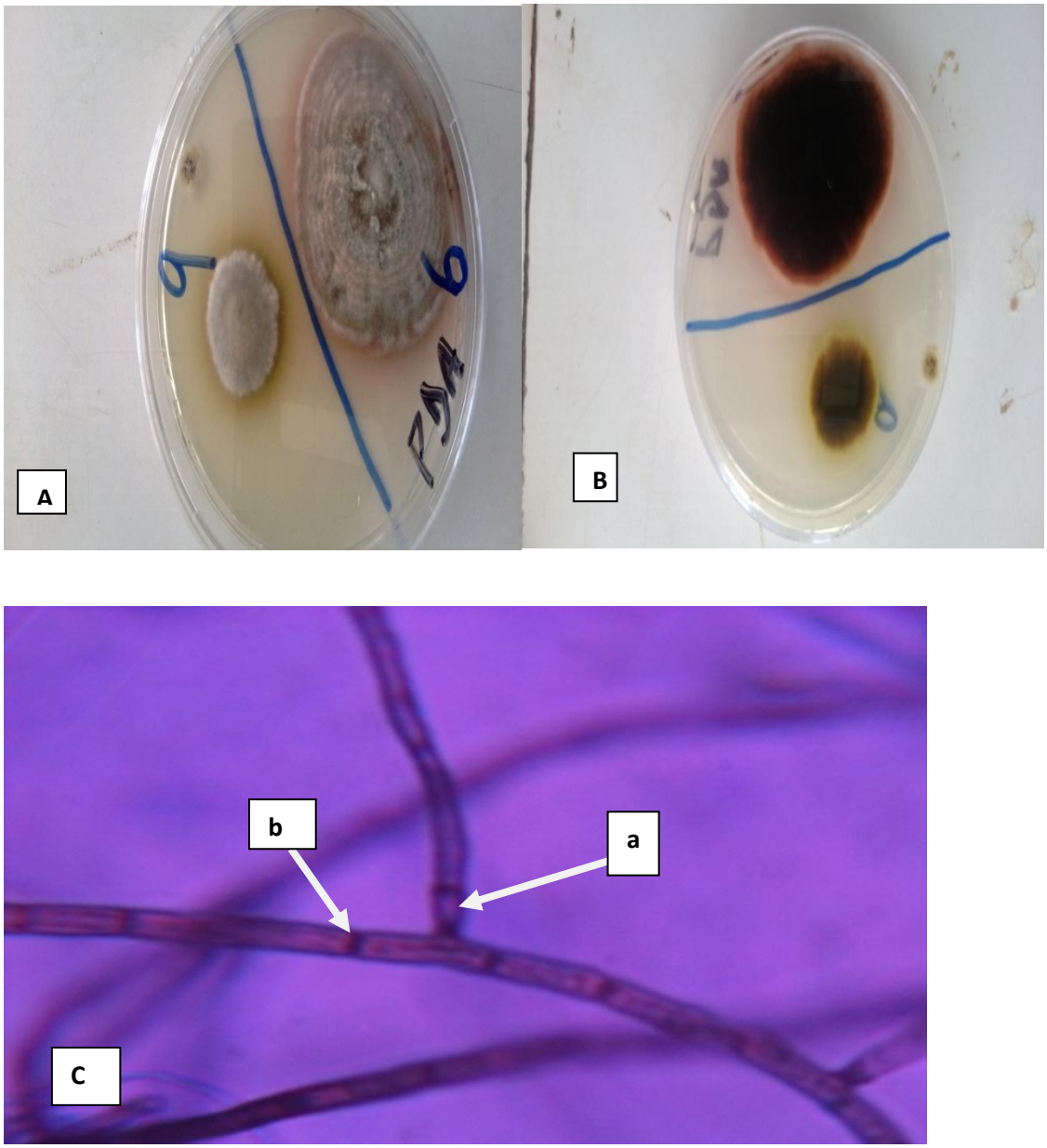


Plate 7: *Rhizoctoniasolani*.Mg x40

A6= Upper surface grey with pink wave edge. Stain; Lactophenol cotton blue

B= Reverse dark brown.

Ca=Branched hyphae, Cb= point of septation.

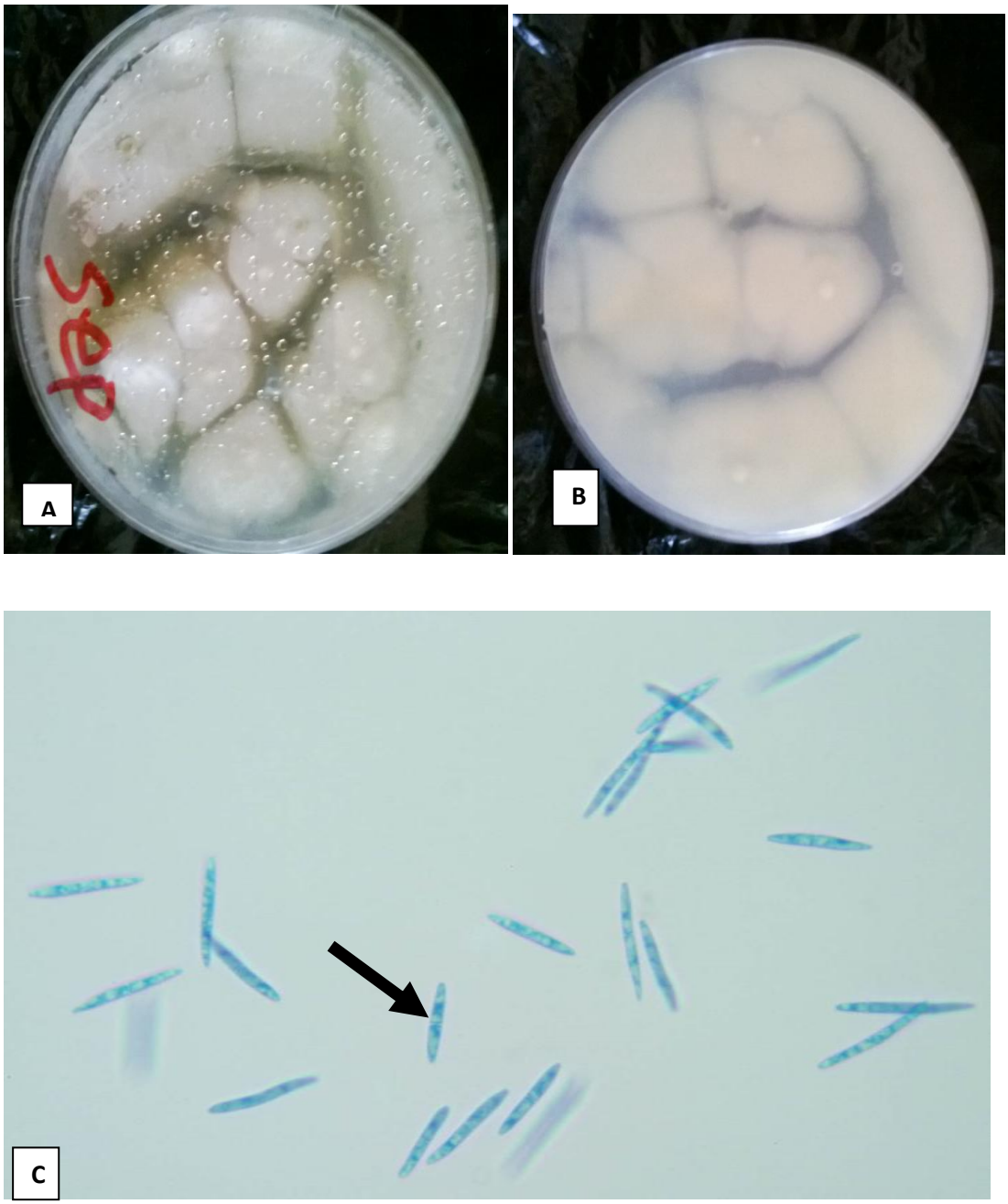


Plate 8: *Septoria* sp. Mg x40

A= Upper surface white Stain; Lactophenol cotton blue

B= Reverse white.

C = conidia hyaline elongated with several cells.

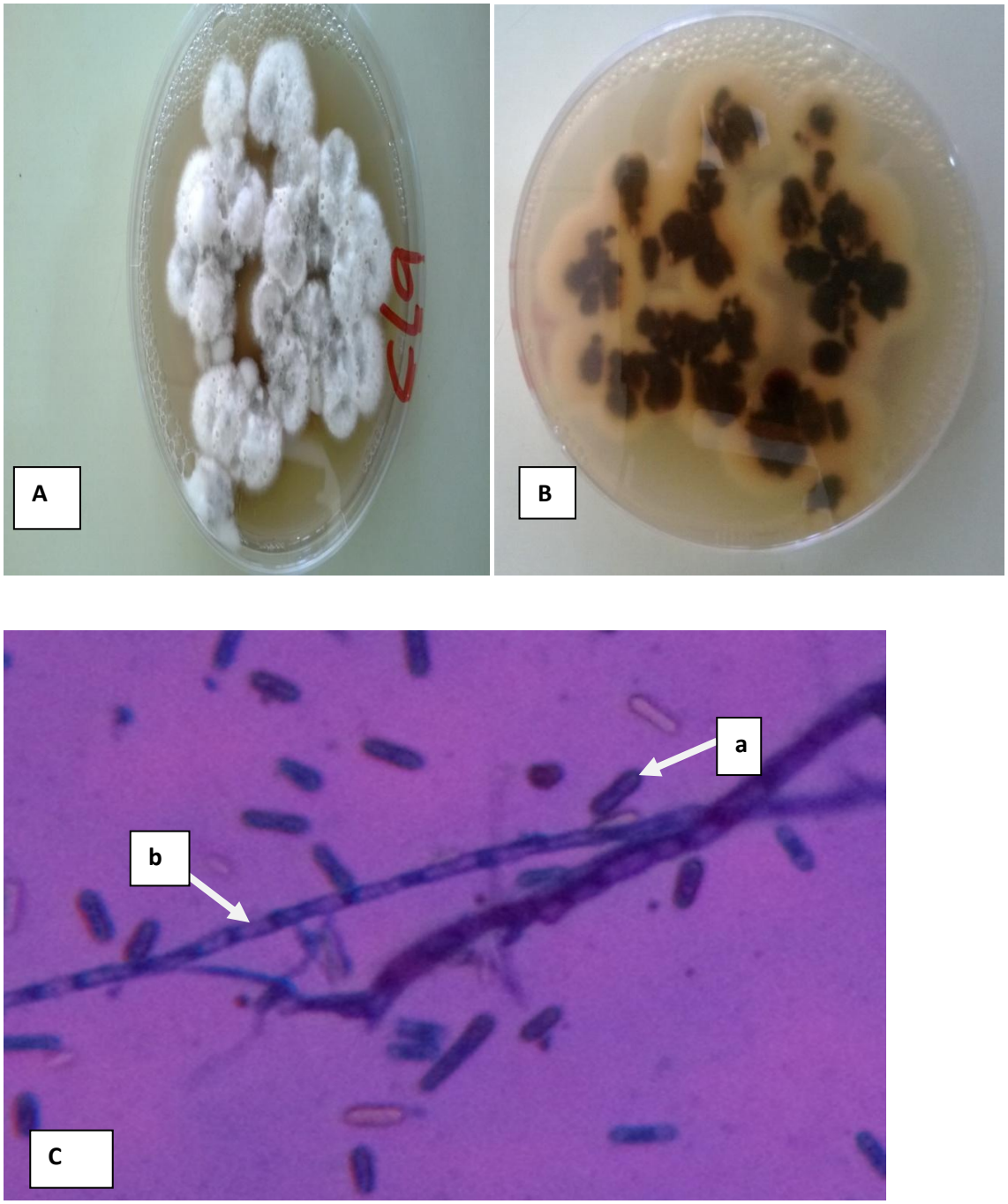
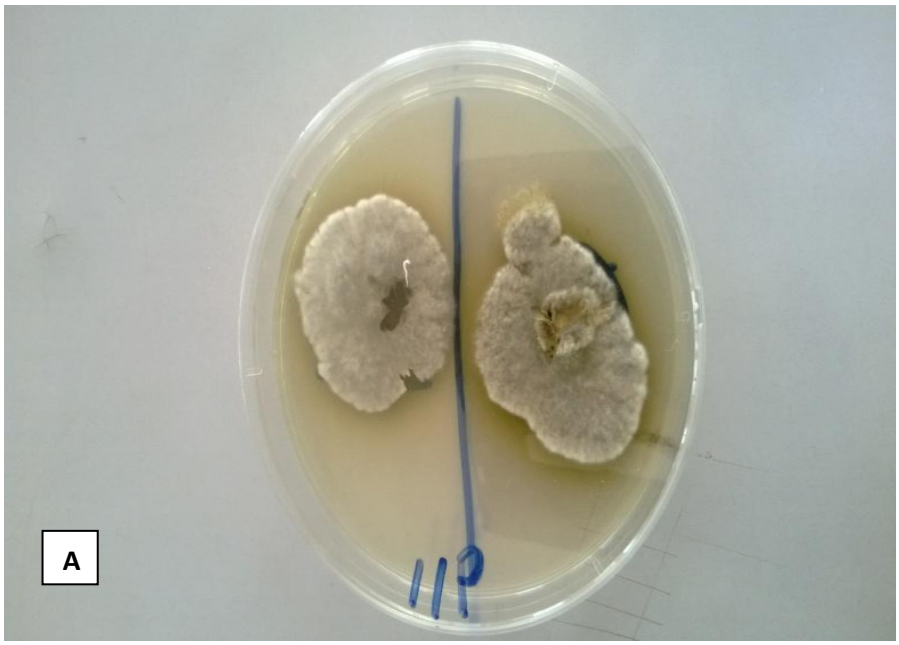


Plate 9: *Cladosporium* sp. Mg x40

A=Upper surface white Stain; Lactophenol cotton blue

B= Reverse black.

Ca= Conidia ovoid dark two celled, Cb= Hyphae.



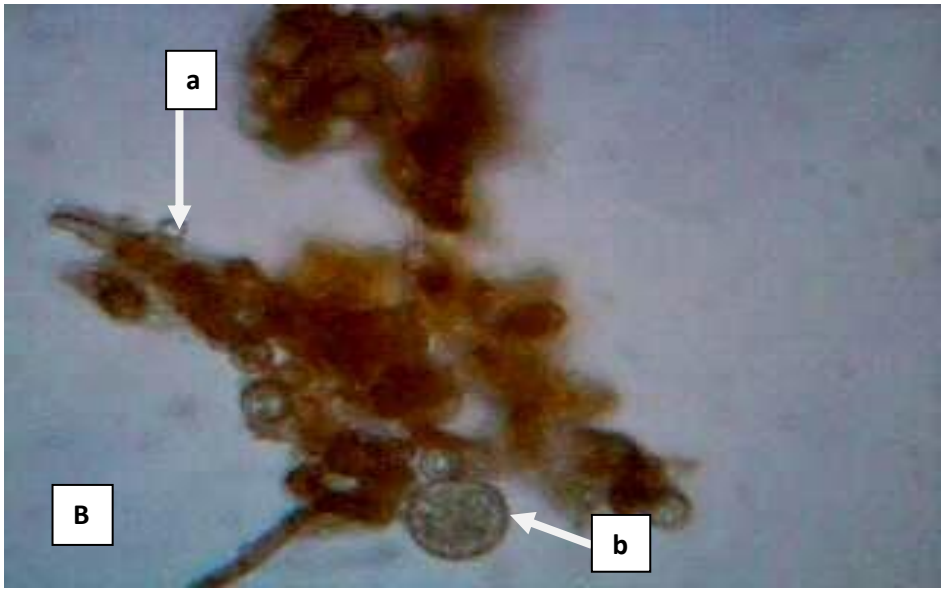


Plate 10: *Mycogone* sp.Mg x40

A=Upper surface grey. Stain; lactophenol cotton blue

Ba=Branched conidiophores with, Bb= globose conidia

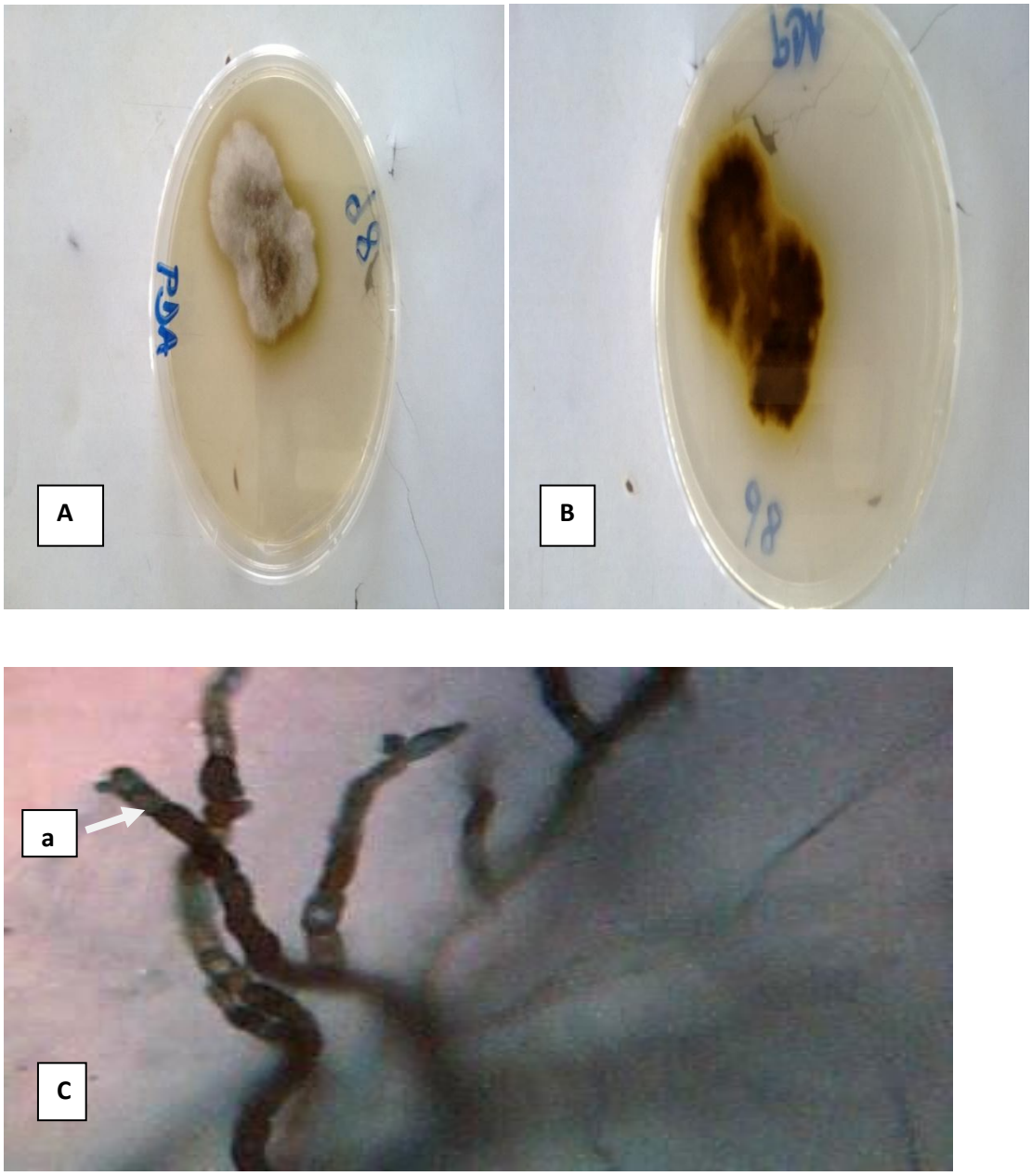


Plate 11: *Bispora* sp. Mg x40

A= Upper surface grey. Stain; Lactophenol cotton blue

B= Reverse brown.

Ca= conidiophores dark and short.

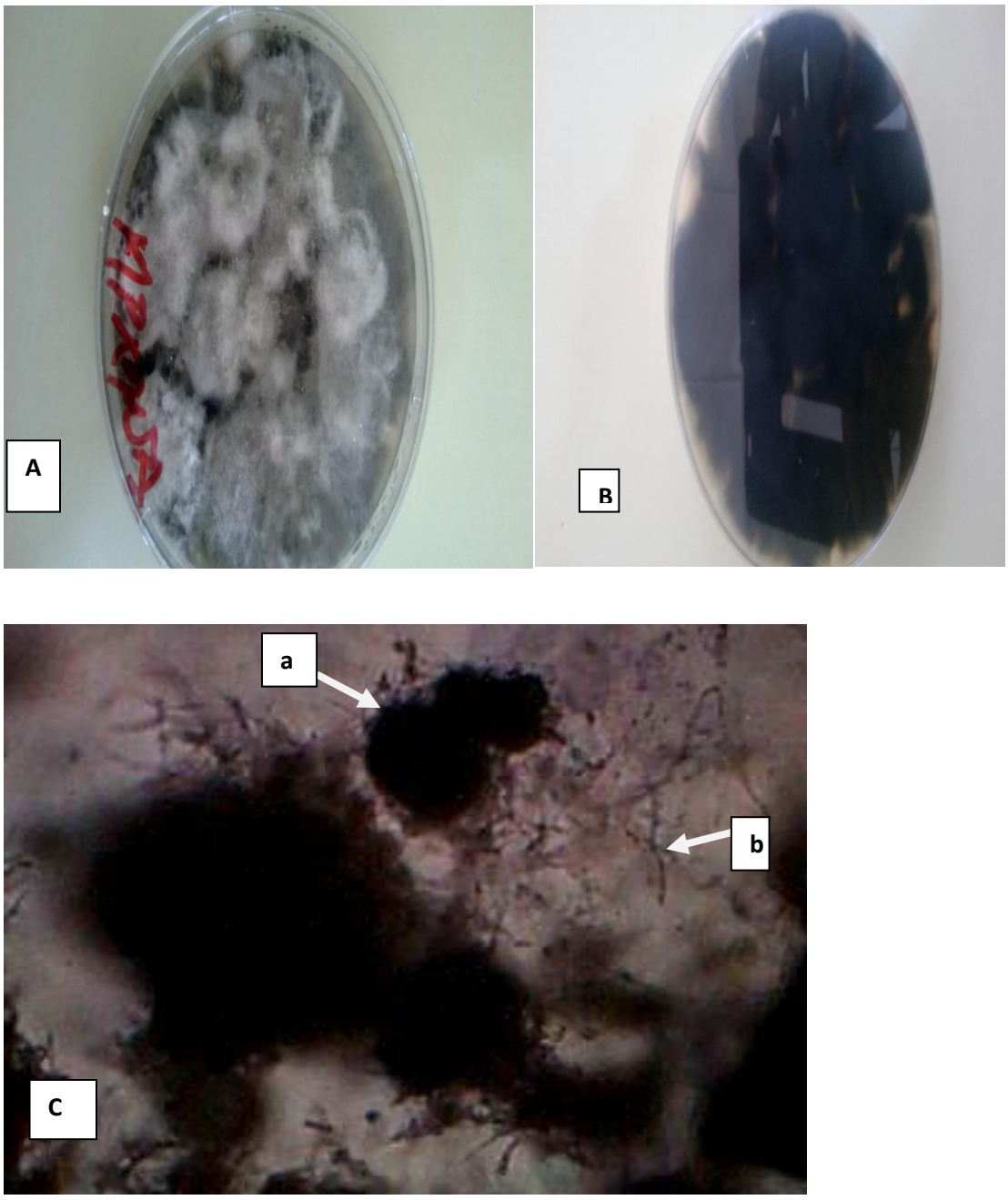
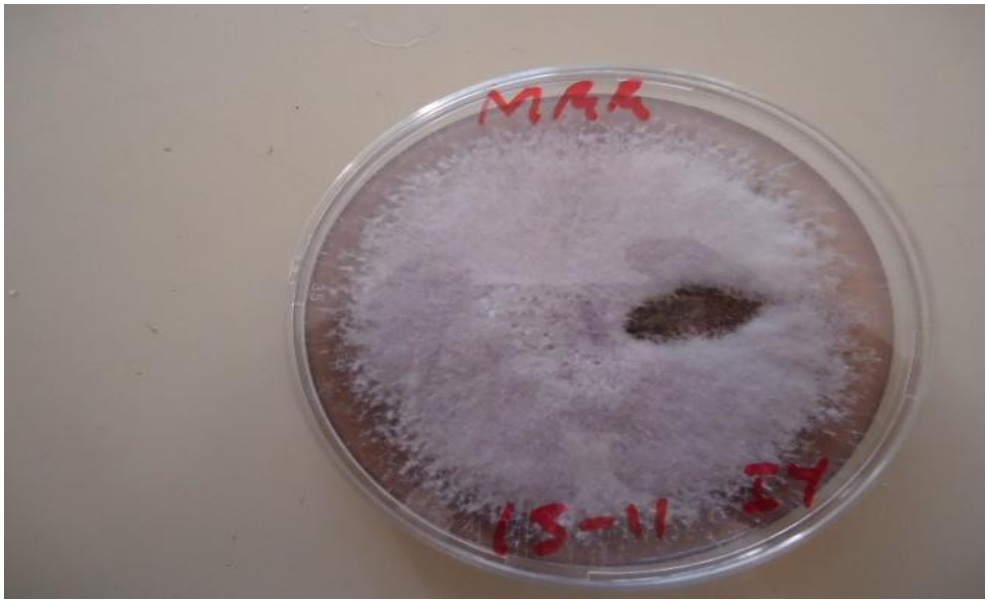


Plate 12: *Macrophomina* sp. Mg x10

A= Upper surface grey. Stain; Lactophenol cotton blue

B= Reverse black.

Ca=Pycnidia, Cb=Hyphae



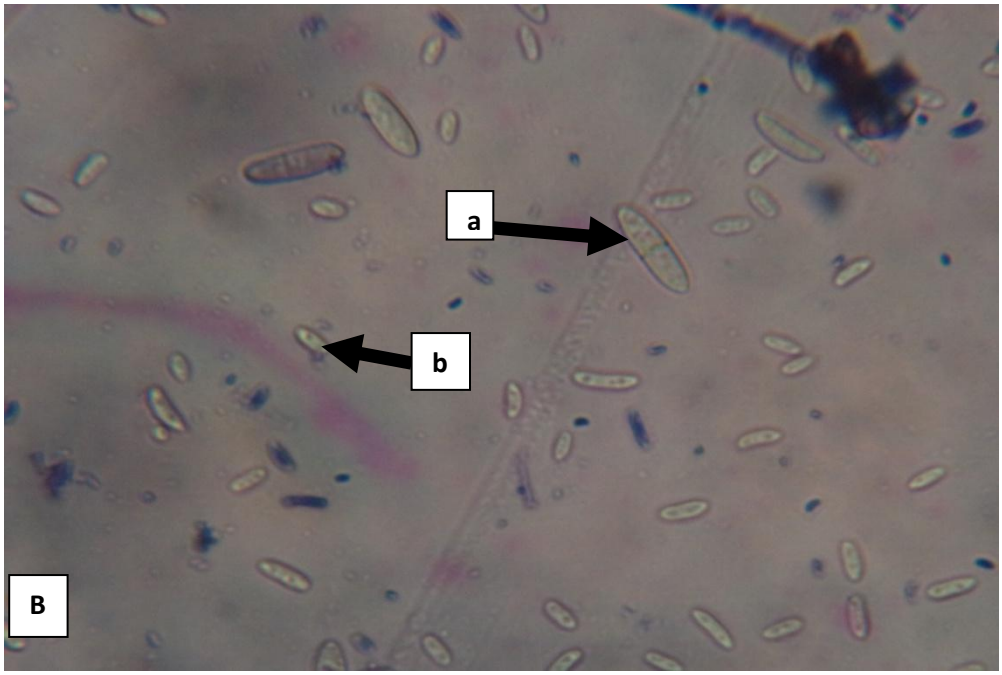


Plate 13: *Gleosporium*sp. Mg x40

A= Upper surface pink, Stain; Lactophenol cotton blue

Ba and Bb= Hyaline ovoid conidia variable in length.



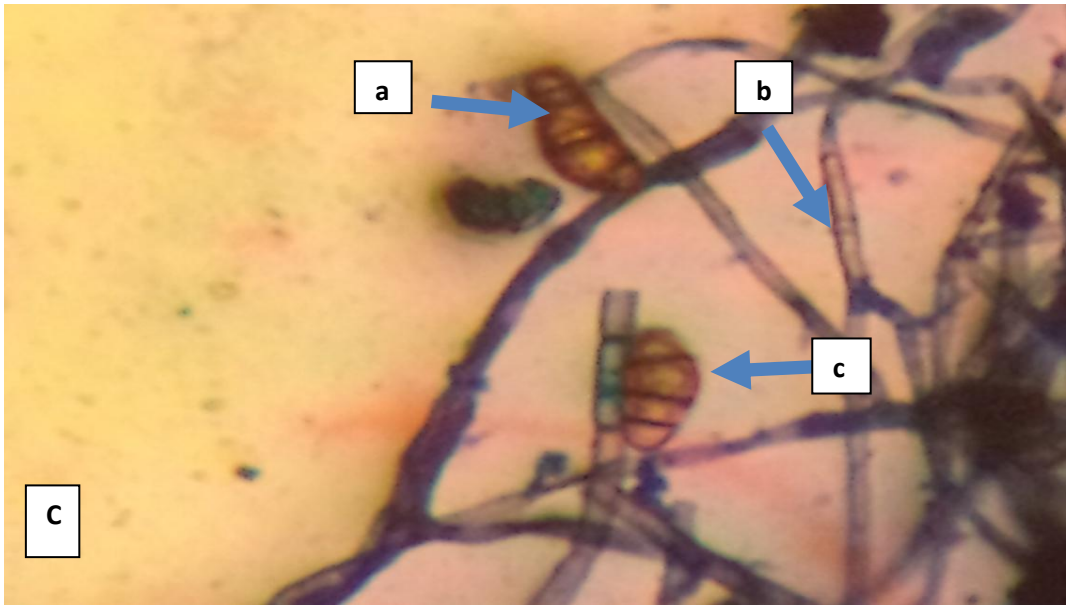


Plate 14: *Curvularia*sp. Mg x40

A=Upper surface black. Stain; Lactophenol cotton blue

B= Reverse black.

Ca= Conidia with four cell, Cb= Hyaline mycelia, Cc= enlarged cell of conidia

Table 4.3: Percentage Occurrence of Fungi from Cultivated plants

Fungi isolated	**Numberofoccurrence**	**Percentageoccurrence**
Rhizoctonia solani	6	35.29
Sclerotium sp.	2	11.76
Septoria sp.	1	5.88
Cladosporium sp.	2	11.76
Bispora sp.	1	5.88
Macrophomina sp.	1	5.88
Mycogone sp.	1	5.88
Fusarium equiseti	1	5.88
Curvularia sp.	1	5.88
Gleosporium sp.	1	5.88
Total	**17**	**100**

4.4Types of Symptoms Observed on Leaves Uncultivated Plants

Eight different types of symptoms that differ in size, color and shape were observed on eight different species of uncultivated plants. *Spigelia anthelmia*havecircular spots with white center and brown margin leads to isolation of*Macrophoma* sp., *Nigrospora aerophila* and *Trichoderma* sp.*Trianthemaportulacastrum*have circular white spot with brown margin leads to isolation of*Monocheatia* sp.*Chlorophytum blepherophyllum*havelarge circular spots with grey spot with brown margin,*Fusarium equiseti* and *Choanephora* sp. were isolated. *Ipomoea nil*have small circular spots with white center brown margin*Rhizoctonia solani* was isolated.

Clematis hirsute shows circular spots with grey center brown margin that leads to isolation of*Nigrospora aerophila. Senna singuena*havedark brown scattered spots with irregular shape, *Nigrospora aerophila* was isolated. *Combretum molle*withgrey spots that make hole when older leads to *Rhizoctonia solani. Porphyrostemma chevalieri*withbrown spot dispersed with irregular shapes leads to isolation*Macrophomina* sp., *Alternaria* sp. and *Helicosporium* sp.Thesesymptoms were described in Table 4.4and shown in Plates 15and 16.

4.5 Identification of FungiIsolated from Spot Symptoms on Uncultivated plant

Ten different types of fungi were isolated from spot symptoms on uncultivated plants. Each fungus was found to produce a definite symptom except *Nigrospora aerophila* which was associated with three types of symptoms and *Rhizoctonia solani* which was associated with two types spot symptoms.

Macrophoma sp.macroscopic character shows upper surface grey, reverse black, microscopic character shows darkpycnidia. *Nigrospora aerophila*macroscopic character showsupper surface light brown, reverse light brown, microscopic character shows globose black conidia with vesicle and hyphae.*Trichoderma* sp. macroscopic character shows upper surface green, microscopic character shows Conidia. *Monochaetia*sp. macroscopic character upper surface brown, reverse brown, microscopic character shows conidia of different shapes with slender conidiophore.*Choanephora* sp. macroscopic character shows upper surface black with white center, reverse black, microscopic character shows one celled ellipsoid brown conidia.*Alternaria* sp. macroscopic character shows upper surface black with grey margin, reverse brown, microscopic character shows conidiophores with both cross and longitudinal septa.*Helicosporium* sp. macroscopic character shows upper surface grey reverse grey, microscopic character shows conidiophores tall slender. *Fusarium equiseti*macroscopic character showsupper surface white, reverse pink, microscopic character shows macroconidia with septation.*Rhizoctoniasolani* macroscopic character shows *u*pper surface grey with pink wave edge, reverse dark brown, microscopic character showsbranched hyphae with point of septation. These characters are describe in Table 4.5 and shown in Plates17 to 23.

These were compared with already described species using keys and already described isolates at IAR, ABU, Zaria.To ascertain the identity of *N. aerophila* and *R. solani* the isolates were send toCommonwealth Agricultural Bureaux International (CABI), UK

Table 4.4: Fungi Isolated from Leaves Spot Symptoms on Uncultivated Plants

Nameofplant	**Descriptionofsymptoms**	**Fungi Isolated**
Spigelia anthelmia	Circular spots with white center and brown margin.	*Macrophoma* sp. *Nigrospora aerophila* *Trichoderma* sp.
Trianthema portulacastrum	Circular white spot with brown margin.	*Monocheatia*sp.
Chlorophytum blepherophyllum	Large circular spots With grey spot with brown margin.	*Fusarium equisetum* *Choanephora* sp.
Ipomoea nil	Small circular spots with white center brown margin	*Rhizoctonia solani*
Clematis hirsute	Circular spots with grey center brown margin	*Nigrospora aerophila*
Senna singuena	Dark brown scattered spots with irregular shape	*Nigrospora aerophila*
Combretum molle	Grey spots that make hole when older	*Rhizoctonia solani*
Porphyrostemma chevalieri	Brown spot dispersed with irregular shapes	*Macrophomina* sp. *Alternaria* sp. *Helicosporium* sp.

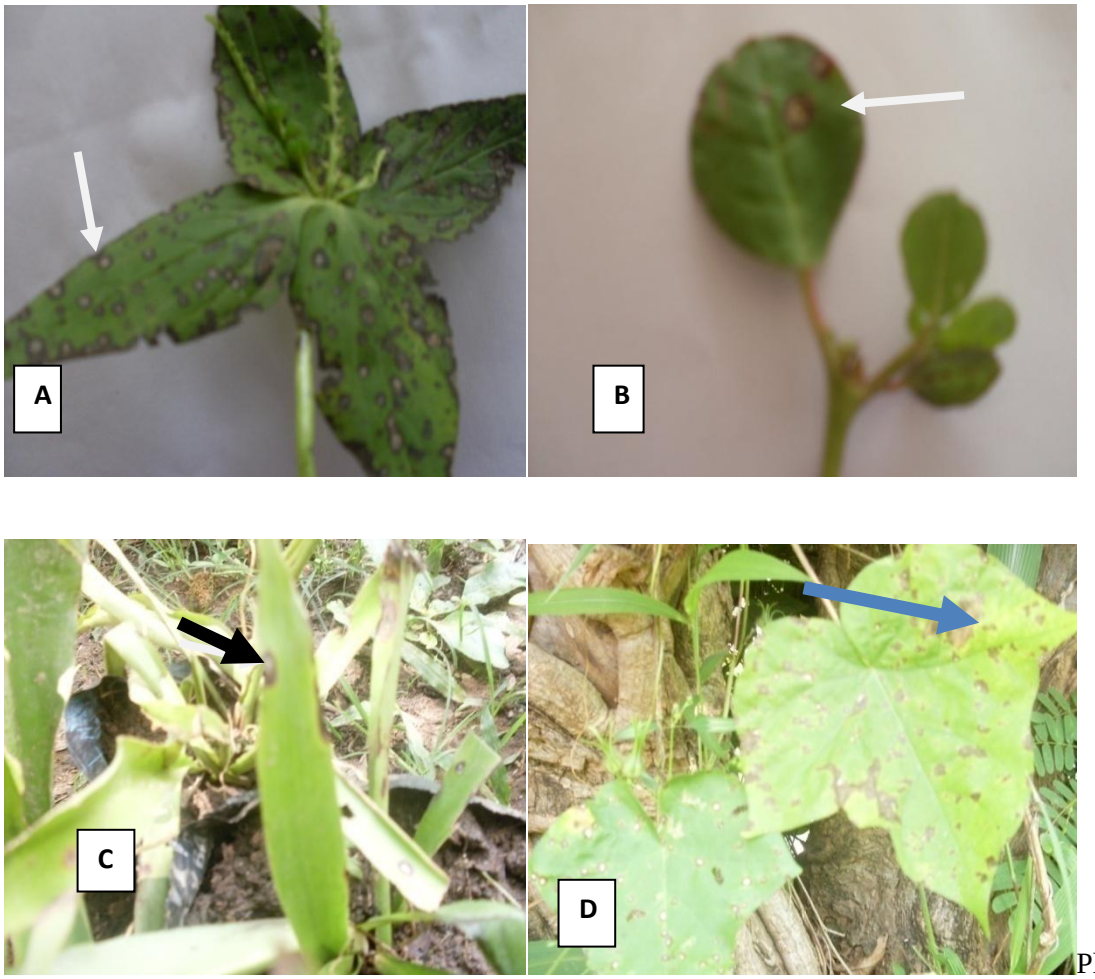


Plate 15:Uncultivated Plants showing spot Symptoms I.A: *Spigelia anthelmia.*circular spots with white center and brown margin.*Macrophoma* sp.,*Nigrospora aerophila* and *Trichoderma* sp.B*:Trianthema portulacastrum.*Circular white spot with brown margin.*Monocheatia* sp. was isolated.Plate C:*Chlorophytum blepherophyllum*large circular spots with grey spot with brown margin.*Fusarium equisetum,*and *Choanephora* sp. were isolated. Plate D: *Ipomoea nil.*small circular spots with white center brown margin. *Rhizoctonia solani* was isolated.

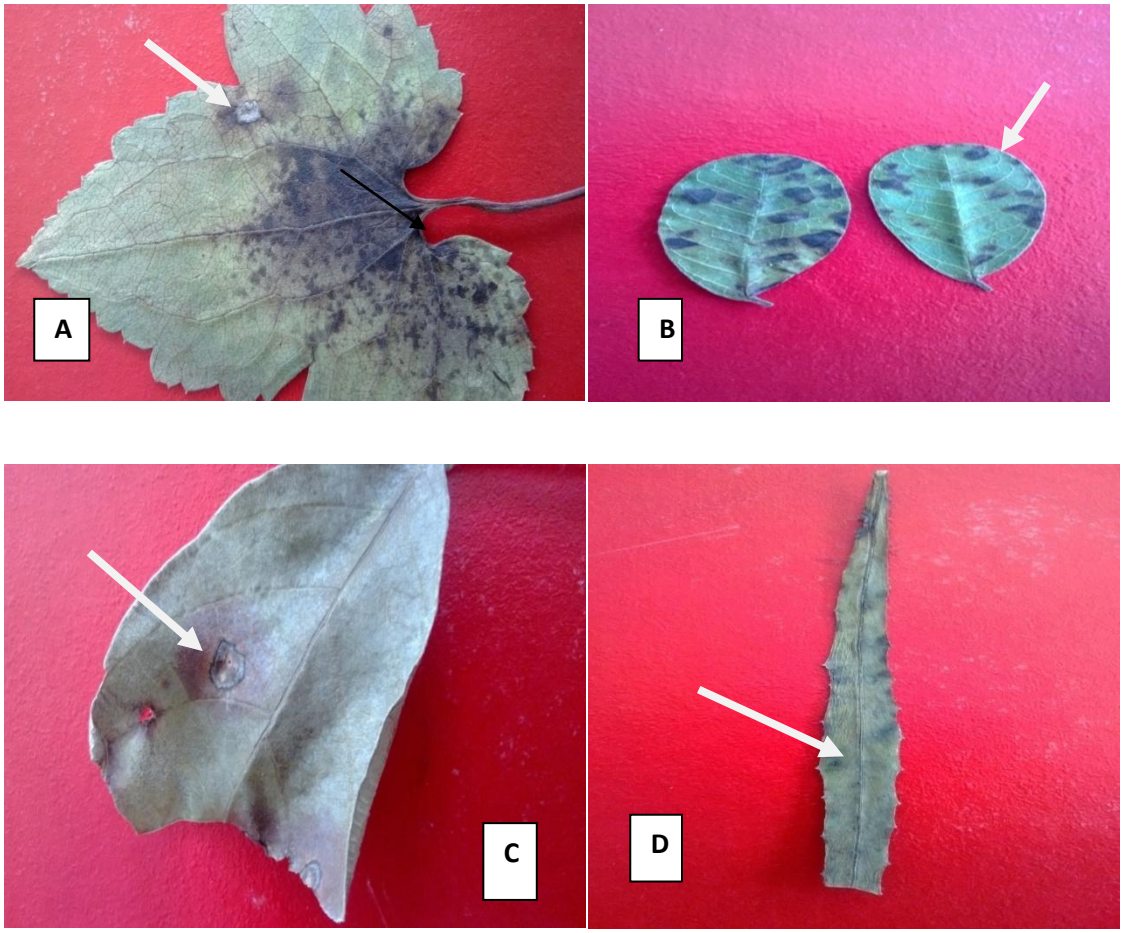


Plate 16:Uncultivated Plants showing spot Symptoms II.A: *Clematis hirsuta.*Circular spots with grey center brown margin, *Nigrospora aerophila* was isolated.B*: Senna singuena.*Dark brown scattered spots with irregular shape, *Nigrospora aerophila* was isolated.Plate C: *Combretum molle*. Grey spots that make hole when older, *Rhizoctonia solani* was isolated. D:*Porphyrostemma chevalieri*. Brown spot dispersed with irregular shapes. *Macrophomina* sp., *Alternaria* sp. and *Helicosporium* sp. were isolated.

Table 4.5: Cultural and Microscopic Characters Identification of Fungi Isolated from Uncultivated plants

Fungi	Macroscopic character	Microscopic character
Macrophoma sp.	Upper surface grey, Reverse black.	Dark pycnidia.
Nigrospora aerophila	Upper surface light brown, reverse light brown.	Short conidiophores, black conidia, 1-celled globose, vesicle present.
Trichoderma sp.	Upper surface green, reverse green.	Conidiophores branched, conidia 1-celled ovoid
Monocheatia sp.	Upper surface brown, reverse brown.	Conidia dark, several celled with pointed end cell.
Choanephora sp.	Upper surface black with white center, reverse black.	Conidia one celled ellipsoid brown.
Alternaria sp.	Upper surface black with grey margin, reverse brown.	Conidiophores with both cross and longitudinal septa.
Helicosporium sp.	Upper surface grey, reverse grey.	Conidiophores tall slender.
Fusariumequiseti	Upper surface white cottony, Reverse pink.	Macroconidia with septation and Microconidia.
Rhizoctonia solani	Upper surface grey with pink wave edge, Reverse dark brown.	Branched hyphae with point of septation.
Macrophomina sp.	Upper surface grey cottony, reverse black.	Presence of hypha and pycnidia

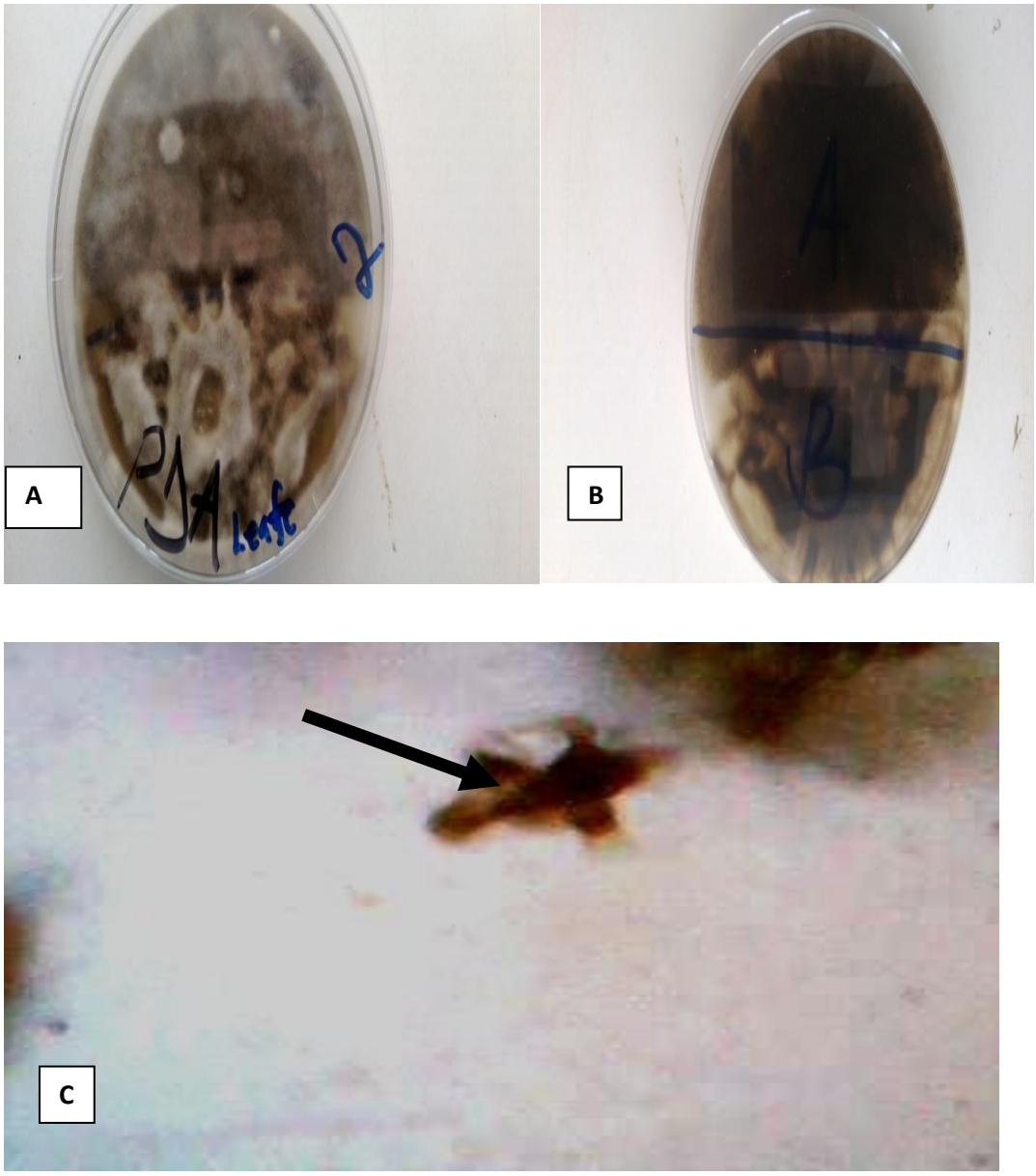


Plate 17:*Macrophoma* sp. Mg x40

A= upper surface grey, Stain; Lactophenol cotton blue.

B=Reverse black,

C=Dark pycnidia.

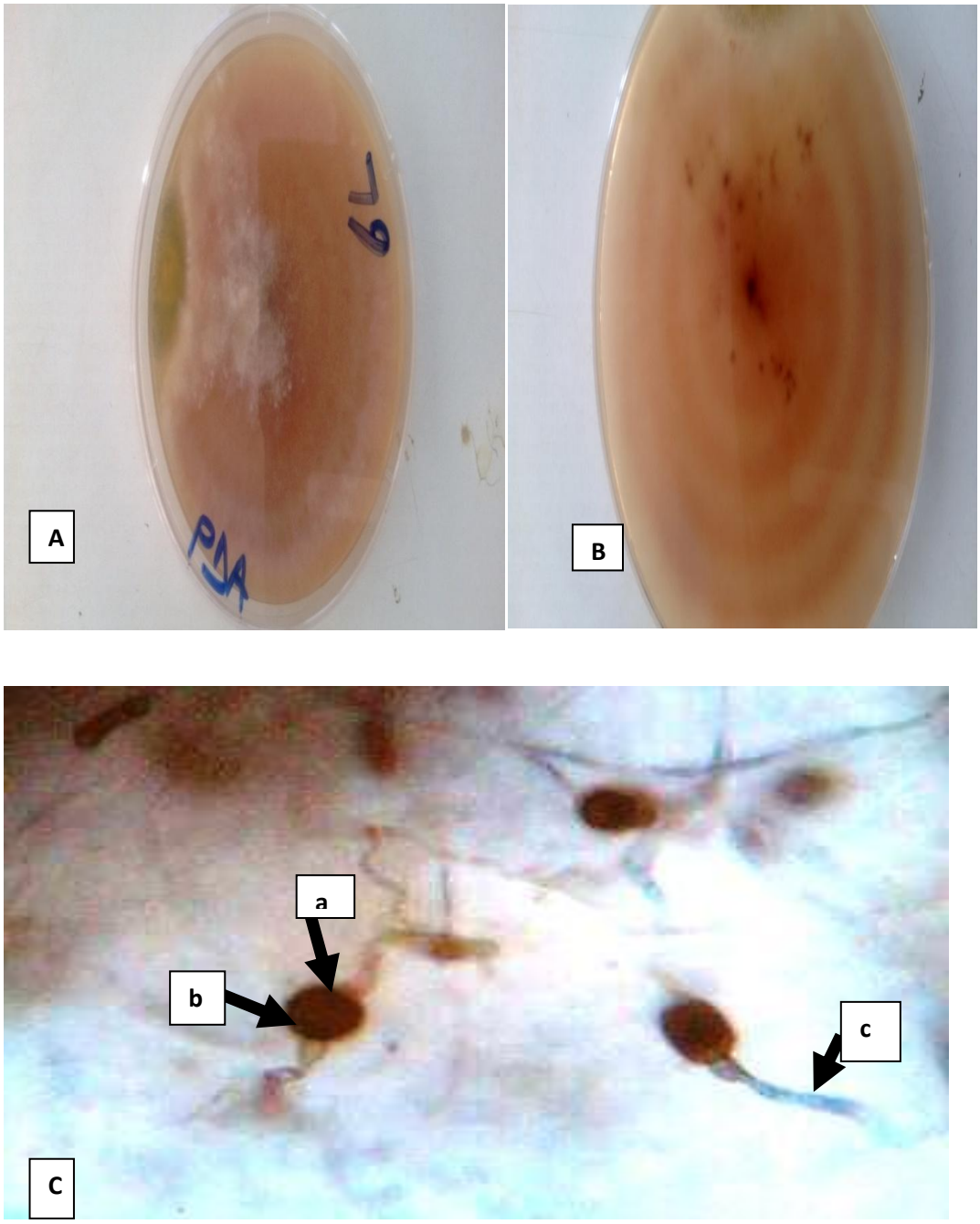
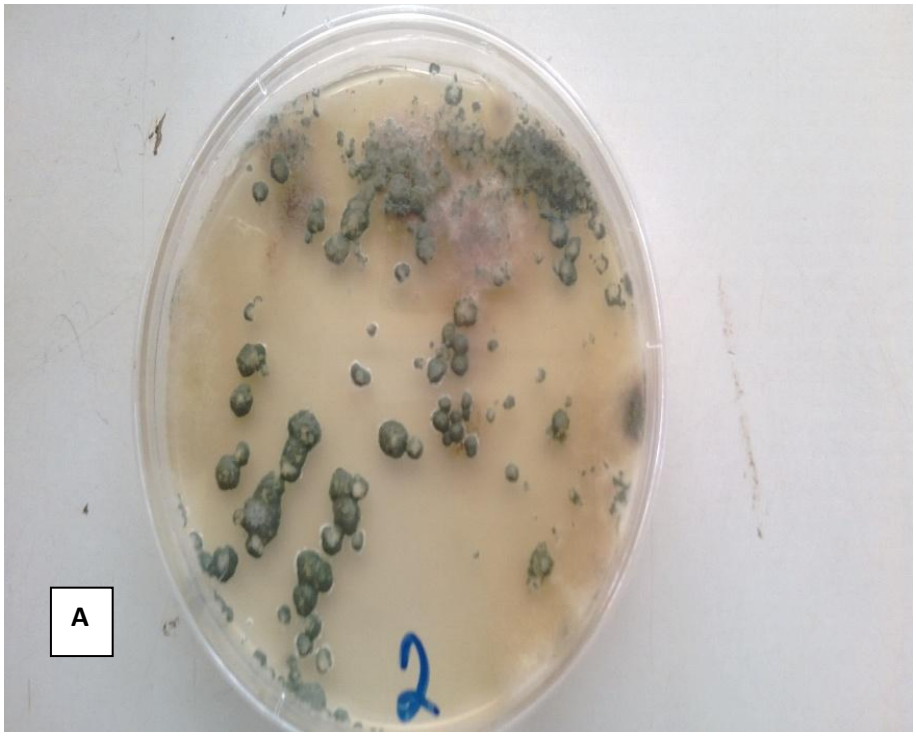


Plate 18:*Nigrospora aerophila*. Mg x40

A= Upper surface light brown, Stain; Lactophenol cotton blue

B=Reverse light brown,

Ca=Vesicle, Cb= Globose black conidia, Cc= Hyphae.



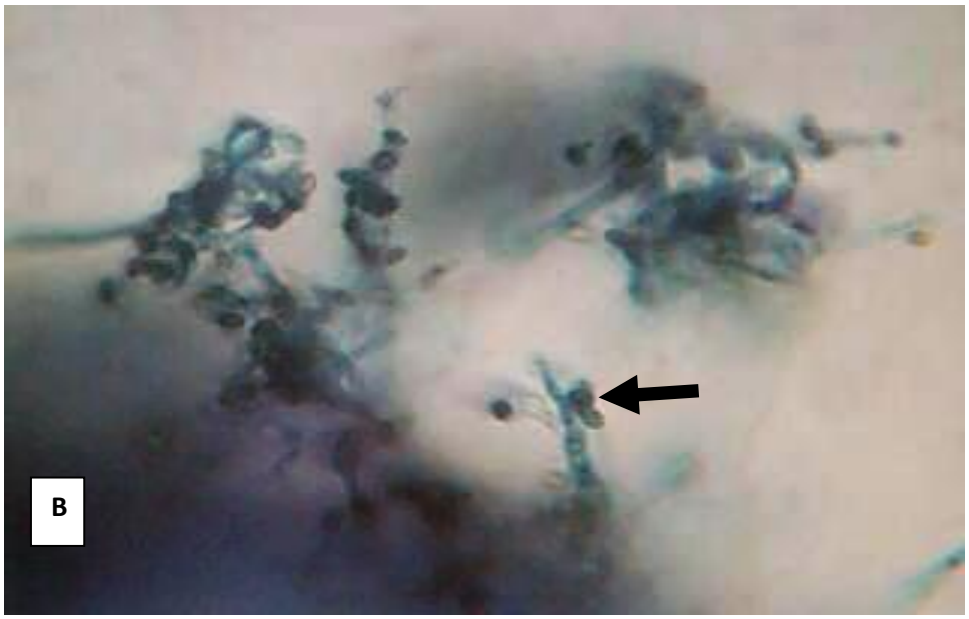


Plate 19: *Trichoderma* sp. Mg x40

A= Upper surface green, Stain; Lactophenol cotton blue

B= Conidia



Plate 20: *Monochaetia*sp..Mg x40

A= Upper surface brown, Stain; Lactophenol cotton blue

B= Reverse brown,

Ca = Conidia dark, several celled with pointed end cell..

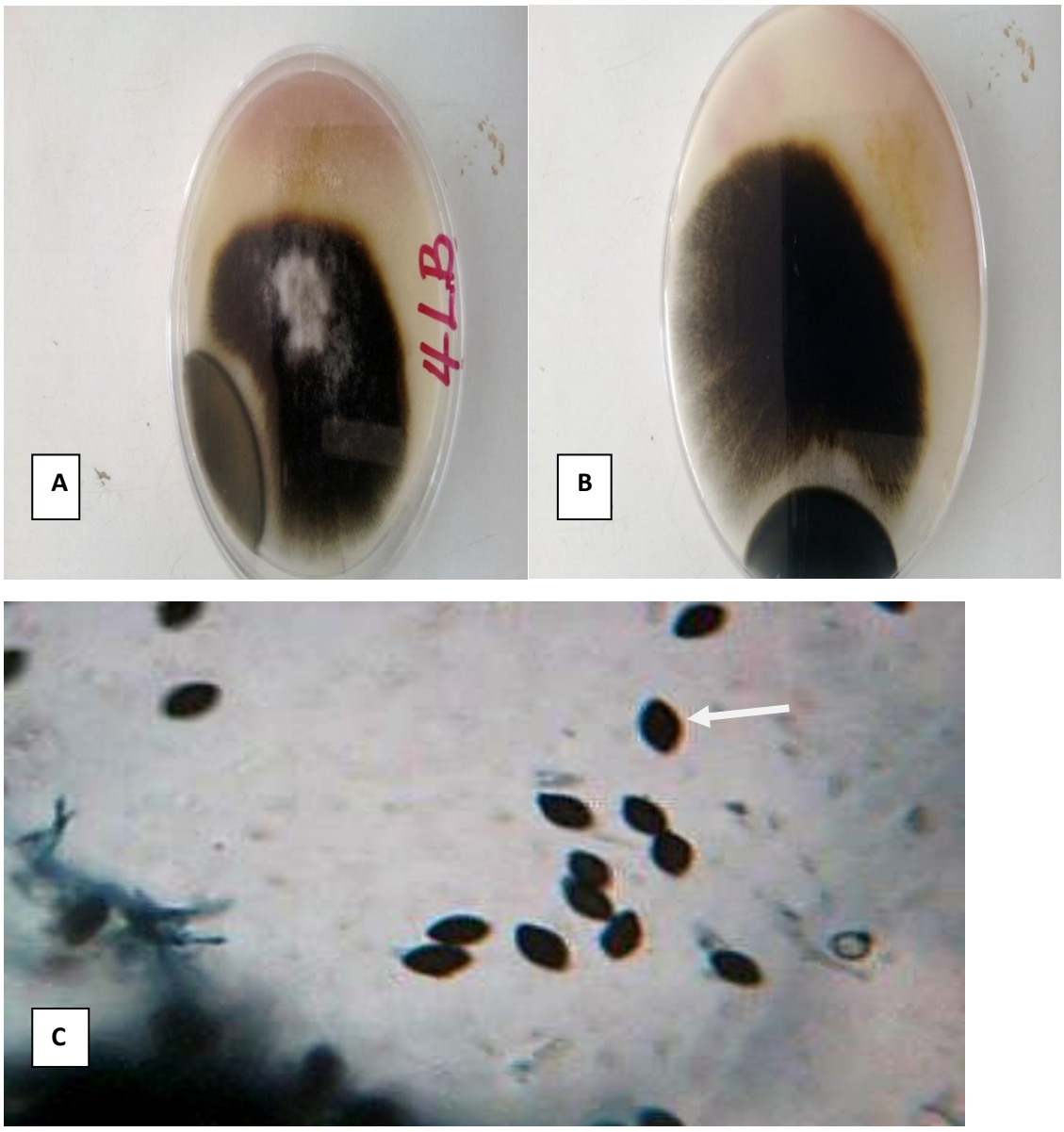


Plate 21: *Choanephora* sp. Mg x40

A= Upper surface black with white center. Stain; Lactophenol cotton blue

B=Reverse black,

C= Conidia one celled ellipsoid brown.

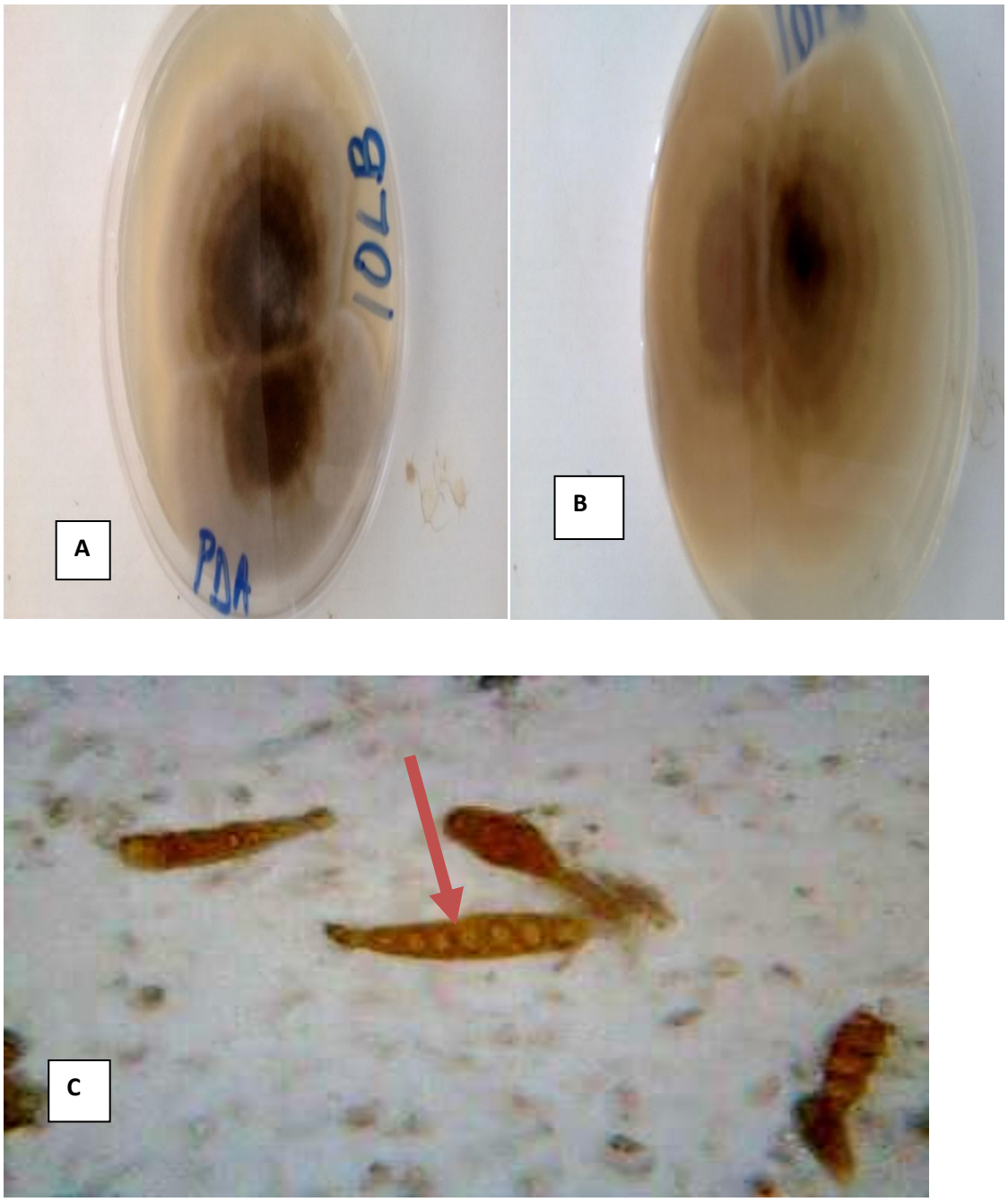


Plate 22: *Alternaria* sp. Mgx40

A= Upper surface black with grey margin. Stain;Lactophenol cotton blue

B= Reverse brown.

C= Conidiophores with both cross and longitudinal septa.

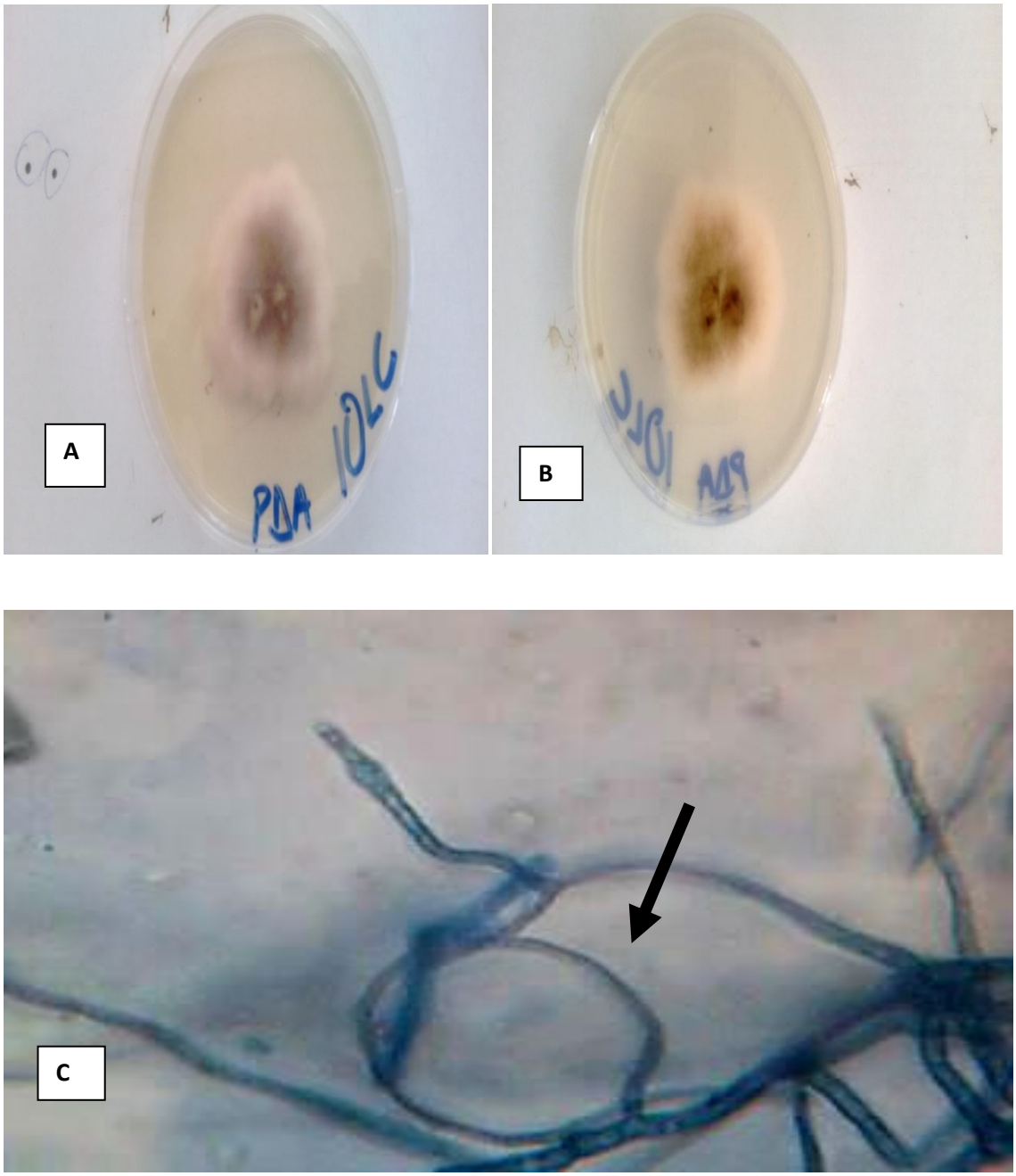


Plate 23: *Helicosporium* sp. Mg x40

A= Upper surface grey. Stain; Lactophenol cotton blue

B= Reverse grey.

C= Conidiophores tall slender

4.6Percentage Occurrence of Fungi from Uncultivated Plants

Nigrospora aerophila has the highest percentage occurrence of 23.08 %, followed by *R. solani*with 15.39 %. *Fusarium equiseti, Macrophoma, Choanephora, Macrophomina, Alternaria, Helicosporium, Trichoderma* and *Monocheatia* spp. have the lowest percentage occurrence of 7.69 %. These are shown in table 4.6.

4.7Diversity of Fungi Isolated from Cultivated and Uncultivated Plants and their Ecological and Epidemiological relationship

4.7.1 Diversity of fungi isolated from cultivated and uncultivated plants

A total of thirteen different fungi were isolated from two cultivated plants and eight uncultivated plants. *R. solani* was isolated from cowpea, *T. occidentalis,Ipomoea nil,* and *Combretum molle,*the fungus can be found on either of these plants. *Cladosporium*sp was isolated from cowpea and *Telfairia. occidentalis. Fusarium equiseti* was isolated from cowpea and *Chlorophytum blepherophyllum.* The remaining fungi were isolated from only one plant. This is shown in Table 4.7.

4.7.2 Ecological and epidemiological relationship between fungal isolates, cultivated and uncultivated Plants

Principal component analysis was use to determine the ecological and epidemiological relationship between fungal isolates, cultivated and uncultivated plants. It shows that *Rhizoctonia solani* is closely related to cowpea,*T. occidentalis, Chlorophytum blepherophyllum,*and *Combretum molle,Fusarium equiseti, Cladosporium, Curvularia* and*Choanephora* spp. were related to cowpea, *T. occidentalis*, and *Chlorophytum*

blepherophyllum,Porphyrostemma chevalieri.Sclerotium and *Septoria*spp. were only related to cowpea, *Alternaria* and *Macrophomina*spp. were only related to *Porphyrostemma chevalieri. Macrophoma* sp. was related to *Spigelia anthelmia. Monocheatia*sp. is related to *Trianthema portulacastrum* and *Chlorophytum blepherophyllum, Nigrospora aerophila* is not related to any cultivated plantsbut to uncultivated plants i.e*Spigeliaanthelmia*and*Senna singuena.* This is shown in Fig 1.

4.8PathogenecityTest on Three Cowpea Varieties

Fusarium equiseti,Rhizoctonia solani,Sclerotium, Septoria,Cladosporium, and*Macrophomina*spp. were tested on three cowpea varieties which shows no symptoms as shown in Table 4.8

4.9*Invitro*Pathogenecity using Leaf Disk

*Fusarium equiseti,Rhizoctonia solani,Nigrosporaaerophila, Choanephora*and *Macrophoma*spp.were tested on leaves of uncultivated plants found to be producing the same symptoms when inoculated as shown in Table 4.9 and Plate24.

Table 4.6: Percentage Occurrence of Fungi Isolated from Uncultivated Plants

Fungi isolated	Numberofoccurrence	Percentageoccurrence
Nigrospora aerophila	3	23.08
Rhizoctonia solani	2	15.39
Trichoderma sp.	1	7.69
*Monocheatia*sp.	1	7.69
Fusarium equisetum	1	7.69
Macrophoma sp.	1	7.69
Choanephora sp.	1	7.69
Macrophomina sp.	1	7.69
Alternaria sp.	1	7.69
Helicosporium sp.	1	7.69
Total	**13**	**100**

Table 4.7: Fungi Isolated from Cultivated and Uncultivated Plants

Examinedplants										
Fungi Isolated	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Rhizoctonia solani	+	+	-	-	-	+	-	-	+	-
Nigrospora aerophila	-	-	+	-	-	-	+	+	-	-
Fusarium equisetum	+	-	-	-	+	-	-	-	-	-
Septoria sp.	+	-	-	-	-	-	-	-	-	-
Macrophomina sp.	+	-	-	-	-	-	-	-	-	+
Macrophoma sp.	-	-	+	-	-	-	-	-	-	-
Cladosporium sp.	+	+	-	-	-	-	-	-	-	-
Gleosporium sp.	-	+	-	-	-	-	-	-	-	-
Choanephora sp.	-	-	-	-	+	-	-	-	-	-
Alternaria sp.	-	-	-	-	-	-	-	-	-	+
Curvularia sp.	-	+	-	-	-	-	-	-	-	-
*Monocheatia*sp.	-	-	-	+	-	-	-	-	-	-
Sclerotium sp.	+	-	-	-	-	-	-	-	-	-
Mycogone sp.	+	-	-	-	-	-	-	-	-	-
Bispora sp.	+	-	-	-	-	-	-	-	-	-
Helicosporium sp.	-	-	-	-	-	-	-	-	-	+
Trichoderma sp.	-	-	+	-	-	-	-	-	-	-

Key:1=cowpea, 2=*T. occidentalis*, 3= *Spigelia anthelmia*, 4 = *Trianthema portulacastrum*, 5= *Chlorophytum blepherophyllum*, 6= *Ipomoea nil*, 7= *Clematis hirsuta*, 8=*Senna singuena*, 9=*Combretum molle*, 10= *Porphyrostemma chevalieri.*+ = present - = absent

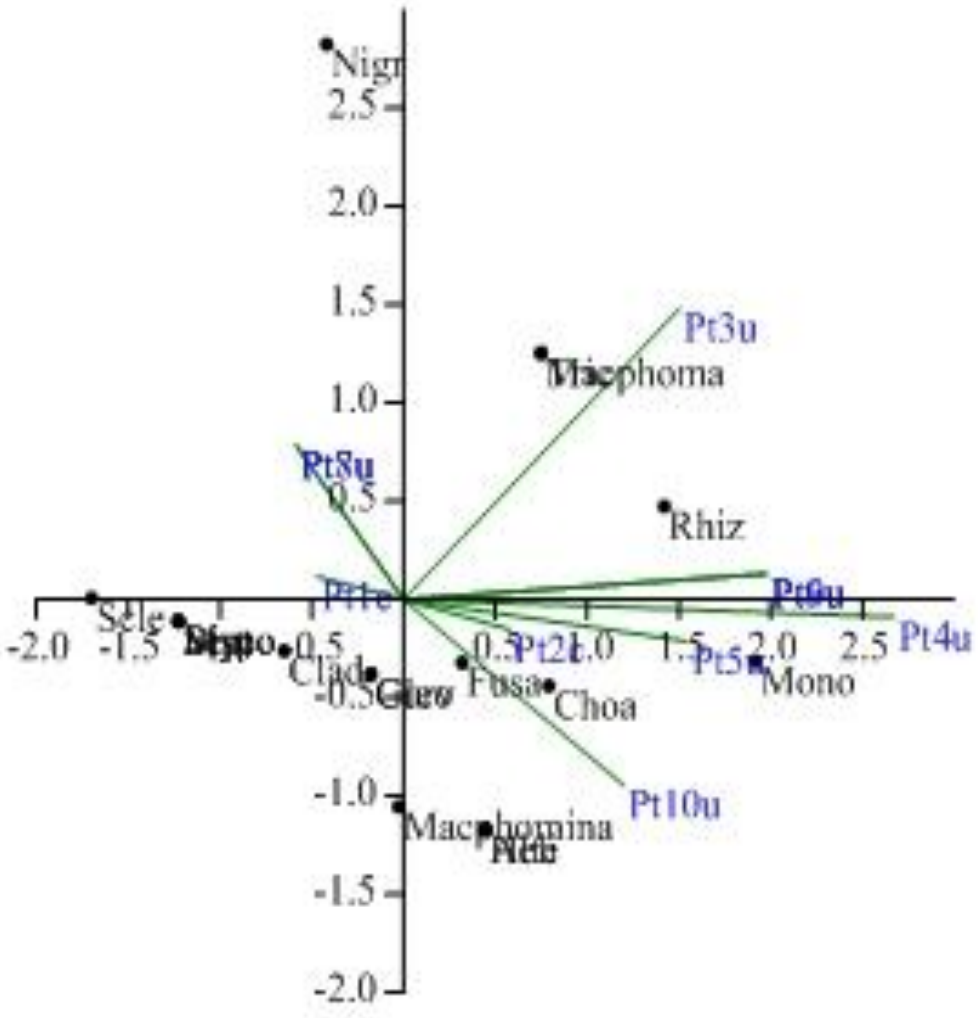


Figure 1: Ecological and epidemiological relationship between fungal Isolates, cultivated and Uncultivated.

Key: pt1=cowpea, pt2=*T. occidentalis*, pt3= *Spigelia anthelmia*, pt4 = *Trianthema portulacastrum*, pt5= *Chlorophytum blepherophyllum*, pt6= *Ipomoea nil*, pt7= *Clematis hirsuta*, pt8=*Senna singuena*, pt9=*Combretum molle*, pt10= *Porphyrostemma chevalieri*.C= Cultivated plants, U= uncultivated plants

Scle= *Sclerotium*sp., Sep= *Septoria* sp., *Cla= Cladosporium*sp., Cur= *Curvularia* sp., Choa= *Choanephora*sp., Mono= *Monocheatia*sp., *Rhiz=Rhizoctoniasolani*, Fusa= *Fusariumequiseti, Nigr= Nigrosporaaerophila, Alte= Alternaria*sp.

Table 4.8:Reaction of Cowpea Varieties to some InoculatedFungi

	Cowpea Varieties		
Fungi	**SAMPEA 8**	**SAMPEA 7**	**KANANNADO**
Fusarium equiseti	-	-	-
Sclerotium sp.	-	-	-
Rhizoctonia solani	-	-	-
Septoria sp.	-	-	-
Cladosporium sp.	-	-	-
Macrophomina sp.	-	-	-

+ =Pathogenic - = Nonpathogenic

Table 4.9: *Invitro*Pathogenecityusing Leaf Disk on leaves of Uncultivated Plants

	Uncultivated Plants				
Fungi	***Chlorophytum blepherophyllum***	***Spigeliaa nthelmia***	***Senna singuena***	***Ipomoea nil***	***Combretu m molle***
Fusarium equiseti	+	-	-	-	-
Choanephora sp.	+	-	-	-	-
Macrophoma sp.	-	+	-	-	-
Nigrosporaaerophila	-	+	+	-	-
Rhizoctonia solani	-	-	-	+	+

+ = Pathogenic. - = Not pathogenic

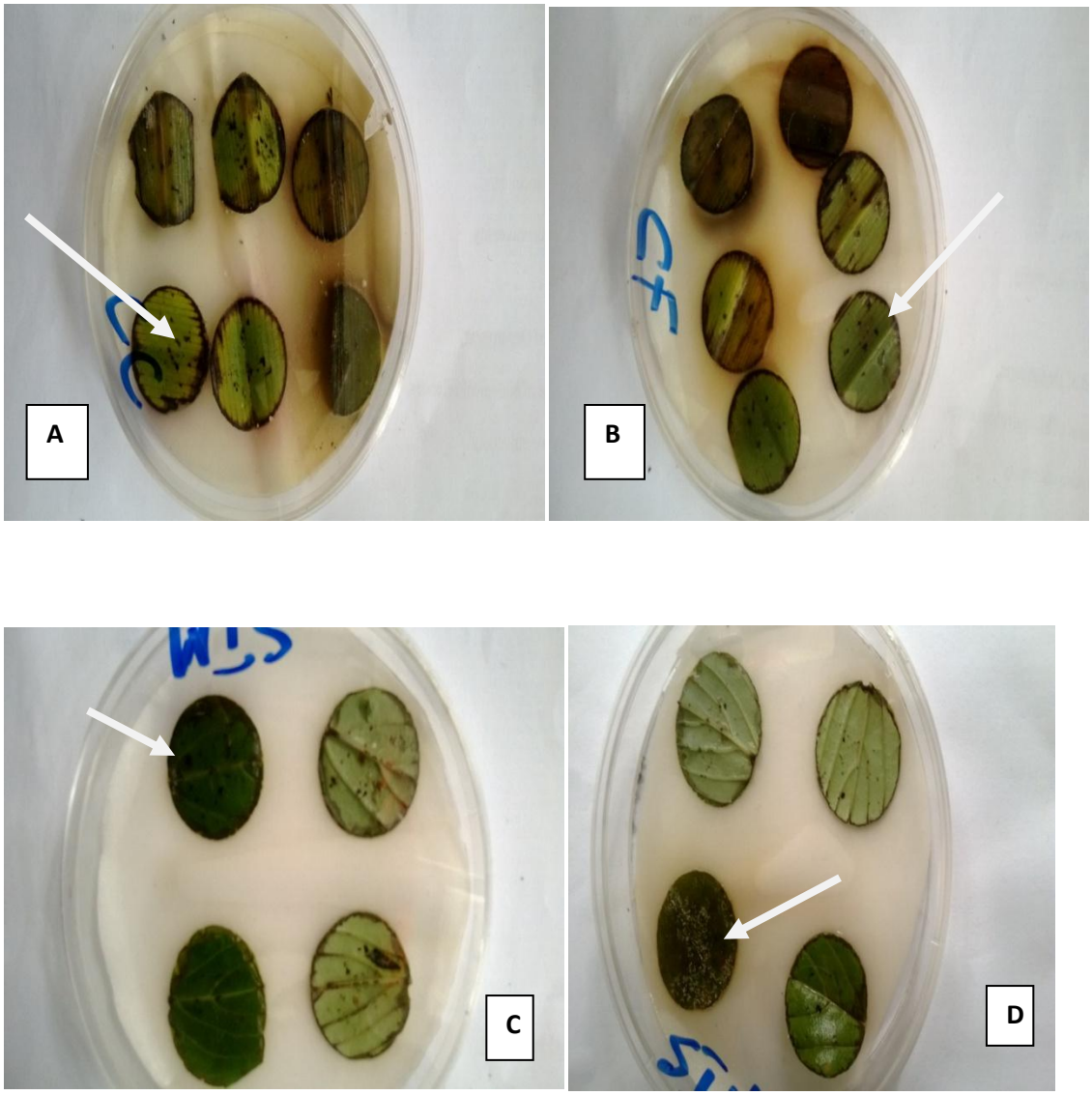


Plate 24:Leaf of uncultivated plants inoculated with fungishowing symptoms. A: Leaf of *Chlorophytum blepherophyllum* inoculated with *Choanephora* sp.showing symptoms. B:Leaf of *Chlorophytum blepherophyllum* inoculated with *Fusarium aerophila*showing symptoms. C: Leaf of *Spigelia anthelmia* inoculated with *Macrophoma* sp.showing symptoms. D:Leaf of *Spigelia anthelmia* inoculated with *Nigrospora aerophila*showing symptoms.

CHAPTRE FIVE

5.0 DISCUSSION

5.1 Fungi from Cultivated Plants

A total of fourteen different types of spot symptoms have been observed in these studies. Different types of spot symptoms observed varied in shapes and colors depending on the plant.

Rhizoctoniasolani was isolated from five different types of symptoms (circular dark brown, raised circular dark brown spots, lesions along sutures having grey centre, oval spots with white centre with brown margin and spots on peduncle which had grey centre brown margin) on cowpea pods and peduncle. Adebite and Amusa(2008) reported similar symptoms on cowpea pods in South Western Nigeria,i.e cowpea pod having circular dark brown spot.Raised circular dark brown, lesions along sutures having grey centre, oval spots with white centre with brown margin and spots on peduncle which had grey centre brown margin spotswere also observed on cowpea pod which were different from the spots reported by Adebite andAmusa(2008). These types of symptom have not been reported before in Northern Nigeria. The difference in the symptoms may be attributed to the causal organisms, stage of development and/ or due to varietal differences. In this study *R. solani* was not isolated from any other part other than the pod and peduncle, though young stems and leaves have been reported (Emechebe *et al,* 1980; Allen and Lenne, 1998; Adebite and Amusa, 2008).

Sclerotium sp.was isolated from cowpea pod andpeduncle in these studies, this might be due to varietal difference that is only restricted to pod and peduncle, although (Adejumo and Ikotun, 2003)had earlier reported *Sclerotium* rot on cowpea stem which produced fan of silking mycelium. Emechebe *et al.*(1980) also reported *Sclerotiumrolfsii* on stem of cowpea in Northern Guinea savannah. Occurrence of *Sclerotium* sp. on cowpea pod and peduncle is the first report from Northern Guinea Savannah. Since the species was not identified, it may be a different species as earlier reported byAdejumo and Ikotun(2003) and Emechebe *et al.*(1980).

*Septoria*sp. was isolated from white lesions with concentric ring in this studies which was similar to report of RPD (1999) and Ojiambo*et al.*(2007) which reported *Septoria* leaf spot to produce circular to angular grayish white center with dark margin. It has been reported on cowpea leaves, pods and stem inducing symptoms which appear as whitish lesions with concentric rings (Singh and Allen, 1979).

Cladosporium sp. was found to produce brown coalesced lesions making this to be the first report of this type of symptom on cowpea pods in Zaria area. Howard and David, 2007 reported symptoms of *Cladosporium* leaf spot on egg plant and pepper to have purplish brown on the upper surface of the leaf. Emechebe*et al.*(1980) reported *Cladosporium* sp.to be a seed borne fungus on cowpea in Northern Guinea Savannah.

Macrophomina sp. was isolated from cowpea pod having coalesced dark brown lesion. This type of symptom is being reported on cowpea pod for the first time in Samaru.Amusa*et al.*(2007) reported *Macrophomina* sp. on cowpea in Ibadan Nigeria. It has been reported to induce a wide range of diseases/symptoms such as causing charcoal

rot (damping off), dry root rot, wilt, leaf blight and ashy stem blight (Emechebe and Lagoke, 2002; Singh *etal.*, 1990, Abdon*etal*., 1980). *Macrophominaphaseolina*was reported to cause pod blight of soybean and stem of cowpea Northern guinea savannah (Emechebe *et al.*, 1980).

Fusarium equiseti was isolated from cowpea peduncle. It has been reported on cowpea leaves and seeds causing it to turn yellow followed by defoliation (Singh and Allen, 1979). Emechebe *et al.*, (1980) reported *Fusarium equisetum* to beseed borne in cowpea in the Northern Guinea Savannah.

Isolation of*Curvularia* sp. and *R.solani* from leaf and *Gleosporium* sp. and *Cladosporium* sp. on stem of *T. occidentalis* are reported for the first time in Samaru. These types of symptoms have been reported earlier on other plants (Iken and Amusa, 2004; Fajemisin and Okuyemi, 1976;Olaoye, 2009;Akram*et al* 2014;Emechebe *et al*, 1980; Olson, 1978; Toit and Vernon 2006), but not on *T. occidentalis.*

A total of seventeen fungi were isolated from cultivated plants. *R. solani* followed by *Sclerotium* sp and *Cladosporium* sp were found to be more prevalent. It may be due to the fact that *R. solani* has the capability to induce spot on different plants readily which may be attributed to varying types of enzymes produced by *R. solani*(Bateman *et al.*, 1969).

5.2 Fungi from uncultivated plants

The investigation on fungi causing weed diseases has been reported by many researchers in different part of the world (Adebitan, 1998; Black *et al.*,1996a; Suwanagu*et al.*, 2013). Four different weeds (*Chlorophytum blepherophyllum,Spigelia anthelmia,Trianthema*

*portulacastrum*and*Ipomoea nil*) collectedfrom the vicinity of cowpea farm, all showed spots symptoms which were similar in shape but with minor variations inmargin and centre coloration. From these symptoms however one or more taxonomically different fungi were isolated. So variation in color might be attributed to difference in types of fungi associated with the symptoms. The fungi have been reported on other plant species (Emechebe*et a.l,* 1980; Oladiran, 1980;Blazquiz, 1986;Nelson, 1992; Adejumo and Ikotun, 2003; Verma and Gupta, 2008;Officer *et al.*, 2012; Zheng*et al.*,2012; Marley, 2013; Benson, 1996; Gross *et al*., 1998; Verma*et al.*, 2006). *Fusarium equiseti*and *R. solani* was also isolated from cowpea pod and peduncle and *T. occidentalis* in this study. This shows that *Chlorophytum blepherophyllum*and *Ipomoea nil*may serve as reservoir hosts of these fungi which are a threat to cowpea and *T. occidentalis*.

The four different wild plants(*Clematis hirsuta,Combretum molle,Senna singuena*and *Porphyrostemma chevalieri*)collectedfrom the vicinity of cowpea farm;all had their individual peculiar symptoms.

*Nigrospora aerophila*was isolated from *Clematis hirsuta*and *Senna singuena* althoughthe spot types were different. Thedifference may be attributed plant and/or variation in fungal biological race. Other species of *Nigrospora* had been reported to cause leaf spot on many other cultivated and wild plants(Emechebe *et al,* 1980; Nutsugah*et al.*, 2004;Verma and Gupta, 2008;Zheng*et al.*, 2012). This association of *N. aerophila* is being reported with leaf spot for the first time on *Clematis hirsuta* and *Senna singuena.*

This is also the first time *Rhizoctonia solani*was isolated fromleaves of *Combretum molle* in Zaria. This fungus was isolated from cowpea pods and *Telfairia occidentalis*leaves in

this studies. This shows that *Combretum molle* may be acting as reservoir host of the fungus. This fungus has been reported on other cultivated crops and wild plants (Benson, 1996; Gross *et al.*,2005; Verma*et al,* 2006; Gonzalez *et al.,* 2011).

Three different fungi were isolated from leaves of *Porphyrostemma chevalieri;Macrophomina* sp.*, Alternaria* sp.*, and Helicosporium* sp. It is however not sure if the leaf spot symptoms wereproduce by a single fungus or more than a single fungus.This is the first report of association of these fungi with*Porphyrostemmachevalieri*. *Alternaria* sp.and *Macrophomina* sp. have been reported to cause leaf spot on a number of cultivated and wild plants(Ibrahim *et al*, 1975b; Jackson, 1959; Emechebe *et al.*, 1980;Abdon *et al.*, 1980 Cohen and Rotem, 1987; Singh *et al.*,1990; Kucharek, 2000; Chidambaram, 2007; Reis and Boiteux, 2010;Kehinde, 2011;Motllagh, 2011; Fuhlbohmetal, 2012; Watt, 2013).*Macrophomina* sp. isolated from this plant was also isolated from cowpea pod in this studies, this suggest that this plant may serve as reservoir host for this fungi.

Percentage occurrence of fungi isolated from weeds and wild plant shows *Nigrospora aerophila*followed by *Rhizoctonia solani*. This may be due to the fungi having wide host range.

The close relationship observed between some of the fungi isolated from cultivated and uncultivated plants (*Fusarium equiseti,* and *Rhizoctonia solani*) enhances the understanding of the ecology and epidemiology of fungi inducing spot disease. It indicates that there may be possible transmission between hosts where the uncultivated hosts are found near cultivated fields. It also suggest the change in time of planting the

cultivated plants because the uncultivated plants serve as reservoir hosts of the fungi which will later be transmitted through wind or rain splash to cultivated plants when available. Other fungi isolated (*Nigrospora aerophila* and *Macrophoma* sp.) were only closely related to the uncultivated plants, this indicate that they are restricted to the uncultivated plants studied so they can be use for biocontrol of the uncultivated plants.

Pathogenecity test of fungi on three varieties of cowpea showed that the fungi are not pathogenic to cowpea varieties tested. A single fungus may not be responsible for the spot but a combination of more than one fungus. The varieties used to test pathogenecity were different from the varieties which the fungi were isolated and these may be resistant to the isolated fungi.

*Invitro*pathogenecity test carried out on some leaves of weeds and wild plants showed that *Chlorophytum blepherophyllum* is a susceptible host of *Fusarium equisetum and Choanephora*sp.because inoculation with these pathogens produced symptoms.*Spigelia anthelmia* was found to be susceptible to*Nigrospora aerophila* and *Macrophoma* sp. as these pathogens produced symptoms on inoculated leaf, there is no report on these pathogens infecting this plant. *Senna singuena* was also found to be a host of *Nigrospora aerophila.*

CHAPTER SIX

6.0 Summary, Conclusions and Recommendations

6.1 Summary

Fungi associated with spot symptoms on cultivated plants (*Vignaunguiculata*L Walp, and *Telfairiaoccidentalis*) and uncultivated plants (*Chlorophytum blepherophyllum, Spigelia anthelmia, Ipomoea nil, Trianthema portulacastrum, Senna singuena, Combretum molle, Porphyrostemma chevalieri* and *Clematis hirsute*) were studied.Sixteen different types of spot symptoms were observed on cultivated plants and eight on uncultivated plants. Ten fungi were found to be associated with spot symptoms on cultivated plants and ten fungi were found to be associated with spot symptoms on uncultivated plants. *R. solani* was found associated with both spots on cultivated and uncultivated plants. The symptoms observed on cultivated crops and uncultivated plants in these studies will enhance the differentiation of leaf spot symptoms induce by fungi and other causes of spot symptoms. The occurrence of similar fungi in both cultivated and uncultivated plants, suggest the possible transmission within the hosts which suggest proper management of weed within the cowpea and *Telfairia occidentalis*farms.

6.2 Conclusions

1. Sixteen different types of spot symptoms were observed on cultivated plants and eight on uncultivated plants.

2. Ten fungi were found to be associated with spot symptoms on cultivated plants and ten fungi were found to be associated with spot symptoms on uncultivated plants.There is a relationship between fungi isolated from cultivated and uncultivated plants.

6.3Recommendations

1. The symptoms observed on cultivated and uncultivated plants in these studies will enhance the differentiation of leaf spot symptoms induced by fungi from symptoms caused by pesticides and insecticides.

2. The occurrence of relationship between fungi from cultivated and uncultivated plants, suggest the possible transmission within the hosts which suggest proper management of weed within the cowpea and *Telfairia occidentalis*farms.

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APPENDIX I

ConidiaConcentrations

Concentration of each fungus used is shown below;

Fusarium equisetum =1x 10^6conidia/ml

Choanephora sp. = 3 x 10^5 conidia/ml

Macrophoma sp. = 3 x 10^5 conidia/ml

Nigrospora aerophila = 7 x 10^5conidia/ml

Septoria sp. =3 x10^5 conidia/ml

*Clladosporium*sp. = 1 x 10^6 conidia/ml

Macrophomina sp. = 3 x 10^5conidia/ml