RESPONSE OF *Jatropha curcas* L. TO FUNGAL PATHOGENS IN SOUTHWESTERN NIGERIA

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ABSTRACT

Cultivation of *Jatropha curcas* L. for production of biodiesel as an alternative source of energy is crucial for sustainable development in Nigeria. Pests and pathogens especially fungi have been reported to be a major constraint to commercial cultivation of *J. curcas* in Asia and some West African countries. However, this is yet to be fully investigated and documented in Nigeria. Fungal pathogens infecting *J. curcas* in southwestern Nigeria were therefore investigated.

Two hundred plant samples were randomly collected from each of Ekiti, Lagos, Ogun, Ondo, Osun and Oyo states, Nigeria. Fungi were isolated from these samples and the isolates were identified using standard procedures. Pathogenicity tests of these organisms (n=12) were carried out on five *J. curcas* accessions (Ex-Basirika, Ex-Mbatdiya, Ex-Misau, Ex-Ibadan and Ex-Kano) in the screenhouse using Koch's postulates. The experiment was laid out in a Completely Randomized Design (CRD) with four replicates. The accessions were also grown for three years on the field in Randomized Complete Block Design (RCBD) layout with four replicates. Data were collected on occurrence of fungal organisms. Disease intensity (incidence and severity) were recorded at weekly intervals for 16 weeks after inoculation. Field evaluation of disease incidence (%) and severity (1-5, ranging from no disease to highly susceptible) was carried out at three months interval for three years. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Anthracnose, mildew, canker, dieback, fruit and root rots were common symptoms found on *J. curcas* in southwestern Nigeria. *Colletotrichum gloeosporioides* was the highest occurring fungal species in all the states with the highest occurrence (26.0 %) in Ekiti. *Oidum jatrophae* was the lowest with the least occurrence (7.1 %) in Lagos state. *Colletotrichum gloeosporioides, Curvularia lunata, Fusarium oxysporum, Oidum jatrophae* and *Lasiodiplodia theobromae* among the isolated fungi were pathogenic on *Jatropha curcas* in the screenhouse experiment. Reactions of the accessions to fungal diseases showed significant variations in disease incidence and severity. Susceptibility of accessions was in the order: Ex-Ibadan > Ex-Kano > Ex-Mbatdiya > Ex-Basirika > Ex-Misau (61.4 % > 59.5 % > 43.4 % > 37.4 % and > 28.3 %, respectively). Anthracnose was

the most prevalent disease encountered, with maximum incidence in the screenhouse (26.6 %) and on the field (95.7 %). Disease intensity for *Colletotrichum* leaf anthracnose, *L. theobromae* collar rots, Cankers, *Oidum* mildew, shoot dieback and *F. oxysporum* root rots was 59.7 %, 26.5 % , 16.4 %, 41.9 %, 43.1 % and 38.9 % respectively.

Five pathogenic and seven non-pathogenic fungi were isolated from *Jatropha curcas* in southwestern Nigeria. *Colletotrichum gloeosporioides* was the major isolated fungal pathogen and was the most virulent on *Jatropha curcas*. Ex-Misau was tolerant and it is therefore recommended for intensive cultivation.

Keywords: *Jatropha curcas* accessions, Pathogenicity, Fungal infection, Anthracnose, *Colletotrichum gloeosporioides*

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CERTIFICATION

I certify that this study was carried out by Joy Oluchi Nwogwugwu in the Department of Crop Protection and Environmental Biology University of Ibadan, Nigeria.

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DEDICATION

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INTRODUCTION

Jatropha curcas L. (hereafter referred to as "Jatropha") or Physic nut is a perennial deciduous shrub belonging to the family Euphorbiaceae (Carels, 2009). It originated in Central America and is now widely distributed in the tropics and subtropics (Foidl *et al.*, 2007; Heller 1996; Prueksakorn *et al*., 2006; David *et al.*, 2009). *Jatropha* has several properties such as its hardiness, rapid growth, easy propagation and widely ranging usefulness which led to its spread by the Portuguese traders as a valuable hedge plant and an oil yielding species (Dhilon *et al.*, 2008; Zimbabwe Biomass News, 1996).

A worldwide interest in physic nut has developed since it has been shown that it has an enormous potential to be used as a biofuel feedstock (Achten *et al.*, 2010). It produces seed that can be processed into non-polluting biodiesel. The oil contains 21% saturated fatty acids and 79% unsaturated fatty acids (Gubitz *et al.*, 1999). Its biodiesel has similar characteristics to those of fossil diesel and can be used as a substitute (Makkar and Becker, 2009). The optimistic yield estimate of diesel from physic nut is 1,300 litres oil per hectare, lower than oil palm but higher than canola (Anonymous, 2007; Kochar *et al*., 2005). The oil content of the seeds can be as high as 40 % (Kandpal and Madan, 1995). It is an underutilized, oil-bearing crop which can be exploited to provide opportunities for good returns and rural development.

The seeds contain viscous oil, 50% by weight; which can be used for the manufacture of candles and soap in the cosmetics industry and for cooking and lighting (Liu *et al*., 1997). The wood of Jatropha can be used for numerous purposes including fuel and it has relatively high efficiency of carbon sequestration (Kumar and Sharma, 2008; Francis *et al*., 2005). As it is not a forage crop, it plays an important role in keeping out grazing animals and protecting other valuable food or cash crops. *Jatropha* products from the fruit, seed

coat and seed cake- are rich in nitrogen, phosphorus and potassium (NPK) and are used as fertilizers to improve soil fertility (Flemming, 2009; Rockefeller Foundation, 1998).

J. curcas possesses many medicinal properties, its seed oil is used for treatment of a number of human and veterinary ailments, like piles, snakebites, paralysis, dropsy, rheumatism, sciatica, and skin diseases, such as, scabies, eczemas and ringworm, amongst many other uses (Perry, 1980; Nath and Dutta, 1992; Liu *et al*., 1997; Kumar and Sharma, 2008). Almost all the plant parts of this species exude sticky, opalescent, acrid and astringent latex. The latex of *J. curcas* contains an alkaloid known as '*Jatrophine'*, which has anti-cancerous properties (Mampane *et al*., 1987), the latex is also used as an external application for skin diseases, rheumatism and for sores on domestic livestock (Gübitz *et al.*, 1999). Tender twigs of the plant are used for cleaning the teeth and twig sap is used for dressing wound and ulcers. Extract from the leaves and seed has molluscicidal, insecticidal and fungicidal properties (Solsoloy and Solsoloy, 1997; Nwosu and Okafor, 1995). A decoction of the leaves is used against cough and as an antiseptic after birth (Heller, 1996).

The plant has a wide range of adaptability for climatic and edaphic factors and grows well even on marginal lands, enduring drought, alkalinity/salinity of the soil. It can be grown on land that is normally considered unsuitable for agriculture and so does not have the same potential as the other bio-fuel crops to cause food-price inflation. It serves as good source to green-up barren wastelands and it is also suitable for preventing soil erosion and shifting sand dunes (Friends of the Earth, 2009).

Crude oil (fossil fuel) has been the main source of energy, its damage to the environment and its limited reserves necessitate research on alternative sources of energy such as oleaginous plants. In the 1980s, the concern grew that global warming and the resulting climatic change were enhanced by CO_2 emissions resulting from fossil fuel consumption (Okonkwo, 2001). The use of other alternatives to crude oil has important implications for meeting the demands of rural energy services. These characteristics along with its versatility make it of vital importance to developing countries subjected to decreasing tree cover and soil fertility (Rockfeller Foundation1998; Reinhard and Rothkreuz, 1999). Jatropha has become an example for the tremendous hope placed on novel crops that "offer

all the benefits of biofuels without the pitfalls" (Renner, 2007) to deliver oilseeds from marginal lands and in semi-arid regions without compromising food production, diminishing natural resources or ecosystem services, such as carbon stocks and soil fertility.

However, despite its uses, yield can be constrained by the prevalence of pests and diseases. Several biotic and abiotic constraints limit Jatropha production (Behera *et al.,* 2010). Existing literature indicates that contrary to popular belief, toxicity and insecticidal properties of *J. curcas* are not sufficient deterrent for insect pests and pathogens that may cause economic damage in plantations. Physic nut suffers from several fungal and bacterial diseases and more recently by the Jatropha mosaic India virus (JMIV) (Rangaswamy *et al*., 2005). Foidl *et al*. (2007) reported that pests can cause up to 57 % damage on physic nut. The attack by pests and diseases is a limiting factor in achieving optimum production especially under sole monocultures, where pests and disease control may become inevitable (Otieno and Mwangi, 2009; Xiao *et al*., 2009). Incidence of pests and diseases is widely reported under plantation monoculture and may be of economic significance (Nasibulina, 2010; Hawkins and Chen, 2011). In some regions the incidence of pests and diseases is increasing as the crop is grown more intensively over larger areas and planted throughout the year for bio-fuel processing. Communities and farmers have consistently recounted cases of *Jatropha* pests spreading to other food crops such as cassava, sorghum, maize and peanuts; and an increasing number of experts are also raising similar concerns (FAO, 2010; Achten *et al*., 2010). It is a host to the fungus causing "frogeye" disease *(Cercospora* spp.) which is common on tobacco (Grimm and Maes, 1997). Several other important diseases found on Jatropha among others, are Alternaria spots, rust, Cercospora leafspots, Fusarium wilt, collar rot, root rot, damping-off and bacteria leaf spots (FACT, 2006; Rodriues *et al*., 2011).

Evidence on how environmental benefits and developmental potentials can be achieved through Jatropha cultivation is still lacking (German *et al.,* 2011; Pandey *et al.,* 2012). Intensification of a plant generally tends to increase the threat of pests and diseases (Fry, 1982; Savary and Zadoks, 1992; Agrios, 2005). The change in cultivation pattern of physic

nut into intensive monoculture pattern should be followed by raising awareness about its pests and diseases.

In order to obtain necessary information on *J. curcas* diseases in Nigeria that would serve as baseline information and in creating awareness on the likely threats from fungal diseases that could be encountered on Jatropha plantations in Southwestern Nigeria, the following were the aims and objectives of this study:

1. investigation of fungal organisms associated with *J. curcas* in the six states of Southwestern Nigeria

2. investigation of the pathogenicity of the fungal pathogens associated with the plant in Ibadan, Southwestern Nigeria

3. evaluation of the responses of *J.curcas* accessions to the most prevalent fungal diseases under screenhouse and field conditions in Ibadan.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Current Distribution of *Jatropha curcas* L

The genus Jatropha (Plate 2.1), is native to Mexico, where there are approximately 45 species, 77% of which are endemic (Rodríguez-Acosta *et al*., 2009). The genus includes 175 species in the world (Dehgan and Webster, 1979; Ye *et al.,* 2009). The center of diversity of *Jatropha* is the Mesoamerican region (Mexico and Central America), which is illustrated by the fact that more than 100 out of 175 species of *Jatropha* are native to that region (Rao *et al*., 2008). Jiménez and Vega (2011) reported that there are 41 native species in Mexico, of which 31 are strictly endemic. Several species of *Jatropha* are native to South America (Ovando-Medina *et al*., 2011).

Jatropha curcas is widely present throughout Central America, Africa and Asia, where it is grown as a hedge plant (Basha *et al.,* 2009; Subramanyam *et al*., 2009; Ovando-Medina *et al.,* 2011). *J. curcas* was probably first exported from Central America to Africa around 1800 (FAO, 2010). It was initially introduced to the Cabo Verde Islands and Guinea Bissau in western Africa, and subsequently distributed across other tropical and subtropical Africa and Asia (Heller, 1996; Ratree, 2004).

The tree is well adapted to arid conditions and has gained worldwide economic significance as a potential species for biofuel production (Fact Foundation, 2006). All the exported provenances are reported to be toxic to humans (Heller 1996; Solomon *et al*., 2002). Dispersed non-toxic provenances of this species have been found only in Mexico (Heller 1996, Martínez *et al*., 2006).

Plate 2.1 *Jatropha curcas* plant (a) and fruits (b)

2.1.1 Taxonomy and related species of *J. curcas*

Jatropha (family: Euphorbiaceae) is a morphologically diverse genus comprising 160–175 species of trees, shrubs, rhizomatous sub-shrubs and herbs distributed mainly in the seasonally dry tropics (Dehgan, 1984). Three species of *Jatropha* have naturalised in Queensland: *Jatropha curcas* L., *Jatropha gossypiifolia* L. and *Jatropha podagrica* Hook (Queensland Herbarium records, 2007). Another two species, *J. integerrima* Jacq and *J. multifida* L., are known from cultivated specimens. These two species, along with *J. podagrica* Hook, are currently sold as garden ornamentals. There have been three detailed taxonomic studies of *Jatropha*: Pax (1910), McVaugh (1945) and Dehgan and Webster (1979). The existence of a partially fertile hybrid between *J. curcas* and *J. integerrima* prompted the review by Dehgan and Webster (1979), which resulted in the recognition of two subgenera (*Curcas* and *Jatropha*), 10 sections and 10 subsections. Dehgan (1984) attempted inter-specific hybridisations between 20 species of eight of the 10 sections that explored the genetic relationships between the various taxa.

McVaugh (1945), Wilbur (1954), Dehgan and Webster (1979) and Dehgan and Schutzman (1994) agree that *J. curcas* is the most primitive member of the genus. It appears to be very closely related to a number of congeners since it can interbreed (as maternal parent) with many species in both subgenera *Curcas* and *Jatropha*. *Jatropha* species *(J. berlandieri, J. podagrica, J. nudicaulis*) are ornamental plants aptly called ‘ogiri oke’ in recognition of its common use as boundary mark in Ojoto and other Igbo-speaking parts of South East Nigeria (Nwosu and Okafor, 1995; Das *et al*., 2010).

2.1.2 Propagation and cultivation of Jatropha

Jatropha grows readily from seeds or cuttings; however, trees propagated by cuttings show a lower longevity and possess a lower drought and disease resistance than those propagated by seeds (Matyas, 1986). Trees produced from cuttings do not produce true taproots (hence they are less drought tolerant), rather they produce pseudo-taproots that may penetrate only 1/2 to 2/3rds the depth of the soil as taproots produced on trees grown from seed.

Use of branch cuttings for propagation is easy and results in rapid growth (Jones and Miller, 1992).

Allsopp and Stock, (1994) and Parsons (2008) recommended the use of *mycorrhizae* to enhance yields through increased drought tolerance, facilitating moisture transport, uptake of other essential nutrients and surviving stressful conditions. *Mycorrhizal* associations have been observed with Jatropha and are known to aid the plant's growth under conditions where phosphate is limiting (Jones and Miller, 1992). *Jatropha* though described as having a low nutrient requirement because it is adapted to growing in poor soils. However, growing a productive crop requires correct fertilization and adequate rainfall or irrigation. Equally, high levels of fertilizer and excessive irrigation can induce high total biomass production at the expense of seed yield. Ghosh *et al.* (2007) recorded that on wasteland in India, 3.0 tons per ha. of *Jatropha* seed cake (also known as "press" cake), containing 3.2 percent N, 1.2 percent P_2O_5 and 1.4 percent K_2O, increased yields significantly when applied to young plants of Jatropha.. A trial at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India showed increasing yield with fertilization to an optimum level at 100g Urea + 38g SSP (Single Super Phosphate), but that over-application depressed yields (Achten *et al*., 2008).

The plant has a wide range of adaptability for climatic and edaphic factors and grows well even on marginal lands, enduring drought, alkalinity/salinity of soil and thus, serves as best source to green-up barren wastelands (Tewari, 2007). The wide geographical and climatic distribution is indicative of the fact that there exists a tremendous genetic diversity (Ginwal *et al.*, 2004). Although *Jatropha* can survive precipitation as low as 300 mm, by shedding its leaves, it does not produce well under such conditions.. Rainfall induces flowering as well as drought. The cycle of flowering can thus be influenced using irrigation. High humidity or high rainfall can result in more fungus attacks to which the plant is sensitive (Manurung, 2007).

There are ecological areas suitable for cultivating Jatropha apart from the plant being believed to possessing several properties such as its hardiness, rapid growth (Dhilon *et al.*, 2008). According to Henning (1997), *Jatropha* sheds its leaves during the dry season,

current distribution shows that introduction has been most successful in drier regions of the tropics with an average annual rainfall of between 300 and 1000mm, and occurs mainly at lower altitudes (0-500m). It is not sensitive to day length. It grows best on well-drained soils with good aeration.

Fresh seeds of *J. curcas* have high levels of viability but low levels of germination, suggesting innate (primary) dormancy (AgroForestryTree Database, 2007). In Zimbabwe, germination rates of more than 93% were reported for air-dried seeds kept in uncontrolled room conditions for up to 12 months (Jepsen *et al*., 2004). Similarly, Ratree (2004) found that germination rates of *J. curcas* seeds fell from 90% to 43% after 112 days storage at room temperature. While seed samples older than 15 months are less than 50% viable, a small percentage of seeds can remain viable for up to seven years (Anon, 2005). Dagar *et al*. (2004) observed that germination of *J. curcas* seeds was highest at a soil pH of 8 (90% germination) and most rapid at a soil pH of 6–8 (six days to maximum germination). Seed size is correlated with percentage germination, seeds less than 300 mg failing to germinate. The seed has an important content of protein (25-30%) and fat (55-60%); therefore seed storage should not exceed 10 to 15 months ensuring seed quality and for its oil content (Alfonso, 2007).

2.2 Economic Benefits of *J. curcas*

Although ability to control land degradation and oil production are most important environmental uses of Jatropha, its products provide numerous other benefits that would additionally improve the living conditions of the rural people and offer greater income opportunities through enhanced rural employment (Bradley *et al.*, 2009).

2.2.1 Medicinal uses of Jatropha

It is a multipurpose crop of significant economic importance as a biofuel. Moreover, all parts of the shrub are used in traditional medicine and as raw material for pharmaceutical and cosmetic industries (Liu et al., 1997; Paramathma *et al*., 2006). Fernández (2004) reported that the use of Jatropha plant for traditional medicinal uses (both human and veterinary purposes) is being scientifically investigated. *J. curcas* species belong to the

family Euphorbiaceae which has great significance in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America (Burkill, 1994). It is commonly called physic nut, purging nut or pig nut. The oil is a strong purgative, widely used as an antiseptic for cough, skin diseases, and as a pain reliever from rheumatism. Jatropha latex can heal wounds and also has anti-microbial properties. Previous studies have reported that the plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm, sores and joint rheumatism (Irvine, 1961). The water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity (Matsuse *et al*., 1999). Preparations of all parts of the plant, including seeds, leaves and bark are also used in traditional medicine and for veterinary purposes; it is also used to treat various skin diseases and rheumatism (Heller, 1996). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002). The seeds are laxative, but they partially lose that property when roasted, and are often eaten in some regions of Mexico. This plant has been considered toxic because of the alkaloids found in the seed, known as phorbol esters, which cause the laxative effect and some other symptoms. Only in Mexico, there has been found varieties with very low content of toxins, which are eaten roasted and prepared in traditional dishes by the people (CGIAR**,** 2008).

2.2.2 Fertilizer production from seed of Jatropha

Jatropha seed cake makes an excellent organic fertilizer with high nitrogen content similar to, or better than, chicken manure. As organic manure, the seed cake can make a valuable contribution to micronutrient requirement of crops (Patolia *et al.*, 2007). The cake resulting from the extraction of oil, which comes from toxic varieties, can only be used to produce fertilizers; after extracting the alkaloids, or if the cake comes from edible seeds, it can be used in animal feed. About 50 percent of the original seed weight remains as seed cake residue, in the form of protein and carbohydrates after extracting the oil. The amount of oil left in the seed cake depends on the extraction process. There are trade-offs for the seed cake. It may be used as fertilizer, fuel or, if it is detoxified or if non-toxic varieties are used, it can be used as animal feed (Henning, 2007). However, it is significant that not returning the seed cake to the plantation as fertilizer reduces the utility of Jatropha in improving

degraded land. Jatropha seed cake is high in protein – 58.1 % by weight compared to soy meal's 48 %t – and would be a valuable livestock protein feed supplement if it were not for its toxicity (Gaydou, 1982; Makkar *et al*., 1997).

2.2.3 Commercial raw material for soap and vanish manufacture from Jatropha oil

Jatropha oil has been used commercially as a raw material for soap manufacture for decades, both by large and small industrial producers. Soap from Jatropha oil is being made by small informal industries in rural areas in both Zimbabwe and Mali (NRI, 1998; IPGRI, 1996). It is also used to prepare varnish after being burned with iron oxides, or as an excellent substitute for industrial oils. In Europe, it is used in the threading of wool and textile manufacturing. It is used together with ashes of burned bananas to make a hard homemade soap. The juice of the leaf is used to dye fabrics with an indelible black colour. The crust has 37% tannins that give a dark blue dye. Its latex also has 10% tannin and can be used as ink. The oil is used as lubricants, soap and candle manufacturing. It has also been reported as hair growth stimulant and thus can be used as hair oil (Joshi, 2000).

2.2.4 Pesticide uses of Jatropha oil

Jatropha oil has molluscicidal properties against the vector snails of the *Schistosoma* parasite that causes bilharzia. The emulsified oil has been found to be an effective insecticide against weevil pests and houseflies, and an oil extract has been found to control cotton bollworm and sorghum stem borers (Gubitz *et al*., 1999). Achten *et al.* (2008) reported that the oil extract of *J. curcas* can be used as an insecticide, molluscicide, fungicide and nematicide. Fagbenro-Beyioku *et al.* (1998) investigated and reported the anti-parasitic activity of the sap and crushed leaves of *J. curcas*. For instance, it has been used in the control of insect pests of cotton including cotton bollworm, and on pests of pulses, potato and corn.

Methanol extracts of Jatropha seed (which contains biodegradable toxins) are being tested in Germany for control of bilharzia-carrying water snails (Spencer and Spencer, 2004). And the pesticidal action of the seed oil is also the subject of research of International Crops Research Institute for the Semi-Arid Tropics, (ICRISAT) in India. These potential uses are yet to be commercialized. It has not been reported useful as animal feed, because

the oil and seed possess toxic constituents like curcine, curcasin, resinous texalbumin resembling ricin and a substance which has nauseating and purgative properties and create digestion problems in live-stock (King *et al*., 2009).

2.2.5 Fuel production from Jatropha seed

The seed cake has a high energy content of 25MJ kg-1. Experiments have shown that some 60 percent more biogas was produced from Jatropha seed cake in anaerobic digesters *than* from cattle dung, and that it had a higher calorific value (Abreu, 2008). The residue from the biogas digester can be used further as a fertilizer. Where cow dung is used for household fuel, as in India, the seed cake can be combined with cow dung and cellulosic crop residues, such as seed husks, to make fuel *briquette*. Biogas has been produced from fruit shells. In addition, trials showed that seed husks can be used as a feedstock for a gasification plant (Achten *et al*., 2008). *Jatropha* fruit shells and seed husks can be used for direct combustion. Since the shells make up around 35–40 percent of the whole fruit by weight and have a calorific value approaching that of fuelwood, they could be a useful by-product of Jatropha oil production. The calorific values of *Prosopis juliflora* (a fuelwood species of semi-arid areas) and Jatropha fruit shells are similar.

However, four times volume of fruit shells is required to equal the heating value of fuel wood, due to their lower bulk density. Seed husks have a higher heating value and greater bulk density which makes them more valuable than the fruit shells as a combustible fuel. However, the technology required to separate the seed husk from the kernel is more suited to large processing plants than small rural industry. The fruit shells can be dried and ground to a powder and formed into fuel briquettes. A trial found that 1 kg of briquettes took around 35 minutes for complete combustion, giving temperatures in the range of 525ºC–780ºC (Singh *et al.*, 2008). The ash left after combustion of Jatropha shell briquettes is high in potassium, which may be applied to crops in homestead gardens.

2.2.6 Land conservation and carbon sequestration

J. curcas is widely planted in the tropics as a ‘living fence’ around fields and villages (Heller, 1996). In Africa and India, it is planted for reforestation of eroded areas. The plant

has also been suggested as a commodity that can be traded for 'carbon credits'. *J. curcas* is an eligible species for carbon credit trading under the Kyoto Protocol (1994). Mousumi and Verma (2008) suggests that 8 kg CO_2 can be absorbed per year per *J. curcas* tree. Openshaw (2000) suggests that, at maturity, a *J. curcas* plantation would have 400–500 trees per hectare. Every *J. curcas* tree that is planted can sequester some 8 kg (17.6 lbs) of carbon per year. In a commercial cultivation plants may be 2 meters x 2 meters apart, for 2,500 plants per hectare (2.5 acres). This means that a typical plantation would be able to sequester 20,000 kg (44,100 lbs) of carbon per year per hectare. Similarly, Igboanugo *et al*. (2009) showed that of all the timber and woody species tested *J. curcas* had the highest carbon dioxide sequestration capacity per unit leaf area. The physic nut tree satisfies all the conditions set down under the UN Kyoto convention as a positive sink tree that can be used for afforestation/reforestation projects to accomplish carbon sequestration (increasing the carbon stored by tree in a forest). Under the Clean Development Mechanism (CDM), afforestation and reforestation projects through planting, seeding and /or the human induced promotion of natural seed sources, forested land should be for at least 50 years (Jones and Miller, 1991; Mousumi and Verma, 2008). Under the definition of a forest of a CDM afforestation or reforestation project, a single minimum tree crown cover value should be 10% to 30%. The minimum height value should be between two and five meters. The physic nut tree lasts for over 50 years, and develops a crown cover value of over 30% and the average height of the single tree ranges from 4.5 to 5 meters. The physic nut tree can therefore be used for CDM afforestation and/ or reforestation project.

2.2.7 Biodiesel production from seeds of Jatropha

Special interest has been shown in the cultivation of physic nut for the purpose of biodiesel (Hennenberg *et al*., 2009; Smeets and Faaij, 2010). The oil content of *J. curcas* seeds is high and could be up to 40% (Kandpal and Madan, 1995). At present, there is considerable worldwide commercial interest in the prospect of producing 'bio-diesel' from oil in the seeds of *J. curcas*, with large plantations recently established in India and across other developing nations (Moreira, 2006). Since the oil crisis of the 1970s and recognition of the limitations of world oil resources, this technology has received special attention. Most of the research was carried out in temperate regions with the aim of making available to

farmers possibilities for diversifying in view of the increasing subsidy-driven surpluses in traditional commodities (ADPP and FACT Foundation, 2006; Singh *et al*., 2008).

Biodiesel refers to a vegetable oil- or animal fat-based diesel fuel consisting of long-chain alkyl (methyl, propyl or ethyl) esters Junginger *et al*., 2010). Typical feedstocks for biodiesel are vegetable oils such as rapeseed oil, soybean oil, palm oil, etc. or animal fat (tallow). In a former GTZ project carried out in Mali, it has been demonstrated that physic nut oil is competitive with imported diesel, especially in remote areas, where fuel is often not available (Lutz 1992; Appropriate Technology International, concept paper "Mali vegetable motor fuel production"). Economic work conducted at the University of Missouri estimated the benefits of producing Bio-diesel in a metropolitan region and concluded that 100 million gallons of Bio-diesel production could generate an estimated $8.34 million increase in personal income and over 6,000 additional temporary or permanent jobs for the metropolitan region (Junginger *et al*., 2010).

World biodiesel production increased six-fold from about 1.8 million tonnes in 2004 to about 10.6 million tonnes in 2008 (Martinot and Sawin, 2009). The EU produces about two-thirds of this, with Germany, France, Italy and Spain being the top EU producers. European biodiesel production rose to 7.8 million tonnes in 2008, equivalent to a 35.7% increase compared to 2007 and 2008. However, EU production declined by 7% in 2009 because of strong competition from abroad (FAPRI, 2009). Other main biodiesel producers include the United States, Argentina and Brazil. Biodiesel consumption in the EU amounted to about 9.2 million tonnes (EurObserv'ER, 2009; Taylor, 2009), with Germany alone consuming 2.9 million tonnes.

2.3 Importance of Plant Diseases

Plant disease as an injurius alteration to plant's physiological processes has negative consequencies on crop production. The significance of plant diseases does not only apply to the countries in which the crops are grown but the introduction of exotic plant pathogens cause great crop losses and additional exotic threats to places where plant propagules are exported to (Roux *et al*., 2005). Fry (1982) noted that plant diseases cause great economic losses to growers, increase price of product to consumers; sometimes cause direct and

severe pathological effects on humans and animals that eat diseased plant products; destroy the environment by damaging plants and trees. In trying to control the diseases, people release tons of toxic pesticides that pollute the water and the environment; they may limit the kinds of plant that can grow in a large geographic area; may also determine the kinds of agricultural industries and the level of employment in an area by affecting the amount and kind of yield available for local consumption or process. EDPO (1994) reported that diseases reduce the quantity and quality of plant production and may make plants dangerous to humans and animals (mycotoxins); cause financial losses; and the cost of controlling plant disease is also direct loss from diseases.

Plant pathogenic fungi are destructive agents causing losses of agricultural commodities in many areas of the world, ranking alongside insects and weeds for crop loss or yield reduction. They can occur on growing in-field crops as well as harvested commodities, leading to damage ranging from rancidity, odour, flavour changes, loss of nutrients, and germ layer destruction. This can result in a reduction in the quality as well as gross spoilage and possible mycotoxin production (Wareing, 2010)

Estimates on crop losses that can be directly attributed to fungi vary. For example, Oerke and Dehne (2004) estimated that, worldwide, weeds caused up to 32% losses, animal pests 18% and pathogens about 15%; for individual crops, fungal losses can be up to 100% if a susceptible cultivar is planted or the climate is favourable in any year.

In the tropics, the high ambient temperatures and high humidities combine to cause major disease problems (Cutler, 1991; FHIA, 2008). The list of crops affected by fungi are broad ranging from, cereals, oilseeds, fruits and vegetables, tree crops, and even animal feeds. Direct crop losses in the field are caused by plant pathogens by reducing crop yields. Some plant pathogens may produce mycotoxins, either as incidental products, or as a chemical associated with the infection process, for example, in some species of Fusarium. Plant pathogens may also render the crop of lower grade by causing blemishes, blights or other quality problems (Gour, 2006). Spoilage fungi may not be able to attack crops in the field, but cause problems once the crop is harvested, if conditions allow. Some spoilage fungi can also produce mycotoxins, for example *Penicillium* and *Aspergillus*.

Wareing *et al*. (2010) reported that *Fusarium* species among other fungal pathogen are implicated in wilts, blights, root rots and cankers of many plants such as legumes, coffee, pine trees, wheat, corn, carnations and grasses. *Aspergillus* species tend to be associated more with tropical and warm temperate crops, for example oilseeds and nuts, since they prefer to grow at relatively high temperatures. *Alternaria* species are both plant pathogens and toxins producers in both pre- and post-harvest commodities (Moss, 2006).

Mycotoxin contamination of crops has been a worldwide problem for thousands of years. However, the significance of the mycotoxins present in foods, the effect on human health and the impact on the economy has been assessed only over the last few decades. Plant fungal pathogens or food spoilage moulds are the source of this type of toxin. The Food and Agriculture Organisation (FAO) estimated that 25% of the world's food crops are affected by mycotoxins during growth and storage.

2.3.1 Disease assessment

Nutter *et al*. (2006) reported that plant disease estimation either by human activity or some equipment can ensure good measurements with different degree of precision and accuracy plant disease measurement plays key role in diagnosis. It represents the basis for: epidemiological studies, assessment of crop losses, plant disease survey, development and application of models, correct management of crop protection, screening of resistance, evaluation of protection methods and other experiments (Heller, 1997). It is therefore a tool in determining the prevalence and extent of damage caused by a disease. It is important in developing effective management strategies (Daamen, 1986; Kranz, 1988; Nutter, 2001). Disease is measured in terms of intensity and can be expressed either as disease incidence or disease severity (Madden, 1983; Madden *et al*., 2007).

2.3.2 Biotic constraints against *J.curcas* cultivation

The assumption that Jatropha is relatively resistant to pests and diseases might strongly rely on the fact that current knowledge is generally based on experimental plots or small scale experiments. Growing *Jatropha* in large scale monocultures will increase the risk for pests and diseases. A group of organisms likely to affect *Jatropha*, such as fungi (e.g.

Phytophthora, *Mucoraceae*), insects (e.g. stem borer, leaf miner, caterpillars,and scale) and diseases (e.g. mosaic virus) was defined by M¨unch and Kiefer (1986); Grimm (1999); Narayana *et al.* (2006); Shanker and Dhyani (2006); ¨Ullenberg (2008).

Physic nut is also susceptible to a range of insect pests, notably the whitefly, *B. tabaci*, the leaf and capsule borer *Pampelia morosalis* and the Scutellarid bugs, *Scutellera nobilis* and *Chrysocoris purpureus.* Sustainable and ecologically acceptable means of control of these pests and diseases are of paramount importance, if large scale *Jatropha* plantations are to be developed successfully in under-utilised, semi-arid regions. The shield-backed or scutellera bug pest of plantation stands of *Jatropha* in Nicaragua **(***Pachycoris klugii*) and India (*Scutellera nobilis*), causes flower fall, fruit abortion and seed malformation. Other serious pests include the larvae of the moth *Pempelia morosalis* which damages the flowers and young fruits, the bark-eating borer *Indarbela quadrinotata*, the blister miner *Stomphastis thraustica*, the semi-lopper, *Achaea janata*, and the flower beetle (Kaushik *et al*., 2001; Xiao *et al.*, 2010).

Lozano (2007) listed pathogens infecting the plant without mentioning their geographical distribution, including *Phytophthora* spp., *Pythium* spp., and *Fusarium* spp., *Helminthosporium tetramera, Pestalotiopsis paraguarensis, P. versicolor*, and *Cercospora* spp. It has also been reported that several pathogens infect physic nut in Indonesia: *Ralstonia solanacearum*, *Rhizoctonia bataticola* (Republic of Indonesia Department of Agriculture, 2006a), *Phytophthora nicotianae* (Republic of Indonesia Department of Agriculture, 2006b), *Alternaria* spp. *Cercospora* spp., *Fusarium* spp., *Botrytis* and *Xanthomonas campestris* (Hambali *et al.*, 2006; Prihandana and Hendroko, 2006). Latha *et al*. (2009) reported the occurrence of root and collar rot diseases of physic nut in India. A recent survey in Karnataka State, India, showed that the occurrence of *Jatropha* Mosaic Virus Disease (JMVD) ranged between 13 - 47%, causing significant yield loss. *Jatropha* Mosaic Virus (JMV) is transmitted by the whitefly, *Bemisia tabaci* and there are other close similarities between this pathosystem and that of cassava mosaic virus (Padilla and Monterroso, 2003; Colvin, 2009)). Severe mosaic disease accompanied by yellow spots was noticed on 15% of *J. curcas* growing in the experimental plots of NBRI, Lucknow,

India, during October of 2006. Inoculations with sap from symptomatic plants resulted in systemic mosaic on three of seven *J. curcas* seedlings (GISP, 2008).

2.3.3 Fungi associated with seeds of *J. curcas*

Neves *et al*. (2009) reported that *Fusarium, Rhizoctonia and Alternaria* spp. were the major fungi detected in the blotter test of Jatropha seeds. Worang (2008) isolated sixteen fungal species from physic nut seeds (jatropha) during six months of storage. At the beginning of storage, most of the fungi that infect seeds of *J. curcas* were the field fungi such as *Colletotrichum, Cladosporium* and *Fusarium* spp. and after three months of storage, *Aspegillus* and *Fusarium* dominated other species in the store (Jayaraman *et al.,* 2011).

Prabha *et al*. (2012) reported the occurrence of nine storage fungi on Jatropha namely, *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Curvularia pallescens, Fusarium solani, Penicillium sublateritium, Rhizopus nigricans* and *Trichoderma harzianum.* The fungi detected from stored seeds were same as in fresh seeds except *Penicillium sublateritium* which was absent in stored seeds. There were variations in the occurrence of different fungal species in fresh and stored seeds. His findings agreed with earlier reports on *J. curcas* seeds by Srivastava *et al.*(2011); Sahab *et al.* (2011) and Singh *et al.* (1996) that jatropha seeds are heavily deteriorated during storage, as they act as a source of stored nutrients for fungi such as *Aspergillus flavus, A. fumigatus, A. niger* and *Rhizopus nigricans* (Chelkowski, 1991; Hegde *et al*., 2009).

Jayaraman *et al.* (2011) observed the occurrence of *Aspergillus flavus, A. fumigatus, A. niger, Penicillum* sp. and *Rhizopus* sp. from four samples of jatropha seeds from Chennai. Fungal deterioration was reported in jatropha seeds collected from central India which also decreased oil yield and quality (Singh *et al.,* 1996). Worang (2008) reported similar observation on *J. curcas* seeds. Hedge *et al*. (2009) isolated *Sclerotium rolfsii* from seeds and seedlings of *J. curcas*. Melo *et al.* (2007) and Neves *et al.* (2009) reported that *Fusarium* spp. and *Aspergillus* were the most frequent isolated fungi on Jatropha seeds.

Tanaka (2011) and Neves *et al*.(2009)) have also noted that important plant pathogenic field fungi such as *Fusarium* spp, *Colletotrichum* spp. and *Rhizoctonia solani* causing

damping –off, root rot, anthracnose and wilt, are consistently found associated with seeds of different plant species and have the potential of causing severe plant damage . Species of *Fusarium* have also been found in association with seeds of caster bean (*Ricinus communities* L.), another important plant for production of biodesel and belonging to the family Euphrbiaceae, like Jatropha (Mariotti *et al.*, 1987).

2.4 Fungal Diseases of *J. curcas*

2.4.1 Folial Diseases of *J. curcas*

2.4.1.1 Passalora leaf spot

Passalora leaf spot (*Passalora ajrekari* (Syd.) U. Braun and *Passalora jatrophigena* U. Braun and F.O. Freire) was reported on *J. curcas* by many researchers. The disease was first described in Brazil by Braun and Freire (2004) and later by Freire and Parente (2006) on leaves of *Jatropha curcas* and *Jatropha podagrica*, and in other countries by Crous and Braun (2003). The primary symptoms of this disease are rounded leaf lesions that are creamy to light brown in color, with a narrow dark brown halo, which later became limited by leaf veins and darkened. Lesions measure 1-2 cm in diameter and rarely coalesce (Freire and Parente, 2006). The genus *Passalora* is a cercosporoid fungus, previously included in the genus *Cercospora* that has its teleomorph as *Mycosphaerella.* Species share taxonomic characteristics such as branched, septate, smooth, hyaline to pigmented hyphae; absent to well-developed stromata; solitary or fasciculate to synnematous conidiomata, conidiophores, arising from stromata or hyphae, internal or superficial, pluriseptate, subhyaline to pigmented; conspicuous conidiogenous loci, with scars that are somewhat thickened and darkened; conidia that are solitary to catenate in simple or branched chains, amerosporous to scolecosporous, aseptate to pluriseptate, and pale to distinctly pigmented and hila that are somewhat thickened and darkened (Crous and Braun, 2003). Although it has been reported in several countries, to date this disease has not presented risk to physic nut cultivation.

2.4.1.2 Cercospora/Pseudocercospora leafspot

This disease is caused by the fungi, (*Cercospora jatrophicola (*Speg.) Chupp, *Cercospora jatrophigena* U. Braun), the pathogen has been observed in some Jatropha growing regions. *Cercospora* is reported to cause leaf spot on physic nut and the species is reported to be *C. ricinella* (Hambali *et al*.,2006) and *C. jatrophae* (Prihandana and Hendroko, 2006). This disease manifests in the form of leaf spots that consist of well-delimited brown irregular necrotic spots (Dianese *et al*., 2010). The genus *Cercospora* groups, anamorphs of *Mycosphaerella* with hyphae that is colourless or near-colourless to pigmented, branched, septate, and smooth to faintly rough-walls. Stromata are lacking to well-developed, subhyaline to usually pigment. Conidiophores are solitary to fasciculate, arising from internal hyphae or stromata, erect, sub-hyaline to pigment. Conidiogenous loci (scars) are conspicuous, thickened and darkened. Conidia are solitary, scolecosporous, cylindrical-filiform, hyaline or sub hyaline, mostly pluriseptate, and smooth and hila are thickened and darkened (Crous and Braun, 2003). Crous and Braun (2003) cited the occurrence of five species of cercosporoid on physic nut. However, few studies have examined fungi cercosporoid in this crop. As a result, there is no information about favorable conditions, symptoms or disease control. To date, this disease has not been reported to present risk to the cultivation of physic nut.

2.4.1.3 *Alternaria* leaf spot

Diagnosis of alternaria leaf spot on *J. curcas* was similar to that of *cercospora* leaf spot except that all spot areas were brown in color without whitish area. In addition, after 2-day incubation at 100% relative humidity at room temperature, conidia were found in the spot area. The conidia consisted of several dark cells. According to the identification key (Barnett and Hunter, 2001), the conidia were formed by *Alternaria*. *Alternaria ricini* was reported to be the causal agent of leaf spot of physic nut (Hambali *et al*., 2006).

2.4.1.4 Foliar necrosis/Anthracnose

Jatropha Anthracnose caused by species of *Colletotrichum* (*Colletotrichum gloeosporioides* (Penz.) Sacc. and *Colletotrichum capsici* (Syd.)Butl.and Bisby) was first described on

Physic nut by the USDA (1960) in the USA; in Brazil by Viégas (1961), later by Freire and Parente (2006) and Sá *et al*. (2011). Currently, the disease is present in all areas where physic nut is cultivated. The most commonly observed symptoms are brown to black necrotic lesions that are irregularly shaped and appear on the edges and center of the leaf and which may contain a yellow halo. The lesions appear in the form of small, isolated points that coalesce and subsequently cause the complete destruction of the leaves. The fruit can also become infected, which leads to the appearance of dark brown lesions.

Torres-Calzada *et al*. (2011) reported that in fields of physic nut (located in the Yucatan Peninsula in Mexico symptoms of foliar necrosis, crown canker and apical death of seedlings, and leaf blight causing 70% defoliation which affected about 25% of the production in Yucatan, caused by *Colletotrichum capsici*. Leaves infected by this fungus developed crown canker and apical death in seedlings. *C. capsici* (Syd.) E. J. Butler and Bisby was also reported an important pathogen with a worldwide distribution and is involved in diseases of economically important hosts such as pepper (Than *et al*., 2008) and papaya (Tapia-Tussell *et al*., 2008).

Colletotrichum spp. is known to infect a large range of hosts and to cause various symptoms, the most common of which is anthracnose. *Colletotrichum* is a fungus anamorph of the phylum Ascomycota and teleomorph genus Glomerella. The species of this genus have the following characteristics: conidiomata that are acervular, subcuticular or epidermal, and may contain setae; conidiophores that are hyaline to brown; conidiogenous cells that are enteroblastic, phialidic and hyaline; conidia that are hyaline, aseptate (except prior to germination), straight or falcate, smooth and thin-walled; and appressoria that are brown, entirely or with crenate to irregular margins produced with germination of conidia (Sutton, 1980). These fungi can survive in seeds, crop residues, infected plants, and in soil as saprophytes. Although the disease occurs in various regions of the world, it is more severe in regions with a hot and humid climate (Agrios, 2005).

2.4.1.5 Powdery mildew on *J. curcas*

Powdery mildew caused by the fungus *Pseudoidium jatrophae* (Braun and Cook, 2012) was previously described as *Oidium heveae* Steim by Viégas (1961) in Brazil. *Oidum*

jatrophae Hosag., Siddappa, Vijay and Udaiyan (Braun and Cook, 2012) in India. This disease occurs commonly in physic nut plantations and it has been frequently observed in various regions of Brazil and the rest of the world. The most common symptoms of the disease are the production of abundant white or gray mycelia in leaves, petioles, stems, flowers and fruits (Dianese and Cargnin, 2008). With the evolution of the disease, infected plants may show necrotic lesions, which cause leaf fall, underdevelopment, death of buds and young fruit deformation (Bedendo, 2011). The fungus that causes this disease is a typical biotrophic pathogen of the phylum Ascomycota, order Erysiphales. This pathogen may be characterized by white or grayish colonies, septate and branched mycelia; conidiophores that are erect or ascending, cylindrical, hyaline, septate and forming conidia singly; conidia that are usually large in proportion to the diameter of the conidiophores, simple, smooth, ellipsoid-ovoid deiform, hyaline, single celled (Braun and Cook, 2012). The disease generally favors warm temperatures, humidity of 75-80% and reduced light. Heavy rains are generally unfavorable to the pathogen (Furtado and Trindade, 2005). In Brazil, the disease usually occurs in the dry season, apparently without causing extensive losses, because its occurrence coincides with the plants' period of natural defoliation (Saturnino *et al*., 2005).

Powdery mildew is a fungal disease that affects a wide range of plants. *Oidium* species have been found to attack *J. curcas* in Kenya (Hawksworth, 2001), with a nationwide distribution. Leaves, shoots and fruits are covered with a white floury colour, which turns into black patches with severe infection after some time. The disease is enhanced by an alternation of rainy days and sunny days and a degree of environmental humidity. Ploughing in or removing plant residues after harvest, crop rotations, and the use of resistant cultivars are recommended for control of the disease. Excessive nitrogen fertilization results in more severe infection of pigeon peas by a similar fungus. Currently, there are no fungicides recommended for culture, but some studies cite that spraying sulfur fungicides works to control this fungus. Another measure is to control alternative hosts, especially plants of the family *Euphorbiaceae* (Furtado and Trindade, 2005; Saturnino *et al*., 2005; Dias *et al*., 2007).

2.4.1.6 Rust on *J. curcas*

The first report of rust disease on *J. curcas,* described its cause as *Uredo jatrophicola* Arthur, (Arthur, 1915). In Brazil, this disease was first found in 1936 in São Paulo (Viégas, 1945). Currently, it is widely distributed throughout Brazil (Dias *et al*., 2007) and several other countries. The fungus that causes this disease was previously classified as *Phakopsora jatrophicola* (Arthur) Cummins; however, it was reclassified as *Phakopsora arthuriana* Buriticá and Hennen (Hennen *et al*., 2005). This disease was also reported among other pathogens attacking physic nut in Kenya by FHIA (2008). The symptoms are orange to light brown pustules observed on the underside of the leaves. Yellow stains can be seen in the area of the visible pustules on the underside. These pustules contain large number of spores which can be spread by wind, water, or insects. The symptoms manifest on the leaves, initially in the form of small chlorotic points on the upper surface, which correspond to the underside of the leaf, and then small protruding pustules, which after breaking, release a powdery mass of uredospores of orange color, giving a ferruginous aspect. In severe infections, pustules coalesce to form necrotic spots, which are reddish brown and irregularly shaped and can destroy the leaf (Dias *et al.*, 2007; Carneiro *et al*. 2009).

The *Phakopsora arthuriana* belongs to the phylum Basidiomycota, class Pucciniomycetes. It is characterized by uredinia hypophyllous, occasionally epiphyllous, in small groups opening by a pore, surrounded by numerous not septate paraphyses that project outside the host; urediniospores, ellipsoid, to obvoid, sessile, closely and finely echinulate, germ pores obscure; telia hypophyllous, suberpidermal in origin, closely around the uredinia; teliospores irregularly arranged, cuboid, ellipsoid to polygonal (Hennen *et al.*, 2005). Currently there are no fungicides recommended for this culture. However, according to Dias *et al*. (2007), protective copper fungicides can control this disease.

2.4.2 Vascular/root disease on *J. curcas*

Root rot caused by *Rhizoctonia bataticola* has been recorded as one of the most devastating disease of jatropha (Sharma and Kumar, 2009 and Kumar *et al*., 2011). Pathogenicity test, along with the culture appearance and microscopic structure of the isolated fungus causing

root rot disease on *J. curcas* in China was identified as *Fusarium solani* and its teleomorph (*Nectria Haematococca*) (Yue-kai *et al.*, 2011; Wu *et al*., 2011). This was different from that of root rot disease in India, which was identified as *Fusarium moniliforme* (Kaushik *et al*., 2001). Yue-kai *et al.* (2011) observed serious root and collar rot disease on *J. curcas* nurseries and plantations resulting in great damage and economic losses. They reported up to 83.5% seedling mortality due to attack of this pathogen. The main symptoms of the root rot disease were leaf yellowing, wilting and finally leaf fall, as well as blackening and rotting of the roots and collars, eventually leading to plant death. The time from infection to death of infected plants was about three to four months.

J. curcas is a succulent and vigorous plant species with great potential for root regeneration, even if underground parts (roots, collars) were rotted, the above ground parts (stem, top leaves and buds) remained healthy for a limited amount of time. This characteristic makes detection of the disease difficult during the early stages of infection, making the disease hard to predict and manage. Rot disease was mainly found on 0–3 year old seedlings and was usually associated with over watering or water logging and root rot was found to be more common than collar rot, which was seen usually in the later stages of the disease. *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl was also reported to infect *J. curcas i*n India and Brazil (Latha *et al*., 2009; Pereira *et al*., 2009).

BLAST analysis from diseased *J. curcas* plant parts also indicated most potential fungal isolates such as *Macrophomina phaseolina, Phomopsis longicolla, Fusarium oxysporum, Alternaria alternate, Botryosphaeria dothidea* in India (ICRISAT, 2010; Alexander and Olinto, 2013). Srinivasa *et al*. (2011) observed 80% plant infection and loss of whole plantation due to black rot infection caused by *Botryosphaeria dothidea* in India. The affected *Jatropha* plants showed reddish brown gummy exudation from the infected (shriveling) parts of the stem, leading to the death of the plant. Occurrence and spread of this disease was mostly observed in summer months, when a severe water stress and drought conditions prevailed. Microscopic examination of the affected plant tissues revealed the presence of scattered globose pycnidia near the margins of the black lesions.

Marieke *et al.* (2012) in Senegal reported an outbreak of vascular infection on Jatropha caused by fungi. The disease was mainly characterised by collar and root rot, which caused foliage to yellow and wilt, before the plant eventually dies. Mortality increased from 16% of all dead plants across the entire field in the second year to 36% in the third year and 65% in the fourth year. At the end of the observations, two thirds of the six hectares of the plantation were ravaged. No stabilisation of the disease was detected. The disease spread from clearly localised outbreaks to the various plots, which were added over the years. Similar damage was observed on other rain-fed Jatropha crops on plantations in eastern Senegal and west of the Groundnut Basin. However, the mortality rate there was generally lower than that observed in Bokhol. It is possible that the prevailing environmental conditions in northern Senegal stress the plants and make them more vulnerable to attacks. In addition, the irrigation system put into place created favourable conditions for the development and spread of the pathogen, by maintaining constant humidity in the soil. These findings were in line with those reported earlier that four pathogens cause root rot in Jatropha: *Fusarium oxysporum* Schlecht. f. (Hu *et al*., 2009), *Macrophomina phaseolina* (Tassi) Goid, *Phomopsis longicolla* Hobbs, *Alternaria alternata* (Fr.) Keissler (Kumar *et al*., 2011, Rao *et al*., 2011).

Attacks by *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. have been observed in India and Brazil, respectively by Latha *et al*.(2009) and by Pereira *et al.*(2009). Similar symptoms were observed in India, following attacks by *Fusarium moniliiforme* Shel. (Kaushik *et al*., 2011) and *Botryosphaeria dothidea* (Mougeot: E.M. Fries) Cesati and De Notaris (Rao *et al*., 2011) and in China following attacks by *Nectria haematococca* Berk. and Broome (Wink *et al*., 2000). Marieke *et al.* (2012) recorded that the threats from vascular attack could increase if far larger areas were planted with Jatropha and its development pose serious threat to Jatropha crops.

Vascular infections are known, all over the world, to cause major damage to crops. Haggag (2014) reported canker and collar rot attack on *J. curcas* in Egypt. The disease was characterized with shedding of the leaves, blackening and decaying of the collar regions of the stems and necrotic lesions on branches. A fungus was consistently isolated on potato dextrose agar (PDA) from symptomatic branches with single-spore fungal cultures

produced white, aerial mycelium that became dull gray after a week in culture. The mycelium was fast spreading, branched and septate. Pycnidia from 30-day-old pure cultures produced dark brown, oval conidia that were two celled, thin walled, and oval with longitudinal striations. The average size of the conidia was 23.64.12.73 µm with a length/width ratio of 1.86. Conidia were initially unicellular, hyaline, thick-walled with granular content. Based on conidial morphology, the fungus was identified as *Lasiodiplodia theobromae*. This finding on *L. theobromae* causing cankers and dieback on physic nut was reported in Malaysia (Sulaiman and Thanarajoo, 2012) and India (Latha *et al*., 2009).

Root rot problem caused by *Phythophthora* sp. has only been reported in a plantation at Kiambere in Mwingi North of India by (Barnard *et al*., 1992). *Phytophthora* spp. are relatively host-specific parasites and many of the species are of considerable economic importance. High humidity and temperatures of 28-32°C are conducive to the rapid build-up of the disease. It was characterized by yellowing of foliage reduced leaf size and eventual death of the whole plant. As the disease progresses, patches of diseased plants become conspicuous in the field and were visible from a distance.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Survey and Sampling Collection

Leaf, stem, fruit and root samples were collected from six South-Western states of Nigeria (Ekiti, Lagos, Ogun, Ondo, Osun and Oyo) between May and August, 2009 (Fig.1). Five distantly located areas each were sampled in the Northern and Southern parts of each state. Random sampling technique was adopted where plants with disease symptoms and apparently healthy plants were collected from each sampling location adopting the method of (Elazegui *et al*., 1990). Samples were collected from ten diseased and ten healthy plants in each town and preserved in paper bags for laboratory analysis. Total of 200 samples were collected per state; 100 each from the northern and southern parts of each state.

3.2 Experimental Site of the Study and Laboratory Protocols

The studies were conducted in the field, screenhouse, and laboratories of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan and Forestry Research Institute of Nigeria (FRIN), Ibadan.

3.2.1 Sterilization of laboratory materials

The glasswares used were well washed in sterilized in teepol detergent and rinsed with tap water. Before the pipettes were sterilized, the ends usually placed in the mouth, were plugged with cotton wool in such a way that it did not jut out. This was to prevent the unsterilized air from getting inside the pipettes. Petri plates were placed in canisters while pipettes were wrapped in aluminum foil. All glasswares were then sterilized in a Gallenkamp oven at 160°C for at least 3 hours. The inoculating needles were flame sterilized on spirit lamp. Liquid medium and distilled water used were sterilized in an autoclave at 121°C and 1.05 kg/cm^2 pressure for 15 minutes. . The inoculating chamber was disinfected by swabbing with cotton wool soaked in 70% ethanol. The ultra-violet light in the inoculating chamber was put on for at least 30 minutes before each use. All

isolations and inoculations were carried out under sterile laminar flow and the UV light was put on for at least 30 min after each use of the chamber.

3.2.2 Potato Dextose Agar preparation

Potato Dextose Agar (PDA) was used for this study. Thirty-nine grams of PDA was weighed into 1000 ml of distilled water. To achieve homogenous solution, the agar was heated in a water-bath for 5 minutes and then dispensed into 250 ml flasks. The flasks were then covered with non-absorbent cotton wool wrapped with aluminum foil. All the flasks were placed in an autoclave and sterilized at 121°C and 1.05kg/cm^2 pressure for 15 minutes. After sterilization, the medium was allowed to cool to 45°C and then dispensed aseptically under laminar flow chamber into sterilized Petri dishes and allowed to solidify.

3.2.3 Culturing for identification of fungal flora associated with *J.curcas.*

Jatropha plants showing disease symptoms that were collected during the survey were brought to the laboratory for examination. Each of the specimens was observed in the laboratory after arrival or at the next day. The samples were thoroughly washed with tap water. Small pieces (2-5) cm were sliced off from the advancing edges of the infected part. They were surface-sterilized in 10% of commercial sodium hypochlorite (NaOCl) solution in distilled water for one minute and rinsed in five successive changes of sterile distilled water in watch glasses. Potato dextrose agar (PDA) supplemented with 1.4 ml lactic acid per liter of medium was used for fungal isolations.

They were blotted dry with sterile filter paper and aseptically placed on 9cm Petri plates containing acidified PDA to inhibit bacterial growth. The plates were incubated at 28 ± 2 $^{\circ}$C and were monitored daily for fungal growth for 15 days. The isolations made were from the diseased leaves, stems and dieback symptoms on shoots and branches and cankers following standard laboratory procedures on isolation of fungi from plants (Agrios, 2005). Pure cultures were obtained by sub-culturing into fresh PDA plates. Stock cultures were maintained on agar slants in McCartney bottles.

3.2.4 Laboratory isolation and fungi identification

Fungal colonies that grew out of the cultures were observed and later identified. Cultural and morphological characteristics were observed and identification was made to genus level using the method of Barnett and Hunter (2001). Each emerging fungal colony was marked, assigned a unique number and isolated onto fresh culture medium. The number of times each colony was encountered per sample was counted. Fungal isolates were then grouped based on colony color and mycelial texture using a sterozoom microscope. The isolates were purified by isolating single spores as described by Slippers *et al*. (2004). Spores were mounted on microscope to further group and identify the fungi using established protocols (Booth, 1971; Ellis, 1976; Barnett and Hunter, 2001; Leslie and Summerell, 2006; Philips *et al*., 2008). Representative cultures from single spore isolates of each morphological group were selected for further studies. The morphological and cultural characteristics of each isolate were studied by removing small fragments of mycelia and fruiting structures from a fresh culture (seven days old). This was then mounted, teased in lacto-phenol cotton blue on a microscope slide and observation made with the aid of a compound microscope. The photomicrograph of the isolated organisms was taken using a microscope camera attachment.

3.2.5 Source of Jatropha accessions used for seed health test and in screenhouse and field studies

Five *J. curcas* accessions were used for this study. The accessions had been previously selected from trial plots of Forestry Research Institute of Nigeria in the northern Nigeria and Ibadan and were assembled in the FRIN Gene bank at Ibadan, namely: Ex-Kano, Ex-Basirika, Ex-Misau, Ex-Mbatdiya and Ex-Ibadan.

3.2.6 Jatropha seed pathology and postharvest quality of seeds from different accessions of *J. curcas*

The objective of this study was to examine the survival of different pathogens on Jatropha seeds. Fresh seeds stored for four weeks after harvesting were used for the experiment. The seed health test technique used was the agar plate method (ISTA, 2008). Each of the seed (25 seeds /accession) was surface-sterilized by immersing in 10% commercial sodium hypochlorite (NaOCl) for one minute and rinsed in 3 changes of sterile distilled water. Solidified PDA which had been poured in 9cm Pyrex Petri dishes were cut with a sterile 10 mm cork borer and aseptically transferred with sterile forceps into empty sterile plates. Each Petri plate contained five PDA cut discs equidistant from each other. The surface-sterilized seeds were dried with sterile filter paper to remove excess moisture, after which they were aseptically transferred with sterile forceps onto the cut PDA discs. The equidistance maintained between each seed was to ensure that organisms from one seed do not cross-contaminate other neighboring seeds. Each Petri plate was inoculated with five seeds in five replications arranged in a completely randomized design (CRD). The plated seeds were incubated for eight days at room temperature of 28±2^{o}C. Examination of the plates for growth of fungi from the seeds and identification were made using binocular compound microscope. Records taken on the seeds infected by the fungi and the percentage infection were calculated.

3.2.7 Studies on seed transmission of pathogens

Infected seeds from the above experiment were used in this study. Seedlings were raised in nursery trays. The seeds were sown in steam-sterilized top soil and the soil watered at two days intervals until seedling emergence. At four weeks, the seedlings were uprooted washed and taken to the laboratory for pathogen isolation and identification.

3.3 Screenhouse Evaluation of Jatropha Accessions for Fungi Diseases

3.3.1 Preparation of fungal inocula for pathogenicity test

Fungal *inocula* were prepared by using seven day old cultures made from each isolate. A small piece of inoculum from the actively growing edge of a culture plate was transferred using 5mm cork borer and aseptically inoculated into a freshly prepared PDA plate and incubated at the temperature of 28 ± 2 °C for eight days. When the culture was nine centimeters in diameter, 50ml sterile distilled water was added to each plate and the spores and mycelia brushed gently with a camel's hair brush to form mycelia-spore suspension. The suspension was filtered through two layers of muslin cloth to remove mycelia and then adjusted with the aid of a haemocytometer to 10^6 spores/ml of distilled water.

3.3.2. Pathogenicity test of fungal organisms isolated from *J. curcas* accessions

Seeds from five Jatropha accessions were raised in pots containing steam-sterilized soil in the screenhouse. The pots were arranged in a completely randomized design with five pots per replicates per treatment. Seedlings were maintained in screenhouse for four weeks before inoculation. During the growing and experimental periods, seedlings were watered once daily and observed for 12 months. All the fungal organisms earlier isolated from Jatropha plants were subjected to pathogenicity test. The time taken by each plant to show early symptoms were recorded and the number of seedling showing various diseases symptom types and the number of dead seedlings were counted in each treatment. Three seedlings were randomly selected from each treatment. Slit longitudinally and the lengths of internal lesion if any were measured upwards and downwards from the point of inoculation and were recorded, the proportion of the leaf showing symptoms was also recorded.

3.3.3 Inoculation techniques used for pathogenicity screening of *J. curcas* seedlings

Two methods of inoculation techniques were used for this study in the screenhouse experiments:

(a) Leaf spray technique

The test plants were inoculated by spraying with spore suspensions as inocula on leaflets the spaying was done until run-off by delivering the suspension from a master hand sprayer. A drop of Tween-80 per 100ml inocula was added to the spore suspension as a surface wetting agent. For each plant, average of 15 leaves per shoot was inoculated from the plant portion above the fourth fully developed leaves from the apex of four-week old healthy plant. A 1000ml spore suspension containing 10^6 spores/ml for each fungal isolate used for each plant. The control plants were sprayed with sterile distilled water (SDW) without fungal inocula. The inoculated plants were covered with transparent polythene bags immediately after spraying for 72 hours. This was to maintain high humidity around the inoculated plants that enhanced spore germination.

(b) Stem puncture technique

Five cuts (one centimeter deep) were made on the stems of four-week old Jatropha seedlings. The cuts were made on (5-10cm) from the apex region with sterilized surgical blade. Five millimeters actively growing plugs of fungal mycelia/spores on agar were inserted into the wounds (Muimba, 1982). The cuts were then sealed immediately with parafilm to avoid contamination and death of adjacent tissues. The parafilm was then removed from the stems after two weeks of inoculation (WAI).

Solidified PDA plugs without pathogen was inserted into the cuts made on the control plants. Symptoms were visually observed on plants from five days after inoculation (DAI). Data were collected based on different disease symptoms expressed days after inoculations (DAI).

3.3.4 Re-isolations of fungal pathogens after inoculation

At the end of the experiment, three plants each from the five inoculated and five uninoculated (control) were randomly selected for re-isolation of the pathogens. A $1cm^2$ piece was cut off from the discolored regions between the infected and healthy tissues (disease leading edge) of each sample. They were surface sterilized and rinsed with distilled sterile water and thereafter plated on PDA. The plates were incubated at 28°C for

14 days after which fungal identity was confirmed by observing the culture characteristic visually and conidia morphology using compound microscope as described previously.

3.4 Screening for Disease Resistance

This study was conducted to evaluate the best accession, in terms of resistance to observed diseases and % germinability.

(a) *In vitro* Selection

Seeds from the five different accessions collected were used for this study. Parameters for selection were based on seeds that germinate out of 50 seeds plated, germination percentage and number of inherent pathogens isolated from seeds during incubation period of 5-7 days. Scale of 0-4 was used:

0 = poor % germination (0-25%)
1 = Low % germination (30-49%)
2 = Moderate % germination (50-74%)
3 = High % germination (75-89%)
4 = Very high % germination (90-100%)

(b) Screenhouse screening for disease resistance

Fifty seeds from each accession were raised in polythene bags filled with steam-sterilized top soil in the screenhouse. Watering was done at two-day intervals. The seedlings were monitored for four months. Observation and readings were taken on disease development. Seedlings with varying degrees of resistance to different fungal diseases were selected and later transferred to the field. Parameters for selection of accessions were; germination % (GA), based on the number of germinated jatropha seeds per accession in the replication and plant vigour (PV), with a developed scale of (0-4) according Fokunang (1995) where;

0 = low % germination (0-29%), severe leaf damage and stunting of plant.
1 = low % germination (30-49%), stunting of plants and leaves / stem infection
2 = high % germination (50-74%), leaf / stem infections.
3 = high germination % (75-89%), low disease infection of leaf / stem.

4 = high % germination (90-100%), no disease infection of leaf / stem.

(c) Field screening test for disease resistance

The seedlings from the above treatment were monitored in the field for development of disease symptoms. Data on different symptoms, incidence, severity and disease infection index were collected at 3 and 6 months after planting.

3.4.1 Disease assessment of different fungal pathogens in screenhouse and natural field infections

Seedlings were raised from the five accessions at the sreenhouse of CPEB. Ten plants were used per accession in four replications arranged in a RCBD in the screenhouse and five plants randomly sampled were assessed. They were monitored for disease expression from the first day of seedling emergence. The pathogens were assessed at single inoculation and under combined or mixed inoculations. The potential fungal pathogens used for disease assessment were:

A ===>*Colletotrichum gloeosporioides.*

B== =>*Fusarium oxysporum.*

C===> *Lasiodiplodia theobromae.*

D===>*Oidum jatrophae.*

E===>*Curvularia lunata*

3.4.2 Development of screening methods and evaluation of resistance to fungal diseases

Five accessions of *J.curcas* from Forestry Research Institute trial plots were raised in the screenhouse. These accessions were inoculated at four weeks after planting (4WAP) with the five mentioned potential fungal pathogens isolated ealier from Jatropha. A total of eight plants per accession were raised in polythene pots; treatments were replicated four times for each fungal pathogen. Assessment of fungal disease severity was based on observation of disease symptoms on inoculated plants. Scale of (0-4), adopted from Latha *et al.* (2009) was used for scoring the severity. Disease symptoms were observed and recorded from five days after inoculation (DAI).

1 = No symptoms

2 = angular chlorotic spots and/ or irregular greyish necrotic spots on leaves and petioles

3 = extensive leaf blight and leaf chlorosis

4 = wilting, defoliation, anthracnose/necrotic lesions on twigs and lateral shoot, fruit and root rot and eventual die-back.

The data were collected from one month till 12 months after planting and host reactions were classified into susceptible, moderately susceptible and resistant.

3.4.3 Determination of disease incidence and severity

Disease incidence (I): DI = n/N x 100%

$$I = \frac{\text{Number of plants affected (n)}}{\text{Number of plants observed (N)}} \times 100\%$$

Where DI = disease incidence,

n = number of plants showing symptoms, and

N = the total number of plants observed

Disease severity (DS): $\frac{\Sigma(ni.vi)X\ 100\%}{N \times V}$

ni = Number of plants affected at category level i

vi = Damage category level i

N = Number of plants observed

V = Highest damage category value

Damage category value:

0 = No symptom

1 = $0 < x \leq 25$ %

2 = $25 < x \leq 50$ %

3 = $50 < x \leq 75$ %

4 = $x > 75$ %.

3.4.4 Disease assessment in the field under natural infection

The assessment of incidence and severity of diseases were based on the observed disease symptoms in the field under natural infection by fungal pathogens. Hundred healthy seeds each were planted in five rows in three locations (CPEB Crop Garden, FRIN garden and FRIN Arboretum) from each of the five accessions. Two seeds were planted per hole at the planting distance of 1mx1m in a row and 1.5m across row. They were ten stands per row with two seeds per hole which was later thinned down to one plant per hole, two weeks after germination. The accessions were grown for three years on the field in RCBD layout with five replicates. Ten plants randomly sampled were assessed per accession.

Disease symptoms were recorded on leaves, shoots and roots in case of seedling dieback. Disease incidence (I) was calculated by dividing the number of infected part by the total number of parts assessed and were expressed as percentage.

Disease incidence (DI) and severity (S) were calculated on the observed symptoms. Different severity scales for different diseases were adopted:

(a) Shot-hole and Chlorosis, the severity scale by Wokocha and Opara (2004) was adopted where:

1= No visible symptoms on leaf
2 = 1-3 leaves infected
3= 4-6 leaves infected
4= 7-9 leaves infected
5= ≥ 10 leaves infected

(b) Anthracnose disease was scored on the scale of 1-5 as described by Muyolo, (1984) where:

1= No symptoms
2= Development of shallow chlorotic lesions on shoots
3= Development of successive lesions and superficial cankers on young shoots
4= Development of dark-brown lesions on green shoots, petioles and leaves, young

shoots collapse and defoliate.

5= Wilting, drying up of shoots and leaves, death of part or whole plant.

When the whorls showed complete defoliation it was considered to be 100% infected.

3.4.5 Disease progression on the field

This experiment was conducted to study the natural disease progression in the field. Seeds from the five accessions were planted at the Department of Crop Protection and Environmental Biology's experimental plot and at FRIN Ibadan, arboretum. They were monitored for disease expression from the first day of seedling emergence from June 2011 to June 2013. Data on first day of disease incidence, severity and disease progression were recorded.

3.4.6 Anthracnose disease infection index (DII)

The total number of disease infection (necrotic lesions/canker) on each *J.* curcas accession was determined by counting the number of lesions on infected shoots of the plant. The size of the lesions on the shoots of *J.curcas* was determined by measuring at four weeks interval from 6months after planting for six months. The length and width of the spread were taken using a meter rule, then average for all lesions were calculated. Plants were grouped into the following categories for anthracnose evaluation according to the method of Moral and Trapero (2009);

Score	Number of lesions per plant
1	No lesion
2	1-30
3	31-60
4	61-90
5	more than 90

3.5 Morphology of *Colletotrichum gloeosporioides* Spore

The spore suspension for the morphological studies was prepared by mixing a drop of spore suspension and one drop of lacto-phenol on a microscope slide. Each preparation

was warmed on a spirit lamp flame for few seconds and a cover slip affixed. Observation was made on the characteristics of the isolate such as pigmentation, presence or absence of setae, media colour change, and conidial length. The conidial length was estimated by measuring 100 spores using a graduated occular eyepiece calibrated with the microscope stage.

3.5.1 Characterisation of *Colletotrichum gloeosporioides* (Penz.) Penz. et Sacc causing symptoms of anthracnose on *J. curcas*

A seedling sample of 40 plants was raised in the field to determine pathogenic fungi causing symptoms of chlorosis and blighting. Only representative samples of plants with anthracnose symptoms were taken from the field to the laboratory for isolation and confirmation of *C. gloeosporioides* presence by microscopic examination.

Investigations were carried out on one-year-old seedlings. Isolations of pathogen were performed from infected organs of infested shoots and leaves. They were washed under running water, after which diseased tissues were scraped off with a scalpel, infected plant parts were disinfected by immersion in 10% commercial NaOCl for 30 seconds and afterwards rinsed in three changes of sterile distilled water.

After surface sterilization, small pieces of plant tissue were dried with filter paper and then placed on potato dextrose agar (PDA). Plates were incubated at 28±2°C. Colonies growing out of the inoculated points were transferred to freshly prepared PDA medium and pure cultures were then isolated after seven days of incubation and then identified.

Conidia and acervuli of *C. gloeosporioides* were identified morphologically using a compound microscope. Seed samples were obtained from freshly collected fruits from the Ibadan location of the four accession indicated in section 3.2.5. Mycological analysis of 50 seeds and 20 fruits were performed by plating on PDA medium and in blotter tests. They were incubated at 28±2°C under light and darkness for 7 and 12 days, respectively. Fungi were identified on the basis of the appearance of their colonies and spore / conidia morphology.

3.5.2 Effects of planting season on disease incidence and severity

Seeds from the five accessions were planted in June and November, representing wet and dry seasons respectively, of 2010 and 2012 at the Crop garden of CPEB and FRIN. Twenty viable seeds were planted in ten plants per row in two replications arranged in a RCBD. Data on disease incidence and severity were collected on different disease symptoms observed on the leaves, shoots, and roots of the seedlings for the period of 12 months.

3.5.3 Data analyses

Field experiments were laid in RCBD. Data were analysed using analysis of variance (ANOVA) and significantly different means separated using LSD.

CHAPTER FOUR

RESULTS

4.1. Diseased Samples Collected During Survey and Field Studies

Jatropha leaf, shoot, fruit, seed and root infections, were observed in all the sampled areas in the six States of South-western Nigeria (Fig.1.) and at the experimental plots in Ibadan, Oyo state. A total of 1200 samples from diseased plants (leaf spots and blights, diebacks of shoots, stem necrosis and cankers, infected fruits and seeds and apparently healthy tissues were examined.

4.1.1. Fungal species isolated from Jatropha samples

Many fungal colonies were observed on incubated jatropha. Many isolates had no identifiable propagules and were termed indeterminate species. Isolations made from different types of symptoms viz: shot-hole, leaf blight, irregular spots, seed and fruit rot symptoms consistently yielded *Colletotrichum, Fusarium, Lasiodiplodia, Curvularia, Oidum, Aspergillus* and many opportunistic fungi. Cultural studies on the pure cultures obtained showed that the colonies obtained from infected tissues showing the same symptoms, were similar to one another; however, they were sometimes different from those isolated from other types of symptoms. The leaves haboured most of the fungi observed. *Phythophthora*, a fungi-like organism (FLO) was isolated only from infected roots (Table 4.1).

Fig. 4.1 Map of six states in Southwestern Nigeria showing locations sampled

Table 4.1 Fungal species isolated from *J.curcas* in South-Western Nigeria

Isolates	Plant part				
	Leaf	Seed	Stem	Fruit	Root
Curvularia lunata	√	-	-	-	-
Colletotrichum gloeosporioides	√	√	√	√	√
Rhizopus sp.	√	√	√	√	√
Fusarium sp.	√	√	√	√	√
Pestalotia sp.	√	-	-	-	-
Aspergillus sp.	√	√	√	√	√
Penicillium sp.	√	√	√	-	√
Lasiodiplodia sp.	√	-	√	-	√
Corynespora sp.	√	√	-	-	-
Cladosporium sp.	√	-	-	-	-
Oidum spp.	√	-	-	√	-
Phytophthora sp.	-	-	√	√	√
Indeterminate sp.	√	√	√	√	√

√ = present; - = absent

4.1.2 Percentage occurrences of fungal species isolated from Jatropha samples collected from the different states of S/W Nigeria.

In the the six states of South-western Nigeria sampled, five major fungal organisms isolated from the samples were *Colletotrichum*, *Rhizopus*, *Curvularia. Fusarium* and *Aspergillus* spp. The highest percentage occurrences were: 26.0, 25.2, 23.5, 24.2 and 22.0 % respectively. The lowest occurring fungal isolate was *Oidum* sp. with the least mean (7.1%) occurrence in Lagos State. *Colletotrichum, Curvularia* and *Fusarium* were the most frequently encountered fungal pathogen isolated from the infections, while Rhizopus, *Penicillum, Aspergillus, Corynespora* and *Cladosporum* which are opportunistic pathogens were observed on both healthy and diseased tissues. Oyo and Ekiti states had the highest fungal organisms occurring on the samples for most isolates and did not differ significantly (Table 4.2).

4.1.3 Percentage occurrence of seed-borne fungal pathogens associated with five *J. curcas* accessions collected from the gene-bank of FRIN

There were significant differences in the occurrences of all the fungi observed, with maximum mean percentages for *C. gloeosporioides*: 41.1 % on Ex-Ibadan, 38.4 % on Ex-Mbatdiya, and 35.3 % on Ex-Basirika and for *Aspergillus* spp.: 40.8 % on Ex-Kano, 39.3.4 % on Ex-Ibadan. The least incidence was observed on Ex-Ibadan for *Lasiodiplodia* with mean value of 8.1% (Table 4 .3).

4.1.4 Disease symptoms observed in the field at Ibadan

Many disease symptoms were observed on Jatropha plants at the experimental plot in Ibadan after one year of field establishment. These symptoms were similar to those observed from the surveyed areas in the Southern states of Nigeria. The symptoms were leaf spots, blights, defoliation, chlorosis, diebacks of shoots, stem necrosis, cankers, mildew, fruit and seed rots and total death of plants. Fungal pathogens isolated from the diseased samples were also similar to those from the survey sample (Figure 4.2).

Table 4.2 Occurrence of fungal sp. on *J. curcas* from six states of Southwestern Nigeria

State	% Occurrence of fungal species									
	Co	Rh	Fu	Cu	As	La	Pes	Pen	Cl	Oi
Ekiti	26.0	25.2	24.2	23.5	21.3	14.0	14.0	9.8	9.1c	8.5
Lagos	25.4	22.4	23.3	23.4	22.0	12.6	13.1	9.5	8.2	7.11
Ogun	25.3	23.5	20.0	23.3	17.5	12.7	14.8	10.3	9.1	8.5
Ondo	25.0	24.0	22.2	23.2	20.0	15.0	15.8	9.7	11.2	9.4
Osun	25.0	22.6	20.5	22.2	19.6	14.0	14.2	9.5	9.2	10.0
Oyo	25.5	25.1	24.0	23.5	21.3	14.3	14.5	10.6	12.7	8.7
$LSD_{0.05}$	0.81	0.81	1.34	0.77	0.61	0.70	0.61	0.94	1.12	0.87

Co = *Colletotrichum*, Fu = *Fusarium*, Cu = *Curvularia* , As = *Aspergillus*, Rh = *Rhizopus*, La = *Lasiodiplodia*,Pes = *Pestalotia*, Pen = *Pennicillium*, Cl= *Cladosporum*, Oi = *Oidum*

Table 4.3 Incidence of seed-borne fungal organisms associated with five *J. curcas* accessions

	% Occurrence						
Jatropha Accession	*Co*	*Fu*	*Cu*	*Rh*	*Pen*	*As*	*La*
Ex-Misau	14.6	15.4	13.8	14.9	18.4	36.4	11.1
Ex-Kano	30.5	15.6	24.1	22.8	19.4	40.8	11.6
Ex-Basirika	35.3	16.2	12.3	29.5	14.7	30.5	8.5
Ex- Mbatdiya	38.4	32.8	25.7	17.1	20.1	23.9	13.4
Ex-Ibadan	41.1	14.5	23.9	15.2	15.9	39.3	8.1
$LSD_{0.05}$	6.4	11	8.5	8.5	8.7	12.3	6.5

Co = *Colletotrichum,* Fu = *Fusarium,* Cu = *Curvularia* , As = *Aspergillus,*

Rh = *Rhizopus,* La = *Lasiodiplodia,* Pen = *Pennicillium,*

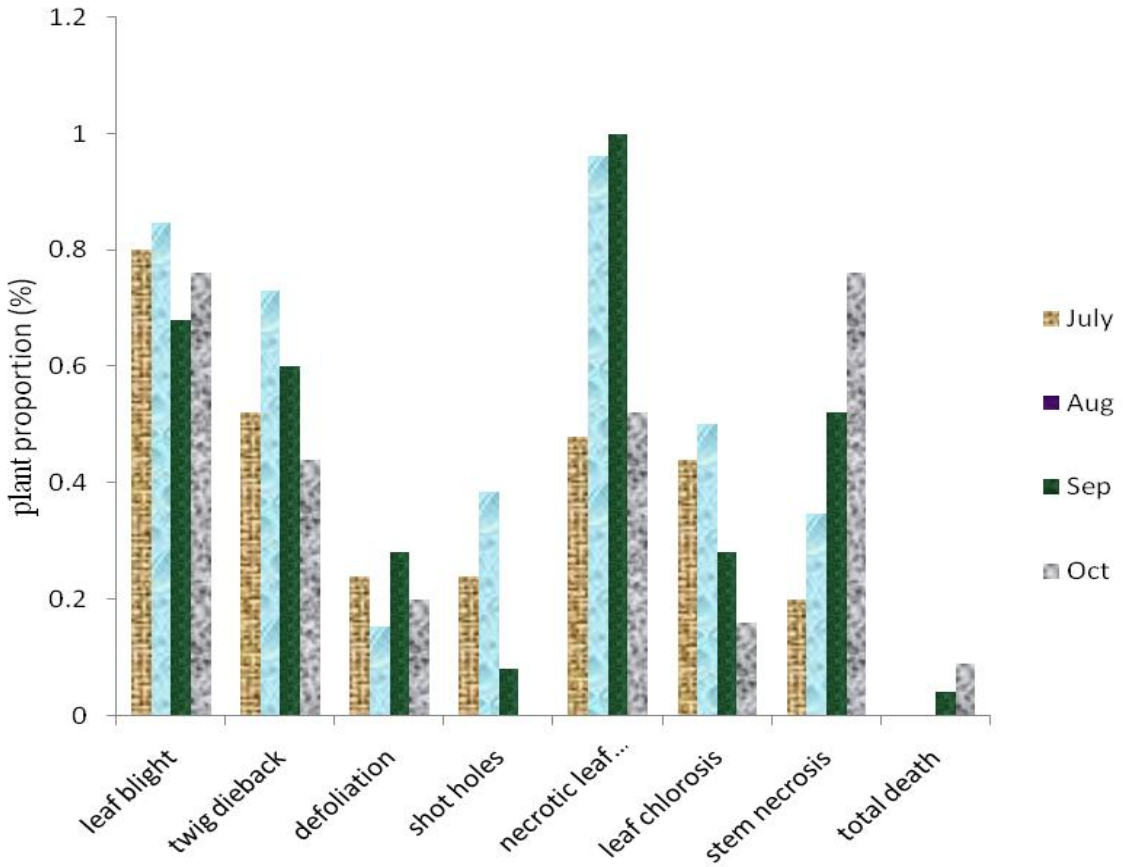

Fig.4. 2 Percentage (%) of plants with disease symptoms on *Jatropha curcas* in the field from July to October, 2009

4.2 Disease Symptoms Observed in the Field

4.2.1 Leaf spots

Leaf infections mainly, (leaf spots) caused by a variety of fungi were observed on *Jatropha curcas*. The disease appeared as spots on foliage. Damage on leaves resulted in several spots symptoms on the foliage. Spots were most often brownish, but sometimes tan or black. Dark margin around the spots were sometimes present. Over time the spots coalesced, enlarged and formed blotches. Spots or blotches were angular and located around the veins and were generally referred to as anthracnose. Leaf spots were found all over the surveyed areas and in the experimental plots. The major fungi isolated from the spots were *Curvularia* sp., *Colletotrichum* sp., *Pestalotia* sp., *Rhizopus* sp., *Cladosporium* sp., and *Aspergillus* sp., causing characteristic brown dead portions on leaves. Infected leaves turned yellow and were shed prematurely, causing defoliation of plants. (Plate 4.1a and b).

4.2.2 Severe leaf blight and chlorosis

The first symptomatic infection was noted at the later stage of the plant life from six months and above. The lesion first appeared as minute yellowish specks, and later discernible as circular reddish brown lesions with a chlorotic halo. As the infection progressed, three clearly distinguishable foliage symptoms viz. shot-hole, irregular spot, chlorosis and severe necrosis were apparent on the affected seedlings. The few yellowish spots on infected leaves later coalesced and turned gray or large brown necrotic lesions on the leaves; they became blighted and eventually dropped-off. Acervuli with characteristic, numerous dark setae of *C. gloeosporioides* and perithecia of the teleomorph, *Glomerella cingulata* were consistently isolated from infected tissues. The isolation of *C. gloeosporioides* without surface disinfection of plant material was most convenient. The pathogen inhabited tissues mainly externally, and surface disinfection during isolation of pathogen caused sterility of the conidia (Plate 4.2).

Besides the pathogen, *Fusarium* spp. and other indeterminate colonies of fungi and bacteria were also observed.

Plate 4.1a Initial stage of leaf spot showing (a) chlorotic spots; (b) necrosis caused by *C. gloeosporioides*

Plate 4.1b Chlorotic spots (a) and Necrotic leaf spots (b) on *J. curcas* caused by *C. gloeosporioides*

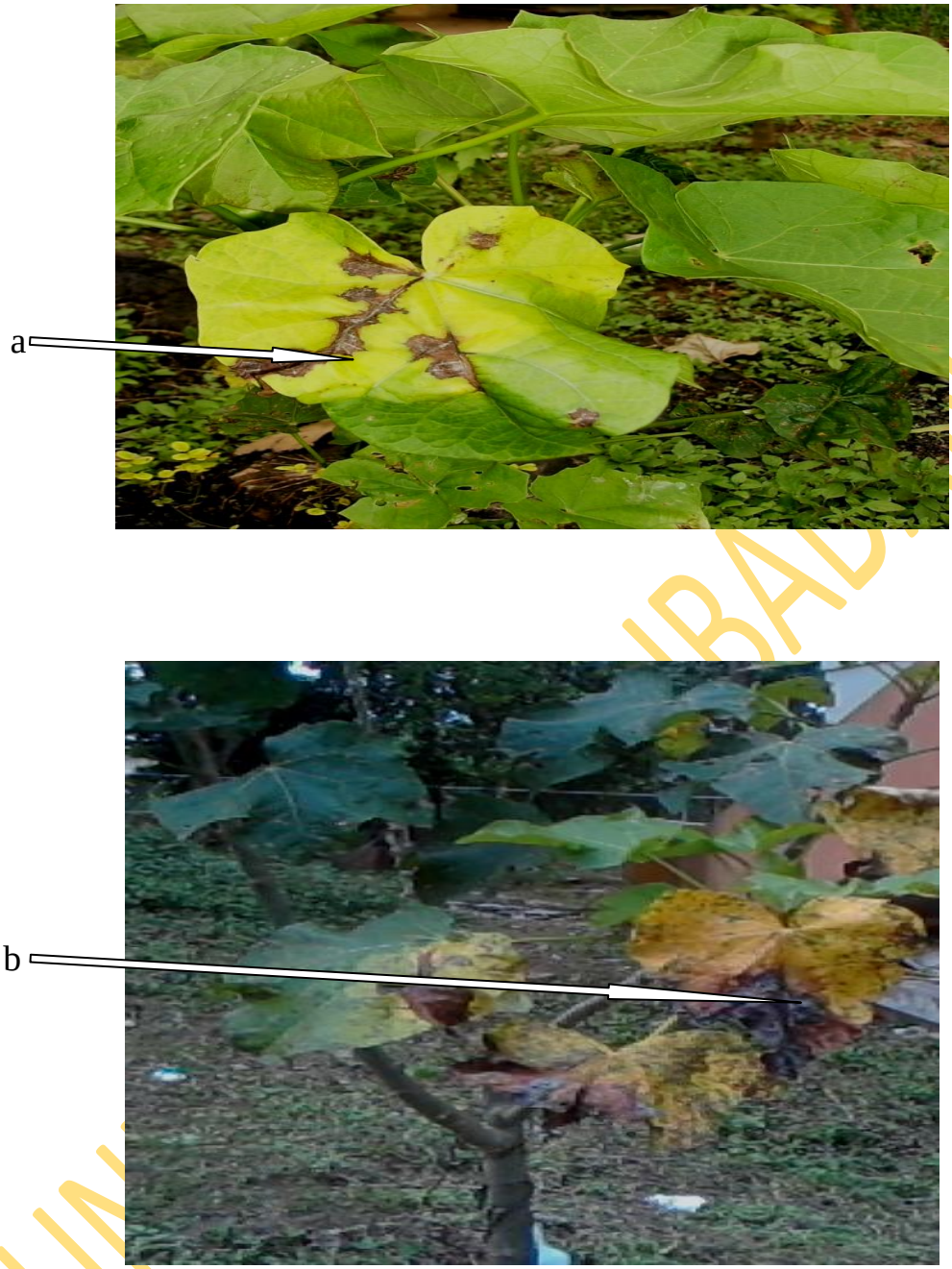

Plate 4.2 Seedling blight showing brown necrotic patches (a) and gray burnt-like patches (b) on *J. curcas* in the field caused by *C. gloeosporioides*

4.2.3 Anthracnose disease on *J. curcas* leaves

Anthracnose disease caused by *C. gloeosporioides* was sometimes confused with other diseases occurring jatropha. Laboratory analysis was the only way that confirmed the causal organism of this disease. Infections often first became apparent at a portion of the leaf margin. The affected plant tissue frequently progresses from chlorotic to necrotic. Infected leaves developed tan to reddish brown lesions that are typically associated with leaf veins; leaves that had already expanded may become cupped and distorted with large areas of dead tissues. In severe cases leave dropped. This pathogen spreads by spores that were easy to splash with irrigation water and rain drops. The spores were somewhat sticky and were not easily spread by simple air movement from the wind. Wounding increased disease severity, it served as first portal for entry of the pathogen (Plate 4.3). Other forms of leaf necrosis were also observed as brown enlarged patches appearing all over the leaf. Isolations confirmed presence of mixed infections of *Curvularia* and *C. gloeosporioides*.

4.2.4 Petiole infection caused by *C. gloeosporioides*.

Incidence of petiole infection observed on *J.curcas* was low in the field. The infection showed as necrotic portion on the petiole of older seedlings causing the petiole to droop and finally led to leaf drop. Isolation from this infection yielded *C. gloeosporioides.* (Plate 4.4).

4.2.5 Symptoms incited on Jatropha plants in later stages of development

Leaf chlorosis caused by *C. gloeosporioides* was observed both in the field and on seedlings inoculated in the green-house. Plants turned chlorotic after four days of artificial inoculation. The yellowish blotches served as early signs for the necrotic lesions which later appeared on the plants at full course of the disease (Plate 4.5.).

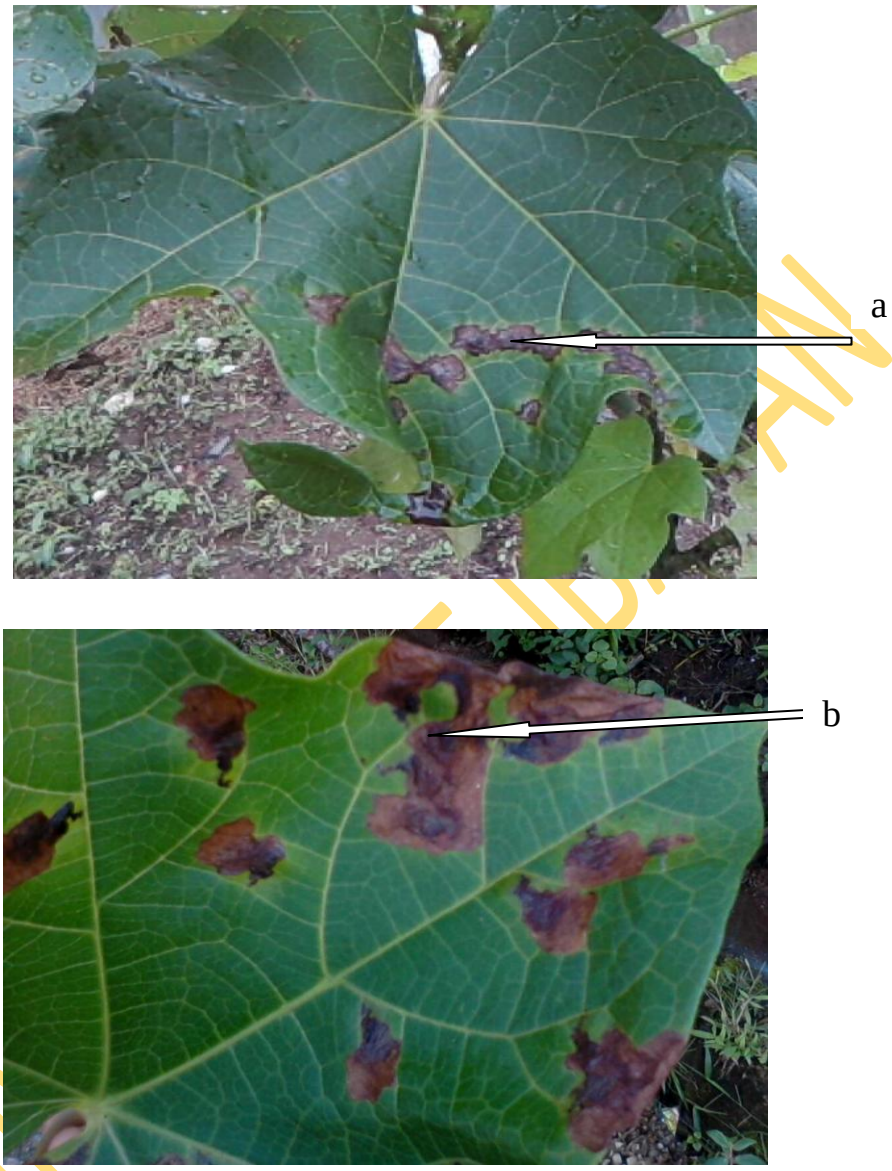

Plate 4.3 Anthracnose disease on *J.curcas* leaves caused by (a) *C. gloeosporioides* and (b) *C. lunata*

Plate 4.4 Petiole infection on Ex- Mbatdiya accession in the field caused by *C. gloeosporioides*

Plate 4.5 Leaf chlorosis on Ex- Misau caused by *C. gloeosporioides* inoculation (14 days after inoculation)

4.2.6 Twig necrosis

On stems, brown to dark-brown lesions distinctly marked from the healthy green portion were observed. The wounds enlarged in size and girdled the stem, increasing rapidly. Eventually, the wounds caused the portion of the plant above the lesion to dry out but remained standing. Stem tissues became red then turned to black and become necrotic; with a large patch of necrotic tissue circumscribing much (60%) of the stem of Jatropha. The necrosis spread through all the internal tissue of the stem when split longitudinally and the discoloration reached into the pith. All the conducting tissues were also discoloured. The death of the conducting tissue prevented the movement of water and minerals from the root upward to the tip of the plant causing the wilting and shedding of leaves. The defoliated upper part still remained green and un-discoloured. Other associated fungal organisms isolated from the infected plants were *Fusarium* sp., *Pestalotia* sp. and other microbial contaminants**.** *C. gloeosporioides* was the major organism implicated for the cause of this symptom after pathogenicity test (Plates 4.6 to plate 4.9).

4.2.7 Shoot canker on *Jatropha curcas* accessions

Cankers or open wounds were observed on older seedlings in this study. This developed from the point of many necrotic lesions incited by *C. gloeosporioides* on the shoots of the plants. The wounds enlarged in size and girdled the stem. The lesions on stem and branches increase rapidly causing the portion of the plant above the lesion to dry out but remain attached to the plant. In some cases of this symptom, plants shed their leaves; shoots broke at slightest wind or rain storm. The wound were ports of entry for further secondary infections as they harboured many opportunistic micro-organisms such as *L. theobromae.* All the accessions were susceptible to this disease but differed significantly in canker expression nine months after planting (9MAP) except Ex-Mbatdiya and Ex – Basirika. Ex – Kano had the highest canker size of 11.3cm average for the two locations. The lowest canker expression was Ex – Misau at a mean of 3.87cm. Canker size was higher on FRIN location than on UI, though, differences were not significant at ($P \leq 0.05$) (Plate 4.10. and Table 4.4).

Plate 4.6 Necrosis (a) and defoliation (b) on twig of *J. curcas* at 12months after planting caused by *Colletotrichum* sp.

Plate 4.7 Length of necrotic portion (A) and the longitudinal section (B) on *J. curcas* shoot *due* to *Colletotrichum* invasion

Plate 4.8 Multiple twig dieback (a) and defoliation (b) due to *Colletotrichum* invasion

Plate 4.9 Lesion caused by *C. gloeosporioides* on *J. curcas* branch resulting in breakage

Plate 4.10 Stem cankers on *J.curcas* seedlings caused by *C. gloeosporioides*

Table 4.4 Incidence of canker on shoots of *J. curcas* accessions under natural infection 9 months after planting (MAP) in two locations

	% incidence	
Accession	UI	FRIN
Ex – Basirika	8.8	10.8
Ex-Mbatdiya	8.6	10.9
Ex – Misau	4.2	3.6
Ex – Kano	9.4	13.2
$LSD_{0.05}$	0.86	2.20

UI = University of Ibadan

FRIN = Forestry Research Institute of Nigeria, Jericho Ibadan

4.2.8 Powdery mildew caused by *Oidum jatrophae*

Powdery mildew disease caused by *Oidum jatrophae* was found to attack *J. curcas* both during the survey and in the field experiment. The damage on leaves shoots and fruits, were dusty white to gray coating which turned into black patches in severe infections. Powdery mildew began as discrete, usually circular, powdery white patches. As these patches expanded they coalesced, producing a continuous mat of mildew (similar to dirt or dust). The major symptoms of the disease were the production of abundant white to gray mycelia on leaves, petioles, stems, flowers and fruits. With the evolution of the disease, infected plants showed necrotic lesions, which caused leaf fall, stunting, death of buds and young fruit deformation.

The fungus that caused this disease was a typical biotrophic pathogen of the phylum Ascomycota, order Erysiphales. The pathogen was characterized by white or grayish colonies, septate and branched mycelia; conidiophores that are erect or ascending, cylindrical, hyaline, septate and forming conidia singly; conidia were large in proportion to the diameter of the conidiophores; simple, smooth, ellipsoid-ovoid deiform, and hyaline, single celled. The disease was favored by warm temperatures, humidity of 75-80% and reduced light. Heavy rains were unfavorable to the pathogen. The disease occurred in the dry season which also coincided with the plants' period of natural defoliation (Plate 4.11).

4.2.9 Fruit and seed infection

There were conspicuous rots on the infected fruits; which when sliced open and progressed to seed rot. Some seeds showed symptoms of infection by fungi. Grey-brownish small spots, deformation of seeds and reduction in size were observed. From incubated seeds, *Aspergillus* sp. and *C. gloeosporioides* were the most dominant fungi species isolated, both on blotter and agar tests. *C. gloeosporioides* infected young flowers and fruits. It also infected the stems, causing damping off, and die-back (Plate 4.12).

Plate 4.11 Powdery mildew caused by *Oidum jatrophae* on *J.curcas* fruits

Plate 4.12p Healthy fruits (a), fruit rot caused by *Colletotrichum, Fusarium and Lasiodiplodia* sp. (b) and (c) rotted immature seeds of harvested *J. curcas* fruit *caused* by *Colletotrichum* sp.

4.3 Dry Rot on Jatropha Caused by *Fusarium* sp.

Fusarium verticillioides caused dry rot along the stem region. Stem tissues were red and later turned black and necrotic. Infected shoots dried up causing loss of shoots due to stem damage (Plate 4.13).

4.3.1 Fusarium wilt and root rot

The presence of vascular disease caused the roots, collar and branches to rot as was confirmed by pathogenicity screening. The disease symptoms were yellowing and withering of the leaves. Leaves of the infected seedlings first became pale green; defoliation of the lower part of the plant followed by the upper leaves in severe cases, reduced leaf size and eventual death of the whole plant were all symptoms of this disease. Lateral roots when uprooted were affected and their tips rotted. There was evidence of severe wet rot, mummification of the stem and root portions**.** Two pathogens, *Fusarium oxysporum* and *Lasiodiplodia theobromae* were identified as the cause of the root rot observed on *J. curcas* (Plate 4.14).

4.3.2 Fruit and seed infections

Different fungal colonies grew out of the cultured infected *J.curcas* seeds and fruits. Whitish, dark to pink mycelia were observed from 24 hours after incubation at room temperature 28±2 ^{O}C. *C. gloeosporioides* was the most prevalent pathogen isolated. *Fusarium* spp. and *L.theobromae* were also closely associated with the infected fruits and seeds while many other fungal contaminants such as *Aspergillus* spp., *Rhizopus* and *Penicillium* spp. were severally encountered (Plate 4.15).

4.4 Conidia of *Curvularia lunata* Isolated from Leaf Spot on *J. curcas*.

Brown and simple conidiophores with apical conidia were observed under the microscope when cultures were incubated for 5-7 days. Conidia were dark, end cells were lighter, and and three to five- celled, typically bent, and with one of the central cells enlarged (Plate 4.16).

Plate 4.13 Stem dry rot caused by *F. verticillioides*

Plate 4.14 Fusarium wilt and root rot caused by *F. oxysporum*

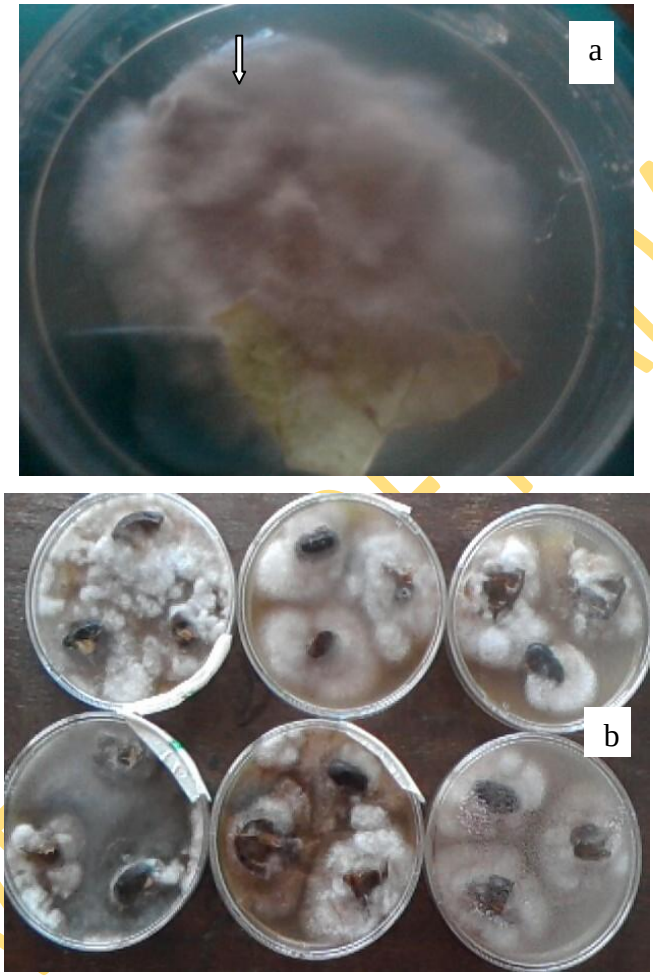

Plate 4.15 Leaf culture showing (a) characteristic whitish to pink coloured colony of *Fusarium* sp. on colonized leaves and (b) fruit culture with multiple fungal colonies on infected *J. curcas*

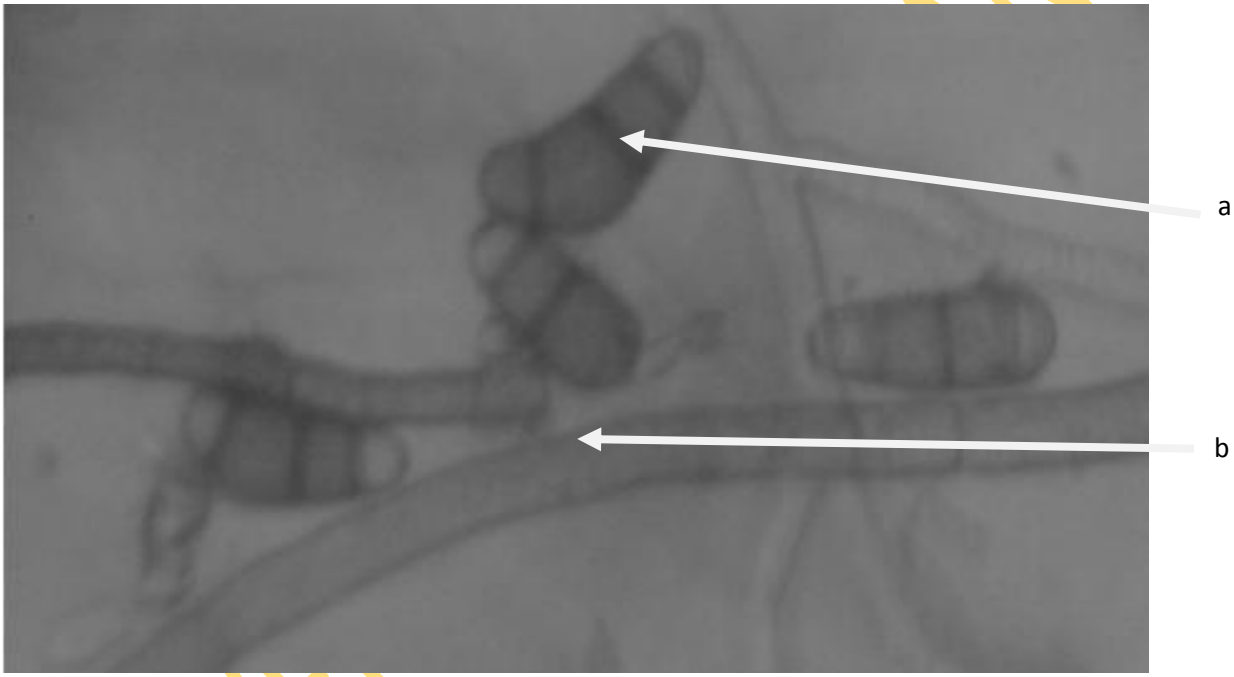

Plate 4.16 Photomicrograph of *Conidia* (a) and hyphae of *Curvularia* (b) isolated from infected leaf of *J.curcas*

4.4.1 *C. gloeosporioides* from infected Jatropha plants

Colony appeared white and gradually turned olivaceous grey in colour as the culture grew older after five to seven days of inoculation on PDA. There were aerial mycelium slightly flocculose with orange conidial pustules evident at the centre of the colony. Reverse of colony appeared smoky grey in colour. Conidia cylindrical with obtuse ends, hyaline, aseptate, uninucleate, formed in setose or globose acervuli or on solitary phialides on mycelium. The acervulus was round to elongate to irregular. There was sparse to profuse setae, dark brown to black, straight to slightly curved, 1-4 septate, swollen at the base and tapering towards the apex (Plates 4. 17 and 18).

4.4.2 *Pestalotia* isolated from leaf spot symptom on *Jatropha*

This fungus was isolated from fresh infected leaves of physic nut. The acervuli were dark and cushion-shaped. The conidia were dark; 3-4 celled, with hyaline, pointed end cells, ellipsoid with two or more hyaline apical appendages (Plate 4.19).

4.4.3 Fungal contaminants (a) (*Cladosporium* sp.) and (b) *Rhizopus* sp. isolated from *J. curcas.*

Cladosporium had distinct dark, upright conidiphores branched near the apex. The conidia were dark, one to two celled, variable in shape and size but mostly cylindrical or lemon-shaped *Rhizopus* had spherical fruiting body which pushed above the surface, with a stalk, a cap and the gills. At first, the caps (heads) were joined all round its edge to the stalk but later collapsed leaving a ring of tissues. Several spores were released from these broken caps through the gills into the air. . The fungi were contaminants and saprobic on decayed leaves and fruits of *J.curcas*. (Plates 4.20 and 4.21).

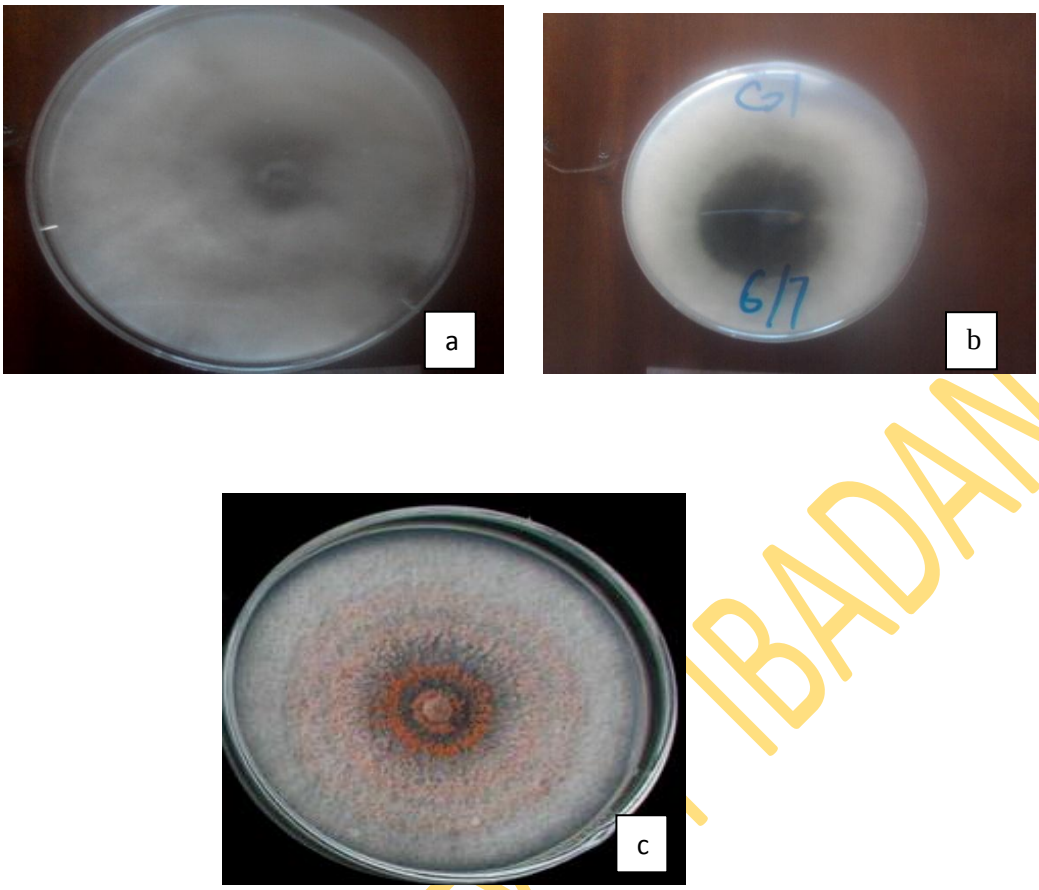

Plate.4.17 Pure cultures of *Colletotrichum gloeosporioides on* PDA isolated from Jatropha samples; (a) Fluffy growth of isolate of *C. gloeosporioides* after three days of inoculation at 28 ± 2°C, (b) colony of *C. gloeosporioides* showing distinct olivaceous grey zonation alternated with rosy buff zonation at the center and (c) culture of *C. gloeosporioides* showing orange conidial pustules apparent at the centre of the colony after five days of inoculation.

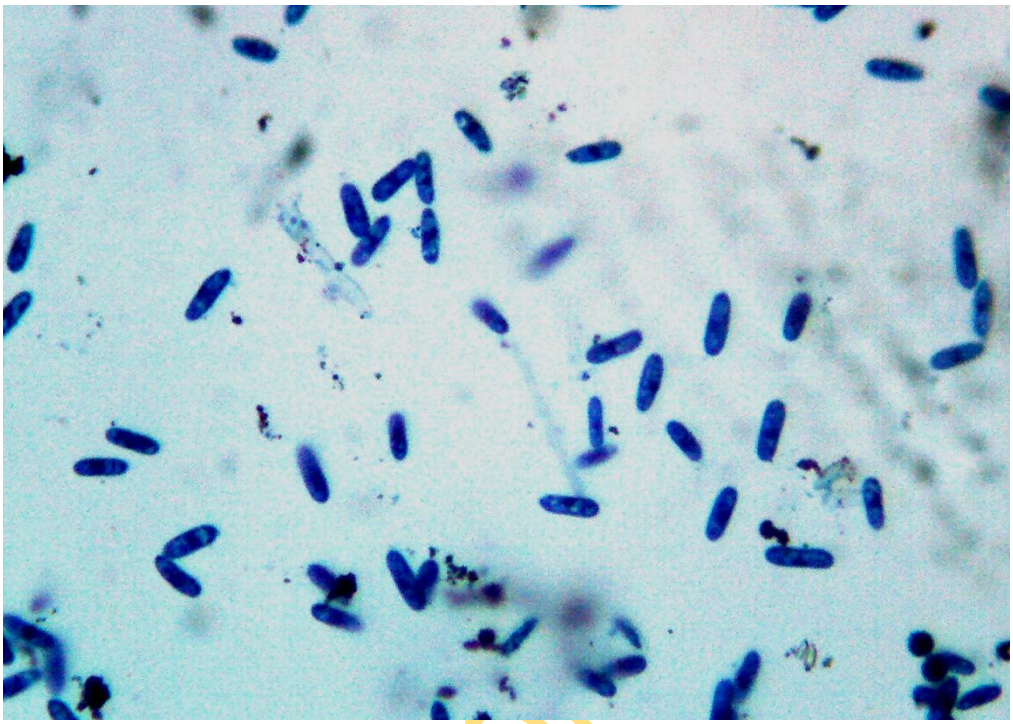

Plate.4.18 Photomicrograph showing cylindrical conidia of *C. gloeosporioides* isolated from *J. curcas* (x 40)

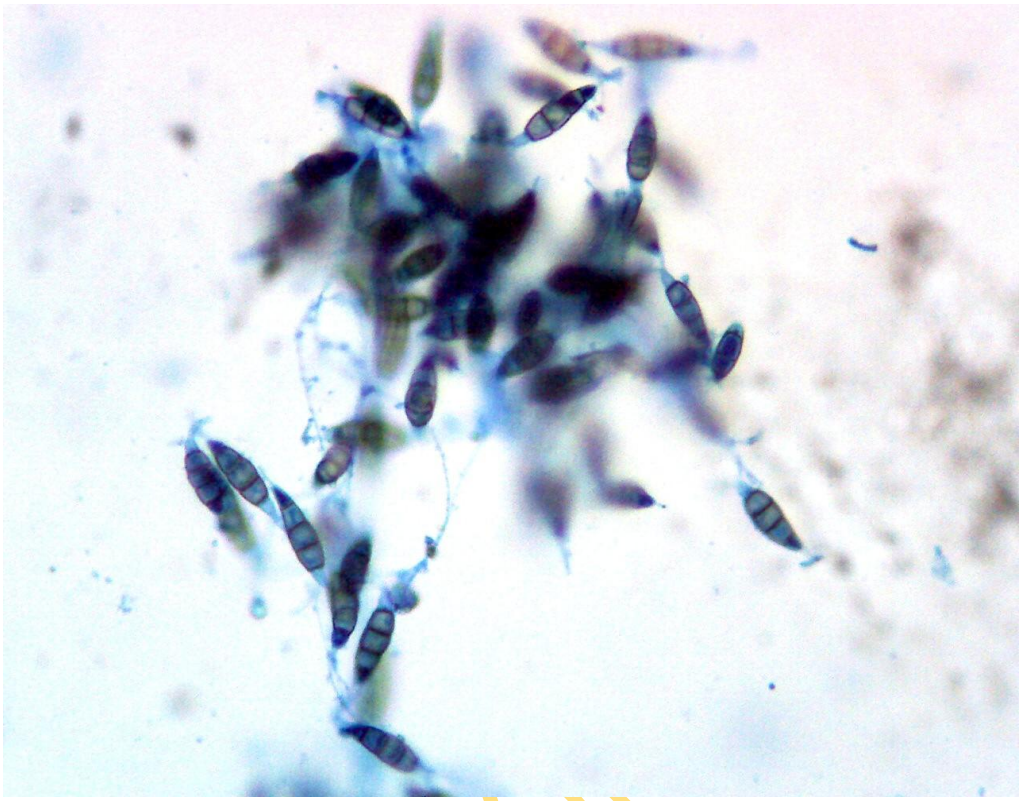

Plate 4.19 Photomicrograph of conidia of *Pestalotia* sp. isolated from *J. curcas* leaf (x40)

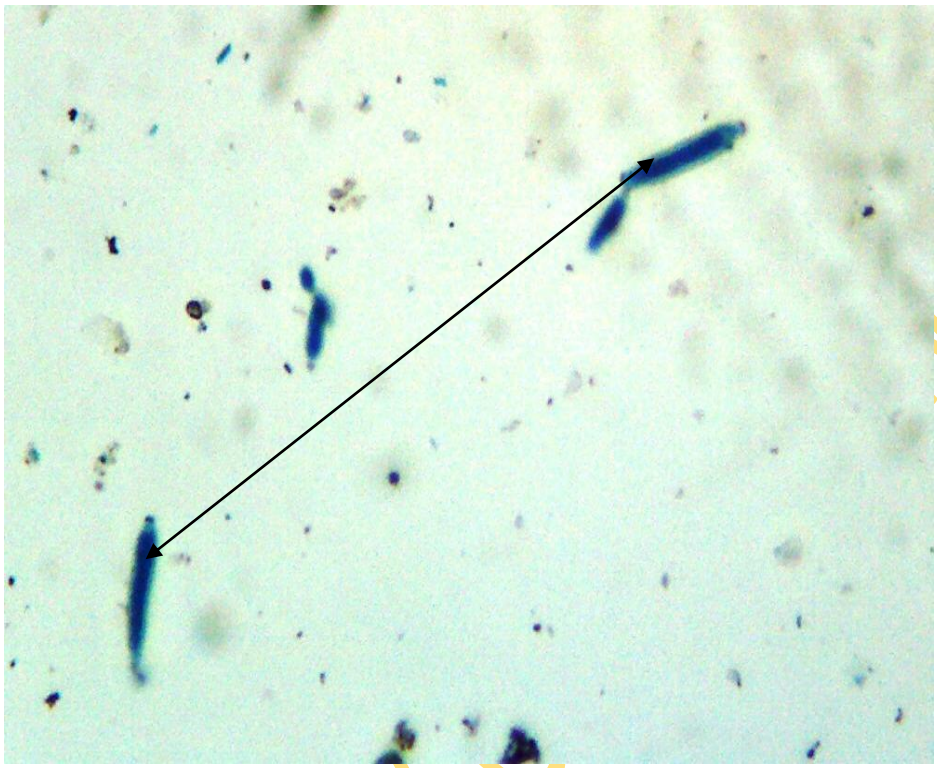

Plate 4.20 Photomicrograph of conidia of *Cladosporium* sp. (x40)

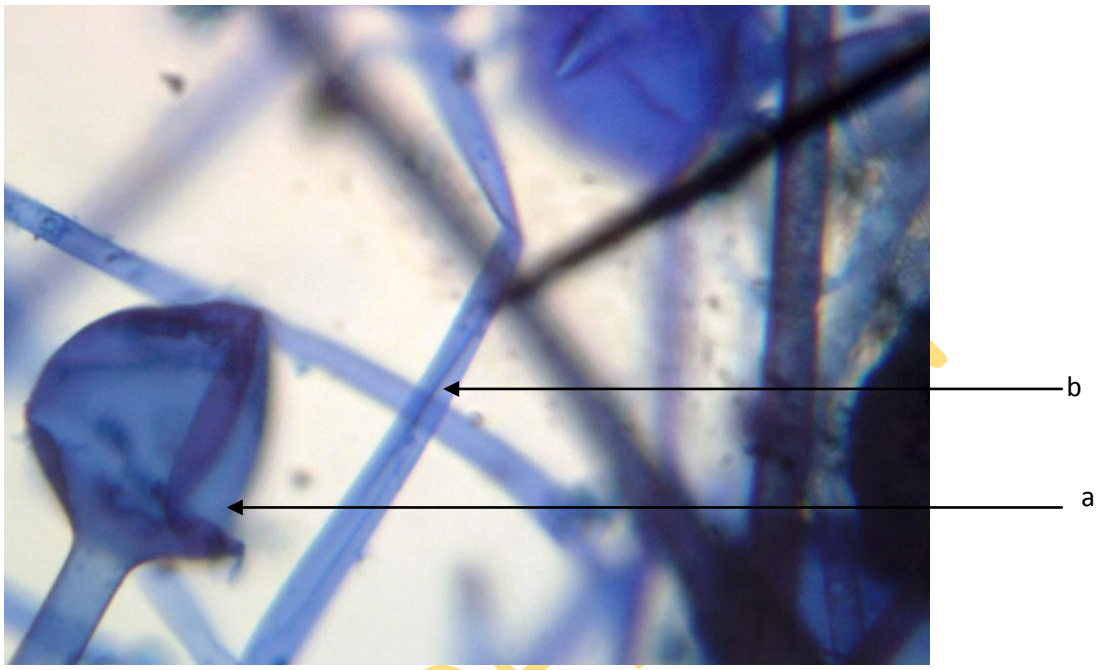

Plate 4.21 Photomicrograph of (a) collapsed cap (head) (b) mycelia strands of *Rhizopus* sp. (x40)

4.4.4 Conidia of *F. oxysporum* isolated from infected parts of *J.curcas.*

Cotton-like mycelium in culture with some tinge of pink or yellow yielded hyaline conidia, one celled (microconidia) and large several-celled (macro-conidia) slightly curved with pointed ends and boat-shaped. Macro-conidia were predominant on seeds of *J.curcas* as latent infection (Plate 4.22).

4.4.5 *Lasiodiplodia theobromae*

Lasiodiplodia theobromae isolated from infected plant parts (roots, fruits and leaves). Samples were cultured and after 4 – 5 days of incubation in PDA at 28 ± 2 °C, the fungus initially produced white colonies, which later turned black (5 – 7 days). The mycelium was fast spreading, immersed, branched and septate. Shiny black pycnidia were produced on the medium after 7 – 8 days in culture. Conidia were initially unicellular, ellipsoidal, hyaline, and thick-walled with granular content. Mature conidia were one-septate, dark brown with longitudinal striations (Plate 4.23).

4.4.6 Pathogenicity test of the fungal organisms isolated from Jatropha plants

Among the several fungal organisms found to be associated with *J.curcas* only five were found pathogenic on Jatropha following Koch's postulate. *C. gloeosporioides* was highly pathogenic compared to all other isolated pathogens. It infected all parts of the plant, the leaves, shoot and the roots. The occurrence of some species such as *Pestalotia* and *Phytophthora* were infrequent and very low therefore were not further investigated (Table 4.5).

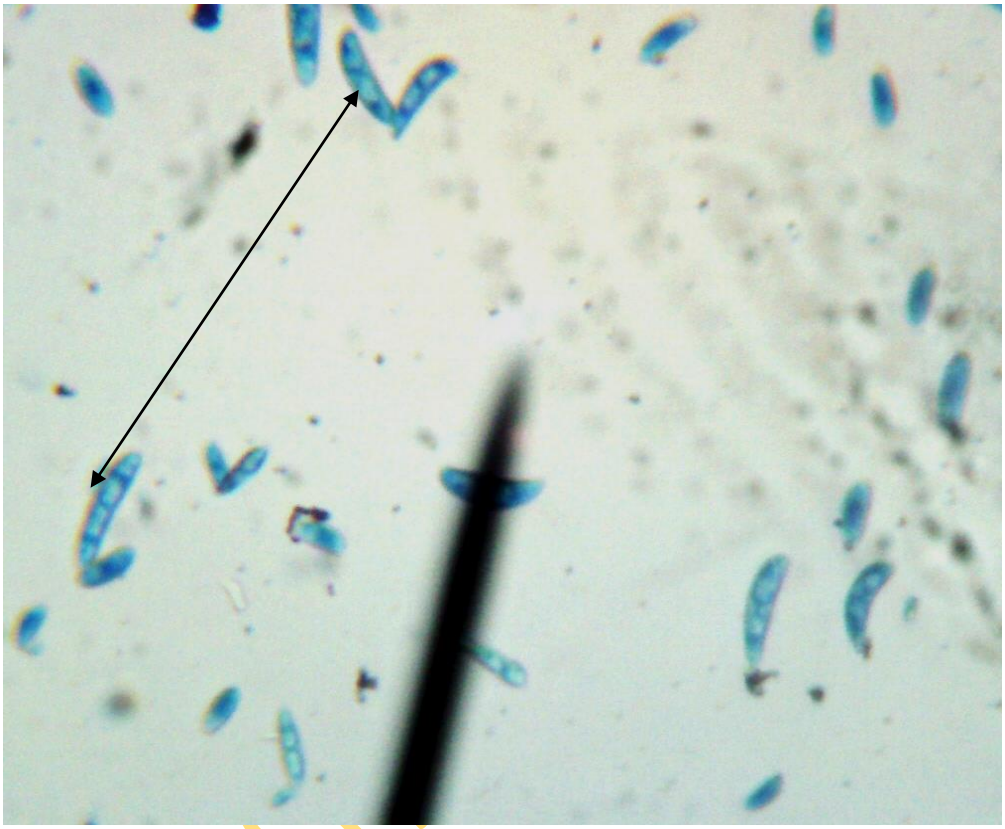

Plate 4.22 Septate conidia of *Fusarium oxysporum* isolated from *Jatropha* samples (x40)

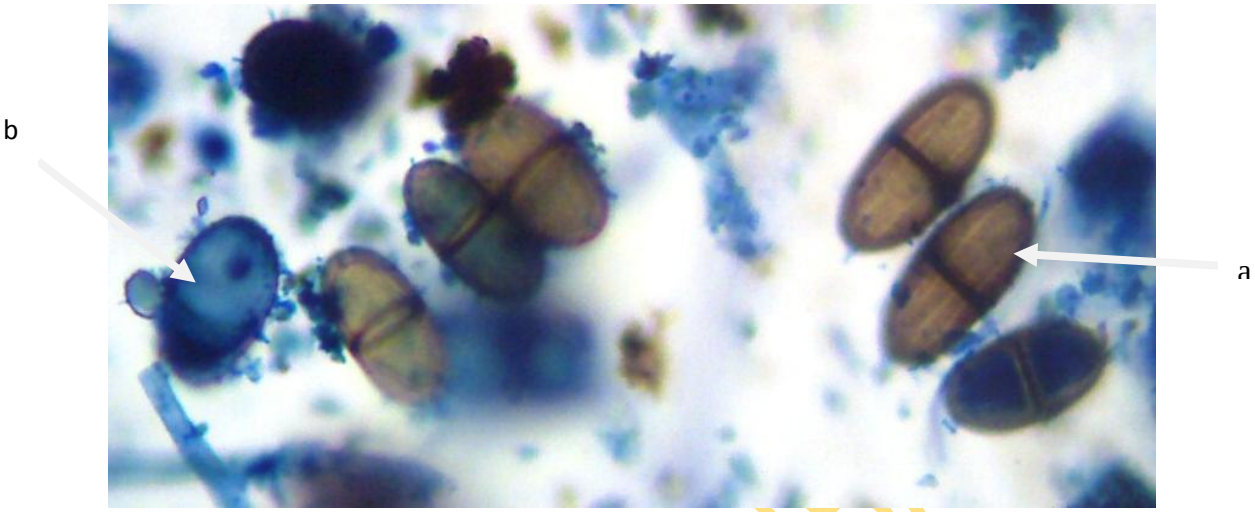

Plate 4.23 Photomicrograph showing septate mature (a) and hyaline unicellular immature conidia (b) of *L.asiodiplodia theobromae* (x40).

Table 4.5 Pathogenicity test of fungal isolates on *J. curcas*

Fungal species plant reactions to inoculation	Plant reactions to inoculation
Curvularia lunata	+
Colletotrichum gloeosporioides	+
Rhizopus sp.	-
Fusarium sp.	+
Aspergillus sp.	-
Penicillium sp.	-
Lasiodiplodia sp.	+
Corynespora sp.	-
Cladosporium sp.	-
Oidum sp.	+
Indeterminate sp.	-

+= Pathogenic on Jatropha

- = Not pathogenic on Jatroph**a**

4.4.7 Reaction of selected *Jatropha* accessions to canker causing pathogen under different inoculation techniques

Canker incidence was significantly (P= 0.05) lower with leaf spray technique in all the Jatropha accessions than with stem puncture. There were no significant differences in canker expression between 8WAI and 12WAI. The degree of infection under the techniques varied significantly from 8WAI and 12WAI with the 12WAI having the maximum incidence value of 16.8 % on Ex-Kano. There were no significant differences observed at P = (0.05) in all the accessions at 8WAI under leaf spray technique but at 12 WAI Ex- Mbatdiya and Ex-Kano differed from the other accessions significancantly in canker expression. The mean percentage incidences in all the inoculated plants were significantly higher from their control counterparts (Table 4.6).

4.4.8 Effect of inoculation of different pathogens on leaf spot expression on *J. curcas*

There were signiffcant differences in disease incidences caused by each pathogen at four weeks after inoculation. Mean maximum values of 18.9, 11.8, 9.3 and 3.0 % were recorded for *Colletotrichum* on Ex- Kano, *Curvularia* on Ex-Mbatdiya, *Lasiodiplodia* on Ex- Kano and *Oidum* on Ex-Basirika respectively. *Fusarium* sp. had the least damage (leaf spot) but did not differ at (P= 0.05) from other accessions. *Colletotrichum* ranked first followed by *Curvularia*, *Lasiodiplodia* and Oidum on leaf spot damage on the inoculated plants (Table 4.7).

4.4.9 Disease incidence incited by combined inoculation of four fungal pathogens on Jatropha seedlings

Disease incidence was significantly high on Ex – Kano, Ex – Basinka and Ex – Mbatdiya. The four fungal pathogens induced wilting on all the accessions at 4WAI. Mixed pathogen inoculation induced significantly higher disease damage with mean maximum value of 20.6 % than single pathogen inoculations. Single inoculation of *L. theobromae* gave highest incidence value of 18.2 % while, *Curvularia*, *Colletotrichum* and *Fusarium* sp. had mean maximum values of 14.1%, 12.3 % and 13.0 % respectively. Ex – Misau had the least incidence with *Curvularia* with a mean value of 5.8 % on (Table 4.8).

Table 4.6 Comparison of two inoculation techniques on the incidence of canker caused by *Colletotrichum* sp. on *J. curcas* accessions

	Incidence (%)			
	Leaf spray		Stem puncture	
Accession	8WAI	12WAI	8WAI	12WAI
Ex-Basirika	4.7	6.3	10.1	16.3
Ex Mbatdiya	4.9	5.4	8.0	14.8
Ex-Misau	4.7	4.9	5.9	10.7
Ex-Kano	6.3	7.0	8.1	16.8
$LSD_{0.05}$	1.81	0.69	1.97	1.65

WAI = Weeks after Inoculation

Table 4.7 Leaf spot incidence on *J. curcas* accessions at four weeks after inoculation

	% incidence at 4 WAI				
Accession	*Colletotrichum*	*Curvularia*	*Lasiodiplodia*	*Oidum*	*Fusarium*
Ex-Basirika	15.2	10.8	7.3	3.0	1.8
Ex-Mbatdiya	13.8	11.8	7.1	2.7	1.4
Ex-Misau	10.1	2.8	3.2	1.0	1.4
Ex-Kano	18.9	11.0	9.3	2.0	1.6
$LSD_{0.05}$	1.29	2.25	1.40	1.59	1.67

WAI = Weeks after Inoculation

Table 4.8 Wilting incidence on *J. curcas* accession after four weeks of combined inoculation with four fungal pathogens

Accession	% Incidence				
	Co	Cu	La	Fu	Co + Cu + La + Fu
Ex-Basirika	10.4	13.8	18.2	13.0	20.2
Ex-Mbatdiya	10.3	14.2	16.7	12.4	20.6
Ex-Misau	6.4	5.8	13.2	7.9	12.8
Ex-Kano	12.3	14.1	16.9	11.2	20.4
LSD $_{0.05}$	1.40	2.25	1.29	1.59	1.67

Co = *Colletotrichum*, Cu = *Curvularia*, La = *Lasiodiplodia*, Fu = *Fusarium*.

4.4.10 Susceptibility scoring on Jatropha accessions after artificial inoculation

Chlorosis, wilting and defoliation were observed on the inoculated seedlings in all the accessions at the end of experiment at 63 days after inoculation.. The time of appearance for the first symptom (T_0) varied from seven days after inoculation in most susceptible accessions (Ex-Kano, Ex – Basirika and Ex- Ibadan) to 25 days after inoculation (DAI) in Ex – Misau and Ex – Mbatdiya. In Ex- Misau, it took 63 days to attain 100% leaf infection with very low disease severity (1.2 %), (Table 4.9).

4.4.11 Major disease symptoms observed on leaves at four to 12 weeks after inoculation

Inoculated plants showed leaf chlorosis, spot and blight. *Colletotrichum* induced multiple symptoms with significant differences observed from the control plants. Ex – Kano had the highest mean disease value of 26.5 % for leaf spot at 12 WAI which was significantly different from only Ex-Misau with the least mean value of 9.0 % for leaf chlorosis at 12WAI. The highest leaf blight incidence was recorded at 12WAI on Ex-Mbatdiya. In all the accessions, there werre no significant differences at 8WAI and 12WAI in most of the symptoms observed (Table 4.10).

4.4.12 Root infection caused by *F. oxysporum*

Accessions showed no significant difference at the inoculation treatments at twelve weeks after inoculation (12 WAI). There was difference between incidence at three months and six months duration with mean maximum value of 21.3 % recorded with Ex-Kano at six MAI. The least incidence mean value of 16.8 % was recorded in accession Ex-Misau at 12 WAI, (Table 4.11).

Table 4.9 Time lag between inoculation and anthracnose expression on selected *J. curcas* accessions inoculated with C*olletotrichum gloeosporioides*

Accession	T_0	T_{50}	T_{100}	%DI	DSI
Ex-Ibadan	6.7	12.8	22.3	53.4	2.3
Ex-Kano	7.6	19.0	21.4	59.6	2.4
Ex – Basirika	17.8	34.8	47.0	61.6	2.3
Ex- Mbatdiya	24.8	32.4	25.3	58.7	3.0
Ex-Misau	25.0	45.5	62.7	8.1	1.2
$LSD_{0.05}$	2.80	3.35	7.55	3.70	0.67

T_0 = days from inoculation until the first leaf with symptom appeared, T_{50} =days from inoculation until 50% of leaves were affected, T_{100}= days from inoculation until 100% of leaves were affected.

DI =Disease incidence, DSI (DII) = Disease severity index or (Disease intensity index)

Table 4.10 Incidence (%) of leaf chlorosis (LC), leaf spot (LS) and leaf blight (LB) on *J. curcas* inoculated with *Colletotrichum sp*.

	Accession											
	Ex-Basirika			Ex-Mbatdiya			Ex-Misau			Ex-Kano		
WAI	LC	LS	LB	LC	LS	LB	LC	LS	LB	LC	LS	LB
4WAI	20.6	23.6	23.0	19.9	21.6	23.3	10.8	10.3	11.6	21.6	25.2	23.4
8WAI	22.5	25.2	24.4	22.1	24.4	25.2	11.2	10.3	9.3	23.1	25.9	25.3
12WAI	22.5	25.8	24.2	22.2	24.8	25.5	9.0	9.9	9.6	24.2	26.5	25.5
$LSD_{0.05}$	1.30	1.49	0.85	1.76	2.27	1.72	1.71	2.15	3.13	1.40	1.24	2.10

LC = leaf chlorosis, LS = leaf spot, LB = leaf blight

WAI = Weeks after Inoculation

Table 4.11 Root rot incidence on *J. curcas* accessions inoculated with *F. oxysporum* in the screenhouse

	(%) incidence	
Accession	3 MAI	6 MAI
Ex –Basirika	17.3	19.2ns
Ex – Misau	16.8	19.1
Ex- Ibadan	17.2	22.7
Ex- Kano	17.5	21.3
Ex-Mbatdiya	17.0	19.2
LSD $_{0.05}$	NS	2.00

MAI = months after inoculation

NS = Not significantly different

4.4.13 Lesion development on stems caused by *Colletotrichum* sp. Inoculation

At four-week- after inoculation (WAI), there was lesion development which progressed, girdling the regions of the shoots at the point of inoculation. At 12 WAI, the maximum mean percentage incidence of lesion on the most susceptible accession, Ex- Kano was 4.7 % and this was significantly different from the mean lesion observed at four weeks after inoculation. All the accessions were susceptible to *Colletotrichum*. All the accessions did not differ significantly in their lesion expression under four and eight WAI. At 12 WAI Ex-Mbatdiya had the minimum mean lesion size of 1.9 %, and was significantly different from the other accessions (Table 4.12).

4.4.14 Disease reaction on Jatropha accessions after L. theobromae treatment

The accessions were susceptible to collar rot pathogen, *Lasiodiplodia*. Disease expression on the accessions was highest at 12 WAI. Incidence at four WAI was significantly lower than at eight and twelve WAI which did not differ significantly at (P= 0.05) in all the accessions. Ex-Misau had the least disease incidence, 9.2 % significantly lower than in all the other accessions (Table 4.13).

4.4.15 Disease expression on Ex-Misau inoculated with *Colletotrichum* and *Curvularia* species.

Spraying leaves of Ex-Misau, with *Colletotrichum* and *Curvularia* produced foliar disease symptoms. Simultaneous inoculation of *Colletotrichum* and *Curvularia* had the highest wilt incidence (20.1 %). There were significant variations in disease expressions observed on all the accessions after treatment. Incidence of chlorotic spots, necrotic lesions, petiole infection, defoliation and wilt were high. Petiole infection had the least mean incidence value among the symptoms expressed with mean value of 4.9 % for *Curvularia* single inoculation. Wilting, defoliation necrotic lesions were high with all types of inoculations (Table 4.14).

Table 4.12 Stem lesion caused by *C. gloeosporioides* on *J.curcas* shoots at four, eight and twelve weeks after inoculation

	Lesion (%)		
Accession	4 WAI	8 WAI	12 WAI
Ex –Basirika	2.1	2.5	3.7
Ex-Mbatdiya	2.2	3.3	1.9
Ex – Misau	1.7	2.5	3.8
Ex- Kano	1.8	3.5	4.7
$LSD_{0.05}$	ns	ns	1.73

WAI = Weeks after Inoculation

Ns = not significant

Table 4.13 Collar rot incidence (%) on *J. curcas* accessions inoculated with *L. theobromae* in the screenhouse

	Accession			
WAI	Ex-Basirika	Ex-Kano	Ex-Misau	Ex-Mbatdiya
4 WAI	23.6	25.2	11.6	21.6
8 WAI	25.2	25.9	9.2	23.1
12 WAI	25.8	26.5	9.6	24.2
$LSD_{0.05}$	1.49	1.24	3.13	1.40

WAI = Weeks after inoculation

Table 4.14 Reaction of Ex- Misau accession to double pathogen inoculation of *C.gloeosporioides* and *Curvularia lunata* at (12 WAI)

Inoculation treatment	% Incidence				
	Chlorotic spots	Necrotic lesion	Petiole infection	Wilt	Defoliation
Co	9.5	14.7	6.7	17.5	13.8
Cu	6.7	9.2	4.9	19.1	15.0
Co + Cu same time	9.5	15.4	7.9	20.1	12.6
Co One week before Cu	10.0	16.2	7.8	19.5	14.7
Cu, one week before Co	9.5	15.1	8.8	19.4	15.0
Control	2.5	1.7	1.3	3.0	4.3
LSD $_{0.05}$	2.23	3.25	2.26	3.70	2.36

. Co = *Colletotrichum*, Cu = *Curvularia,*

WAI = Weeks after inoculation

4.5 Plant Weights of Inoculated Jatropha Accessions with their Control Counterparts

The results indicated that in all the accessions, inoculated plants differed significantly from their control. Fresh weights of the inoculated plants were lower due to the loss of roots, leaves and stunting caused by the pathogen. Plant weights of inoculated Ex-Misau and Ex-Mbatdiya showed no significant difference but were different from Ex-Basirika and Ex – Kano. The highest plant weight of 51.4g in the inoculated plants was observed from Ex-Misau. There were no significant differences (P= 0.05) in fresh weight among the uninoculated plants (Table 4.15).

4.5.1 Anthracnose expression on *J. curcas* under natural infection

Observation in the field showed that there was low incidence of leaf chlorosis at three months after planting in all accessions. Leaf blight and wilt were significantly high in all the test plants. Leaf blight among the disease symptoms observed recorded the highest incidence both at three and six MAP. Ex-Mbatdiya had the highest disease incidence of 33.9 % at nine MAP for leaf chlorosis. However wilting incidence was higher at three MAP than at nine MAP in all accessions. Ex-Kano was the most susceptible at nine MAP with mean highest (32.9 %) for leaf blight (Table 4.16).

4.5.2 Performance of five *J. curcas* accessions in the field under natural infection by *C. gloeosporioides*

All the accessions were susceptible under field infection. They differed significantly in their disease responses. Ex-Misau had least infection. There were yellowish patches on the shoots which later turned brownish and necrotic; girdling the whole stem. These led to wilting and defoliation on the affected twigs or whole shoot causing them to break off or had tip diebacks. This resulted in loss of branches on the affected plants. In severe infections, there were open wounds (cankers) which developed from the point of the necrotic lesions on the stem. Ex – Kano was highly susceptible with maximum mean values of leaf infection, shoot lesion and canker incidences (59.5 %, 41.4 % and 16.8 %) respectively. (Table 4.17).

Table 4. 15 Fresh plant weights of *Jatropha* accessions inoculated with *C. gloeosporioides*

	Fresh weight (g)		
Accession	Inoculated	Uninoculated	$LSD_{0.05}$
Ex - Misau	51.4	97.6	1.52
Ex – Mbatdiya	51.0	91.1	2.03
Ex –Kano	48.3	83.3	3.42
Ex – Basirika	41.1	84.0	4.40
$LSD_{0.05}$	4.07	2.46	

Table 4.16 Disease incidence on *J. curcas* accessions at 3 and 6 months after field establishment

Accession	% incidence								
	Leaf chlorosis			Leaf blight			Wilt		
	3	6	Mean	3	6	Mean	3	6	Mean
Ex-Basirika	21.1	24.2	23.15	20.1	23.0	21.55	15.2	15.0	15.1
Ex-Mbatdiya	22.0	34.0	28.0	20.1	23.7	21.9	16.1	15.8	24.0
Ex-Misau	21.2	21.3	21.25	22.4	22.8	22.6	12.6	11.0	18.1
Ex-Kano	22.0	24.5	23.25	12.2	33.0	22.6	16.4	16.0	16.2
$LSD_{0.05}$	1.22	1.09		1.82	1.65		2.0	2.6	

MAP = Month after planting

Table 4.17 Disease severity on *J. curcas* accessions for resistance to anthracnose under natural infection at 9 and 12 MAP

	Severity score					
	9 MAP			12 MAP		
Accession	Leaf lesion	Shoot Lesion	Canker	Leaf lesion	shoot Lesion	Canker
Ex – Misau	14.7	11.6	4.8	28.3	33.5	8.9
Ex-Mbatdiya	17.0	22.1	5.1	43.4	35.5	9.4
Ex – Basirika	33.6	19.6	5.0	58.4	37.4	10.4
Ex – Kano	35.3	20.7	5.6	59.5	41.4	16.8
Ex-Ibadan	35.9	21.0	6.1	59.3	40.5	15.9
$LSD_{0.05}$	1.80	1.2	0.91	2.52	1.21	0.61

MAP = Months after planting

4.5.3 Foliar infections in early and later stages of plant growth of Jatropha accessions

Blight, spots and shot-hole symptoms were observed on younger leaves. However on older leaves the incidence was low but was dominated with irregular spots. The first evidence of infection was noted four days after germination. The symptoms caused by *C. gloeosporioides were* undistinguishable at the initial stage of infection. The lesion first appeared as minute yellowish specks, and later discernible as circular reddish brown lesions with a chlorotic halo. As the infection progressed, three clearly distinguishable foliage symptoms viz, shot-hole, irregular spot and blight were apparent on the affected seedlings. All the four accessions were susceptible at various stages of observation. There were no significant differences in disease incidence from four, eight MAP but at 12 MAP anthracnose expression on the accessions differed significantly with Ex-Misau performing better than all the accessions in terms of disease expression with a least mean infection of 28.3 % recorded at 12MAP (Table 4.18). Plants reaction to shot-holes was generally low while defoliation symptom was the highest observed leaf infection. Leaves showing chlorosis dropped earlier than the normal leaf life. Shot -hole symptom persisted in the field enlarging to form ragged leaf appearance. In all the accessions, Ex- Kano was significantly more susceptible than the other accessions having maximum mean value of 34.9 % defoliation (Table 4.19).

4.5.4 Expression of shoot dieback symptoms on seedlings of *J. curcas* at six and 12 months under natural field infection

All the accessions showed symptoms of both tip and shoot dieback as a result of necrotic lesions found earlier on affected plants in the field. This was as a result of the damage done by the pathogen on the conducting tissues of the plants. Ex – Kano had the highest disease incidence of 43.1 % at 12 MAP which differed significantly from the Ex – Misau with mean incidence of 26.0 %. There was no variation among the accessions at six MAP (Table 4.20).

Table 4. 18 Resistance evaluation of *J.curcas* accessions for anthracnose resistance at 4, 8 and 12 months after planting

	% incidence		
Accession	4 MAP	8 MAP	12 MAP
Ex - Misau	22.5	23.2	28.3
Ex - Basirika	21.9	24.3	37.4
Ex - Mbatdiya	18.0	18.5	43.4
Ex - Kano	22.9	23.7	59.5
Ex-Ibadan	23.7	24.6	61.4
$LSD_{0.05}$	2.03	3.2	1.8

MAP = Months after planting

Table 4.19 Incidence of leaf chlorosis, shot–hole and defoliation on *J. curcas* accessions at 9 months after planting in the field

	Incidence %		
Accession	Leaf chlorosis	shot-holes	defoliation
Ex-Misau	21.3	24.3	31.3
Ex – Kano	28.0	29.8	34.9
Ex – Mbatdiya	28.7	29.0	31.1
Ex – Basirika	30.1	27.2	32.3
Ex-Ibadan	31.4	28.5	32.1
LSD $_{0.05}$	2.43	2.6	1.87

Table 4.20 Shoot dieback on *J. curcas* at 6, 9 and 12 MAP under natural field infection by *C. gloeosporioides 1*

	Disease incidence (%)		
Accession	6 MAP	9 MAP	12 MAP
Ex-Misau	23.0	26.3	26.0
Ex – Mbatdiya	29.4	42.1	36.5
Ex – Basiriki	30.2	43.6	35.2
Ex-Ibadan	35.7	42.8	36.3
Ex – Kano	37.4	37.2	43.1
$LSD_{0.05}$	5.10	1.55	1.35

MAP = Month after planting

4.5.5 Severity scoring of powdery mildew on Jatropha accessions under field infection

Plants showed significant variations on disease severity at six, nine and twelve months after planting. Among the accessions there were also differences in performance with Ex-Basirika performing better with the least mean disease score of 5.1 % at six MAI. Ex-Mbatdiya and Ex-Ibadan were highly susceptible and had the highest disease score values of 19.2 % and 20.2% at nine MAP among the other accessions (Table 4.21).

4.5.6 Screening of Jatropha under three seasons of field observation for powdery mildew infection

The results indicated that the incidence of grey mildew was high among all the accessions. A low incidence was however recorded in 2010 and 2011 in field. A significant outbreak of grey mildew occurred in the field in 2012 during the dry periods of August – September (Table 4.22). There was inoculum build-up in the field from 2010 to 2012 which caused significantly high disease values in the year 2012. There were no variation in mildew incidence among the accessions in year 2010 and 2011 however, in 2012, Ex-Basirika and Ex-Mbatdiya (41.5 % and 41.9 %) significantly (P=0.05) differed from Ex-Misau, Ex-Ibadan and E-Kano (25.2 %, 23.7 and 20.6 %) respectively.

4.5.7 Root rots expression on *J.curcas* plants in the field

Plants showed high incidence of root rot leading to high mortality in the first 3 months after planting. The mummified shoots when uprooted showed symptom of decayed roots which were smelly and water soaked. The leaves first became chlorotic and later defoliated. There were significant differences in the incidence and severity of rot among the accessions. Ex-Ibadan, Ex-Mbatdiya and Ex- Kano (33.8, 36.7 % and 35.7 %) were statistically different from Ex-Misau (28.5 %) and Ex-Basirika (33.2 %) at P=0.05 in disease incidences. However, disease severity index was significantly different among the accessions.Ex-Ibadan and Ex-Basirika had the highest mean score (40.2 and 38.9 %); the least value of 19.5 % was recorded on Ex-Misau (Table 4.23).

Table 4.21 Disease severity on leaves of *J. curcas* plants infected by *Oidum* sp. at different seedling ages under natural infection

	% leaf area affected / Accession				
Seedling age	Ex-Mbatdiya	Ex-Kano	Ex-Misau	Ex-Ibadan	Ex-Basirika
6months	9.2	7.7	8.8	8.4	5.1
9months	19.2	14.0	14.4	20.2	10.5
12months	18.1	14.0	13.4	18.3	10.1
$LSD_{0.05}$	2.50	1.76	3.0	2.11	2.70

Table 4.22 Incidence of grey mildew caused by *Oidum* sp. on *Jatropha* accessions under three observation seasons

	Mildew incidence %		
Accession	2010	2011	2012
Ex - Kano	0.3	3.2	20.6
Ex-Ibadan	0.5	3.3	23.7
Ex - Mbatdiya	0.6	2.4	41.9
Ex - Misau	1.7	5.4	25.2
Ex - Basirika	1.9	4.4	41.5
$LSD_{0.05}$	ns	1.64	2.36

Table 4.23 Incidence and severity of root rots on *J. curcas* caused by *Fusarium oxysporum* and *L. theobromae* at three months after planting (MAP)

	% root rot	
Accession	Incidence	Severity
Ex – Misau	28.5	19.5
Ex - Mbatdiya	36.7	25.3
Ex - Kano	35.7	30.5
Ex - Basirika	33.2	38.9
Ex-Ibadan	33.8	40.2
$LSD_{0.05}$	2.3	1.9

MAP = Months after planting

4.5.8 Incidence and severity of shoot/tip dieback symptoms.

All the accessions were susceptible in the field to shoot and tip dieback caused by *C. gloeosporioides.* There were higher disease scores in months where insect pest (mealybug) infestation was higher. These wound-causing insects predisposed the plants to secondary infection and desiccation. There was no definite trend in the susceptibility of the accessions though there were significant differences observed among the accessions. These did not take specific pattern as to which accession performed better in terms of field resistance to shoot dieback. However Ex-Kano had maximum incidence value of 69.4 % at the 3rd year of data collection and the highest severity score (79.4 %) was recorded on Ex-Basirika at the second year (Table 4.24).

4.6 Performance of *J. curcas* Seedlings to Anthracnose Caused by *C. gloeosporioides* in Two Field Locations at Ibadan South-West Nigeria

Incidence of flower abortion on *J. curcas* accessions at third year of field observation varied significantly among different accessions. The highest incidence was recorded on Ex – Basirika (16.0 %) after six weeks of onset of flowering. The least disease incidence mean value recorded was on Ex-Misau, 8.5 % after six weeks of flowering. The disease first infected the leaves and shoots then the flowers causing severe loss of inflorescence. Shriveling of young fruits observed at later stage of the disease caused poor fruit set.(Tables 4.25).

4.6.1 Inoculum potential of *C. gloeosporioides* from Jatropha seeds in the field

There was seed to seedling carry-over of infection on plants raised from seeds, without surface disinfection. Infected plant samples collected at four, eight, twelve and 16 WAP had different percentage values of inoculum load. Ex – Kano gave the highest frequency of isolated pathogen (4.0 %) at 16 WAP. The mean occurrence increased with increasing time after planting. There was a lag stage between eight and twelve WAP which later increased at 16 MAP with the highest mean incidences recorded in all the accessions (Table 4.26).

Table 4.24 Disease incidence and severity scoring of shoot dieback caused by *C. gloeosporioides* on *J.curcas* accessions under natural field infection.

Year	Accession							
	Ex-Basirika		Ex-Mbatidiya		Ex-Kano		Ex-Misau	
	DI %	ISS	DI %	ISS	DI%	ISS	DI %	ISS
2010	26.0	37.8	20.8	41.2	14.4	32.6	10.6	14.6
2011	33.6	79.4	30.6	60.5	56.4	70.0	33.5	42.0
2012	38.9	33.6	73.4	30.5	69.4	32.2	60.0	14.0
$LSD_{0.05}$	4.0	3.3	2.7	3.5	4.2	5.0	3.8	2.2
	VS		SS		SS		MS	

DI% = Disease incidence percentage, ISS = Index of severity of symptoms on plant;

SS = susceptible; MS = moderately susceptible; VS = very susceptible.

Table 4.25 Flower abortion incidence on *J. curcas* accessions caused by *Oidum jatrophae* after six weeks of flower initiation

Accession	2wks	4wks	6wks
Ex- Misau	9.7	9.2	8.5
Ex-Mbatdiya	12.0	14.3	15.6
Ex – Basirika	13.5	13.3	16.0
Ex – Ibadan	12.7	14.0	13.5
Ex – Kano	14.9	13.7	15.6
$LSD_{0.05}$	2.02	3.18	2.29

Wks = weeks of flower initiation

Table 4.26 Survival of *Colletotrichum gloeosporioides* on *J.curcas* seedlings at 4, 8, 12 and 16 weeks after planting (WAP)

	Fungi survival (%)			
Accession	4 WAP	8 WAP	12 WAP	16 WAP
Ex – Misau	1.6	2.1	2.3	2.4b
Ex-Mbatdiya	2.1	2.1	2.5	3.5a
Ex – Basirika	2.1	2.3	2.5	3.1c
Ex – Kano	3.3	2.8	3.1	4.0a
$LSD_{0.05}$	0.99	1.11	1.06	0.81

WAP = Weeks after planting

4.6.2 Performance of seedlings raised from three months stored *J. curcas* seeds

There was significant variation in disease incidence and severity for leaf spot, leaf chlorosis and leaf blight. The least leaf spot incidence of 19.2 % was recorded on Ex – Misau and it differed significantly (P=0.05) from the other accessions. Leaf chlorosis was the least recorded symptom both in incidence and severity and did not differ among the accessions. Defoliation did not differ in the severity scores among the accessions also blight incidence and severity were high on Ex –Basirika with mean index of 16.5 % and 18.3 % respectively (Table 4.27).

4.6.3 Shape and growth rate of different *Colletotrichum* sp. isolated from *J. curcas*

Among the isolates of C .*gloeosporioides* isolated from the field and green house experiments, there were differences in their cultural characteristics slight differences in conidia morphology though possessing the major shape of this species. The culture ranged from white to black and the conidia from cylindrical, falcate to elliptical in shape. The isolates from infected ripened fruits were the fastest to sporulate after two days of incubation while the isolates from fresh young leaves took ≥ seven days before sporulation. Direct isolations made from old leaves with severe spot and blight symptoms yielded conidia of this fungus. Isolates from ripe fruit and infected seeds had the highest radial growth of 6.8cm and 6.3 cm in 48hrs after inoculation. Isolates from leaf had the lowest radial growth (4.6cm) in 48hrs. There was significant variation in the growth rate of these isolates from different plant samples and the number of days before sporulation and production of acervuli (Table 4.28).

4.6.4 Correlation analysis between incidence and severity of foliar anthracnose on *J.curcas.*

There was significant positive correlation between leaf spot and leaf chlorosis, leaf blight and leaf spot, leaf chlorosis and defoliation and leaf blight and defoliation incidences. Correlation analysis between leaf spot incidence and leaf blight severities was also positive and between leaf spot and leaf blight severities also, (Table 4.29).

Table 4. 27 Anthracnose expression on five accessions of *J.curcas* seedlings raised from 12 weeks old stored seeds

	% incidence				Severity score			
Accessions	LS	LC	LB	LD	LS	LC	LB	LD
Ex-Basirika	23.0	4.6	16.5	16.0	27.0	6.0	18.3	35.0
Ex–Mbatdiya	21.3	4.6	15.5	14.0	25.1	5.6	14.0	14.1
Ex- Misau	19.2	4.0	13.0	7.4	24.0	5.0	13.0	13.5
Ex – Kano	23.5	5.0	15.6	10.4	29.0	5.6	18.0	15.6
Ex – Ibadan	23.0	4.0	15.0	11.2	28.3	6.4	16.3	15.1
$LSD_{0.05}$	0.80	1.73	2.26	3.11	1.59	1.58	1.83	22.6

LS: Leaf spot, LC: Leaf chlorosis, LB: Leaf blight, LD: Leaf defoliation

Table 4.28 Conidia shape and growth rate of C. *gloeosporioides* from infected plant parts of field-grown *J. curcas;* cultured on PDA at room temperature

Plant part	Conidia Shape	Radial Growth (cm)/48 hrs
Ripe fruit	Elliptical	6.8
Seed	Cylindrical	6.3
Leaf	Cylindrical	5.5
Shoot	Cylindrical	5.4
Young leaf	Cylindrical	4.6
Immature fruit	Cylindrical	4.6
Necrotic leaf	Falcate (curve, sickle)	4.6
$LSD_{0.05}$		0.61

Table 4.29 Pearson Correlation Coefficient between incidence and severity of disease symptoms measured in the field

	LC	LB	LD	LSS	LCS	LBS	LDS
LS	0.6*	0.53*	0.57**	0.67**	0.18	0.56**	0.09
LC		0.15	0.50*	0.09	-0.1	0.15	-0.01
LB			0.64**	0.50*	-0.01	0.50*	0.30
LD				0.04	0.15	0.33	0.31
LSS					0.26	0.63*	0.10
LCS						0.30	0.01
LBS							0.26
LDS							0.00

*,** Significant at P < 0.05 and P < 0.01, respectively

LS: Leaf spot Incidence
LSS: Leaf spot Severity
LC: Leaf chlorosis
LCS: Leaf chlorosis Severity
LB: Leaf blight Incidence
LBS: Leaf blight Severity
LD: Leaf defoliation
LDS: Leaf defoliation Severity

4.6.5 Leaf spot expression on *J. curcas* accessions during dry season planting trial (2010)

All the *J. curcas* accessions used in this experiment were susceptible to leaf spot pathogen. FRIN-B, the second location at Forestry Research Institute arboretum had the highest disease index (16.4%) on Ex-Kano and least mean incidence value on Ex-Misau, 7.0 % at both at UI and FRIN-A. Severity scores were high in all the locations; however FRIN-B location had the highest disease scores in all the accessions with maximum mean value of 14.0 severity score on Ex-Kano at FRIN-A location. There were significant differences observed among the accessions in all the locations on their severity responses to infection in the field (Table 4.30).

4.6.6 Leaf spot incidence and severity in the wet season (May – June 2011)

Disease incidence and severity were generally higher during the wet season. A mean maximum leaf spot incidence of 41.3 % and severity score (34.0 %) was recorded in Ex – Kano accession at FRIN-B location. Necrotic lesion was least in Ex-Misau without significant variation in all the locations. Disease incidence and severity in both locations, UI and FRIN-B were not significantly different among the accessions. Disease intensity was higher in wet season than the dry season due to favourable weather conditions (Table 4.31).

4.6.7 Leaf spot expression during the dry season (Nov. Dec. 2011)

Record taken at the beginning of the dry season in 2011 before the defoliation in January – February, showed that leaf spot incidence was generally low in all the accessions compared to that observed in the year, 2010. Incidence was significantly higher in FRIN -B location with maximum mean % incidence of 17.1 % and severity score of 12.5 % on Ex-Kano. In comparing incidence and severity in all the accessions, there were significant differences (P=0.05), however Ex-Misau showed very high tolerance to infection in all the locations (Table 4.32).

Table 4.30 Leaf spot incidence and severity on *J. curcas* accessions in three locations (May- June, 2011)

Accession	% Incidence			Severity		
	FRIN-A	FRIN-B	UI	FRINA	FRINB	UI
Ex-Misau	7.0	9.2	7.0	7.0	9.0	6.4
Ex-Basirika	10.0	15.0	12.3	10.0	11.2	8.4
Ex-Mbatdiya	11.5	15.0	12.2	11.5	11.3	10.2
Ex-Kano	14.0	16.4	13.0	14.0	12.3	11.0
$LSD_{0.05}$	1.18	1.17	1.89	1.63	1.17	1.79

FRIN- A= FRIN plant nursery

FRIN- B= FRIN-arboretum

UI= Crop Garden of the Department of Crop Protection and Environmental Biology, University of Ibadan

Table 4.31 Leaf spot incidence (%) and severity on *J. curcas* accessions in three locations (May- June, 2012)

Location	Ex-Basirika	Ex-Mbatdiya	Ex-Misau	Ex-Kano
	% Incidence			
FRIN- B	40.0	40.6	29.0	41.3
FRIN- A	36.4	36.0	28.0	38.0
UI	34.0	34.5	28.0a	37.0
$LSD_{0.05}$	1.74	3.10	1.89	2.27
	Severity score			
FRIN- B	12.6	12.6	10.3	14.0
FRIN- A	10.4	10.0	7.4	10.3
UI	10.7	10.0	7.0	11.6
$LSD_{0.05}$	1.66	2.39	2.12	1.37

FRIN- A= FRIN plant nursery

FRIN- B= FRIN-arboretum

UI= Crop Garden of the Department of Crop Protection and Enviromental Biology, University of Ibadan

Table 4.32 Leaf spot incidence and severity indices on Jatropha accessions in three locations during the dry season (Nov. – Dec. 2012)

Accession	UI		FRIN A		FRINB	
	% Incidence	Severity score	% Incidence	Severity score	% Incidence	Severity score
Ex-Misau	6.1	5.2	6.0	5.0	9.0	8.5
Ex-Basirika	12.0	8.0	13.5	9.6	15.6	11.1
Ex-Mbatdiya	12.3	9.1	13.2	10.2	16.0	11.2
Ex-Kano	13.3	10.0	14.3	12.0	17.1	12.5
$LSD_{0.05}$	1.23	1.09	1.05	0.95	0.89	0.82

FRIN- A= FRIN plant nursery

FRIN- B= FRIN-arboretum

UI= Crop Garden of the Department of Crop Protection and Enviromental Biology, University of Ibadan

4.6.8 Leaf blight expression on Jatropha seedlings at different planting seasons

There was generally low blight incidence in dry season of 2011 – 2012 significantly lower than what was observed in wet season of same year in all the accessions. There was no record of foliar blight at the peak of dry season (Jan. – Feb.) when the records were taken in both years as the plants shed their leaves for moisture conservation. Foliar blight was higher in 2011 wet season with a high percentage incidence of 43.6 % on Ex – Kano. The least incidence was observed on Ex-Misau (13.7 %) in the dry season 2012 and did not differ significantly from year 2011 incidence (Table 4.33).

4.6.9 Comparison of differences between wet and dry season blight severities in four Jatropha accessions

There were no significant differences in disease severities among the accessions in the wet seasons of 2011 and 2012 however, the accessions performed significantly different (P=0.05) during the dry seasons of 2011/2012, recording very low severity scores in all the accessions. The maximum score value was recorded on Ex-Kano (34.4 %). There were no variation between 2011 and 2012 in the performance of Ex-Basirika and Ex-Misau. The least mean score was also recorded on Ex-Misau (1.5 %) in 2012 (Table 4.34).

Table 4.33 Influence of wet and dry seasons on the incidence of foliar blight on *J. curcas* in wet and dry seasons of 2011 and 2012

		% incidence/Accession			
Season	Year	Ex-Basirika	Ex-Mbatdiya	Ex-kano	Ex-Misau
Wet	2011	41.13	39.5	43.6	30.2
	2012	39.4	38.0	42.3	38.2
	$LSD_{0.05}$	2.38	1.83	2.00	2.6
Dry	2011	19.7	20.2	22.1	15.3
	2012	18.0	18.6	20.1	13.7
	$LSD_{0.05}$	1.90	1.26	1.65	3.90

Table 4.34 Severity scoring of leaf blight for different seasons in 2011 and 2012

		Severity score /Accession			
Season	Year	Ex-Basirika	Ex-Mbatdiya	Ex-kano	Ex-Misau
Wet	2011	33.1	31.1	36.1	21.4
	2012	31.0	29.3	34.4	22.1
	$LSD_{0.05}$	2.78	1.94	2.50	1.41
Dry	2011	12.9	1.7	14.4	11.0
	2012	12.0	3.2	13.0	1.5
	$LSD_{0.05}$	1.01	0.72	1.07	0.96

CHAPTER FIVE

5.0 DISCUSSION

This study has debunked the myth that *Jatropha curcas* has no pests and diseases owing to the fact that they were toxic. This same assumption was held for cassava another plant from the same family as Jatropha. This assumption was proved wrong in the 1970s (Fokunang *et al*., 2003), confirming cassava to be susceptible to at least thirty different diseases of fungal, bacterial, viral and mycoplasma origin.

There is no doubt that Jatropha diseases were wide spread in the whole areas of investigation. Therefore, the present study emphasized survey to know the distribution and occurrence of fungal diseases in the South-Western region of Nigeria and to evaluate the intensities of these diseases in the field and screen-house in Ibadan, South-West Nigeria. Field and laboratory studies were conducted to observe and record the symptomatology, incidence, intensity and transmission, at the Department of Crop Protection and Environmental Biology, University of Ibadan and Forestry Research Institute of Nigeria Ibadan.

Roving survey was conducted to know the distribution of *Jatropha* fungal diseases in different parts of the six states during the months of July and August 2009 in the northern and southern parts of each state.

Jatropha fungal diseases were wide spread in the whole area investigated. The sampling results indicated the presence of foliar, shoot, fruit and root diseases. There were significant differences in the percent occurrences of the fungal organisms isolated from the different states. The results also revealed that five fungal diseases viz. anthracnose, shoot necrosis, powdery mildew, fruit and root rots were prevalent in the surveyed areas. More than twelve fungal organisms were found to be associated with *J.curcas.* Out of them, twelve were identified and subjected to pathogenicity tests. *Colletotrichum, Curvularia, Fusarium, Oidum* and *Lasiodiplodia* species were found pathogenic on *J. curcas.*

Most of the fungi occurred on diseased leaves followed by the shoots, fruits and roots. All the isolates showed greater abundance on diseased tissues compared to the healthy tissues.

Penicillim, Aspergillus, Corynespora, and *Cladosporium* did not show significant difference between healthy and diseased tissues. The occurrence of some species such as *Pestalotia* and *Phytophthora* was infrequent and very low; these species were therefore not investigated further because they were probably not involved in the disease observed on *J. curcas.*

The variation in percentage occurrence of the different pathogens isolated from the plant samples from each state indicated significant differences for the states and this might be because of the prevalence of different climatic condition in the states. Ekiti state had the highest incidence of fungal pathogens from the samples followed by Oyo state with the least incidence from Lagos state. This may be due to differences in prevailing agroecological climatic conditions.

The result of the survey was also confirmed by the observations and records obtained from the fields and screenhouse studies in respect to the fungal pathogens that attack *J.curcas* and their resultant symptoms.

In all the experiments carried out in this study, all the accessions showed susceptibility to the major disease symptoms observed and recorded in this work. Ex-Misau performed significantly better both in the nursery and in the field than the other accessions in terms of resistance to most of the pathogens encountered in the study. This attribute can be harnessed in further work in breeding and selection for disease resistant *J.curcas* lines.

Plant disease otherwise defined as the series of invisible and visible responses of plant cells and tissues to a pathogenic organism or environmental factor that result in adverse changes in the form, function, or integrity of the plant and may lead to partial impairment or death of the plant parts or the entire plant (Agrios, 2005). When the ability of the cell of a plant or plant part to carry out one or more of its metabolic functions is interfered with by either pathogen or adverse environmental factor, a disease condition occurs. This may explain the

reason for the different disease symptoms observed in this study considering the abundance of fungal pathogens and insect pests encountered in this study.

Early leaf spot symptoms mostly was dominated with *Curvularia* attack and other opportunist organisms such as pestalotia and cladosporium and was observed at the beginning of the wet season (May-June); but late leaf spot symptoms was predominanted by *C. gloeosporioides* attack which also caused severe foliar infections such as shot-holes, gray spot, heavy leaf blight, chlorosis and defoliation. As a result, maximum leaf infections corresponded with the beginning of July and lasted till the dry season in January when the plant shed all the leaves as a mechanism for drought tolerance.

Investigations carried out from 2009 on *J.curcas* showed that the plant was infected by *C. gloeosporioides* more frequently than by any other pathogen and the percentage of infected plants increased each year. *Colletotrichum* seemed to be the most virulent on all the accessions. It caused leaf and shoot infections, cankers, dieback, fruit and seed rot. Results support earlier findings by Ferreira-Pinto *et al*. (2011) that anthracnose caused by *C. gloeosporioides* is an important physic nut disease causing damages on leaves, stems and fruits and decreasing seed quantity and quality. Freire and Parente (2006) and Sá, *et al*. (2011) reported the presence of this disease in all areas where physic nut is cultivated.

Stephen (2012) reported that one of the most common diseases of plants is *Colletotrichum* leaf spot or anthracnose and that the *Colletotrichum* sp. that is most often present is *C. gloeosporioides*. Several other species have been identified in infections of various hosts Fokunang *et al*., 2000). Frequently, the perfect stage of the pathogen, *Glomerella* spp., is also often present in infected tissues. Anthracnose was the major symptom observed throughout this study. Daniel *et al*. (2009), reported the anthracnose of *Jatropha* caused by this fungus, *Colletotrichum gloeosporioides*. Symptoms were large and irregularly shaped necrotic sports on the leaves. Symptoms usually started at the edges moving toward the center of the leaf. Fungal structures were observed on the underside of the leaves, in the form of dark brown or black spots known as *acervuli*.

The fact that the pathogen is seed-borne and is transmitted to seedlings is at least in part the cause of epidemic spread of the disease. A principal role in infection during the

growing season may be attributed to conidia because of their abundant production. The present study indicated that in Ibadan, the plant infection by *C. gloeosporioides* may occur during the period from May / June to September (one-year-old plantations). Fokunang *et al*. (2005) also reported that temperature affects the survival and infectivity of pathogens. The presence of numerous insect pest on shoots and nematodes on the roots of *J.curcas* plants with anthracnose may have aggravated their infection because of mechanical injuries caused, thereby predisposing them to other secondary pathogens.

Anthracnose symptoms caused by *C. gloeosporioides* were observed in all stages of plant development. The disease infected the young growing shoots and tips, causing spotting, necrosis and distortion of young, expanding leaves and shoots. On leaves neighboring spots coalesced to form large irregular lesions, which often developed along one edge resulting in distortion and twisting of the leaf. Rapidly expanding fungal lesions with distorted leaves, premature leaf shedding and formation of cankers on young stems were the later stages of this disease. Heavily infected plants were often stunted and multi-branched and were characterized by shoot dieback. The main damage came from the development of cankers that killed the cambium and girdled branches causing shoot dieback. Affected trees often broke in high winds, causing loss of twigs and main branches. Trees could grow through the damage, but some remained suppressed. The characteristic fruit-rot caused by the pathogen on *Jatropha* fruits also agreed with the findings of Moral and Trapero (2009)

that *C. gloeosporioides* caused characteristic fruit-rot and mummification syndrome on olive fruits, with abundant production of conidia in a gelatinous matrix (“soapy fruit”) under wet conditions. Great disease severities on seedlings caused their decline. Plant infection during mature stages sometimes did not inhibit setting seeds but they were often infested and became source of primary infection. Symptoms caused by *C. gloeosporioides* and other isolates of this pathogen were leaf and shoot infections, cankers, fruit and seed rot and dieback.

This result is in line with Barranco *et al*. (2003) who reported that *Colletotrichum* spp. caused wilting of leaves and dieback of shoots and branches, as well as characteristic fruit rot syndrome on many annual and perennial plants. Graniti *et al*. (1993) reported that

anthracnose of olive, caused by the fungi *Colletotrichum acutatum* J.H. Simmonds ex Simmonds and *C. gloeosporioides* (Penz.) Penz. and Sacc., was the most destructive disease of olive fruit and was widely distributed in many olive growing regions of the world .

Anthracnose disease was also reported on physic nut by the USDA, (1960) in the USA, in Brazil by Viégas (1961), and later by Freire and Parente (2006) and Sá, *et al*. (2011). Currently, the disease is present in all areas where physic nut is cultivated. The most commonly observed symptoms were brown to black necrotic lesions that are irregularly shaped and appear on the edges and center of the leaf and which may be surrounded by a yellow halo. The lesions appeared in the form of small, isolated points that coalesced and subsequently caused the complete destruction of the leaves. The fruits were also infected, leading to the appearance of dark brown lesions. Torres-Calzada *et al*. (2011) also indicated that the fungus *Colletotrichum capsici* caused stem canker and apical death of seedlings of *J. curcas* in Mexico.

Under field conditions, susceptibility to anthracnose caused by *Colletotrichum sp*. differed greatly among accessions, and years. Madden *et al*. (2007) reported that the incidence of anthracnose depends greatly on cultivar susceptibility and weather.

The ranking of accessions, however, did not change markedly with location or year, suggesting a dominant effect of genotype over the environment (weather, pathogen population, level of disease). Assessments of artificial inoculations complemented field assessments to characterize anthracnose susceptibility in *J.curcas.* Therefore, anthracnose may become a great problem for this oil bearing plant grown in Nigeria, as it is in other countries (Schwarczinger and Vajna 1998; Schmatz *et al*., 2000).

There were very high indices of leaf infections recorded in all the accessions in the field and from the survey samples. Isolation of the disease pathogen from the respective types of foliar symptoms viz., shot-hole, leaf blight and irregular spot and pod rot yielded *Colletotrichum* isolates. These isolates were identified as *Colletotrichum gloeosporioides* (Penz.) Sac. Cultural and morphological studies showed no distinct differences in characteristics among the C. *gloeosporioides* isolates. Leaf spot diseases produced spots

ranging in size from 3-12mm. The colour, appearance and location of these spots varied on different host species. Under heavy infection spots coalesced often causing the leaves to crinkle, resulting in premature leaf fall. The pathogens implicated were *Colletotrichum* and *Curvularia spp.* Disease expression was higher during the wetter months as spores were easily spread by rain splash which favoured disease development during the humid conditions. The disease caused irregular to circular yellow-brown spots, principally on older leaves of *J.curcas.* Spots initiated from the leaf margin or any other part of the leaf and spread to form large blights, with small black fruiting bodies visible on the upper surface. Heavily infected leaves were completely covered by these blights and were shed prematurely. Talhinhas *et al*. (2005) reported similar observations on Olive plant and Muimba (1982); Fokunang *et al*., 2006, 2003, 2001 and 2000) reported similar symptoms on cassava. Spotting of older leaves caused little damage; however, infection on newly formed young leaves could result in impairment of the functional photosynthesis and leaf fall, producing bare tips which could subsequently be invaded by *Botryodiplodia theobromae* Pat. (Yee and Sariah, 1993).

Leaf wilt and branch dieback are important symptoms associated with anthracnose in many plants as was reported by (Barranco *et al.*, 2008; Moral *et al.,* 2009). In some countries, such as Greece, Italy, and Portugal, these symptoms have been considered as a primary anthracnose syndrome caused by a direct attack of the pathogen on leaves and branches of many plants (Zachos and Makris, 1963; Graniti *et al.*1993). In Spain, however, branch dieback has been considered a secondary syndrome resulting from phytotoxins produced in affected parts (Moral *et al.,* 2009). In this study, the pathogen was isolated from affected leaves, shoots, and branches of all sampled accessions, but the recovery of *Colletotrichum* was low after artificial inoculation, supporting the phytotoxin hypothesis. Results and field observations verified the lack of resistance of *J.curcas* to anthracnose and related diseases attacking other crops in the same family such as cassava (*Manihot esculenta*). Heavily infected plants showed stunting and general loss of vigour. This is due the fact that leaves are the major organ in photosynthetic functioning of the plant. When severely infected, the normal metabolism in the plant is distorted leading to nutrient deficiency and stunting (Agrios, 2005).

Studies on pathogenicity and virulence of *C. gloeosporioides, Fusarium, Curvularia, Lasiodiplodia and Oidium species* under artificial inoculation on *J.curcas* accessions indicated that all the isolates were pathogenic on *J.curcas* .They caused necrotic lesions by leaf spray and stem puncture .There was a gradual increase in the size of necrotic lesions at indicated periods of inoculation. The reactions of the *Jatropha* accessions were identical to all the fungal inocula tested. It was however observed that there were significant differences in the virulence of the isolates from one host to another.

Under artificial inoculation, *C. gloeosporioides* caused necrotic lesions on leaves and shoots which later led to open wounds (cankers) on the affected shoots. In addition to the differences in the lesion and symptoms produced, their patterns of pathogenesis on the *Jatropha* accessions showed variations. Invasion of wounded stems by the *Colletotrichum* sp. resulted in severe to slight canker development depending on the accession. However, on non-inoculated (control), stems showed only small, raised lesions which failed to enlarge. These findings suggested that the pathogen must have colonized the damaged tissues at an early stage to cause development of stem cankers.

The differences observed in canker sizes among different accessions inoculated with *C. gloeosporioides* may be as a result of the different inherent genetical constitutions that enabled them react differently to different diseases. The different areas from where the plants were collected may have been responsible also for the different reactions of the accessions to same disease infections. The differences in disease incidences and intensities recorded for different disease symptoms and between accessions under different inoculation methods may be due to the passive resistance on the leaves, the pathogens must first overcome the cuticular barrier of the leaf. The relative humidity and the leaf wetness must also favour the pathogen at the period of spray to avoid desiccation of the inocula and enhance probable germination of the spore to produce infection. This is not necessarily the case in stem puncture technique as a wound is already created favouring the growth of secondary organisms that degrade cells. The results from the sequential inoculation suggest that there is synergistic interaction among the pathogens and this agrees with the finding of Jefferies *et al*. (1990) which recorded that the synergistic interaction among plant

pathogens varies with factors such as host differential variety and the sequence of inoculation.

The results of these studies also demonstrated that, at least on susceptible accessions, the damage caused by anthracnose on branches should be considered an important aspect of the disease. A comparison between susceptible and resistant accessions evaluated in this test and earlier in the trial stations of Forestry Research Institute of Nigeria, suggests the selection of Ex-Misau, as some promising resistant materials to foliar infection and anthracnose disease were observed in this accession.

Colletotrichum seemed to be the most virulent on all the accessions, supporting earlier findings (Than *et al.*, 2008; Torres-Calzada *et al*., 2011)), that *Colletotrichum capsici* caused foliar necrosis, crown canker and apical death of seedlings, and leaf blight causing 70% defoliation on *J. curcas* in Mexico. Ferreira-Pinto *et al*. (2011) has reported anthracnose disease caused by *C. gloeosporioides* as an important physic nut disease causing damages on leaves, stems and fruits and consequently a decrease in seed quantity and quality. Kwon et *al.* (2012), Nicolas *et al.* (2008), also reported on leaf spot and stem canker on *J. curcas* in Korea caused by *C. gloeosporioides.*

Cultural and morphological studies showed no distinct differences in characteristics among the C. *gloeosporioides* isolates which could facilitate strain differentiation within the species, except for light variation in culture colour and consistency of the mycelium. All the C. *gloeosporioides* isolates produced hyaline, aseptate, uninucleate, cylindrical conidia with obtuse ends, which were formed in setose or globose acervuli. The shape of the acervuli ranged from round to elongate to irregular. Setae were sparse to profuse, dark brown to black, straight to slightly curved, septate, swollen at the base and tapering towards the apex.

Spore shape was similar for each of the *C. gloeosporioides* isolates. However, spore size was different for some *C. gloeosporioides* groups. This calls for more investigation to really ascertain the relatedness of these isolates of *C. gloeosporioides.*

Curvularia, Lasiodiplodia, Fusarium and *Oidum* were moderately virulent on all the *J.curcas* accessions tested. Early anthracnose development gave a quick response, which became clearer as the factors such as canker development and death were later signs of infections. Ex-Kano and Ex-Mbatdiya were more sensitive to infection by all the pathogens tested while in most cases Ex-Misau showed tolerance both in the field and in the green house: This is a clear indication that Ex-Misau can be suited to all localities since the spore load on the seeds were less on Ex-Misau. Misau is a less humid area hence there is less prevalence of diseases there. The most prevalent pathogen of *Jatropha* from this study was *C. gloeosporioides* and it was seed-borne. Reed *et al*. (1996) and Than *et al*. (2008) reported that *Colletotrichum* species cause anthracnose, which can cause considerable damage in a large number of crops such as cereals, coffee and legumes. Even greater economic losses are due to post harvest anthracnose disease of tropical and subtropical fruits such as avocado, banana and mango (Mordue, 1967; Jeffries *et al*., 1990). They have been reported to colonize decaying wild fruits also (Tang, *et al*., 2003). *Colletotrichum* species that cause serious plant disease are also commonly isolated as endophytes from healthy plants, and have been identified as saprobes on dead plant materials (Photita *et al*., 2001a; Promputtha *et al*., 2002; Toofanee and Dulymamode, 2002; Kumar and Hyde, 2004).

Brown *et al*. (1998), Bussaban *et al*. (2001), Photita *et al*. (2001b) and Promputtha *et al*. (2002), all described the endophytic, saprobic and many pathogenic strains in the genus as being frequently classified as *Colletotrichum gloeosporioides*. *C. gloeosporioides* is a commonly isolated endophyte from a range of plant species (Rodrigues, 1994; Brown *et al*., 1998; Bussaban *et al*., 2001; Photita *et al*., 2001b). Jeger *et al*. (1995) described strains of *C. gloeosporioides* and *Colletotrichum musae* isolated from banana as latent pathogens.

There is a conclusive evidence to suggest that Ex- Kano is highly susceptible and if it must be recommended to farmers, seed treatment prior to planting must be recommended to avoid disease spread while Ex-Misau has shown some degree of resistance to the major pathogens encountered in this study.

The primary cause of the disease both on the leaves and on shoots was *Colletotrichum gloeosporioides*, the result from this study therefore confirmed that the anthracnose, canker and dieback of *J.curcas* were caused by this fungus and it is the most important pathogen on *J.curcas* in South-West Nigeria. It was also clear that the pathogens isolated from *J.curcas* were also virulent as indicated from the pathogenicity studies.

The result obtained from this study is in line with Moral and Trapero, (2009) who reported that the use of less susceptible or resistant cultivars is the best way to control many plant diseases. Although this control method has not been considered as a priority for *J. curcas* anthracnose and other diseases, it will become important as many plantations are being established. These new plantations are more vulnerable to anthracnose epidemics because the high density of trees favours pathogen dispersal and environmental conditions for infection (Trapero, 2007). Differences in virulence of pathogen populations also could explain some discrepancies for accession reaction in different locations, but variation in virulence has not been clearly studied in this work.

Powdery mildew posed a threat to *Jatropha* in this work. It was more commonly found during the dry season and could build up rapidly extending to both flowers and fruits causing fruit rots and floral abortion. It typically affected the foliage and new shoots of both old and young trees and is characterised by the association of gray to dark, dusty patches, rapidly-expanding fungal spores on the leaf and shoot, sometimes causing premature shading of leaves. The fungi belonging to order *Erysiphales* and family *Erysiphaceae* are commonly known as powdery mildew fungi. The powdery mildew caused by the fungus *Pseudoidum jatrophae* was reported by Braun and Cook, (2012) and was previously described as *Oidum heveae* Steim by Viégas (1961) in Brazil and *Oidum jatrophae* Hosag, Siddappa, Vijay. and Udaiyan (Braun and Cook, 2012) in India. This disease occurs commonly in physic nut plantations and it has been frequently observed in various regions of Brazil and the rest of the world.

The most common symptoms of the disease were the production of abundant white or gray mycelia on leaves, petioles, stems, flowers and fruits (Dianese and Cargnin, 2008). The observation from this work corresponds with that of Alexandre and Olinto (2011) who

reported that at the onset of the disease, infected plants show necrotic lesions, which caused leaf fall, underdevelopment, death of buds and young fruit deformation

The fungi are highly pathogenic to the variety of Angiospermic plants causing the disease, powdery mildew. The fungi grow ecto-parasitically on the surface of the infected plant parts. The superficial mycelium of the fungi produces enormous number of conidia usually on the leaf surfaces, which appear like a mass of white powder, hence the name powdery mildew. As a group, powdery mildew fungi infect many species of plants, including many trees, shrubs, crops, vegetables, cereals, grasses, numerous ornamental and even weeds. Nearly 7187 host species which are all Angiosperms spread all over the globe is attacked by powdery mildew fungi (Sharma and Khare, 1995). Powdery mildew is more common on cultivated plants and grows luxuriantly in dry, cool seasons. Hosagoudar *et al*.(1997) noted that depending upon environmental conditions the powdery mildew disease may cause significant destruction and loss in plant yields, with the onset of summer they began to disappear and the plants become free from the infection during scorching heat and rainy season. The disease was moderate to severe during 2010 and 2012. The mean incidence ranged from 0.26 to 1.86% and from 20.65% to 41.88% during the corresponding years. It was very high during the subsequent years.

Lasiodiplodia theobromae was implicated in the yellowing, wilting, collar and root rot of *Jatropha* in this work, confirming earlier claims about this pathogen that L. *theobromae* is a ubiquitous pathogen of tropical woody trees. The fungus caused shoot blight and dieback of many plant species (Mohali *et al*., 2005) including the dieback and gummosis of mango (Khanzada *et al*., 2004); black branch and dieback disease of cashew in Brazil (Cardoso *et al*., 2002); and collar rot of peanut in Virginia and North Carolina, USA (Phipps and Porter, 1998). *L. theobromae* has also been reported to cause gummosis of *Jatropha podagrica* in China (Fu *et al*., 2007). It has been noted that species such as, *Botryosphaeria rhodina* (Berk. and M.A.Curtis) Arx anamorph *Botryodiplodia theobromae* Pat. syn. *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl, are often associated with canker/dieback diseases of a wide range of tree species (Sulaiman and Thanarajoo, 2012).

L. theobromae is one of the most abundant species with a wide host range throughout the tropics and the temperate regions occurring on over 500 hosts species (Punithaligham, 1980). It is well known as a latent pathogen on many host species and is known to cause serious disease symptoms when the host is stressed (Shoene- Weiss, 1981; Slippers and Wingfield, 2007). Other studies have reported that *Lasiodipodia species* are prevalent within the African continent (Palvic *et al*., 2004; Damm *et al*., 2007; Begoude *et al*., 2010). The roles of this fungi *J.curcas* need to be investigated further. Given that the Botryosphaeriaceae are indicated to be seed borne (Gure *et al*., 2005), it is possible that these pathogens may have been transferred to the seedlings through seeds. It has been recorded that this group of fungi attack their hosts when grown under stress conditions, and the onset of the disease was linked to stressful environments that reduce the growth vigour of the host plants (Desprez-Loustau *et al*., 2006; Slippers and Wingfield, 2007). This explained the case with the sequence inoculation of this pathogen with *Colletotrichum* sp. in this study.

Results from this work showed that *Jatropha* harboured many potential pathogens and other deteriorative mycoflora). Prabha (2012) reported that seeds are important means for transporting plant pathogens over long distances. Therefore seed fungal mycoflora are of considerable importance due to their influence on the overall health, germination, vigour, oil yield and final survival percentage of the plantations.

The high incidence of seed borne fungal pathogens that occurred on the seed stock also explains the early field infections recorded for all the accessions. *Aspergillus* spp. was the most frequent organism isolated from all the accessions with 38.5% incidence followed by *Colletotrichum*, 34.3%. Ex-Mbatdiya gave the highest percentage for isolated organisms and was significantly different from Ex-Misau with 12.0 % incidence. However, *Aspergillus* sp. is always a contaminant, unable to cause any disease on its own. Some of the seed-borne fungi were found to be very destructive, caused seed rot, decreased seeds germination and also caused pre and post germination death as was also reported by (Bolkan *et al.,* 1976; Elarosi, 1993) in other different host species. Dharmaputra *et al.* (2009) reported some irreversible degenerative changes in the quality of *jatropha* seeds during storage, thus making the seed unfit for oil extraction, export purpose or sowing. This

result also supported the findings of Singh *et al.* (1996); Worang (2008); Worang et al., 2008; Srivastava *et al.* (2011) and Sahab *et al.* (2011) that *jatropha* seeds are heavily deteriorated during storage, as they act as a source of stored nutrients for fungi species.

Jayaraman *et al.* (2011) also isolated *Aspergillus flavus, A. fumigatus, A. niger*, *Penicillum* sp. and *Rhizopus* sp. from four samples of jatropha seeds from Chennai. Singh *et al.* (1996) also reported fungal deterioration in jatropha seeds collected from central India which also decreased the oil yield and oil quality. Prabha *et al.* (2012) found that the occurrence percentage of some fungi on *Jatropha* seeds such as *Alternaria alternata, Fusarium solani, Penicillium sublateritium* and *Trichoderma harzianum* decreased in both blotter and agar seed-health methods when compared to the fresh seeds during storage.

Oilseeds are one of the most difficult commodities to store because it contains lipids which are more prone to spoilage by microorganisms during storage (Subramnyam and Rao, 1974; Prasad and Sahay, 1986). The storage fungi develop and infest seeds during storage even when the moisture content is as low as less than 16%. The risk of seed to field carryover is a factor to be considered in the choice of planting materials for this plant. Contamination of seeds by different mycoflora causes loss of viability, increase in seed rate and high cost of labour in poorly managed seed-lot. *Jatropha* as an oil seed goes rancid especially when colonized by pre and post harvest fungal organisms. The health of seed has also a significant influence on the incidence of diseases in stores as well as in the soils before plant emergence or during plant development (Priou and El Mahjoub, 1999). It was observed from the present study that, different species of storage fungi were isolated in all the samples even at low moisture content at 3 months after harvest. This shows the xerophilic nature of storage fungi, they can grow at low water activity substrate. It is also ascertained that, when the seeds are stored, there is a possibility of continuous growth in certain species of the storage microorganisms and increase in the moisture content (Chakrabarty, 1987). However, in this study, there is no significant increase in the individual species of fungi as observed, which may be due to a very minor level of variation in the moisture content even after long storage. The slight moisture content increase against long term storage period might be due to the hydrolysis or breakdown of lipid, carbohydrate and proteins and release of water molecules. The release of free fatty

acids from lipid degradation by fungi is well known and hydrolysis of starch and proteins for utilization by the microorganisms also, has been studied from the earlier findings (Jayaraman *et al*., 2011). Abulude *et al*. (2007) and Hanny, (2008) who earlier reported losses in oilseeds and oils due to the increase in Free Fatty Acids (FFA), which degrades the fatty acid fraction and decrease of viscosity. Hence, the energy value of biodiesel may be expected to be less during the storage period and due to storage fungi (Ashwani and Satyawati, 2008). Bothast (2009) and Hanny, (2008) reported that even after the extraction of oil from the seeds, the oilcake may contain unwanted microbial products like mycotoxins and further development of fungi due to contamination in the oil mills during the operation of oil extraction process and this indicates the spoilage of nutrients like protein, carbohydrate, fats which pose health hazard for use of these byproducts for poultry and animals. Therefore, suitable storage conditions and period of storage have to be ascertained and recommended through research evidences to avoid these losses. The important observation in the present investigation is the predominant occurrence of *Aspergillus* spp., *Colletotrichum* spp. in the order of dominance, which shows that these fungi may be having the ability of utilization of oil present in the *Jatropha* seeds and further losses on biodiesel value in seeds, if stored in poor conditions and after improper drying (Ginwal *et al*., 2004; Kaushik *et al*., 2006).

The susceptibility of *J. curcas* to root rot fungi was significant among all the accessions tested. *Fusarium oxysporum was* implicated in wilting and root rot of Jatropha seedlings in this study. Hambali *et al.* (2006) reported that *F. oxysporum* could cause wilt symptoms in physic nut in the seedbeds as well as in the fi eld. Ginting and Maryono (2009) also reported the susceptibility of *J. curcas* to *F. oxysporum* in Indonasia. *L. theobromae* was often encountered causing root and collar rot and was often not distinguishable from the rots caused by *Fusarium* spp. on Jatopha. Both organisms were sometimes isolated together from the same infected samples. In India *J.curcas* has been reported to suffer heavy losses due to a root disease caused by *Lasiodiplodia theobromae* (Khanzada *et al*., 2004). The symptoms observed were yellowing, drooping and shedding of leaves, blackening and decaying of the collar region of the stem and rotting of the roots. The root rot caused by *Fusarium sp* .in this study were sometimes mistaken for symptoms of rot caused by *Lasiodiplodia* and were both isolated together from the same infected samples. The

association of these rot causing fungi need to be investigated further. *Fusarium verticillioides* was also implicated in dry rot of shoots but more frequently in necrosis and root rot which was not prevalent in this study, may be because of the dry nature of the soil in which the experiment was carried out. Root rot was more prevalent during the wet season among seedlings under 6 months after planting (MAP) and in the over watered potted seedlings. This pathogen was frequently isolated from seeds confirming the seed-borne nature of this organism and the reason for the early infection in the field. Analysis of the results showed that there was no interaction between the accessions and the locations both in respect of incidences (I) and the severities (S). Generally, I and S were very high in all the locations for different seasons. Disease incidence is an error free measurement as it determines only the presence or absence of the disease (James. 1974). However, estimation based only on a single factor i.e. incidence or severity does not seem to be satisfactory for a meaningful evaluation of disease resistance under field conditions since both incidence and severity are complimentary indices. Therefore estimates based on both parameters could offer a better estimate of the field resistance of the clones.

Pathogenic fungi can affect the survival, growth rate and form of individual trees, as well as the quality and value of yield produced (Wylie and Peters, 1993; Speight and Wylie, 2001). Severe and/or repeated damage from disease attacks can kill trees outright, or make them more susceptible to other mortality factors. Defoliation reduces a tree's ability to photosynthesize, ultimately restricting growth (Sharma, 2008). Leaf and shoot blight diseases cause necrosis of leaf tissues, shriveling and distortion of twigs and growing points (Fokunang *et al*., 2004).

Root rots destroy the root system leading to chlorosis, defoliation and death. Loss and damage of shoots or main branch can result in the formation of multiple shoots or twigs, distorted stems and bushy form as was observed in this work. Diseases, including cankers, blights and bacterial wilts weaken and deform stems (Sharma and Kumar, 2009).

The impacts of diseases need to be considered at all stages in the development of a plantation industry (Speight and Wylie, 2001). Apart from having a primary impact on tree health, diseases pose secondary impacts by compounding existing problems. Trees that are

stressed may be more susceptible to, and less able to recover from, a subsequent disease infection. Often more than one pathogen or disease can be active at any one time as was observed in this work on *J.curcas.* The cost of eradication or control of any damaging exotic agent could cost millions of dollars added to the loss of resources (Wingfleid *et al*., 2001). It is now recognized in many countries that early detection is vital in preventing huge economic and resource losses, enabling sustainable eradication and containment measures to be initiated (Sharma and Saraf, 2007).

It has long been recognized that a realistic approach to crop protection should consider the various pests, including diseases that affect a crop (McRoberts, *et al*., 2003). A survey may provide the necessary overview of the pathosystem; adequate methods for analyzing survey data can produce preliminary information on its behaviour including major interactions. In this context, surveys can be considered as part of a systems approach. Epidemiologists are frequently confronted with large data sets representing information on the characterization, the dynamics, or the behavior of a pathosystem (Jongschaap *et al*., 2007).

Change of planting pattern could increase the importance of some plant diseases. Intensification of a plant generally tends to increase the threat of pests and diseases (Fry, 1982; Agrios, 2005). Plants that are grown less intensive usually do not suffer severely from pests and pathogen attack (Coughlin, 2006; GFU and GTZ, 2004). However, when they are cultivated intensively such as in intensive monoculture patterns, plants are of high risk to be attacked severely by pests and diseases, especially when they are not properly managed. Accordingly, the change in cultivation pattern of physic nut into intensive monoculture pattern should be followed by raising awareness about its pests and diseases. Information about physic nut diseases in literature is very limited. Though it has been proven that the crop is known to suffer from infections by different pathogens, among them *Jatropha* anthracnose disease caused by *Colletotrichum spp.* has been widely reported as a major trait to *Jatropha* cultivation as was observed prevalently on *Jatropha* in SouthWest Nigeria. The assumption that *Jatropha* is relatively resistant to pests and diseases might strongly rely on the fact that current knowledge is generally based on experimental plots or small scale experience (Dehgan and Schutzman, 1994).

J. curcas is a valuable oil bearing species, with comparatively little recorded information on its health problems. Adequate pest and disease surveys have not been conducted earlier in Nigeria on this crop especially in Southern regions of the country. Research has only been done in the early stages of plantation development, and there are, no doubt, many other fungal pathogens yet to be identified. Long-term management of these pests and diseases requires knowledge of their biology; impact and management options for those species already present, and those that threaten from offshore.

In establishing trials and plantations in this region, the following measures will promote tree health and contribute to our understanding of the distribution and impact of its pests and diseases:

Ensuring accurate matching of tree accessions to site and carrying out regular site maintenance to promote tree vigour and reduce stress. This will improve tree tolerance and resistance to pest and disease attack. Weed control and fertilizer application can also significantly improve tree health following pest or disease damage episodes (Stone and Birk, 2001; Carnegie, 2002). Routine health surveillance of plantations to assess the incidence and severity of pests and diseases over time and evaluating the impacts on plantation productivity will be necessary. This may provide early warning of problems as they arise.

There is need for establishing a comprehensive health data base to record pests, diseases, impacts, geographic and temporal occurrence and other factors in relation to the Jatropha species grown (FACT, 2007; FAO, 2003). Any plantings in this area provide an opportunity to increase our knowledge of pest and disease interactions with other plants, particularly in plantation situations. Such a database will be crucial for planning future plantations, example, matching accession to site, and developing a plantation risk rating.

This is the first comprehensive study on the incidence and severity of Jatropha anthracnose and other fungal infections in SouthWest Nigeria; Thus we suggest that future studies, especially related to yield loss assessment and disease management should be conducted across the country and should give due attention to local landraces that may serve as good sources of resistance.

Occurrence of anthracnose at a severe level in this study justifies the need for further studies to confirm if there are other pathogens with an additive effect to this disease apart from the major pathogen *Colletotrichum sp.* implicated in this study. Associated yield loss and the economic importance of this disease at least, across South-Western Nigeria need to be investigated. The pathogen, *Colletotrichum* sp. isolated from the diseased samples in this study is currently being used to characterize the isolates.

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION

Contrary to popular opinion, the toxicity and insecticidal properties of *J. curcas* failed to protect it against attacks by insect pests and diseases. The different parts of the plant were affected: roots, branches, leaves, flowers and fruits.

This review emphasizes the diversity of pathogens associated with this plant and the damage that they cause. Most of these diseases may become a serious problem for Nigerian farmers, due to thier severity and the lack of registered chemical products for these pathogens. Further studies should be carried out in order to know the environmental conditions that favor the prevalence of these diseases on *J. curcas*, as well as the development of control strategies and resistant varieties.

Reliable predictions of the productivity of *Jatropha* are necessary to make reasonable decisions, on yield and investment. The study therefore confirms that:

(i) Mixed planting using *J.curcas* faces challenges because the species is under threat from widespread anthracnose, canker, dieback, root rot, mildew, fruit and seed rot diseases.

(ii) A connection between symptoms on the trees and occurrence of fungal pathogens established that five fungal pathogens, *Colletotrichum, Fusarium, Oidum, Curvularia* and *Lasiodiplodia spp.* were individually capable of causing disease symptoms on leaf, shoot, fruits and roots of *J.curcas*.

(iii) In the pathogenicity tests, the five accessions, Ex-Basirika, Ex-Mbatdiya, Ex-Misau, Ex-Kano and Ex-Ibadan were susceptible to diseases and insect pests.

(iv) *J. curcas* was host to over twelve fungal species, some of which caused serious diseases of other woody as well as agricultural species. The endophytic nature of some of the pathogens identified in this study enabled them to be transferred easily through seeds to seedlings and to new areas without being noticed.

(vi) The emergency of unspecific pathogens coupled with stressful environmental factors for the host can mean that *J.curcas* whether in a tree mix or as monocultures can be vulnerable to infection because new environments may add to stress factors and therefore, increase their susceptibility to opportunistic pathogens.

The result forms a baseline on which other studies could be carried out in Nigeria. The intensification of jatropha cultivation for biodiesel and reforestation, and the lack of clear understanding of the biology of this introduced tree species in new environments may lead to the spread of plant pathogens. *J.curcas* leaves and other bi-products of the plant are used as mulch and bio-fertilizers on annual agricultural crops; this is likely to increase the inoculum level of the pathogens thereby, posing a threat to other susceptible crops.

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Appendix

Plate.1 *Jatropha curcas* plant attacked by Mealybug insect pest in the screenhouse causing drooping and leaf chlosis Leaf chlorosis

Plate.2 *Jatropha curcas* plant attacked by Mealybug insect pest in the screenhouse causing total death of seedling

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