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FIRST REPORT OF *Thielaviopsis ethacetica* CAUSING NECK BENDING/ INCLINATION OF THE UPPER REGION OF OIL PALMS IN NIGERIA

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Abstract — Plant diseases are among the major challenges faced by the oil palm cultivation in Nigeria. The major plantation diseases of the oil palm include Fusarium wilt (*F. oxysporum fsp. elaeidis*), dry basal rot (*Ceratocystis paradoxa*), *Ganoderma* trunk and recently neck bending/inclination of the upper region of the palm noticed in some Southern and Western States of Nigeria. Pathological and molecular investigation into the etiology of the disease was found to be caused by *Thielaviopsis ethacetica*. Depending on the disease severity on the oil palm, the symptoms include gradual inclination of the palm, yellowing of fronds, drying of the leaves of the palm from the base, production of a fermented fruit odor, and finally the collapse and death of the palm. *T. ethacetica* was consistently isolated from the diseased oil palms; produced numerous spores of two types (microconidia and macroconidia) and were further characterized using spores sizes, morphology and sexual compatibility test. The aggressive nature of *T. ethacetica* on the oil palm was confirmed by the sexual compatibility test resulting in the presence of sexual spores in the tissues of the severely affected palms while asexual spores were present in tissues of the mildly affected palms. The outcome of this study confirmed *T. ethacetica* to be the causal agent of neck bending/inclination of the upper region of the oil palm in Nigeria.

Keywords — **Neck bending/inclination of upper region, Nigeria, oil palm, *Thielaviopsis ethacetica***

INTRODUCTION

The oil palm in the wild appear to be free from serious pests and diseases not until after the World War II when effort was been made to make it a plantation crop (Hartley, 1988). Some of the major plantation diseases of the adult palm in oil palm plantations include the Fusarium wilt (*F. oxysporum fsp elaeidis*), dry basal rot (*Cerastocystis paradoxa*) and the Ganoderma trunk rot. National survey carried out in Nigeria between 1947 and 1984 showed that the incidence rate of the Fusarium wilt (*F. oxysporum fsp elaeidis*) disease ranged between 0.03 to 47% and can destroy up to 47% of field palms (Oritsejafor,1989). Effort towards management of the Fusarium wilt disease of the oil palm was screening of oil palm progenies against different species of *F. oxysporum fsp. elaeidis* to obtain tolerant palms.

The *Ganoderma* truck rot and the dry basal rot diseases caused by *Ganoderma* and *Thielaviopsis* spp. respectively have been effectively managed using good agronomic practices and chemical base fungicides. These diseases have been under control since their outbreaks. However, there was a sudden outbreak of the *Thielaviopsis* spp. disease in some oil palm plantations in the year 2019 resulting in a rare disease symptom (inclination of the upper region of the palm) in the oil palm. Survey studies showed that the source of the oil palms was not indigenous. The outbreak of the disease was first recorded in Southern part of Nigeria in a commercial oil palm plantation of 11,000 hectares, affecting oil palms between the ages of 2-3 years and later in some other plantations in Western part of Nigeria. The disease has a characteristics symptom of neck bending/ inclination of the upper portion of the oil palm (Esiegbuya, 2019). The disease is observed to be common in oil palms between the ages of 2-3years, with incidence rate of between 65%-75% in affected plantations. The severity of the disease on the palm results in complete death of the palm within a month of symptom manifestation. Asymptomatic palms sometimes later suffer from fruits abortion during its bearing stage thereby brings about huge loss to the farmers.

Neck bending disease or inclination of the upper portion of palms has been previously reported in date palms Mirzaee et al. (2014); Abdullah et al. (2009). According to Abdullah et al. (2009) *T. paradoxa* and *T. punctulata* isolated from the soil of date palm plantations were responsible for the disease during the process of transplanting offshoot of the palm to the field. Suleman et al. (2001) also implicated the pathogens *C. paradoxa* and *C. radicicola* colonizing the date palm that are under stress also resulting in neck bending and eventual death of the palms.

Due to the huge economic importance of the oil palm and this being the first report of this disease symptom with the oil palm in Nigeria, and also the ability of the pathogen to cause dry basal and fruit abortion at later stages of the oil palm development necessitated the need to carry out a study on the etiology of the pathogen on the oil palm with the aim of providing a management strategy for the disease.

MATERIALS AND METHODS

Study locations

The outbreak of the neck bending/ inclination of the upper region of the palm was reported in several plantations in Nigeria, however, the two study locations used in the study was selected based on plantation size and availability of weather data.

Plantation A, where the outbreak of the disease was first observed is a private-owned plantation of 11,000 ha situtated in Edo State, Benin City, Southwestern Nigeria, is bounded approximately by latitudes 6.08° and 6.30°N and longitudes 5.01° and 5.27°E. The climate of the region is characterized by a double maximal year-round rainfall pattern with a mean monthly rainfall of about 2000mm which peaks between May and October and a mean monthly temperature of 27°C.

Plantation B where the incidence of the disease was not recorded is an agricultural research institute located in Edo State, Southern Nigeria in the heart of the oil palm belt region. It is on latitude 060 0331N and longitude 050 37 1E and on altitude 149.4m. The region is characterized with high rainfall and temperature. Rainfall is over 2066mm per annum, and temperature of 30 – 33°C.

Other plantations where the outbreak of the disease was recorded but with no weather station were in Egbedore, Ondo and Ikole Ekiti, Ekiti States of Nigeria.

Soil Water Deficit

The water deficit was determined according to the methods of Chaillard et al. (1983); Corley (1996) and Roslan and Haniff (2004) cited in Bakoumé et al. (2013). Evapotranspiration (ETc) of oil palm in the month was considered to be 150 mm/month in a month with less than 10 days of rain and 120 mm/month for 10 days and above of rain. Soil water deficit was calculated by the difference between the monthly rainfall (ER) values and ETc.

Collection and Isolation of Fungal Strains

Ten samples each were collected in 2019 from roots, stems, meristem, bud tissue (under the meristem), spear leaves, leaflets from old leaves, petiole bases of young leaves, inflorescences, fruit peduncles and soils near oil palms (*E. guineensis*) affected by the neck bending/ inclination disease. After tissue collection, the samples were washed with water, surface sterilization was performed by the immersion of the previously washed plant tissue in hypochlorite (1%) for 1 min, then in ethanol (70%) for 1 min which was then rinsed with water.

Isolates from soil samples were obtained by serial dilution (10-1, 10 -2 and 10-3) with sterilized distilled water; 0.1 ml of each dilution was plated on potato dextrose agar (PDA) and incubated at 250C. Where fungal growth was detected, subsequent 0.1ml spore dilutions were transferred into water agar (20g Difco agar, 1L distilled water) plates and incubated at 25°C until isolations of single culture was obtained.

Morphological Identification of the Different Isolates of *Thielaviopsis* spp. Isolated from the Soil and Inner Tissues of the Oil Palm

Preliminary identification of each of the isolated fungus was done using its cultural and microscopic features according to methods described by Borges et al. (2019). The preliminary identified isolates using cultural and microscopic features were also sent to Commonwealth Mycological Institute (CMI), Surrey, England, UK for identification.

a. Pathogenicity testing of fungal isolates

The thirty isolates of *Thielaviopsis* spp. obtained were individually inoculated onto approximately 6-month- old oil palm *E. guineensis* (highly susceptible species) seedlings of ten replicates each which were at the 6-10 true-leaf stage, by inoculating with an agar plug (5mm in diameter) containing mycelium of *Thielaviopsis* spp. at the base and upper stem of the palm. Prior to inoculation, the base and upper stem of the palms were surface sterilized with 75% ethanol and mechanical injury was created with a surfaced-sterilized 3mm cork borer. The control consisting of ten oil palm seedlings were similarly treated except that they were inoculated with agar plug without fungal growth. The inoculated plants were kept in a humid chamber and environmental conditions such as rainfall, humidity and sunlight were measured. To satisfy Koch postulates, inoculated points in the oil palm seedlings showing disease infection resulting from the effect of *Thielaviopsis* spp. was inoculated on freshly prepared PDA on triplicates plates and growth was recorded.

b. Sexual compatibility test

The sexual compatibility test was carried using the methods described by Borges et al (2019). Monosporic strains were cultivated in PDA with sterilized oil palm frond fragments. The isolates were placed in Petri dishes, 3cm apart in between the sterilized oil palm frond, and incubated for 2 to 4 weeks in the dark at 25°C. Thereafter, the dual culture plates were examined for the presence of sexual fruiting bodies (Mbenoum et al. 2014).

RESULTS AND DISCUSSION

Symptomatology and Soil Water Deficit in the Study Locations

Field observation of the symptoms of the neck bending/inclination of the upper portion of the oil palm disease of the oil palm begins with a characteristic symptom of gradual inclination of the palm (Figure 1), yellowing of fronds, drying of the leaves of the palm from the base (Figure 2) and finally the collapse and death of the palm during the dry season (Figure 3). Transverse section of the base of affected oil palm seedling was characterized with production of a fermented odor (Figure 4).

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 1. Gradual inclination of the upper region of the palm.
Fig. 2. Yellowing of the fronds.
Fig. 3. Final collapsed of the oil palm.
Fig. 4. Transverse section of the trunk of the palm characterized with the production of a fermented fruit odor.

The outbreak of the disease was first noticed in the February 2019 with young palms between the ages of 2 and 3 years. The affected young palms in the affected plantation were cultivated in both loamy and laterite soils followed with good agronomic practices recommended for oil palm cultivation. The high incidence of the disease among the young palms resulted in the gradual collapsing and death of the young palms within a month of symptom manifestation. Transverse of affected trunk palm was characterized by a fermented fruit odor.

The soil water deficit in the year 2018 (Figure 5) in the plantation where the incidence of the disease was first recorded has to a total of 575.2mm soil water deficit accruing for a period of six months while in 2019 it was 451.9mm for six months. The

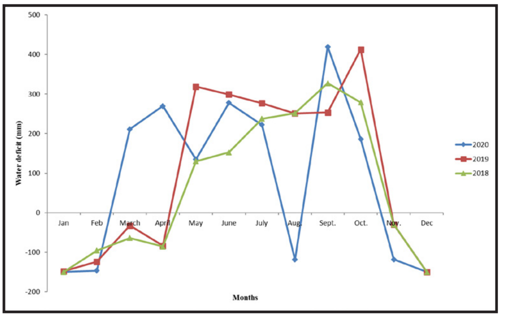

Fig. 5. Soil water deficit for locations were the neck bending disease/inclination of the upper region of palm was recorded.

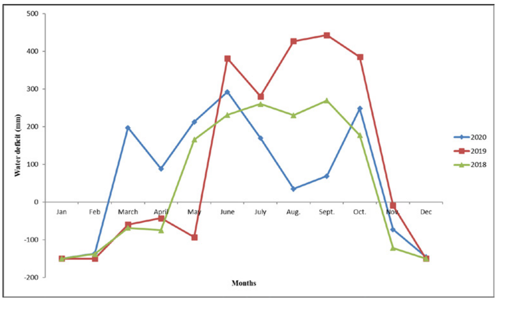

Fig. 6. Soil water deficit for station were the neck bending disease/inclination of the upper region of palm was not recorded.

year 2018 has a total rainfall of 1703.5mm while the year 2019 has a value of 2,145mm.

In the plantation where the outbreak of the disease was not recorded, the total soil water deficit was for the year 2018 (Figure 6) was 703.2mm for a period of six months and 655.6mm for 2019 for also a period of six months. The total amount of rainfall for 2018 and 2019 was 1530.8mm and 2312.1mm respectively.

Morphological and Microscopic Description of Fungi Associated with the different Portions of the Diseased Oil Palm Tissues and Soil Samples

a. *Cultural and microscopic characteristics of the isolates*

Colonies initially presented white coloration, turning dark after four days of growth (Figure 7). Primary conidia were hyaline, unicellular and cylindrical while the secondary conidia were brown, unicellular, became darker with age. Aleuroconidia were unicellular, dark brown, had rough cell wall (Figure 8). There was no sexual compatibility between the isolates.

Figure 7. Culture plate of *T. ethacetica* on PDA.

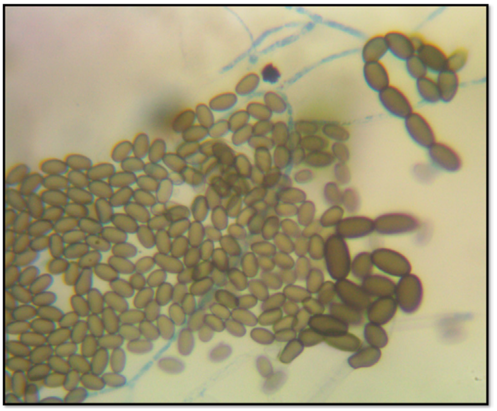

Figure 8. *T. ethacetica*: Secondary conidia in clusters (yellow arrow), Aleuroconidia in chains (black arrow), Primary conidia in chain (red arrow).

b. *Molecular identification and confirmation of the pathogen by CMI*

Molecular identification of the pathogen using the CMI identification protocol revealed the presence of *Thielaviopsis* spp. where there were similar matches to sequences of *T. euricoi* and *T. ethacetica* (and synonyms of these species). The three isolates sent for confirmation based on their virulence on the oil palm were assigned CMI No. E0000764001, E0000764002 and E0000764003, respectively.

1. Pathogenicity testing

a. *Pathogenicity testing of the isolates unto the base of the palm and definition*

The results of the pathogenicity test using the different isolates of *Thielaviopsis* spp. unto the base of the palm resulted in the formation of a pathogenic lesion around the point of inoculation within seven days after which, no visible symptom was observed.

b. *Pathogenicity testing of the isolates unto the inner tissues of the palm and definition*

Pathogenicity trials of *Thielaviopsis* spp. into the inner tissues of the palm also revealed a group of the isolates based on the virulence level that showed gradual invasion of the spear leaves of the palm by the pathogen resulting in the necrosis of tissues around the point of inoculation, abnormal spreading of the palm fronds and finally fracture and collapse of the spear leaves at the point of inoculation. Transverse section of the oil palm stem showed brownish coloration of the inner tissues of the palm and gradual yellowing and abnormal spreading and inclination of the fronds.

c. Pathogenicity levels defined

Pathogenicity data were obtained from a total of 30 fungal isolates collected over one-year period. During the pathogenicity testing, symptoms of the disease were evident within a week after inoculation. All isolates caused black necrotic lesions at the point of inoculation, and in some cases, leaf chlorosis. The symptom caused by individual isolates of *Thielaviopsis* spp. differs on the oil palm based on the stage of disease severity. The isolates were classified into three groups or levels according to their pathogenicity on the *E. guineensis*, oil palm seedlings. The control plants remained healthy during the evaluation period while the *Thielaviopsis* spp. strains showed high pathogenic variation between isolates.

Pathogenicity level –The first group consisted of 28 isolates, which caused stem lesions after which no further symptom developed. The isolates in this group were morphologically black on PDA, producing two types of spores (microconidia and macrocondia). The microconidia are secondary conidia in chains while the macroconidia (Aleuroconidia) is a large oblong, thick-walled, brown-black spore borne in chains. This group contains the largest number of *Thielaviopsis* spp. The second and third group also consisted of one isolate each which causes abnormal spreading/inclination of the palm fronds and collapse of the spear leaves at the point of inoculation. The isolates in each group were morphologically similar to those in group one. *T. ethacetica* has not been previously identified to be associated with the oil palm in Nigeria. Mbenoum et al. (2015) believed the pathogen to have its origin from Cameroun. This is due to the high genetic diversity of the four groups of the pathogen in the country with two of the genetic groups of the isolates native to the oil palm and the other two from cacao and pineapple (Mbenoum et al., 2015). Cameroun is among the oil palm producing nations with a production rate of 210, 000 tons of crude palm oil in 2010 (Ngom, 2011) and shares its border with Nigeria. There is possibility of seed and seedling transmission between countries during oil palm plantation establishment. This could be interpreted as the likely source of introduction of the pathogen into Nigeria.

Thielaviopsis paradoxa previously reported to be associated with oil palm in Nigeria causes various diseases such as the dry basal rot (Robertson 1962; Wingfield et al., 1993) and black seed rot (Omamor, 1985). *Thielaviopsis paradoxa* has been previously known as *Spororischima paradoxum* de Seynes, *Chalara paradoxa* de Seynes, *Ceratocystis paradoxa* de Seynes (teleomorph phase of the fungus) Dade, 1928; Mbenoum et al. (2014). Due to the complexity in the identification of the fungus, de Beer et al. (2014) proposed the genus *Thielaviopsis* which accommodates fungi that are typified by *T. paradoxa* sensu stricto. Molecular studies have highlighted increasing species diversity within this genus, distinguishing between discrete homothallic (*T. cerberus*) and heterothallic (*T. paradoxas.*str., *T. musarum* and *T. ethacetica* and *T .euricoi* which were previously treated as *T. paradoxa*)

(Mbenoum et al. 2014; Mbenoum et al. 2015).

There is the possibility of previous misidentification of the genus associated with the oil palm especially in the absence of molecular markers as suggested by Mbenoum et al. (2015) or probably the case of plant pathogens that have been introduced into a new region along with their host resulting in the emergence of devastating new disease (Anderson et al., 2004; Brasier 2008 cited in Mbenoum et al., 2015), as observed in this study. The devastating effect of *T. ethacetica* on the oil palm can result in 65% loss of the palms if adequate measures are not taken in time.

The absence of information in literatures on the devastating effect of *T. ethacetica* on the oil palm in Nigeria led to the interpretation that the findings from this study is the first report on the pathologic effect of *T. ethacetica* on the oil palm in Nigeria.

Studies have confirmed that *T. ethacetica* is different from *T. paradoxa* due to the absence of the synnemata morphological marker. Meanwhile the similarity between the two fungal isolates was inferred by the morphology of the perithecium and the presence of digitate ornamentation at the base of the perithecium (Mbenoum et al. 2014; Melo et al. 2016). Morphological analysis of *Thielaviopsis* spp. complicates taxonomic studies since the same strain produces different types of conidia at different developmental stages (Borges et al., 2019). Apart from describing the taxonomic status of *T. ethacetica*, these authors (Mbenoum et al. 2014; Mbenoumet al. 2015 and Borges et al., 2019) did not attempt to describe the disease impact of *T. ethacetica* on the oil palm thus, probably indicating its endophytic relationship with oil palm.

Thielaviopsis spp. has also been previously identified as an endophytic pathogen affecting susceptible host genotypes under stress (Alvarez et al., 2012). There is the possibility that the exotic oil palms being imported into Nigeria were host to *T. ethacetica*, thus felled to the pathogenic effect of *T. ethacetica* as a result of environmental changes. The second possibility might be that the exotic palms imported into Nigeria were unable to withstand the pathogenic effect of the soil borne *T. ethacetica* in Nigeria.

The amount of rainfall recorded in this study was within the range stated by Hartley (1988) but the soil water deficit in the two study locations was higher in the study location with no record of the disease than the study location where the outbreak was recorded indicating that the soil water deficit has a minimal role on the outbreak of the disease. However, low soil water deficit has been reported to be associated with some physiological disorder in palms (Sime and Darby, 2011). Soil water deficit is however depended on soil water holding capacity (Caliman and Southworth 1998). Olivin (1968) and Van der Vosen (1969) stated that the annual average soil water deficit that is optimal, suitable and favorable and unfavorable for the oil palm is ≤150mm, ≤250mm, ≤400mm and ≥ 400mm, respectively.

Several authors have shown that the isolates of the *Thielaviopsis* spp. varied in aggressiveness on different hosts, such as eucalypts, coffee, gmelina, taro and edible fig (Baker et al., 2003; Marin et al. 2003; Zauza et al. 2004; Guimarães et al. 2010; Harrington et al. 2011). The varied level of aggressiveness was attributed to the genetic variability observed in the genus (Harrington et al., 2000; 2011; Baker et al., 2003; Oliveira et al., 2015) thus constituting a limiting factor in screening for crop resistance and greater use of fungicides for its control (McDonald and Linde 2002). Different level of aggressiveness of *T. ethacetica* was also noticed in this study among the thirty isolates causing the inclination of the upper region of oil palm. The aggressive nature of *T. ethacetica* was attributed to presence of sexual spores in the tissues of the severely affected palms while asexual spores were present in tissues of mildly affected palms. Mbenoum et al. (2015) described *T. ethaceitica* as a heterothallic fungus that requires two compatible partners (MAT 1-1 and MAT 1-2) to produce sexual spores.

The interaction between *T. ethacetica* and the host tissue in this study gave rise to the damage layers and release of a fermented fruit odour which confirmed the invasion of the host by the pathogen through its spores. Kuo et al. 1969; Tokeshi and Rago 2005;Raid, 2009 cited in Borges et al. (2019) confirmed that during tissue colonization by *T. paradoxa* host plant tissues release an odor characteristic of pineapple essence, which is due to the release of ethyl acetate, the toxin responsible for inhibiting buds and roots. Joli (1961) also noted that the micro and macroconidia lie freely in the soil and/ or in buried plant tissues and macroconidia play major role in long term survival of the pathogen in the soil. This study showed that the microconidia was mainly present in the colonized tissues thus indicating its role in the pathogenicity of the fungi.

CONCLUSION

There is an indication that the isolation of *T. ethacetica* associated with the oil palms was from exotic palms imported into the country. This called for proper quarantine measures in order to control the transmission of plant pathogens within borders of the country. However, further molecular studies and pest risk analyses are needed to confirm the origin of the *T. ethacetica* isolated from the oil palm. The devastating effect of *T. ethacetica* on the oil palm also emphasized the need for effective management of the disease.

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REFERENCES

Abdullah, S.K., Asensio, L., Monfort, E., Gomez- Vidail, S., Salinas, J.,Lopez -Lorca, L.V., Jansson, H .B. 2009. Incidence of the two date palm pathogens, *Thielaviopsis paradoxa* and *T. punctulata* in soil from date palm plantations in Elx, South- East Spain. *Journal of Plant Protection Research* .49 (3): 276-279.

Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R. and Daszak, P. 2004. Emerging infectious diseases of plant: pathogen pollution, climate change and agrotechnology drivers. Trends in Ecology and Evolution 19 (10):535-544.

Alvarez, E.A., Llano, G.A., Loke, J.B. and Chacon, M.I. 2012 Characterization of *Thielaviopsis paradoxa* Isolates from Oil Palms in Colombia,Ecuador and Brazil, *Journal of Phyto pathology* 160:690–700.

Baker, C.J., Harrington, T. C., Kraus, U., and Alfenas, A. C.2003. Genetic variability and host specialization in the Latin American clade of *Ceratocystis fimbriata. Phyto pathology*, 93 (10): 1274–1284.

Bakoumé, C., Shahbudin, N., Yacob, S., See Siang, C. and Ali Thambi, M.N. 2013. Improved method for estimating soil moisture deficit in oil palm (*Elaeis guineensis* Jacq.) areas with limited climatic data. *Journal of Agricultural Science*; 5 (8): 57-65.

Brasier, C.M. 2008. The biosecurity threat to the UK and global environment from international trade in plant. Plant Pathology 57 (5): 792-808.

Borges, A.F., Neto, F., Matos, K., Beserra Júnior, E.A., Massola Júnior, N.S., Moreira, S.I., and de Melo M.P. 2019 *Thielaviopsis ethacetica* the etiological agent of sugarcane pineapple sett rot disease in Brazil. Tropical Plant Pathology 44:1-7.

Chaillard, H., Daniel, C., Houeto, V., and Ochs, R. 1983. Oil palm and coconut irrigation. A 900 ha “Experiment” in the Benin People’s Republic. Oléagineux, 38(10): 519-533.

Corley, R. H. V. 1996. Irrigation of oil palms - a review. Journal of Plantation Crops, 24 (supplement), 45-2.

Dade, H. A. 1928. *Ceratostomella paradoxa*, the perfect stage of *Thielaviopsis paradoxa* (De Seynes) von Hohnel, Transactions of the British Mycological Society13 (3-4), 184-193.

de Beer ZW, Duong TA, Barnes IBD, Wingfield B.D, Wingfield M.J 2014 Redefining Ceratocystis and allied genera. Studies in Mycology 79:187 –219.

Esiegbuya D.O. 2019. Monitoring, Detection and Management of a New Disease Symptom (Neck Bending) of the Oil Palm caused by *Thielaviopsis paradoxa* (De Seynes) submitted to OKOMU extension planation 2, Benin City, Edo State, Nigeria.

Guimarães, L. M. S., Titon, M., Lau, D., Rosse, L. N., Oliveira, L. S. S., Rosado, C. C. G., Christo, G. G. O., and Alfenas, A. C. 2010. *Eucalyptus pellita* as a source of resistance to rust, ceratocystis wilt and leafblight. Crop Breeding and Applied Bio technology, 10, 124 –131.

Harrington, T. C. 2000. Host specialization and speciation in the American wilt pathogen *Ceratocystis fimbriata.* Fitopatologia Brasileira, 25 (Suppl.), 262 – 263.

Harrington, T. C., Thorpe, D. J., and Alfenas, A. C. 2011. Genetic variation and variation in aggressiveness to native and exotic hosts among Brazilian populations of Ceratocystis fimbriata. Phyto pathology, 101 (5), 555 –566.

Hartley, C.W.S. 1988. The oil palm. Third edition. Longman group limited. London. New York. 761p.

Joli, P. 1961. Researchers surle *Thielaviopsis paradoxa* musarum Mitch. Bulletin for Mycological Society, France, 77,219-288.

Kuo TT, Chien MM, Li HW 1969 Ethyl acetate produced by *Ceratocystis paradoxa* and *Ceratocystis adiposum* and its role in inhibition of germination of sugarcane buds. Canadian Journal of Botany 47:14 59–1463.

Marin, M., Castro, B., Gaitan, A., Preisig, O., Wingfield, B. D., and Wingfield, M. J. 2003. Relationships of *Ceratocystis fimbriata* isolates from Colombian Coffee-Growing regions based on molecular data a patho genicity. Journal of Phytopathology, 151 (7-8), 35-405.

Mbenoum M, de Beer ZB, Wingfield MJ, Wingfield BD, Roux J 2014 Reconsidering species boundaries in the *Ceratocystis paradoxa* complex, including a new species from oil palm and cacao in Cameroon. Mycologia 106 (4): 757–784.

Mbenoun, M., Wingfield, M.J., Letsoalo, T., Bihon, W., Wingfield, B.D. and

Roux, J. 2015 Independent origins and incipient speciation among host -associated populations of *Thielaviopsis ethacetica* in Cameroon, Fungal Biology, Retrieved from: http://dx.doi.org/10.1016/j.funbio.2015.05.009.

McDonald, B. A., & Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology, 40: 349 –379.

Melo MP, Matos KS, Beserra JEA Jr, Moreira SI, Silva FF, Neto FA, Alves E, Pereira OL 2016. *Thielaviopsis musarum* causes posthar- vest crown and fruit rot of banana in Northeastern Brazil. Tropical Plant Pathology 41: 258–263.

Mirzaee, M.R.,Tajali, I.H and Javadmosavi, S.A 2014. *Thielaviopsis paradoxa* Causing Neck Bending Disease of Date Palm in Iran Journal of Plant Pathology, 96 (4): 113.

Ngom E. 2011. Oil palm in Cameroon. Communication at the event 'Sharing what works in sustainable and equitable oil palm development'. Bogor, Indonesia: CIFOR. 21–27.

Oliveira, L.S.S., Harrington, T. C., Ferreira, M.A., Damacena, M. B., Al-Sadi, A. M., Al-Mahmooli, I. H. S., Alfenas, A. C. 2015. Species or Genotypes? Reassessment of four recently described species of the Ceratocystis wilt pathogen, *C. fimbriata*, on *Mangifera indica*. Phytopathology, doi:10.1094/PHYTO-03-15-0065-R.

Omamor, I.B. 1985. Black seed rot of sprouted seeds of the oil palm, *Elaeis guineensis* Transaction of British Mycolological Society 84 (1): 159-161. DOI: 10.1016/s0007-1536(85)80230-8

Oritsejafor, J.J. 1989. Status of the oil palm vascular wilt disease in Nigeria. International conference on Oil Palm and Palm Products Benin City: Nigeria.

Raid RN 2009. Pineapple disease of sugar cane. Agronomy Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Florida Sugarcane Handbook.

Robertson, J. S. 1962. Dry Basal Rot, a new disease ofoil palms caused by *Ceratocystis paradoxa* (Dade) Moreau. Transactions of the British Mycological Society 45: 475-478.

Roslan, M. M. N., and Haniff, M. H. 2004. Water deficit and irrigation in oil palm – a review of recent studies and findings. Oil Palm Bulletin of Malaysia Palm Oil Board, 49: 1-6.

Suleman, P., Al Musallam, A and Menezes, C.A.2001. The effect of solute potential and water stress on black scorch caused by *Chalara paradoxa* and *Chalara radicicolaon* date palms. Plant Disease. 85:80-83.

Tokeshi H, Rago A. 2005. Doenças da canadeaçúcarhíbridos de Saccharum spp. In: KIMATI, H.; Amorim L, Rezende JAM, Bergamin FA, Camargo LEA. Manual de fitopatologia: Doenças das plantascultivadas. São Paulo SPAgronômica Ceres, pp185–196.

Wingfield, B. D., Grant, W. S. Wolfaardt, J. F. and Wingfield, M. J. 1994. Ribosomal RNA sequence phylogeny is not congruent with ascospore morphology among species in *Ceratocystis sensustricto.* In: Molecular Biology and Evolution. 11: 376-383.