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Incidence of red rust (alga leaf spot) in cashew orchards in Ghana

Abdulai Muntala[1*], Patrick Mawuenyengan. Norshie[1], Kwadwo Gyase Santo[1], Stephen Larbi-Koranteng[2], Daniel A. Ntiamoah[1], Frank Ankomah-Boamah[1], Annor Shilla[1]

[1]Department of Horticulture and Crop Production, School of Agriculture and Technology, University of Energy and Natural Resources, Sunyani, Ghana.
[2]Akenten Appiah Menka University of Skills Training and Entrepreneurial Development, Kumasi-Ghana

***Corresponding author: muntala.abdulai@uenr.edu.gh**
ORCID: 00 0000-0001-5128-3299

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Abstract: Cashew (*Anacardium occidentale* L.) production is under threat in the Dormaa area of the Bono region of Ghana due to a green algae pathogen. The objective of this study was therefore to determine the incidence and severity of red rust disease in cashew orchards in Dormaa Central Municipality. A survey was conducted in the 2019 and 2020 seasons in 30 selected orchards in six cashew-growing communities in Dormaa municipality. The identification of the putative alga was done both *in vivo* using visual assessment and *in vitro* using microscopic observation at a magnification of 40x. Koch's postulate was completed to confirm the pathogen's identity. The result of the test produced symptoms similar to those observed before the test, i.e., circular, raised, or flattened velvety/fuzzy orange-brown or burnt-orange or rust-pigmented or greenish-grey spots with dimensions ranging from specks to spots with average sizes of up to 3mm. The incidence and severity of the disease varied significantly ($P < 0.001$) according to the community and orchard. Based on the morphological analysis and pathogenicity test, the algal pathogen was identified as *Cephaleuros* spp.

Keywords: Anacardium occidentale; severity; incidence; orchard; red rust; Ghana.

Introduction

The cashew tree (*Anacardium occidentale* L.) belongs to the family Anacardiaceae and has its origin in northeastern Brazil. It is an economic cash crop grown not only in Ghana, but also in other tropical regions (Kenya, Bangladesh, Southeast Asia, India, Mozambique, Tanzania, etc.) of the world (Banchhor and Baid, 2007). The Anacardiaceae family consists of more than 700 species, found in 82 genera, most of which are grown in tropical areas, with a few exceptions found in temperate zones. The cultivation of this economic tree is gaining popularity worldwide due to its picturesque effects, the edible nature of the fruit when ripe or transformed into beer, wine, or juice, as well as its wood properties (Mazzetto et al., 2009; Afouda et al., 2013).

Cashew and its by-products, such as Cashew Nut Shell Liquid (CNSL), have wide industrial applications such as surfactants (Scorzza et al., 2010), lubricants (Rios et al., 2009), flame-resistant materials (Ravichandran et al., 2011), doping agents (Radhakrishan et al., 2009), resins (Pillai et al., 1990), plasticizer production (Anilkumar and Jayakannan, 2007), and other medicinal attributes such as controlling rhinitis and anti-termite, and antimicrobial properties (Toyomizu et al., 1993; Cardoso et al., 2017). Cashew nuts are believed to contain good cholesterol (HDL), low-density lipoproteins (LDL), and more monounsaturated fats, which make people gain less weight when eaten at least twice a week (Dendena and Corsi, 2014).

Globally, the cultivation of cashew nuts and the total area of production are on the rise each year, with global annual production within the period of 20 years, from 1993 to 2013, recording an increase of more than 400%, from 1,053.232 to 4,439.960 metric tons, with a corresponding total harvested area rising from 2,089.710 ha to 5,457.009 ha (FAOSTAT, 2015).

The world production of cashews is threatened by biotic factors, resulting in a substantial reduction in the quantity and quality of cashew nut yield. *Cephaleuros* infections, commonly called red rust, algal leaf spot, green scurf, and algal fruit spot, are caused by aerophilic, branched filamentous green algae. The genus *Cephaleuros*, which consists of 15 species belonging to the kingdom Archaeplastida, class Ulvophycetes, order Chlorophyta, and family Trentepohliaceae (Sunpapao, 2016), and whose growth and survival are dependent on conducive environmental conditions, is reported to cause diseases on several plants in tropical and subtropical areas of the globe (Malagi et al., 2011). It has also been reported to decrease the quantity and quality of guava and mango production (Scot, 2008; Batista et al., 2016).

The red rust caused by *Cephaleuros* species is characterized by spots on the upper surfaces of leaves and fruits, with a corresponding reduction in the photosynthetic leaf surface areas of the economic host plants. Under severe infection

conditions, tissue necrosis, damage to the stem, and defoliation have been recorded (Ponmurugan et al., 2009; Pereira et al., 2020).
The species in this genus possess an irregular disc thallus that penetrates and grows below the epidermal cells of the plant host. The filamentous body of this alga is found to be freely branched and coalesced with rainfall, favoring the production and dispersal of its zoospores (Chanthapatchot and Satjarak, 2019). The life cycle of *Cephaleuros* species consists of an alternation of heteromorphic generations in which the sporophyte metamorphosizes into a dwarf plant. The species has both sexual and asexual reproduction phases, in which the sexual phase results in the production of the dwarf sporophyte (zygote). The asexual phase is considered the most important in host infection and disease processes (Scot, 2008).
Common important horticultural species such as *Cephaleuros parasiticus* and *Cephaleuros virescens* have been reported in other parts of the world (Sunpapao, 2016; Malagi et al., 2011; Scot, 2008). *Cephaleuros virescens* has been reported as the most frequently occurring species, with a broad host range, attacking a number of higher plants (at least 287 plant hosts and cultivars) worldwide. The typical symptoms of the disease on the plant leaves may vary based on the particular *Cephaleuros* species involved in the infection process. The distinctive symptoms known to be caused by *Cephaleuros virescens* on a number of hosts are characterized by raised, flattened, and burnt-orange to brown-coloured growth on the upper leaf surface (Scot, 2008). Cashew farmers in the study area are now expressing concern about the occurrence of this algal disease in their orchards. The objective of this study was therefore to determine the incidence and severity of red rust disease as well as morphologically identify the causal agent of the disease in cashew orchards in Dormaa Central Municipality in the Bono Region of Ghana.

Results

Red rust symptoms description
The infected cashew trees suspected to be caused by *Cephaleuros* species in the various orchards visited showed varying degrees of symptoms on the leaves. The symptoms observed in the fields occurred mostly on the adaxial or upper part of the leaves, with very little occurrence on the twigs and petioles. The spots were circular, raised or flattened, velvety/fuzzy, orange-brown or burnt-orange or rust-pigmented, or greenish-grey spots of different dimensions ranging from specks to spots with average sizes of up to 3mm with filamentous margins (Figure 1). The symptoms were not seen on the abaxial or lower parts of the cashew leaves.

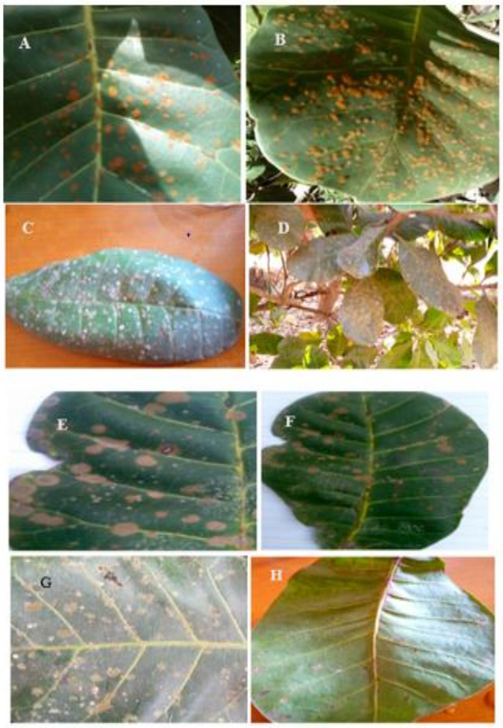

Fig 1. Symptoms of algal leaf spot (red rust); A-B: leaves showing small, numerous orange-brown or burnt-orange symptoms; C-D: small greenish-grey spots; E-F: large rust-pigmented symptoms; G: large greenish-grey spots; H: abaxial part of the leaf showing no symptoms

Incidence and severity of red rust on cashew
The incidence of red rust disease was recorded in all the orchards visited. The incidence, as well as the severity, varied significantly ($P < 0.001$) according to the community sampled (Figure 2) and orchard (Table 1). The incidence ranged from 5.96% to 18.08% at Tonsuano and Kyeremanso, respectively. The higher incidences ($P < 0.001$) were recorded at Kyeremanso, Atesikrom, and Aboagyeasu. The severity was higher in Antwirifo, Tonsuano, Badukrom, and Kyeremanso. The incidence at the orchard level ranged from 2.2% to 25.6%, whereas the disease severity ranged from 0.2 to 5.0. The least severity was recorded at Aboagyeasua and Atesikrom, with Kyeremanso recording the highest severity.

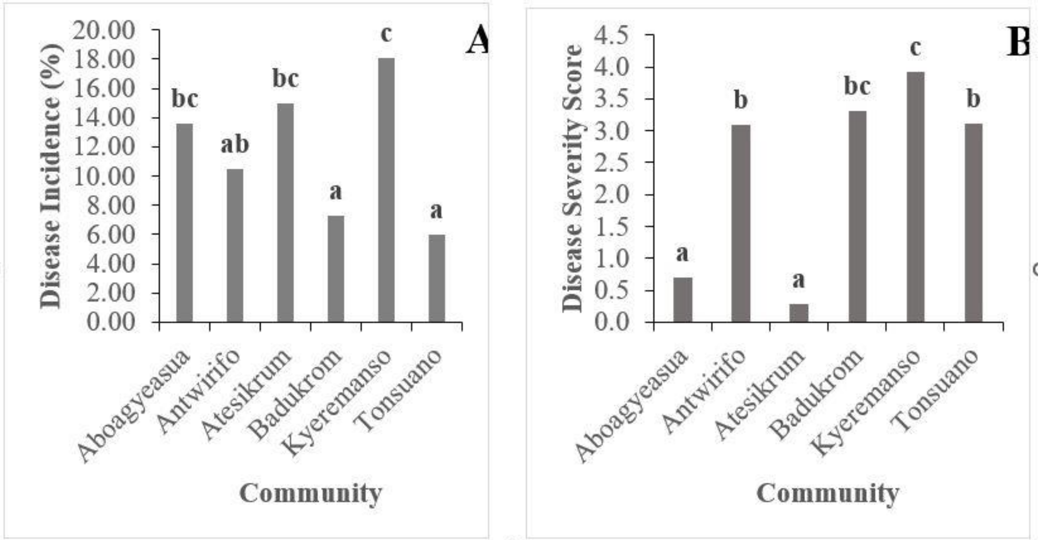

Fig 2. Incidence (A) and severity (B) of red rust on cashew trees in six communities in the Dormaa Central Municipality of the Bono Region of Ghana. Bar charts with the same letter are not significantly different

Table 1. Incidence and severity of red rust on cashew trees at individual orchard level in the Dormaa Central Municipality of the Bono Region of Ghana

Farm	**Incidence (%)**			**Severity**		
Orchard 1	5.2	(17.7)	abc	3.2	(0.3)	a
Orchard 2	4.0	(8.2)	abc	3.2	(2.3)	a
Orchard 3	4.8	(12.9)	abc	3.2	(0.3)	a
Orchard 4	5.0	(14.8)	abc	3.2	(0.4)	a
Orchard 5	4.9	(14.3)	abc	3.2	(0.3)	a
Orchard 6	4.5	(10.6)	abc	3.2	(0.4)	a
Orchard 7	5.0	(15.4)	abc	3.2	(0.2)	a
Orchard 8	5.2	(17.0)	abc	3.2	(0.3)	a
Orchard 9	5.2	(20.6)	abc	3.0	(0.2)	a
Orchard 10	4.6	(11.4)	abc	3.2	(0.3)	a
Orchard 11	5.8	(24.2)	c	3.9	(4.9)	cd
Orchard 12	4.8	(15.6)	abc	3.6	(2.9)	b
Orchard 13	4.3	(8.8)	abc	3.6	(3.0)	b
Orchard 14	5.8	(25.6)	bc	3.7	(3.8)	bcd
Orchard 15	4.9	(16.2)	abc	3.9	(5.0)	d
Orchard 16	3.5	(2.2)	a	3.6	(2.7)	b
Orchard 17	4.3	(9.8)	abc	3.6	(3.2)	bcd
Orchard 18	4.1	(7.0)	abc	3.7	(3.4)	bcd
Orchard 19	4.3	(8.6)	abc	3.6	(2.9)	b
Orchard 20	3.5	(2.2)	a	3.7	(3.3)	bcd
Orchard 21	4.6	(12.6)	abc	3.7	(3.8)	bcd
Orchard 22	5.4	(19.2)	abc	3.6	(2.8)	b
Orchard 23	3.9	(5.2)	abc	3.6	(3.2)	bcd
Orchard 24	4.0	(7.6)	abc	3.5	(2.4)	b
Orchard 25	4.2	(7.6)	abc	3.6	(3.3)	bcd
Orchard 26	5.2	(17.6)	abc	3.6	(3.1)	bc
Orchard 27	3.8	(4.8)	abc	3.7	(3.5)	bcd
Orchard 28	3.7	(4.2)	ab	3.6	(3.3)	bcd
Orchard 29	3.7	(4.0)	ab	3.7	(3.7)	bcd
Orchard 30	4.0	(6.0)	abc	3.6	(3.0)	bc
F. pr.	<0.001			<0.001		
SED **(df = 116)**	0.5349			0.06564		
CV (%)	18.6			3.0		

∗The raw data is shown in parentheses. ∗Means followed by the same letters are not significantly different. ∗Orchard 1-5 (Aboagyeasua community), 6-10 (Atesikrum), 11-15 (Kyeremanso), 16-20 (Tonsuano), 21-25 (Antwirifo), 26-30 (Badukrom)

Morphological description of alga

The microscopic structure of the putative alga as observed under the microscope was branched filament tufts of orange to red-brown ornamentation with erect cylindrical cells and hairs that are unbranched. The sporangiophores occurred either as a single structure or in clumped form and consist of more than one well-defined cross walls (septa) separating each of the structural segments. The sporangiosphore consists of one or more terminal head cells that produce a variable number of lateral sporangia (3–8) and clustered zoosporangia released from the membrane. The sporangium, which is attached by a cell suspensor, forms a crown at the terminal end of the sporangiophores. The clump produced a variable number of sporangiophores (Figure 3). Based on the field symptoms observed on the cashew and the microscopic characteristics of the alga pathogen as observed under a microscope, the pathogen was identified as *Cephaleuros* species.

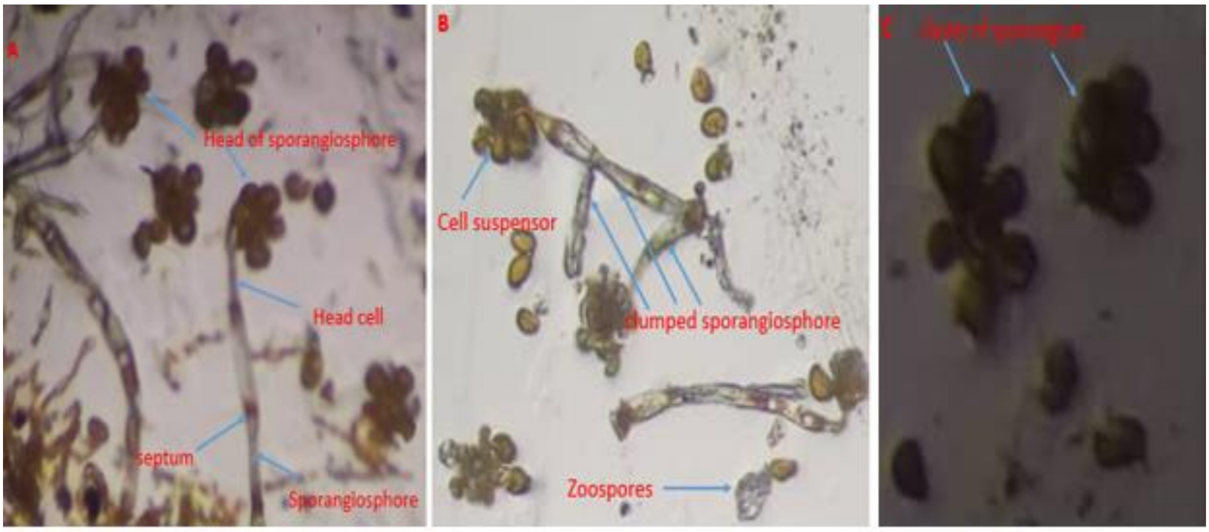

Fig 3. Microscopic characteristics of *Cephaleuros* species as viewed under a microscope (40X).

Pathogenicity test
The *in vitro* pathogenicity test on the cashew leaves confirmed that the putative pathogen was responsible for the red rust disease observed in the field. It produced symptoms similar to those recorded in the various orchards in the study area over a month of inoculation (Figure 4). The spots were re-isolated from the diseased inoculated leaves, thereby confirming Koch's postulate after microscopic examination. The water-inoculated leaves used as a positive control failed to produce any visible spots until the experiment was terminated.

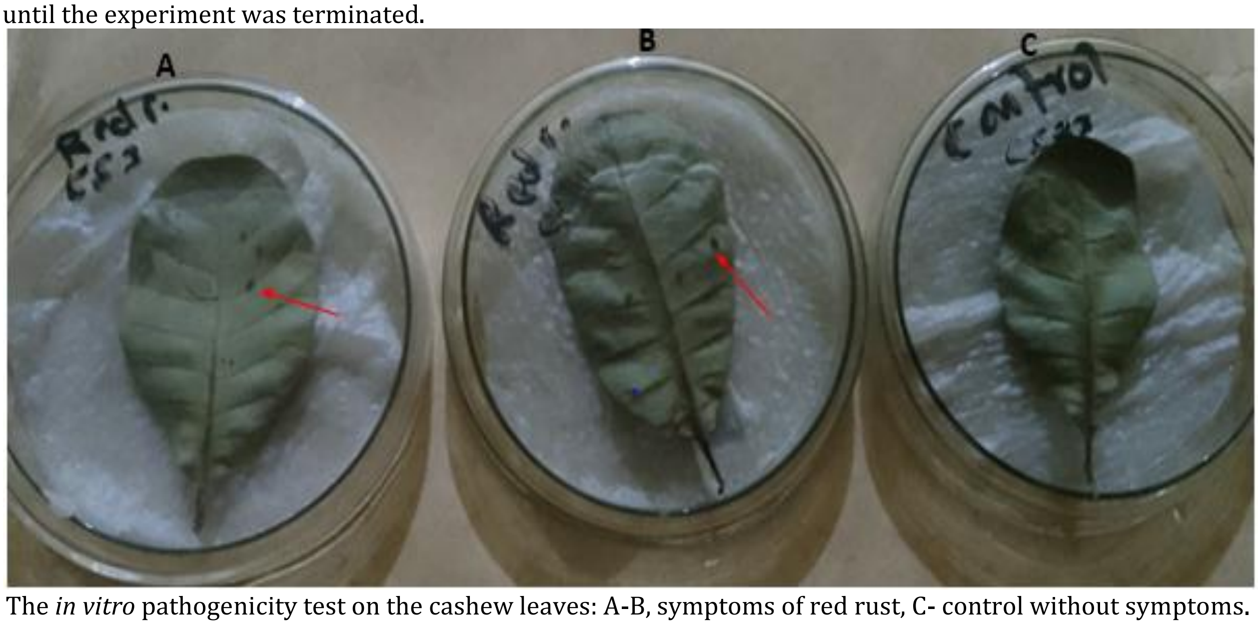

Fig 4. The *in vitro* pathogenicity test on the cashew leaves: A-B, symptoms of red rust, C- control without symptoms.

Discussion

Cephaleuros species, aerophilic, filamentous green algae, are responsible for causing red rust disease on cashew and a number of other plants in different parts of the world, including Ghana, where prevailing environmental conditions favour pathogen development. There is no solely in-depth research on the red rust disease of cashew in Ghana; this is the first of its kind. The disease has been found to register its presence in every single cashew orchard in the study area, and this could present a threat to cashew production in the near future. The red rust or algal leaf spot caused by *Cephaleuros* species has been reported to limit the quality, quantity, and commercial value of mango produce as well as other crops like cashew in other parts of the globe (Batista et al., 2016). The red rust as a disease is now becoming a major concern to cashew production to Ghanaian farmers, and is known to cause reductions in the photosynthetic leaf area and, under severe situations, damage to the stem, necrosis of the leaves, and defoliation (Ponmurugan et al., 2009). Similar observations, such as die-back of branches, distortion of leaves, and leaf fall, were also made in this study. Additionally, the spots were only observed on the upper leaf surfaces during the field visit, and this was validated by Malagi et al. (2011) and Pereira et al. (2020).

In terms of incidence and severity, the disease was found to occur in all the orchards sampled in the study area, with varying occurrences. The incidence of red rust associated with different host plants in different countries of the world has also been reported (Han et al., 2011; Wonni et al., 2017; Khatoon et al., 2017; Majune et al., 2018; Vasconcelos et al., 2019; Pereira et al., 2020). The incidence of the disease on the cashew was very high during the rainy season, and it ranged from 5.96% to 18.08% at Tonsuano and Kyeremanso, respectively, with higher incidences at Kyeremanso, Atesikrom, and Aboagyeasu. The incidence was found to be high in closely planted orchards in the study area. A survey conducted in several orchards during the dry season recorded zero incidence, or some creamy white marks left on the leaf surfaces that were believed to be the remains of the algal matrix of the previous season's infection, and this explains the role of environmental conditions, especially rainfall, in pathogen growth and disease development. The probable reason for the zero incidence during the dry season could be that the pathogen might require a film of wetness to complete its life cycle. In an affirmative stance, Malagi et al. (2011) reported adequate temperature and humidity as necessary factors that influence the growth and reproduction of the pathogen. The role of rainfall in the dispersal of the zoospores of *Cephaleuros* species has also been documented (Chanthapatchot and Satjarak, 2019). A report on the incidence of *Cephaleuros virescens* attacking both mango and cashew, which all belong to the Anacardiaceae family, has been well recorded (Chanthapatchot and Satjarak, 2019). The severity was higher in Antwirifo, Tonsuano, Badukrom, and Kyeremanso. The higher severities in these communities could be attributed to differing weather conditions during the study season. The severity of red rust caused by *Cephaleuros* species was reported by Nelson (2008) and Brooks et al. (2015), and was based on factors such as environment, the host, and the pathogen, coupled with warm weather and regular rains that favour their development.

Identification of the pathogen causing red rust in cashew is paramount to its control or management. The alga pathogen was identified based on its morphological characteristics both in the field using macroscopic structures and microscopically in the laboratory with the aid of a microscope. Macroscopic analysis of the *Cephaleuros* species revealed circular, raised, or flattened velvety or fuzzy orange-brown, burnt-orange, rust-pigmented, or greenish-grey spots of different dimensions with fine strands on which sporangiophores and zoosporangia are borne. *Cephaleuros* species usually show two diverse growth habits, and this is one of the distinctive features by which they can be identified or characterized (L´opez -Bautista et al., 2002; Han *et al*., 2011; Vasconcelos et al., 2018). The yellow or orange pigmentation of the *Cephaleuros* species structures is attributed to the accumulation of carotenoids (L´opez-Bautista et al. 2002). The symptoms observed in the cashew trees are in line with the mycological identification protocols described by several researchers (Thompson and Wujek, 1997; Vasconcelos et al., 2019; Pereira et al., 2020). This, together with the pathogenicity test, confirmed the identity of the causal agent of red rust disease at the genus level as a *Cephaleuros* species.

Management or control of the red rust is very important for farmers in the study area to improve their economic livelihood. Since the disease is found to be high in closely planted orchards, planting the cashew at appropriate spacing will help reduce humidity levels that favour disease development and also improve air circulation. Cultural practices such as improvement of plant nutrition and soil drainage to reduce plant stress and increase plant vigor against the rust pathogen, orchard sanitation to clear off debris that harbours algal inoculum, timely weeding and pruning of affected cashew tree branches, clearing of twinning or climbing weeds around cashew orchards, avoiding too much shade, prevention of mechanical injury during farming operations, and use of tolerant or resistant cashew varieties are all paramount in controlling the red rust disease (Brooks et al., 2015; Ramya et al., 2013; Suto and Ohtani, 2013).

Akter et al. (2016), in their work to determine the effect of diverse chemical control measures on the prevalence and severity of algal rust on guava trees in Kazi Payara, reported BAU-Biofungicide as the best among all the treatments tested. Management of red rust with copper oxychloride and copper sulfate at a rate of 0.3% and 0.25%, respectively, applied 3–4 times at a 15-day interval after the initiation of the disease has also been recommended (Horticulture Year, 2012). Some fungicides, such as the Bordeaux mixture, have also proved effective against red rust disease.

In conclusion, the study has established that the causal agent of the red rust disease in cashew in Dormaa communities is *Cephaleuros* species. The disease was found to occur in all the orchards sampled, with varying degrees of incidence and severity. The two major factors identified to favour the rapid spread of the disease in the study area were rainfall and the close spacing of the planted cashew trees.

Beyond the morphological identification used in this study, molecular analysis of the algal pathogen causing red rust disease of cashew in the study area and all other cashew-producing communities is recommended to find the specific algal pathogen responsible for the disease.

Materials and methods

Study area

The study was conducted in the Dormaa Central Municipality and its surrounding communities during the 2019 and 2020 seasons in the Bono Region of Ghana. Dormaa Ahenkro lies within longitudes 30 West, 70 North, and 70 30'. It is bound on the north by the Jaman South Municipality and in the east by the Dormaa East District; in the south and southeast by the Asunafo North Municipality and Asutifi North District; in the west and southwest by Dormaa West; and in the west and northwest by La Cote d'Ivoire (Figure 5). The population of the municipality, according to the 2021 population and housing census, stands at 112,111, with 33,336 males and 34,563 females (Ghana Statistical Service, 2022).

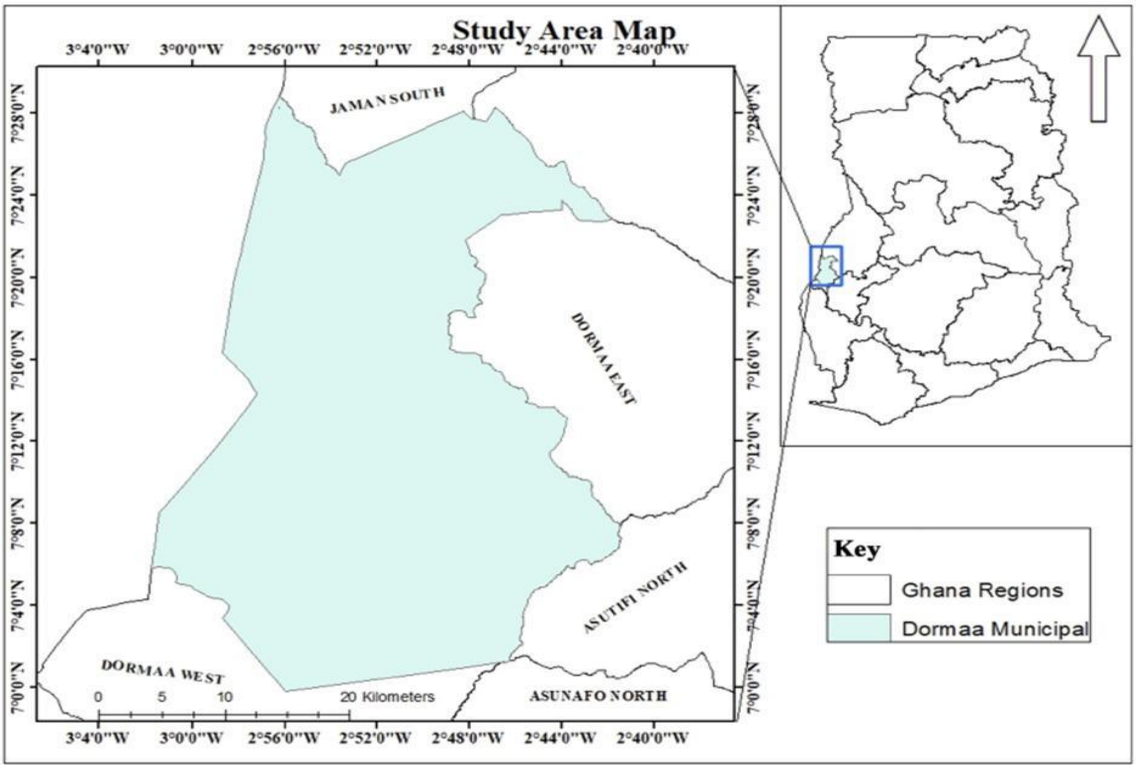

Fig 5. Map of Dormaa Central municipality- the study area.

Plant materials

Survey of the cashew orchards

Thirty (30) cashew orchards were selected using a simple random sampling technique from six (6) different communities in the Dormaa Central Municipality and its environs to determine the incidence and severity of red rust disease. The surveyed communities included Aboagyeasua, Atesikrum, Kyeremanso, Tonsuano, Antwirifo, and Badukrom. Some selected cashew trees that displayed the symptoms of the disease in each of the orchards were randomly tagged for the study. The incidence and severity of the disease were measured on the tagged trees. Some infected samples were collected and sent to the main laboratory of the University of Energy and Natural Resources, Sunyani, for critical analysis of the algal morphological structures using a microscope.

Incidence and Severity Evaluation

Algal disease incidence was determined in the various orchards and expressed as the number of diseased plants in each orchard per the total number of plants assessed and expressed as a percentage. The diseased plants with symptoms were visually and critically assessed.

$$\text{Disease incidence (\%)} = \frac{\text{number of diseased plants}}{\text{Total number of plants assessed}} \times 100$$

In each cashew orchard, five (5) trees were randomly tagged for assessment of the incidence of the disease, and on each tree, a branch was randomly selected, and 100 leaves on each branch were counted, and the percentage of the red rust-infected leaves was recorded to ascertain the degree of incidence of the disease.

For the assessment of disease severity, which is usually expressed as how much plant part is damaged by the disease, five infected leaves showing varying symptoms of the red rust disease were randomly collected from the tagged cashew trees, packed in a brown envelope, and sent to the laboratory for critical measurement of the various algal spots on each leaf. The number of spots on each leaf was also visually assessed and counted using a modified reference scale of 0–9 (Afouda et al., 2013; Soro, 2011) before the data were analyzed.

$$\text{Disease severity} = \frac{\sum (number\ of\ plants\ scored\ for\ each\ rating \times the\ rating\ value)}{total\ plants\ scored\ per\ farm}$$

Statistical analysis

The disease incidence and severity data were square root transformed (transformed data = $\sqrt{(x+1)}$) before being subjected to analysis of variance using GenStat version 12 Edition (Payne et al., 2009). The treatment means were separated using Tukey's test at a probability level of 0.5%.

Morphological Identification of the Alga

The morphological identification of the putative alga started during the field survey of the orchards by observation of orange-brown rust-colored spots that occurred either as clusters or scatted on the cashew leaf surfaces. The observed symptoms were compared to identification protocols as described by Pereira et al. (2020), Han et al. (2011), and Vasconcelos et al. (2019). Diseased samples were taken to the laboratory, and a slide mount was prepared using the scrap method. In the scrap method, a flamed pin was used to dislodge the slightly raised spots on the infected leaves onto a clean slide containing a drop of sterile distilled water, covered with slide slip, and then critically examined with the aid of a compound microscope (Olympus CX 23) at 40X. Structures such as algal thallus, sporangium, and sporangiophore, among others, were used in the identification of the putative pathogen according to the available protocols (Thompson and Wujek, 1997; Malagi et al., 2011; Atsumi et al., 2017; Vasconcelos et al., 2018).

Pathogenicity test

To confirm Koch's postulate, two methods were used. First, the algal spots were scraped from the infected cashew leaves using a sterilized pin. The scrapped colonies were then placed on the surfaces of the healthy cashew leaves in autoclaved Petri dishes containing Whatman filter papers, soaked with sterile distilled water. In the second method, plugs of leaves with spots were carefully cut from the infected leaves and directly placed upside down on the surface of the healthy leaves in the Petri dishes. For the control, sterile distilled water was used instead of algal colonies or leaf plugs. The experiment was set up under normal ambient temperature conditions for observation of the disease progression. Three replicates were maintained for the setup.

Conclusion

The red rust disease is increasingly becoming a major concern to farmers in the study area, and this triggered this research. The study determined the incidence and severity of red rust disease of cashew trees in the Dormaa communities in the Bono region of Ghana, and this is the first of its kind in Ghana. There were varied incidences and severities in the various orchards visited. The causal agent was morphologically identified using a light microscope at the resolution of 40X, and with the aid of various literatures and established protocols, the pathogen was successfully identified as *Cephaleuros* species. The disease is known to reduce the photosynthetic ability of cashew, thereby decreasing yield output.

Acknowledgement

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