CHARACTERIZATION OF RED RUST DISEASE CAUSED BY *CEPHALEUROS VIRESCENS* KUNZE ON CASHEW NUT IN THE SUDANO-SAHELIAN ECOLOGICAL ZONE OF CAMEROON

aNgoh D. J. Patrice*, bMboussi S. Bertrand, cHeu Alain, dKuate T.W. Norbert, eAsta D. Baba, fTchoupou T. D. Brice, Djile Bouba, Ambang Zachée

a Department of Biological Sciences, Faculty of Science, University of Maroua, PO Box 814 Maroua, Cameroon.
bUniversity of Douala, Laboratory of Plant Biology Po Box 2701.
cHigher Technical Teacher’s Training College, Department of Agriculture and Agropastoral Po Box 886 Ebolowa.
dLaboratory of Biotechnologies, Phytopathology and Microbiology Unit, University of Yaoundel, PO Box, 812, Cameroon.
*eHigher National Polytechnic School of Maroua, University of Maroua, PO Box 1450 Maroua.
fDepartment of Plants Genetic and Biotechnology, Institute of Agricultural Research for Development of Maroua (IRAD), PO Box 33.

A B S T R A C T

Cashew nuts (*Anacardium occidentale* L.) is one of the plants adapted in North and Far North of Cameroon after cotton. Nevertheless, the crop is susceptible to several pests and diseases like Red rust. The objective of this work was to characterize red rust disease and the micromorphology of the algal causal agent. Leaves with symptoms of each attacked tree (AB05-08 and AB 29 varieties) were randomly extracted for lesion characterization according to its size and number, in the three mains production sites, Maroua, Yagoua (Far North) and Garoua (North). The pathogen structures were characterized under light microscope. Incidence, severity and prevalence of disease were evaluated during the dry and rainy seasons. The most obvious symptom was small spots lesion, more or less circular with orange color on the upper leaf surfaces. The dimensions obtained for sporangia were 41.32–51.65 μm in length and 25.82–30.95 μm in width. Number of sporangia varied from 0 to 6 depending on the season. Based on symptoms, micromorphology and according to literature, it was found that the disease was caused by the algae *Cephaleuros virescens*. Incidence was very high in Maroua, whatever the season 51.67 and 64.17 % respectively. Severity was very low (less than 16%) whatever the site and season. The results of this study represent an important baseline data for the implementation of strategies against red rust in Cameroon. According to our knowledge, this is the first report of red rust on cashew in Cameroon.

**Keywords**: *Anacardium occidentale*, red rust, *Cephaleuros virescens*, algae, incidence, severity.

INTRODUCTION

With the globalization of trade and the need to respond to new challenges linked to the socio-economic crisis that has affected Africa in recent years, African agriculture has to produce new crops, perennial or annual, food and/or energetic. In most developing countries, agriculture employs 70 % of the workforce and provides between 20 and 60 % of gross domestic product (GDP), and contributes between 10 and 90 % of exportation of goods (Didier, 2001).

In the three northern regions of Cameroon, Adamawa, the North and the Far North, cotton is and remains, the only cash crop. Thus in 1975, the Cameroonian state introduced cashews (*Anacardium occidentale* L.) in the northern region to fight against deforestation by distributing seeds to local populations. Some farmers in the North and Far North regions have started to cultivate cashew for its broad ecological spectrum and to

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* Corresponding Author: Email: ndjuliopat@yahoo.fr
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associate it with vegetable and food crops (Nohia et al., 2017).

In Far North Cameroon, many projects are underway to expand cashew production. However, many orchards associated with mango trees belong to rural farmers. Cashew production represents a new source of income for the rural population (Adeniyi et al., 2019; Djongmo, 2016; Díaz et al., 2003).

The cashew tree is very exported for its nuts whose almond, rich in phenol, oil and sugars, is used in the food industry (Lautié et al., 2001). It is also cultivated for the apple (cashew apple) which contains a sweet, acidic and astringent juice. This apple is directly edible when ripe or then transformed into juice, wine, syrup or beer in Burkina Faso, Senegal and Ivory Coast (Afouda et al., 2013).

However, many problems are found in the chain of production of this crop, such as diseases caused by microorganisms, which in many cases are limiting their commercial exploitation due to the degradation of its qualitative and quantitative statues (Viana et al. 2006; Assenga et al. 2020). In addition to pests, more than twelve diseases have been described on cashew in some countries (Afouda et al., 2013; Wonni et al., 2017; Nakpalo et al., 2017; Khatoon et al., 2017). In Cameroon, few diseases including powdery mildew and anthracnose have been suspected based on symptoms described in literature (Tandjiékpon et al., 2003; Mingue, 2019). Many development programs underway in order to extend the production and marketing areas for cashew nuts. It is therefore necessary to survey the various phytosanitary problems that may jeopardize the productivity of the cashew tree in area.

Red rust is one of the diseases that infect Anacardium occidentale (Binoy et al. 2016). However, its pathogen is out of the ordinary as it is reported to be a parasitic algae belonging to the genus Cephaleuros Kunze ex Fr. that causes red spots on the upper surface of the leaves and fruits (Atsumi et al., 2017; Pereira et al., 2020). In fact, these red spots cause a reduction in plant photosynthetic surface area. These red spots are usually harmless in low density, however, when they are severe or very dense, they can lead to defoliation, tissue necrosis, and loss of marketable fruit (Ponmurugan et al., 2009).

Occurrence of Cephaleuros species on different plant species has been reported in various tropical and subtropical parts of the world including Africa (Benin, Ivory Coast), Asia (India, Korea, Thailand), Australia, South (Brazil, Panama) and North America (Suto and Ohtani, 2009; Han et al., 2011; Muthukumar et al., 2015; Pitaloka et al., 2015; Vasconcelos et al., 2016; Sunpapao et al., 2016; Shukla et al., 2017). However, these algae have not yet been reported in Europe. As climatic conditions seem to influence the occurrence and distribution of Cephaleuros species (Suto and Ohtani, 2013; Suto et al. 2014).

The species Cephaleuros virescensKunze ex Fr. have been reported on many plants such as tea (Camellia sinensis), kava (Piper methysticum), pepper (Piper nigrum), magnolia (Magnolia grandiflora), coffee (Coffea arabica), oil palm (Elaeis guineensis), avocado (Persea americana), vanilla (Vanilla planifolia), acacia (Acacia auriculiformis), mango (Mangifera indica), breadfruit (Artocarpus altilis), guava, coconut (Cocos nucifera), cacao (Theobroma cacao), as well some cultivars citrus (Citrus spp.) (Paracer and Vernon, 2000; Sunpapao and Pitaloka, 2015; Thomas et al., 2016; Vasconcelos et al., 2016).

The algae genus Cephaleuros compound of 15 species, consists of branched, free or coalescing filaments. Cephaleuros species are commonly referred to as a mandatory epiphyte, may also be parasitic, where haustoria are sometimes present within the tissues of the host plant (Ponmurugan et al., 2009). In studies on the Cephaleuros, morphological and sporangia are commonly estimated (Vasconcelos et al., 2018). Therefore, the objective of this study was to carry out description of red rust disease of cashew and morphological characterization of his pathogen agent in Cameroon for the first time.

MATERIALS AND METHODS

Study areas: Samples were collected in North and Far North regions of Cameroon for two years (2019-2020). Departments or Divisions of Diamaré, Mayo Danay and Benoue, which are the main cashew production areas, were chosen. Three orchards were surveyed in each of the three localities of the three subdivisions chosen by site. Maroua 1st (10.4236°N and 10.6297°N-14.1961°E and 14.4814°E), Yagoua (10.1159°N and 10.4079°N-15.1679°E and 15.3866°E) and Garoua 3rd (13°26 39-9°28). Pathogen isolation and his characterization where carry out in Biological Sciences Laboratory of Maroua and Laboratory of Biotechnologies, Phytopathology and Microbiology Unit, University of Yaounde I, Cameroon.

Rainfall: There were no rainfall during March and April months at the three sites. The highest precipitation was...
recorded in Maroua in July, August and October with more than 120 mm of water. The highest rainfall was recorded in August, 80 mm, in Yagoua alone. Overall, the rainfall was higher in Maroua (1140.2 mm) than in Yagoua (901 mm) and Garoua (1007 mm) during the season 2019.

**Plant material:** In the different orchards, trees of cashew were at less 10 years old. Two varieties were represented, AB05-08 variety with red fruit and AB 29 variety with yellow fruits.

**Collection of leaves with red rust symptoms:** The sampling was performed during the dry and rainy seasons for two years (2019-2020). From march to april (dry season), and from july to august (rainy season). In each orchard, infected samples of leaves, were collected (in morning) from ten trees chosen randomly following the diagonal of each of the three orchards visited in each site (Garoua, Yagoua and Maroua). 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 in laboratory. Samplings were used immediately for characterization or were stored at 4°C.

**Pathogens isolation, characterization and identification:** Identification of disease was done by observation of symptoms of red rust in field in different orchards and compared to identification keys (Afouda et al., 2013; Nakpalol et al., 2017; Wonni et al., 2017). Characterization and identification of pathogen consisting of observation, counting, measuring and description of algae structure (length and width of sporangium and sporangiophore) in microscope. And by counting number and measuring the diameter of lesions per leaf and variety (Ponmurugan et al., 2009; Uaciquete, 2013; Vasconcelos et al., 2018; Atsumi et al., 2017).

Samples were directly used to characterize algae structures for each season. To obtain these structures, the rust stains were scraped off with needle to remove body structures on a part of the Petri dishes containing the agar water medium (WA) and also directly on a slide containing a drop of distilled water for observation in microscope (Omax). Number and diameter of lesions were counted and recorded from the leaves of each variety according to season. Optical microscope with micrometer was used to count and measure length and width of sporangia and sporangiophore. An average of 50 measurements of each structure (length, width of sporangium and sporangiophore) and 100 leaves of each variety (lesion size and number) were performed.

For the growth (isolation and cultivation) of pathogen, samples were carried out on Potato Dextrose Agar (PDA; containing g l⁻¹: 200 g potato, 20 g dextrose, 15 g agar) and water agar (WA, containing g l⁻¹, 15 g agar) media. Pêtri dishes of 90 mm were used. Sections of 1 cm diameter were cut from leaves with symptoms using sterilized scalpel. Explants were washed in tap water. Then, sterilized in ethanol (70%) firstly for one minute followed by immersion in 1 % of sodium hypochlorite solution for 2 min. The infected section was then washed three times in Sterile Distilled Water (SDW). Sterilized explants were dried and grown on Petri dishes containing WA and PDA medium. Cultures were incubated at 25°C on photoperiod 12/12h.

**Assessment of disease incidence:** Disease incidence was assessed on twenty trees selected randomly in each orchard in 2019 during dry (march-april) and rainy (july-august) seasons. A modified protocol of Shomari and Kennedy (1999) and Afouda et al., (2013). The evaluation was made according to the North, South, East and West sides of the crown (canopy) of each the56 to 60 trees surveyed by site.

Incidence was estimated following the formula:

\[ I(\%) = \frac{n}{N} \times 100 \]

Where n is the number of trees infected by a disease and N, the total number of trees survey.

**Assessment of disease severity:** Severity was assessed by estimating the leaf area occupied by the symptoms of the disease according to the formula:

\[ S = \frac{\Sigma (ab)}{N} \]

Where S is the average severity of the disease in the site, \( \Sigma \) is the sum of the products of the number of diseased plants, (a) the severity index, (b) the number of plants with the index given in % and N the number total trees observed. Data were recorded during dry (march-april) and rainy (july-august) seasons.

The disease severity index was assessed using a visual scale of 0 to 9 (Cardoso et al., 2009; Soro , 2012; Afouda et al., 2013):

- 0 = no symptom
- 1 = 1-4%
- 2 = 5-9%
- 3 = 10-19%
- 4 = 20-29%
- 5 = 30-44%
- 6 = 45-59%
- 7 = 60-75%
- 8 = 76-90%
- 9 = > 91% percentage of average lesion per leaf infected.

**Assessment of the prevalence of the disease:** The
prevalence assessment was carried out on an average of 40 trees for each variety in three of the studied sites (Maroua, Garoua and Yagoua) using the following formula:

\[ P = \frac{n}{N} \times 100 \]

Where \( n \) is the number of tree of the variety infected and \( N \) is the total number of plant individuals of the variety surveyed in the site.

**STATISTICAL ANALYSIS**

Data collected were analyzed using analysis of variance (ANOVA 1) one way. \( p<0.05 \) and averages were compared through Duncan multiple range test with statistical software SPSS 16.0.

**RESULTS AND DISCUSSION**

**Red rust disease symptoms:** Symptoms of The red rust symptoms observed on the leaf are due to parasitical algae *Cephaleuros virescens*. Leaves show a lot of circular lesions. Theses leaf spots are an orange-brown rust in color and usually occur entirely on the upper leaf surface (Figure 1 A), and seldom on the underside of infected leaves (Figure 1 B).

![A and B figures showing red rust symptoms on cashew leaves](image)

**Morphological characteristics of *Cephaleuros virescens***: Microscopic observation showed many sporangiophores soled or clumped (Figure. 2 A). Each sporangiophore has more than one (1) septa (Figure. 2 B) and many sporangia (Figure. 2D). Sporangia appear as a crown at the head of the sporangiophore. Sporangium are attached by cell suspensor contain many zoospores inside (Figure. 2 C). No sporangiophore was present during the dry season. After isolation of algae on to solid media, no culture medium resulted in *C. virescens* development (Figure. 2 E, F).
Figure 2. Micromorphological characteristics of *Cephalaeuros virescens*on: A) clumped sporangiophores (×100), B) septum in sporangiophore C) sporangium (× 400), sc – suspensor cell, D) single sporangiophore (HC: head cell; S: sporangium), E) sporangium in WA medium after 10 days, F) sporangia in PDA medium after two weeks.

The lesion diameters obtained varied from 0.5 to 3 mm for the red variety with average of 1.7 and from 0.5 to 5 mm for yellow variety. Coefficient of variation was 47% and 38% respectively. As for average diameter, no statistical difference (P≤0.05) was observed with number of lesions per leaf of variety (Table 1).

| Variety       | Ø (mm) | Coefficient of variation (%) | Number of lesions per leaf |
|---------------|--------|------------------------------|---------------------------|
| AB05-08(Red variety) | 1.7a   | 47%                          | 99.4 a                    |
| AB29(Yellow)  | 1.8a   | 38%                          | 115 a                     |
| Coefficient of variation (%) | -      | -                            | 57.2 %                    |

(1) Values followed by the same letter in each column do not differ significantly by Duncan test (P≤0.05)
After algae structure measurement, the sporangia were found to have 20.6 - 30.96 μm in length for both varieties and 13.2-26.4, 13.2-20.6 μm in width respectively for red and yellow variety (Table 2). The total number of sporangia carried by each sporangiophore varied from 1 to 6 for the Yellow variety and from 1 to 9 for the Red variety. But in dry season, no sporangia was found in any leaves. The number of sporangiophore produced by clump was 1-12 for red variety and 1-13 for yellow variety. Length of sporangiophore of Red variety varied from 185.25 to 607.2 μm with coefficient variation of 37 %. With, yellow variety, coefficient of variation was 278.91-660.2 μm with coefficient variation of 13 % (Table 2).

Table 2. Characteristics of Cephaleuros different hosts including C. virescens found on cashew trees in Cameroon

| Algal species | Host | Sporangiophores (μm) | Sporangium (μm) |
|---------------|------|----------------------|------------------|
|               |      | Nb/cl | Length | Width | Nb/cl | Length | Width |
| Cephaleuros virecens(1) | Anacardium occidentale | 1- 12(1) | 185.25 | 15.5 | 1-9 | 20.6 | 13.2 |
|                  |      | 1- 12(1) | 607.2 | 15.5 | 1-6 | 30.96 | 20.6 |
| Cephaleuros virecens(2) | Swieteniamacrophylla | 150.0 | 8.0 - 8.8 | 18.2 | - | 16.5 | - |
|                  |      | 407.3 | | | 31.6 | 21.3 | |
| Cephaleuros virecens(3) | Mangiferaindica | 245.5 | 10.5 | 2-7 | 21.4 | 16.3 | |
|                  |      | 545.6 | 19.1 | | 34.2 | 24.7 | |
| Cephaleuros virecens(4) | Ficus benghalensis | 2-5 | 500-1000 | 12.0 | 25.0 | 30.0 | 22.0 |
| Cephaleuros virecens(5) | Citrus sinensis, C. reticulata, C. limetta | 200.4 | 15.9 | | 20.0 | 19.6 | |
| Cephaleuros virecens(6) | Apurpurnata, F. elastica, E. globulus | 71-242 | 10.2 | | 21.4 | 16.6 | |
|                  |      | 23.15 | | | 28.56 | 26.18 | |
| Cephaleuros virecens(7) | Nepheliumlappaceum | 252-430 | 10-20 | | 20-30 | 15-22.5 | |
| Cephaleuros virecens(8) | Persea americana | 307.5-673.8 | 47.7 | 5-10 | 21.7 | 27.7 | |
|                  |      | 14.6 | | | 15.2 | 19.2 | |
| Cephaleuros parasiticus(9) | Camellia sinensis | 880-1256 | 22.5 | | 17.4 | 17.4 | |
|                  |      | 32.2 | | | 27.5 | 20 | |

(1) C. virescens found in Cameroon cashew trees; (2) Pereira et al. (2020); (3) Vasconcelos et al. (2019); (4) Malagi et al. (2011); (5) Han et al. (2011); (6) Pommurugan et al. (2010); (7) Muthukumar et al. (2014); (8) Sunpapao et al. (2016); (9) Vasconcelos et al. (2016). Nb/cl : number per clump. (a) red variety; (b) yellow variety

**Incidence and severity:** The evolution of the incidence of red rust was significantly different (P <0.001) between the studied sites. In the same area it was also highly different between the two seasons. The highest incident was observed in Maroua with a score of 51.67 ± 1.2 and 64.17 ± 0.9 % respectively in the dry and rainy season, and the lowest recorded in Yagoua with 1.67 ± 0.1 % in the dry season and 1.35 ± 0.2 % respectively in the rainy season. The severity remained very low regardless of the site and the season, despite the significant difference (P <0.05) obtained in Maroua (13.88 ± 1.2 % against 1.35 ± 0.2 % respectively in dry season and rainy season).

Table 3. Average incidence and severity (%) of red rust per site and season

|       | Maroua       | Yagoua       | Garoua       |
|-------|--------------|--------------|--------------|
|       | Dry season   | Rainy season | Dry season   | Rainy season | Dry season | Rainy season |
| Incidence | 51.67±1.2a   | 64.17±0.9b   | 1.67±0.1a    | 16.67±0.6b   | 5.1±0.6a   | 30±0.6b    |
| Severity | 13.88±1.2b   | 1.35±0.2a    | 1.65±0.1a    | 1.3±0.2a     | 2.7±0.1a   | 5.1±0.6b   |
Means followed by the same letter on the same site are not significantly different at the 5% threshold according to Duncan’s test.

Prevalence of red rust on varieties: The figure 3 shows that the prevalence rate of both varieties varies according to localities. The prevalence of red rust rate was higher on the red variety (65.75%) than on yellow (63.63%) in the locality of Maroua. In Yagoua and Garoua, the prevalence was low for the two varieties, 20% and 30% for the yellow variety against 7% and 10% for the red respectively. Apart in Maroua, the yellow variety seemed to be more susceptible to red rust than the red variety.

Figure 3. Prevalence of red rust on cashew varieties studied

DISCUSSION

Diagnosis of cashew disease highlighted red rust disease which pathogen is *Cephaleuros virescens* in the main production sites in North and Far North Cameroon. However, infection severity was low despite the high incidence. Similar results were found by different authors who identified red rust among the main diseases that attack cashew in several countries such as Tanzania (Majune *et al.*, 2018), Benin (Afouda *et al.*, 2013), India (Khatoon *et al.*, 2017) and Burkina Faso (Wonni *et al.*, 2017).

The pathogenicity test was not carried out on young plants, because of the absence of the non growth of the algae in the solid culture media used. Vasconcelos *et al.* (2018), Ren *et al.* (2013) and Ponmurugan *et al.* (2010) have shown that solid agar media such as PDA do not develop the parasitic algae *Cephaleuros virescens* because it is rich in sugar and starch. Unlike liquid media such as Trebouxia medium provides nitrogen through proteose peptone, which is an essential nutrient for algal growth (Ren *et al.*, 2013). Otherwise, algal cultivation in solid media can only be possible after isolation in liquid media because of gradual adaptation of algae to artificial growth media, firstly liquid and then solid media, and or because of abundant growth of filamentous cells, which could facilitate the transfer of the microorganism to cultivation in solid media (Vasconcelos *et al.*, 2018).

Few or no work has been done on the size of the structures of the alga attacking the cashew tree. However, based on the morphological characteristics, the measurements obtained of sporangium and sporangiphore for *Cepahleuros* in all the sites, were found to be close to *C. virescence* obtained by Malagi *et al.*, (2011) from *Ficus benghalensi* and Han *et al.*, (2011) from *Citrus sinensis*. However, these measurements, are in contrary, different mainly in length, to *Cephaleuros*, obtained in Brazil by Pereira *et al.* (2020) from *Svieteniasp.* which measures was 18.2–31.6 μm for sporangium and by Ponmurugan *et al.* (2010) from *Camellia sinensis* 17.4 – 20.8 μm.

A hindrance point for the distinction between *C. parasiticus* and *C. virescens* based on morphological characters would be the small overlapping areas in sporangiphore dimensions obtained by some authors (Pereira *et al.*, 2020). However, these overlapping areas are very limited and do not compromise the morphological taxonomy. The minimum sporangiphore dimensions obtained by Han *et al.*, (2011) can be...
mentioned as an example, which do not happen in C. parasiticus. Furthermore, the starting point for sporangiophores C. parasiticus dimensions corresponds to the maximum found for C. virescens (Ponmurugan et al., 2010). Variability of measurements was obtained from C. virescens sporangiophores and sporangia, with variation coefficients of 37 and 13% for sporangiophore length and 20% for sporangium length, were observed. There are no many reports on the dimension variability found in C. virescens. These results show that the sporangiophores length is more variable when compared with other structures measured. 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.

Malagi et al., (2011) claimed that there is higher algae spot incidence on the shaded leaves of the plant. Interestingly, attacked leaves showed on average 90 and 100 lesions according to varieties. This result is closed of that obtained by Silva et al. (2009) on Mycosphaerella citri, which showed on average 131 injuries per leaf. Pereira et al. (2020) obtained on average 19.4, 11.4 and 2.6 lesions (of different diameter) per leaf on Brazilian mahogany (Swietenia macrophylla).

In this study, 98% of the algae spot lesions were smaller than 3 mm in diameter on both red and yellow varieties, this demonstrates higher capacity of the pathogen to reach different points of the leaf limb. Within the same variety, there is little variation among sizes, suggesting symmetry typical of algae spots. According to Keller et al. (2000), the number of lesions is an important fact because it is a variable highly correlated with the disease severity. The occurrence of algae spots is favored by average monthly temperatures around 23°C and average monthly rainfall of 127 mm (Malagi et al., 2011). During the month of March and April, there were no sporangiophore and sporangium. This can explain, the lowest incidence and severity obtained in Yagoua and moreover, precipitation was absent, to favor the disease development. During the rainy season, incidence was increased in the three sites, but disease severity obtained was lower. This occurs because rainy periods with temperature (around 29-31°C) is ideal for the envelopment of membrane of sporangia to break, which facilitates wind dispersion of zoospores (Duarte et al., 2005).

Red rust incidence was lower in Yagoua. This means that consequences are almost negligible in cashew. Our results are in contrast with those obtained by Ghini et al. (2011) who obtained incidence of 73 to 90.0% in Benin. However, precipitation, humidity and temperature are key factors for the pathogen dispersal and infection. Despite the high rainfall (1140.2 and 901 mm), severity was relatively low in the three sites, because the large canopy cannot favor dispersal of zoospores in the entire tree leaves.

The results were collected during the seasons because of the climate in North and Far North regions of Cameroon comprises a long dry season (9 months) and a short rainy season (3 months). The type of season is a very important parameter in the process of evaluating the course of a disease. This parameter was also taken into account by Afouda et al. (2013). Seasonal information provides data on the interval of time when measures must be taken to treat and prevent diseases in the field.

No sporangia were observed during the dry season. The zoosporangia of C. virescens were formed on leaves of five plant taxa both during the monsoon and the summer seasons (Muthukumar et al., 2014). Suto and ohtani (2013) showed that in Matsue Shimane Prefecture, Japan, the infections by different Cephaleuros species on various tree species occurred from April to July, and the zoospores are produced the following year. These observations clearly show that algal spreading, infection, and reproduction are dependent on environmental conditions.

The prevalence of red rust depends on host variability. Soro (2011) in a study on the behavior of cashew genotypes in northern Côte d’Ivoire, had indicated that work on genotypes could facilitate the selection of cashew varieties. This finding may explain the growing cultivation of the yellow variety compared to the red variety by the owners of cashew orchards in the Far North. In addition, some varieties have a natural resistance which confers on them the capacity to overcome diseases during a certain age of their evolution.

**CONCLUSION**

Based on symptomatologic descriptions and morphological characterization, Red rust of cashew, in Cameroon, is due to C. virescensis, a parasitic alga. It attacks leaves and shows on average 80 lesions per leaf and variety. Average length and width of sporangiophore and sporangium are respectively 396.2 × 15.5 μm and 25.78 ×16.9 μm.
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**Contribution of Authors:**

- NGOH D. J. Patrice: Elaborated the protocol and drafted the manuscript
- Mboussi S. Bertrand: Identified and characterized the pathogen
- Heu Alain: Identified and characterized the pathogen
- Kuate T. W. Norbert: Analyzed the data
- Asta D. Baba: Participated in sampling and data collection
- Tchoupou T. D. Brice: Participated in sampling and data collection
- Djile Bouba: Revised the manuscript
- Ambang Zachée: Revised the manuscript