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FIRST REPORT OF RED RUST DISEASE CAUSED BY *CEPHALEUROS VIRESCENS* ON MANGO (*MANGIFERA INDICA*) TREE IN CAMEROON

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ABSTRACT

In August 2020, a disease with symptoms identical to red rust caused by *Cephaleuros virescens* was found in orchards of mangoes besides orchards of Anacardium surveyed in Maroua and Garoua (Cameroon). The objective of this research was to study this disease with characterizing its causal organism using morphological methods. Mango leaves exhibiting clear symptoms of red rust with pathogen somatic and reproductive structures were used for morphological characterization and identification of the causal pathogen. Leaves were collected based on the symptoms, size and number of lesions associated. The survey results indicate that orange coloured, small circular lesions were found on the upper leaf surfaces and coalescing in midrib were observed. Circular lesions on the leaves were of varied diameter from 1 to 5 mm with an average of 1.6 (coefficient of variation 34%). Among symptomatic leaves showing lesions, 60% were exhibiting lesions smaller than 2 mm in diameter. The length and width of sporangiophores and sporangia were 237.62 - 4645.85 × 15.5 µm and 20.6 - 41.32 × 20.6 µm, respectively. Based on the symptoms observed and microscopic morphological studies, the recovered pathogen was identified as *Cephaleuros virescens*. This is the first report of algal leaf disease on *M. indica* in Cameroon.

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INTRODUCTION

Mango is considered as one of the most important tropical fruits cultivated in the world. It is native to India and Southeast Asia and has been cultivated for over 4,000 years. There are hundreds of types of mango, each with a unique taste, shape, size and colour. In 2018, the global production of mangoes was 55.4 million tonnes, led by India with 39 % (22 million tonnes) of the world total. China and Thailand were the next largest producers (FAO, 2019).

After the Asian continent, the African Continent is the second largest fruit producer in the world. Its production is 3.6 million of tons. Mango production (*Mangifera indica* L.) is in great expansion because of its wide acceptance in the domestic markets. Mango is a low-calorie fruit that is high in fiber, and is a great source of vitamins A and C. It also contains folate, B6, iron and a little calcium, zinc and vitamin E. Mango

contains magnesium that can support heart health (Tedihou *et al.*, 2018). In 2009, 43354 mango trees were reported in North and Far North Cameroon (Temple, 2001). In these two regions, mangoes pulp are processed into juices and nectars, as well as jams. On the other hand, the sale of mangoes is a source of income for the producers (Kameni *et al.*, 2003; Sougnabe *et al.*, 2010).

Besides this importance, several factors are affecting the production of mango. Among these, diseases caused by microorganisms such as Cephaleuros are important constraints for the quality and quantity of current mango production in the world which in turn limit the commercial value of the products (Batista *et al.*, 2016).

Among these microorganisms, *Cephaleuros* species are green algae under kingdom Archaeplastida, Phylum Chlorophyta, Class Ulvophyceae, Order Trentepohliales, Family Trentepohliaceae (Sunpapao, 2016) which parasites several host plants causing the disease known as red rust, which occurs in all tropical and subtropical regions of the world. Temperature and humidity are adequate conditions for their reproduction and growth (Malagi *et al.*, 2011; Nelson, 2008).

Red rust is one of the diseases caused due to *C. virescens* described in the literature (Malagi *et al.*, 2011; Ponmurugan *et al.*, 2009; Sunpapao, 2016). However, the pathogen is out of the ordinary (virus, bacteria, fungi, protozoa which are usually known as plant pathogens) because it is reported to be a parasitic alga which causes the disease to characterize by leaf spots on the upper surface of the leaves and fruits (Malagi *et al.*, 2011; Pereira *et al.*, 2020). Otherwise, these leaf spots cause a reduction in plant photosynthetic surface area. While usually harmless, severe causes of these leaf spots can lead to defoliation, tissue necrosis, damage of stems and loss of marketable fruit (Ponmurugan *et al.*, 2009).

The algae genus *Cephaleuros* consist of 15 species, with branched free or coalescing filaments. *Cephaleuros*, commonly referred to as a mandatory epiphyte, may also be parasitic, where haustoria are sometimes present within the tissues of the host plant. Rain is essential for the production and dispersal of zoospores (Chanthapatchot and Satjarak, 2019; Ponmurugan *et al.*, 2009). In studies on the *Cephaleuros,* morphological, sporangiophore and sporangia are commonly estimated to characterize *Cephaleuros* species (Han *et al.*, 2011; Vasconcelos *et al.*, 2018). Therefore, the objective of this study was to carry out an assessment of red rust disease of mango and identify the pathogen or agent in Cameroon. The findings from this research can be a baseline for further study in the area as it is the first time report from the country.

MATERIALS AND METHODS

Study area

Departments of Diamaré and Benoue were chosen as study area. Two subdivisions [Maroua 1st (10.4236°N and 10.6279°N-14.1961°E and 14.4814°E) and Garoua 3rd (13’26 39-9’28)] were chosen by site and three orchards were surveyed in each subdivision.

Pathogen isolation and characterization were carried out in the laboratory of Biological Sciences of Maroua and in the laboratory of Biotechnologies Phytopathology and Microbiology Unit of the University of Yaounde I, Cameroon.

Collection of leaves with symptoms of red rust

During 2020, a survey of mango orchards was done in North and Far North regions of mango growing villages in Cameroon and trees with mango red rust disease symptoms were noted. In each orchard, symptomatic samples of leaves were collected in the morning from ten trees chosen randomly following the diagonal of each of the three orchards visited in each site. For each sample, the locality name, and geographical coordinates were noted. The samples were packaged in plastic bags containing cotton soaked with sterile distilled water and transported to the laboratory for identification. samples were processed immediately or were stored at 4 °C.

Pathogen characterization and identification

Identification of the disease was started by observation of symptoms of red rust in the field on different orchards and compared with identification keys of Pereira *et al.* (2020); Vasconcelos *et al.* (2019); Malagi et *al*. (2011); Han *et al.* (2011). Characterization and identification of parasitic algae involve observation, counting, measurements and description of algal length, the width of sporangium and sporangiophore under the microscope in addition to determining the number as well as the diameter of lesions per leaf (Ponmurugan *et al.*, 2009; Vasconcelos *et al.*, 2018).

Morphological characterization of the algae structure was done by using leaf samples. To obtain these structures, the rust stains were scraped off with needle and placed on a slide containing a drop of distilled water help for observation in the microscope (Omax). An

average of 15 measurements of structure (length, width, sporangium and sporangiophore and 75 leaves (lesion size and number) were performed by the site. Number and diameter of lesions were determined and recorded using an optical microscope with micrometre calibration.

Statistical analysis

Data collected was analyzed using analysis of variance (ANOVA 1) and means were compared through Duncan multiple range test with statistical software SPSS 16.0. $P<0.05$ is considered statistically significant.

RESULTS

Mango red rust disease assessment

Symptoms of red rust were observed on the leaf area due to parasitical algae *Cephaleuros virescens.* Symptomatic leaves showed a lot of circular lesions with different diameter. The leaf spots were orange-brown rust in colour and usually occured entirely on the upper leaf surface (Figure 1 a). Leaf infection was observed at the basal part of the mango tree leaves with Coalescing lesions whic occured in the midrib of leaves (Figure 1 b, c). Some mango orchards were not far from cashew orchards (*Anacardium occidentale*) orchards.

Figure 1. Symptoms of red rust on mango leaves showing a) circular shape spots ; b) coalescing spots ; c) basal part leaves of the mango plant diseased.

Characterization and identification of algal specie

The result of microscopic observations showed that the sporangiophores were single (Figure 2 a) or clumped (Figure 2 b). Each sporangiophore had more than one septa (Figure 2 a) and sporangia attached by cell suspensor (Figure 2 c). Sporangia appeared to have a crown at the head of the sporangiophore. Sporangium attached by cell suspensor (Figure 2 d) that contained zoospores inside the membrane (Figure 2 e).

The circular lesion diameters obtained varied from 0.5 to 5 mm per leaf with an average of 1.6 and 1.7 according to the site. Coefficient of variation was 43%. As for average diameter, no statistical difference ($P\leq0.05$) was found with a number of lesions per leaf of variety (Table 1).

After algae structure measurement, the sporangia were found to have 20.6 – 41.32 µm in length and 20.6 µm in width with the coefficient of variation of 30% (Table 2). The total number of sporangia produced by each sporangiophore varied from 3-10. The number of sporangiophores produced by clump was 1-10. Length of sporangiophore varied from 237.62 to 464.85 µm with an average of 274.32 and coefficient of variation of 42%. The width was 15.5 µm (Table 2).

Table 1. Average diameter (mm) and number of circular lesions of algae spot per leaf of Garoua and Maroua mango trees.

	Sites		Coefficient of variation per site (%)
	Maroua	Garoua	
Number of leaves	75	75	
Number of lesions per leaf	110	93	64%
Lesion average diameter (mm)	1.7 (1-5)	1.6 (1-5)	43%

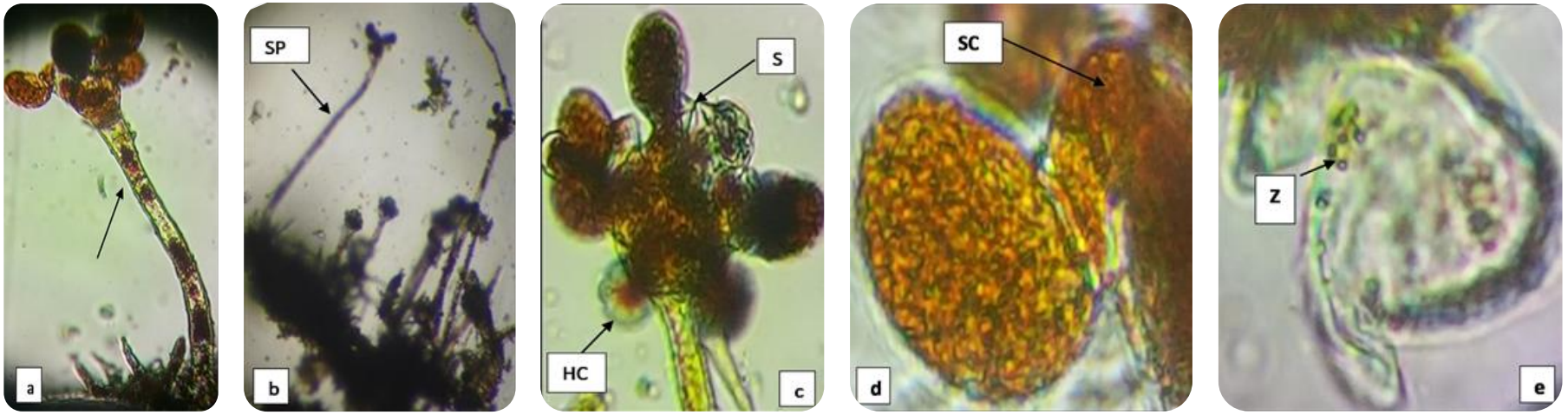

Figure 2. Microscopic characteristics of *Cephaleuros virescens*: a) single sporangiophore (arrow indicates septum in sporangiophore x400), b) clumped sporangiophores. Sporangiophore (SP) x100, c) Head of the sporangiophore. Head cell (SP), sporangium (S), d) sporangium. Suspensor cell (SC), x1000, e) sporangium releasing zoospores. Zoospores (Z).

Table 2. The morphological characteristics obtained from the parasitic algae of red rust of mango trees occurring in Cameroon and compared with some previous works.

Algal species	Host	Sporangiophores (µm)			Sporangium (µm)			Reference
		Nb/cl	Length	Width	Nb/cl	Length	Width	
Cephaleuros virescens [(1)]	*Mangifera indica*	1-10	237.62-464.85	15.5	3-10	20.6-41.32	20.6	
Cephaleuros virescens	*Swietenia macrophylla*		150.0– 407.3	8.0–8.8		18.2-31.6	16.5-21.3	Pereira *et al.* (2020)
Cephaleuros virescens	*Mangifera indica*		245.5-545.6	10.5-19.1	2-7	21.4-34.2	16.3-24.7	Vasconcelos *et al.* (2019)
Cephaleuros virescens	*Ficus benghalensis*	2-5	500- 1000	12.0– 25.0		30.0	22.0	Malagi *et al.* (2011)
Cephaleuros virescens	*Citrus sinensis, C. reticulata, C. limetta*		200.4	15.9		20.0	19.6	Han *et al.* (2011)
Cephaleuros parasiticus	*Camellia sinensis*		880- 1256	22.5- 32.2		17.4-27.5	17.4 - 20	Ponmurugan *et al.* (2010)

[(1)] *C. virescens* found in Cameroon mango leaves. Nb/cl: number per clump.

DISCUSSION

Field assessment of mango disease indicates red rust disease caused by algae is *Cephaleuros virescens* in the main production sites of North and Far North Cameroon. However, no study was done previously in Cameroon about this pathogen. Nevertheless, some author identified red rust disease due to *C. virescens* in different hosts in some countries (Afouda *et al.*, 2013; Han *et al.*, 2011; Khatoon *et al.*, 2017; Majune *et al.*, 2018; Malagi *et al.*, 2011; Pereira *et al.*, 2020; Ponmurugan *et al.*, 2010; Vasconcelos *et al.*, 2019; Wonni *et al.*, 2017).

The occurrence of algae spots is favoured by average monthly temperatures around 23 °C and average monthly rainfall of 127 mm (Malagi *et al.*, 2011). During July and August, rainfall was very high in the sites which was ideal for the envelope membrane of sporangia to break, which facilitates wind dispersion of zoospores (Duarte *et al.*, 2005).

Little or no work has been done on the size of the structures of the alga affecting disease to the mango trees. However, based on the result of morphological characteristics, the measurements of sporangium and sporangiophore of the algae species are 20.6-41.32 × 20.6 and 237.62-464.85 ×15.5 respectively in this study and are found to be close to *C. virescence* identified by Pereira *et al.* (2020) from *Swietenia* sp., Malagi et *al*. (2011) from *Ficus benghalensis* and Han *et al.* (2011) from *Citrus sinensis*. However, these measurements, are in contrary, different mainly in the length of sporangiophore, to *Cephaleuros parasiticus*, obtained by Ponmurugan *et al.* (2010) from *Camellia sinensis*. 800 – 1256 µm respectively. Although *C. virescens and C. parasiticus* have similarities in shape and colouration, the *C. parasiticus* sporangiophores have higher dimensions for length and width: 880-1256 µm × 22.5-32.2 µm (Ponmurugan *et al.*, 2010), mainly for *C. virescens* width (14.0-19.0 µm), as observed by Suto *et al.* (2014). Sporangiophore size is thus, the safer measure to distinguish the two species since Suto *et al.* (2014) have stated that the sporangium size is the same for *C. virescens* and *C. parasiticus*. Results of sporangiophore size in this study agree with that of Vasconcelos *et al.* (2019) obtained 245.5-545.6 µm.

Almost all mango trees nearest the *Anacardium occidentale* were attacked by *C. virescens*. Mango and *Anacardium occidentale* L., are in the same botanica family of Anacardiaceae, therefore can be attacked by the same pathogens (Chanthapatchot and Satjarak, 2019).

Interestingly, attacked leaves showed on average 60 lesions, which can be considered a low value when compared with *Mycosphaerella citri*, which showed on average 131 injuries per leaf (Silva *et al.*, 2008). Moreover, 60 % of the algae spot lesions were smaller than 2 mm in diameter, which demonstrates the greater capacity of the pathogen to reach different points of the leaf limb (Malagi *et al.*, 2011). According to Keller *et al.* (2000), the number of lesions is important because it is highly correlated with the disease severity and incidence of mango red rust must be evaluated in all production areas of Cameroon.

Variability of measurements was obtained from *C. virescens* sporangiophores and sporangia. Pereira *et al.* (2020) obtained coefficients variation of 31.28% for sporangiophore length and 22.06% for that of sporangia. In this study, coefficients variation 42% for sporangiophore length and 30% for that of sporangia were observed. These results show that the sporangiophores length is more variable when compared with other structures measured (Pereira *et al.*, 2020). Finally, it is worth noting that the homogeneity obtained in the measurement of micromorphological structures helps the characterization and standardization of *C. virescens* structure measurement (Carvalho *et al.*, 2008).

CONCLUSION

Based on the observed field symptoms, microscopic characters and description the mango red rust in Cameroon (Maroua and Garoua) is caused by *C. virescens*, parasitic algae. This is the first report of red rust caused by *C virescens* in Cameroon.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

Ngoh Dooh J.P., Ambang Z., Djile Bouba, Heu A., Kuate T.W.N., Kone N.A.N. and Mboussi S.B. participated in the design of the study, pathogens identification and characterization and data analysis. Ngoh Dooh J.P., Tchoupou T.D.B., Jeutsa A.D., Amani G.H. participated in sampling and data collection. Ngoh Dooh J.P. drafted the manuscript. All authors read and approved the final manuscript.