Prevalence, incidence and severity of a new root rot disease of cowpea caused by *Macrophomina phaseolina* (Tassi) Goid in Northern Ghana

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Abstract

Cowpea is an important economic crop in northern Ghana. Following reports of a new cowpea root rot, disease in northern Ghana (Northern, Upper East and Upper West regions), surveys were conducted in 2016 and 2017 with the aim of determining the causal organism, prevalence, incidence and severity of the disease across northern Ghana under rain fed and irrigated conditions. Seventeen locations were surveyed and symptomatic plants were taken to the Plant Pathology laboratory in CSIR- Savanna Agricultural Research Institute (SARI), Nyankpala for pathogen identification. The disease was prevalent in all the locations surveyed. There was no significant difference ($P > 0.05$) between disease incidence and severity (1–9 scale) under rain fed and irrigated conditions for each year. Disease incidence and severity were however significantly different ($P < 0.05$) amongst locations surveyed under both rain fed and irrigated production for 2016 and 2017. Under rain fed conditions for 2016, Nyankpala recorded the highest incidence (43.8%), with Feo recording the lowest (17.8%). For the same period, Manga recorded the highest severity (4.3) with Silbelle recording the least (2.1). Under dry season cultivation for 2016, Asumsapeliga recorded the highest disease incidence (44.5%) with Silbelle recording the lowest (11.4%). Sakpari, however, recorded the highest disease severity (3.8) with Feo recording the lowest for the same period. Under rain fed conditions for 2017, disease incidence was highest in Nyankpala (47.7%) and lowest in Lawra (8.7%). For the same period, however, Manga and Yendi recorded the highest severity (4.3) with Lawra recording the lowest (1.6). Under dry season cowpea production for 2017, Sakpari (64.8%) recorded the highest incidence and Silbelle (7.0%) recording the least. Disease severity for the same period was highest in Nafkluga (4.2) and lowest in Silbelle (2.3). Morpho-cultural characteristics and pathogenicity test confirmed *Macrophomina phaseolina* as the causal organism of the cowpea root rot disease.

Introduction

Cowpea (*Vigna unguiculata* [L.] Walp) is an important legume crop in Ghana particularly, for small-scale farmers (Egbadzor et al., 2013; Owusu et al., 2018). Due to its high nutritional value and early maturity, it is often referred to as the "poor man's meat" (Boukar et al., 2013). Its production does not only ensure food and nutritional security, but also serves as a source of employment and income for smallholder farmers most of whom are women (Wiggins, 2009; Wiggins and Sharda, 2013; Owusu et al., 2018). Although the crop is cultivated in other parts of Ghana, most of the production is, however, in northern Ghana, which falls under the Guinea and Forest-Savanna Transitional agro-climatic zones (Quaye et al., 2009; Egbadzor et al., 2013; Haruna et al., 2018). This may be due to the crop's drought tolerance and ability to thrive and yield under the relatively less fertile soil conditions (Horn and Shimelis, 2020). In northern Ghana, cowpea is primarily produced under rain fed conditions during the main cropping season, however, extensive cultivation of the crop is undertaken during the off-season (dry season) under irrigation or residual moisture along riverbanks and dugouts. Ghana's total production of cowpea was estimated to be 273,000 metric tons produced from 157, 000 ha, in 2018 (MoFA, 2019).

Even though the importance of cowpea cannot be underestimated, diseases are a major constrain to its profitable production in many growing areas of Ghana (Offei et al., 2008; PPRSD, 2020). In 2014, a new cowpea root rot disease was observed in major growing areas in the northern regions of Ghana (CSIR-

SARI, 2015). Symptoms associated with the disease include; damping off, seedling blights, root rots, ashy stem blights (Mayek-Pérez et al., 2002; Mengistu et al., 2007; Romero Luna et al., 2017) and are similar to those induced on cowpea by *Macrophomina phaseolina*, a polyphagous ubiquitous soil borne fungal pathogen which has been described as one of the most important emerging plant pathogens of cowpea (Muchero et al., 2011; Kaur et al., 2012; Boukar et al., 2019). Despite the inadequate statistics associated with *Macrophomina* root rot disease of cowpea, yield loss of up to 10% has been reported in Senegal (Ndiaye et al., 2010). It has also been reported to be one of the most important disease of soybean in the United States of America, with an estimated yield loss of 1.9 to 2.0 million metric tons per year (Wrather et al., 2010; Romero Luna et al., 2017). The pathogen attacks underground parts of susceptible host plants through the colonisation of vascular tissue, the cortex and eventually the xylem vessels resulting in wilting and death of infected plants (Iqbal and Mukhtar, 2014; Yeşil and Baştaş, 2016; Khan et al., 2017). Symptoms of the disease normally express when it is too late to attempt any control measure thus making its control difficult (Pandey et al., 2020).

Under rain fed production during the main season, cowpea is normally the last crop to be planted (May– September) due to the crops early maturing nature (approximately 60–70 days). Its maturity coincides with heavy rains resulting in pod rot if planted earlier than the second week of July. This practice, however, often expose the crop to short dry spells which occur in July–August, characterised by drought and high temperature conditions which predisposes it to conditions favourable for Macrophomina root rot infection (Mayek-Perez et al., 2002; Mengistu et al., 2009; Muchero et al., 2011). Similar drought and heat stress conditions pertain during the dry season when cowpea is planted under irrigated conditions.

Following the outbreak of the new root rot disease of cowpea in northern Ghana in 2014, a preliminary survey revealed incidence up to 30% in some locations in the Upper East region (CSIR-SARI, 2015). Moreover, Songotra, a popular high yielding, early maturing and striga resistant cowpea variety was highly susceptible to the disease and therefore dropped out of the varieties being promoted under a USAID cowpea-upscaling project in Ghana (Atokple, 2017). Even though the disease was reported in many production areas in northern Ghana (CSIR-SARI, 2015; Atokple, 2017), it has not been reported in the pathogen host index for Ghana (Offei et al., 2008; PPRSD, 2020) and thus no scientific study of the disease have been undertaken to determine its prevalence in northern Ghana. Data is therefore lacking on the disease and its importance on the livelihood of resource

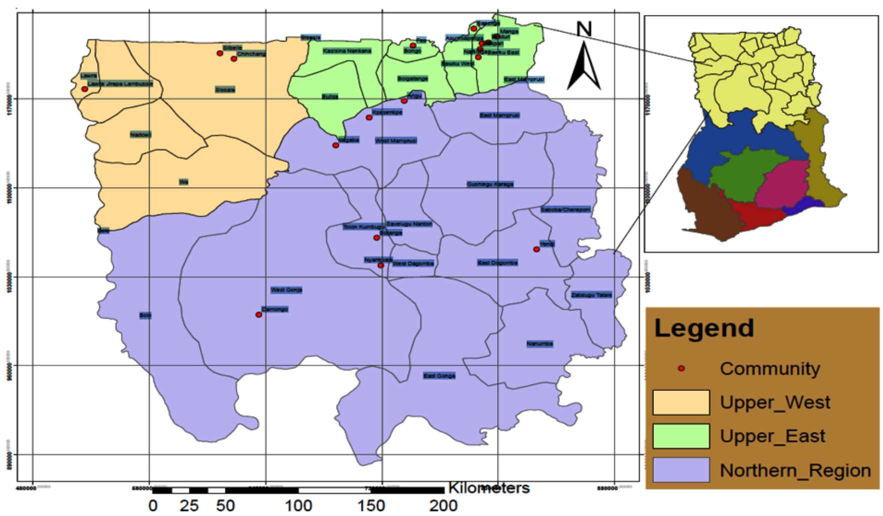

Figure 1. Locations (Communities) (red dots) sampled for Macrophomina root disease of cowpea across northern Ghana during the rainy and dry seasons of 2016 and 2017

TABLE 1
Locations for sampling cowpea root rot disease during the rainy and dry seasons of 2016 and 2017

Region	Community	2016	Season	2017	Season	GPS location coordinates	
Upper East	Arigu	Surveyed	DS only	Surveyed	RS & DS	0°50'55"W	10°33'43"N
Upper East	Asumsapeliga	Surveyed	DS only	Surveyed	DS only	0°21'25"W	10°58'02"N
Upper East	Binduri	Surveyed	RS only	Surveyed	RS only	0°18'57"W	10°58'23"N
Upper East	Feo	Surveyed	RS & DS	Surveyed	RS & DS	0°47'24"W	10°57'18"N
Upper East	Manga	Surveyed	RS & DS	Surveyed	RS & DS	0°15'45"W	11°00'54"N
Upper East	Nafkluga	Surveyed	DS only	Surveyed	DS only	0°22'51"W	10°52'08"N
Upper East	Sakpari	Surveyed	DS only	Surveyed	DS only	0°22'07"W	10°55'35"N
Upper East	Sapeliga	Surveyed	RS only	Surveyed	RS only	0°24'25"W	11°04'18"N
Upper West	Chinchan	Surveyed	RS only	Surveyed	RS only	1°55'05"W	10°52'03"N
Upper West	Lawra	Not surveyed	None	Surveyed	RS only	2°51'25"W	10°39'11"N
Upper West	Silbelle	Surveyed	RS & DS	Surveyed	RS & DS	2°00'21"W	10°54'22"N
Northern	Botanga	Not surveyed	None	Surveyed	DS only	10°01'39"W	9°35'22"N
Northern	Damongo	Not surveyed	None	Surveyed	RS only	1°46'05"W	9°02'42"N
Northern	Nyankpala	Surveyed	RS only	Surveyed	RS & DS	1°00'11"W	9°23'32"N
Northern	Kpasenkpe	Not surveyed	None	Surveyed	DS only	1°04'09"W	10°26'40"N
Northern	Yagaba	Not surveyed	None	Surveyed	DS only	1°16'50"W	10°14'53"N
Northern	Yendi	Surveyed	RS only	Surveyed	RS only	0°01'35"W	9°29'59"N

DS= Dry season; RS= Rainy season

poor farmers. It is expected that, data from this study will guide the development of intervention strategies against the disease that will safeguard livelihood of cowpea farmers in northern Ghana.

- *The objectives of this study therefore were to: Determine and compare the prevalence, incidence and severity of the new cowpea root rot disease in major cowpea growing locations in northern Ghana under rain fed and irrigated conditions.*
- *Identify the causal organism of the cowpea root rot disease in northern Ghana.*

Materials and Methods

Site selection for assessment of incidence and severity of cowpea root rot disease in northern Ghana

A survey was conducted in the then Northern, Upper East and Upper West regions of Ghana in 2016 and 2017 (Figure.1). The survey was conducted during the main cropping season (rainy season) and dry season (Irrigated) of 2016 and 2017. In each region, communities noted for cowpea production were purposively selected based on advice from the Ministry of Food and Agriculture's Agricultural Extension Agents and Field Assistants.

The rainy season's survey was conducted from July–August, whilst the dry season survey was conducted in November–December. In 2016, eight communities were selected during the rainy season and seven in the dry season, while for 2017, eleven communities were selected for both seasons (Table 1). In each selected community, four cowpea farms were randomly selected for assessment of disease incidence and severity. A minimum distance of 500 m was observed between farms under rainy season survey, while a minimum of 200 m distance was observed for the dry season survey. Cowpea farms along roads and hinterlands were surveyed during the rainy season. The dry season survey was, however, conducted along major riverbanks (White Volta River) and irrigation dams in the selected communities. Farm sizes surveyed ranged from less than a hectare to two hectares. Other information such as the cropping history was recorded for each field visited. Global Positioning System (GPS) reading was also recorded for each community sampled with Garmin GPS e Trex 10 and QGIS version 2.8 (QGIS.org, 2018) software was used for map construction of northern Ghana (Figure 1). In all, 148 cowpea farms were surveyed for disease incidence and severity of the cowpea root rot disease.

Description of symptoms of the new cowpea root rot disease in northern Ghana
Cowpea farms where the disease was reported were visited during the rainy and dry seasons of 2016 and 2017. Various stages of the disease were observed on cowpea plants and described by examining diseased roots, stem and leaves with a hand lens (×10). Due to inadequate information of the disease on cowpea, the disease signs and symptoms were compared to description and pictures provided in the compendium of bean diseases (Schwartz et al., 2005). In the course of the survey, stages in the disease development were observed.

Determination of prevalence, incidence and severity of the new cowpea root rot disease in northern Ghana
In each selected community (Table 1), four farms were randomly selected. Within each farm, disease incidence was assessed at five points (A 2 m x 2 m quadrants, was placed at each of the four corners of the almost rectangular fields and in the center). On narrow fields however, disease incidence was assessed along a diagonal with only three quadrants (one at each end and the centre). Disease incidence (percentage) was calculated by counting diseased and healthy plants in each of the quadrants and the incidence value obtained using the formula below:

$$Disease\ incidence\ (\%) = \frac{number\ of\ diseased\ plants}{total\ number\ of\ plants\ (infected\ and\ un\ infected)}\ x\ 100$$

Disease severity was scored on 10 plants randomly selected per farm using a disease assessment key of 1–9 (Table 2) for *Macrophomina* root rot.

Disease prevalence percentage for all the locations surveyed was determined and calculated using the formula:

$$Disease\ prevalence\ (\%) = \frac{locations\ with\ disease\ symptoms}{total\ locations\ surveyed}\ x\ 100$$

Isolation and identification of fungi associated with the new cowpea root rot disease from different locations in northern Ghana

Sample collection and preservation
Symptomatic cowpea plant samples (126) were randomly collected from selected infected fields surveyed across 17 locations in northern Ghana (Table 1). From these fields, three to five plants showing symptoms of the disease including root rot, chlorosis and wilting had their stems cut with a scalpel. After cutting the stems of the infected cowpea plants. The longitudinal sections of the cut stems were inspected for vascular tissue discolouration, which was consistent with the cowpea root rot disease. The diseased samples were then placed in well-labelled paper envelopes of dimensions 11.5 × 22.5 cm and transported to the Plant Pathology laboratory of the Savanna Agricultural Research Institute (CSIR-SARI), Nyankpala for storage at 4°C for at most 24 hours to prevent contamination of plant material.

Preparation of culture media for fungus isolation
Culture medium was prepared according to the directions of the manufacturer (Biolab Diagnostics Laboratory Incorporated, Hungary) A 39 g of Potato Dextrose Agar (PDA) was weighed into a conical flask containing one (1) liter of distilled water. The flask was plugged with cotton wooll and covered with aluminum foil. The solution was heated until it was completely melted following which it was autoclaved at 121°C for 20 min. The PDA was amended with streptomycin sulphate (1.5 g/L) (Demirci and Döken, 1993) and about 20 ml was dispensed into sterile 9 cm Pyrex Petri dishes.

Isolation of fungus
Tissue portions from advancing margins of rot were cut into pieces (4–8 mm) with a flamed scalpel and surface sterilised in 3% sodium hypochlorite solution for two minutes, under a laminar flow chamber following which it was

rinsed thoroughly in three changes of sterile distilled water and dried using a sterile blotter paper. The tissue pieces were then inoculated on the solidified PDA incubated in darkness at 28 ± 1°C for 2–3 days. The isolates were sub-cultured onto freshly prepared streptomycin sulphate (1.5 g/L) amended PDA using hyphal tipping technique (Das et al., 2014) to develop monoxenic cultures. Pure cultures were stored as slants in McCartney bottles at 4°C until needed.

Identification of fungus

Morphological characteristics of mycelial bits mounted in lactophenol were observed with a compound microscope. Conclusive identification was based on culture characteristics on PDA: growth rate and colour, presence or absence of sclerotia and sclerotia structures as described by Barnet and Hunter (2006) for *M. phaseolina*.

Determination of pathogenicity of fungi associated with root rot disease of cowpea

The pathogenic potential of five randomly selected isolates were tested on cowpea (cultivar Songotra) using an artificial inoculation method described by Abawi and Pastor-Corales (1990). Inoculum for pathogenicity test was prepared as described by Abawi and Pastor-Corales (1990). A 50 g polished rice grain was weighed into a 250 ml conical flask containing 100 ml distilled water and allow it to soak overnight. The excess water was decanted and the rice grains emptied onto sterile tray and allowed to dry overnight at room temperature (28–30°C). After 12 hours, the rice grain were emptied into a 250 ml conical flask. The conical flask was plugged with cotton wool and covered with aluminum foil. The contents are then autoclaved (103 kPa, 121°C) for 20 minutes. The rice was allowed to cool to 30°C. Under a sterile laminar flow chamber, the rice grain was inoculated with six, five (5) mm mycelial discs from a 7-day old culture of *M. phaseolina*. The inoculum was then allowed to incubate at room temperature (28 – 30°C) for 15 days in the dark.

Before planting, cowpea seeds were surface disinfected with a 3% concentration of sodium hypochlorite solution for three minutes, followed by three rinsing in sterile distilled water. The seeds were placed on sterile tissue and allowed to dry at 30°C under a laminar flow chamber. Three seeds were planted singly in 25 × 20 × 25 cm plastic bucket containing steam-sterilised top soil arranged in the screen house (temperature of 30–35°C and Relative humidity of 70–75%). Three rice grains inoculated with the five different pathogens were placed with the seed and covered lightly. Control buckets were planted without inoculum. The plants were irrigated when required and observed for characteristic symptoms of Macrophomina root rot. Re-isolation was made from selected symptomatic plants showing similar symptoms of Macrophomina root rot to in fulfilment of Koch's postulates.

Statistical analysis

Means of disease incidence (DI) for the various locations were arcsine transformed prior to analysis. A one-way analysis of variance (ANOVA) with GenStat (12th

TABLE 2

Disease severity key for *Macrophomina* root rot disease of cowpea

Disease scale	Interpretation
1	No visible symptoms on plants
3	Lesions limited to cotyledonary tissue or hypocotyl
5	Lesions have progressed from cotyledons to about 2 cm of stem tissues
7	Lesions are extensive on stem and branches
9	Most of the stem and growing points affected with formation of sclerotia on stem

Source: Modified after Abawi and Pastor-Corales (1990)

TABLE 3
Incidence and severity of *Macrophomina* root rot disease in the dry and rainy season across locations in northern Ghana in 2016

Location surveyed	Rainy season		Location surveyed	Dry season	
	Disease incidence (%)	Disease severity		Disease incidence (%)	Disease severity
Binduri	37.1 (37.5)	3.2	Arigu	33.7 (35.4)	2.8
Chinchan	26.8 (31.0)	3.1	Asumsapeliga	44.5 (41.7)	3.2
Feo	17.8 (24.7)	3.1	Feo	15.9 (22.9)	2.1
Manga	24.9 (29.8)	4.3	Manga	31.6 (34.0)	3.1
Nyankpala	43.8 (41.5)	3.2	Nafkluga	40.4 (39.5)	3.2
Sapeliga	36.4 (37.1)	3.8	Sakpari	41.9 (40.2)	3.8
Silbelle	39.0 (38.5)	2.1	Sillbelle	11.4 (19.4)	2.2
Yendi	35.5 (36.3)	3.9			
2 × SED	(7.9)	1.0	2 × SED	(7.8)	0.8

Means in parenthesis were arcsine transformed

edition) statistical software was used to analyze mean disease incidence and severity (DS) for the various communities. Significant differences ($P < 0.05$) for DI and DS amongst the communities were determined using the standard error of difference (2 x SED). A Student's t-test was used to compare mean DI and DS for rainy season and dry season of each year (2016 and 2017).

Results

Prevalence, incidence and severity of Macrophomina root rot disease of cowpea from different locations in northern Ghana under dry and rainy season conditions

The new cowpea root rot disease was prevalent in all (100.0%) the locations surveyed across northern Ghana under both rainy and dry season cowpea cultivation conditions for both years (Tables 2 and 3).

Significant differences ($P < 0.05$) were observed for incidence and severity of the cowpea root disease amongst the locations surveyed.

Under rain fed conditions for the year 2016, disease incidence ranged from 17.8% to 43.8% (Table 3). The root rot incidence was highest (43.8%) in Nyankpala with Feo recording the lowest (17.8%). Disease incidence in Nyankpala did not vary significantly ($P < 0.05$) from the other locations surveyed, with the exception of Chinchan (26.8%), Manga (24.9%) and Feo (17.8%). For the same period and under the same condition, Silbelle (2.1) recorded the lowest severity. Disease severity was highest in Manga (4.3) and varied significantly ($P < 0.05$) from all the other locations, with the exception of Yendi (3.9) and Sapeliga (3.8).

Under dry season cowpea production for 2016 (Table 3), the root rot disease incidence was highest in Asumsapeliga (44.5%) and lowest in Silbelle (11.4%). The disease incidence in Asumsapeliga was significantly ($P < 0.05$) higher than all the other locations including Sakpari (41.9%) and Arigu (33.7%). It however varied significantly ($P < 0.05$) from Feo (15.9) and Silbelle (11.4%). Significant ($P < 0.05$) variations in disease severity were also observed bettween the locations surveyed for the root rot disease under irrigated conditions for 2016. Even though Sakpari (3.8) recorded the highest severity of the disease under irrigated conditions, it did vary significantly ($P < 0.05$) form Nafkluga (3.2), Asumsapeliga (3.2) and Manga (3.1).

There was no significant difference ($P > 0.05$) between disease incidence for the rainy and dry seasons of 2016 ($t_{0.05}$ (10) = 0.23, $P < 0.05$) and disease severity of the root rot disease for both seasons ($t_{0.05}$ (13) = 1.30, $P < 0.05$).

TABLE 4
Incidence and severity of *Macrophomina* root rot disease in dry and rainy season across locations in northern Ghana in 2017

Location surveyed	Rainy season		Location surveyed	Dry season	
	Disease incidence (%)	Disease severity		Disease incidence (%)	Disease severity
Binduri	38.5 (38.2)	4.0	Arigu	36.6 (37.2)	3.6
Chinchan	24.2 (29.4)	3.7	Asumsapeliga	51.3 (45.8)	3.5
Feo	31.7 (34.2)	3.1	Feo	7.90 (16.1)	2.3
Manga	27.9 (31.8)	4.3	Manga	27.7 (31.6)	3.7
Nyankpala	47.7 (43.7)	4.2	Nafkluga	50.8 (45.4)	4.2
Sapeliga	41.2 (39.9)	4.0	Sakpari	64.8 (54.2)	3.4
Silbelle	40.1 (39.2)	3.5	Sillbelle	7.00 (15.3)	2.3
Yendi	37.0 (37.4)	4.3	Botanga	25.8 (30.4)	3.0
Arigu	19.6 (25.9)	2.1	Kpasenkpe	46.7 (43.1)	2.6
Damongo	13.6 (21.3)	3.0	Nyankpala	30.8 (33.6)	2.7
Lawra	8.70 (17.0)	1.6	Yagaba	55.6 (48.3)	4.1
2 × SED	(6.9)	0.9	2 × SED	(8.4)	0.9

Means in parenthesis were arcsine transformed

Under rain fed cowpea production in 2017, mean incidence of the cowpea root rot disease ranged from 8.7% to 47.7% (Table 4). Disease incidence was highest in Nyankpala (47.7%), but was not significantly ($P < 0.05$) different from Sapeliga (41.2%), Silbelle (40.1%), Binduri (38.5%) and Yendi (37.0%). Incidence was, however, significantly ($P < 0.05$) higher than all the other locations including Lawra (8.7%), which recorded the lowest (Table 4). Severity of the disease for the same period varied significantly ($P < 0.05$) amongst locations with a maximum severity of 4.3 and a minimum of 1.55. Yendi (4.3) and Manga (4.3) registered the highest root rot disease severity, which varied significantly ($P < 0.05$) from all the other locations, with the exception of Manga (4.3), Nyankpala (4.2), Binduri (4.0), Sapeliga (4.0) and Silbelle (3.5).
Under dry season cowpea cultivation for 2017, a disease incidence of 64.8% was recorded as the highest and a lowest of 7.0% (Table 4). Sakpari recorded the highest incidence of the cowpea root rot disease, and varied significantly ($P < 0.05$) from all the other locations, with the exception of Yagaba (55.6%) and Asumsapeiliga (51.3%). Severity values ranging from 2.3 to 4.2. Nafkluga (4.2) recorded the highest severity of the disease but did not vary significantly ($P < 0.05$) from Yagaba (4.1), Manga (3.7), Arigu (3.6) and Sakpari (3.4). Its severity was however, significantly ($P < 0.05$) higher than all the other locations including Feo (2.3) which recorded the lowest (Table 4).
There was no significant difference ($P > 0.05$) ($P > 0.05$) between disease incidence for the rainy and dry seasons of 2017 (t 0.05 (17) = 0.99, $P < 0.05$) and disease severity of the root rot disease for both seasons (t 0.05 (18) = -0.59, $P < 0.05$).

Stages in the development of signs and symptoms of the new cowpea root rot disease in northern Ghana

The general symptoms observed at different stages of the root rot disease development include leaf chlorosis, root rot, collar rot and wilted and dead plants are shown in Figure 2A–D. At the seedling stage, observed symptoms included necrotic lesions on the hypocotyl and cotyledons (Figure 2A); lesions expanded and

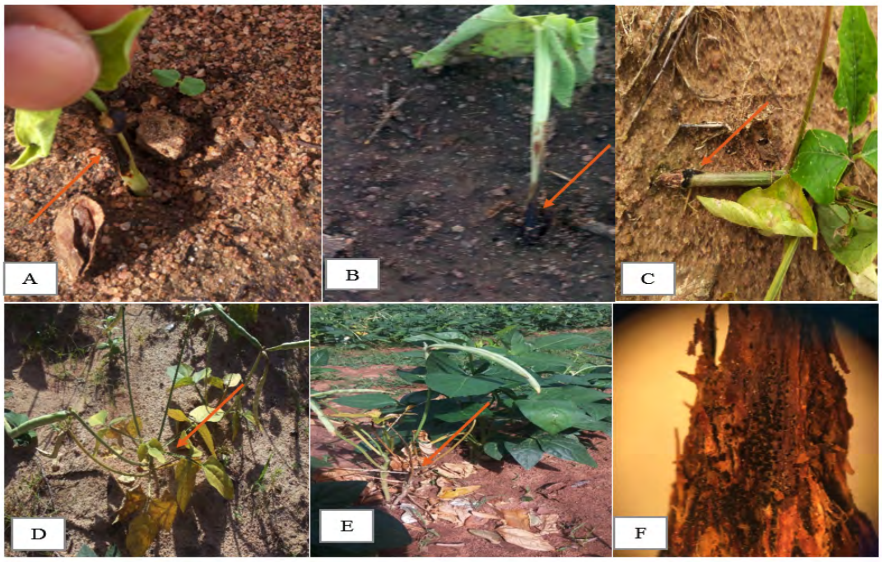

Figure 2: Cowpea plants showing signs and symptoms of *Macrophomina* root rot disease under field conditions: lesions (arrowed) on the hypocotyl shortly after emergence (A), colour rot symptoms (arrowed) (B), collapsed plant with sclerotia (arrowed) at the base of the stem (C), leaf chlorosis(arrowed) (D), premature senescence (arrowed) (E) and sclerotia mass (arrowed) in a portion of stem (vascular tissue) (F)

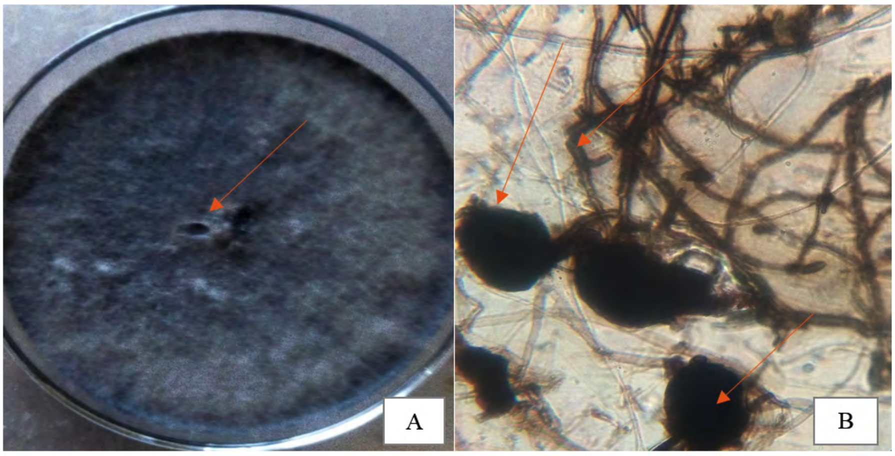

Figure 3: Morphological characteristics of *Macrophomina phaseolina*. A 5-day old culture of *M. phaseolina* associated with cowpea root rot cultured on PDA: greyish fluffy mycelia with submerged black mass (sclerotia) (A). A microscopic view (10 x) of *M. phaseolina* showing (arrowed) irregular and globose shaped sclerotia and dark septate mycelium under a compound microscope (arrowed) (B)

girdled the young seedlings (Figure 2B). At the vegetative and flowering stage, infected plants developed collar rot just above the soil level. The stem was girdled by the disease resulting in development of adventitious roots, and the plants eventually collapsed. Signs included development of sclerotia at the base of stems (Figure 2C). Other symptoms observed on infected plants included leaf chlorosis (Figure 2D) and premature senescence (Figure 2E). Infected plants also showed vascular discoloration and signs of sclerotia in the vascular tissue (Figure 2F).

Confirmation of fungus causing cowpea root rot disease in northern Ghana

Macrophomina phaseolina was consistently isolated from diseased cowpea plants collected from different locations across northern Ghana. Most of the isolates covered a 90 cm Petri dish in72 hours. The pathogen showed greyish fluffy mycelium on PDA on five day old culture (Figure 3A). The mycelium was dark septate with irregular globose shaped

Figure 4: Hypocotyl lesions (arrowed) on 14 days old cowpea seedling artificially inoculated with *M. phaseolina* (A) and symptomless (arrowed) cowpea seedling (14 days old) of non-inoculated cowpea seedlings (B).

sclerotia (Figure 3B) Dhingra and Sinclair 1973; Jain et al., 1973; Barnett and Hunter, 2006).

Isolates of *Macrophomina* root rot were pathogenic to cowpea cultivar (Songotra). Lesions consistent with *M. phaseolina* was observed at seedling emergence and up to 30 days after planting on the hypocotyl of cowpea plants (Figure 4). The non inoculated cowpea were, however, symptomless (Figure 4).

Discussion

The identification of *Macrophomina* as the cause of the new root rot disease of cowpea, in this study should be a cause for concern, for cowpea production in Ghana. From this study, signs and symptoms of the disease including root rot, sclerotia formation on the plant, vascular discoloration and wilting of plants were consistent with descriptions of the disease on Bean (*Phaseolus vulgaris*) as described by Schwartz et al. (2005). A further laboratory analysis of the fungal isolates collected, including cultural characteristics observed on PDA such as mycelial structures, the embedded, irregular globose shaped sclerotia and hyphae pigmentation confirmed the pathogen to be *M. phaseolina* (Dhingra and Sinclair 1973; Jain et al., 1973; Barnett and Hunter, 2006).

Although the disease was not previously reported as an important constrain to cowpea production in Ghana (Offei et al., 2008; PPRSD, 2020), its prevalence as revealed by this study should ignite action on its management. A high prevalence of *Macrophomina* root rot disease on cowpea have similarly been observed in a two year (2013 and 2014) disease survey in India (Mohanapriya et al., 2017). *Macrophomina* root rot has been described as one of the most important emerging threats to profitable production of cowpea in sub Saharan Africa (Ndiaye et al., 2010; Muchero et al., 2011; Boukar et al., 2019).

Macrophomina phaseolina is an important plant pathogen with a wide host range (in excess of over 500 economic and non-economic plant species) and has been reported in other countries (Khangura and Aberra, 2009; Islam et al., 2012; Kaur et al., 2012; Sarr et al., 2014). Its current prevalence have been linked to changing climate due to the high temperatures and drought stress conditions associated with its occurrence. It has been predicted that incidence and severity of *Macrophomina* root rot disease will increase in many areas due to global warming conditions (Medhaug et al., 2017). This call for climate smart mitigation measures against the disease that will help reduce the impact of the disease on cowpea farmers.

Even though production conditions and

seasons did not influence the incidence and severity of the disease, this study revealed varying levels of the disease incidence and severity amongst the locations surveyed under both rain fed and irrigated cowpea production conditions. The observed prevalence, distribution and damage to the cowpea crops should be a major concern requiring urgent attention. Unlike other diseases of cowpea, that the crop may be able to tolerate to produce appreciable yield even under heavy infestation, *Macrophomina* root rot disease mostly affects the roots of cowpea resulting in wilting and eventually death of plant thus resulting in severely reduced yields (Pandey et al., 2020). The incidence and severity across the locations may therefore be an estimate of yield loss on affected fields. The disease may therefore have a severe negative impact on the livelihood of smallholder farmers in northern Ghana who rely on cowpea for nutritional and economic security (Wahaga, 2019). Limited literature currently exist on the impact of *Macrophomina* root rot disease on cowpea. It has, however, been estimated to cause up to 10% yield loss on cowpea in Senegal (Ndiaye et al. 2010). *Macrophomina phaseolina* has also been reported as one of the most important disease causing pathogen of soybean in the United States of America, with an estimated yield loss ranging in excess of up to 2.0 million metric tons (Romero Luna et al., 2017). It is therefore important that, control strategies based on integrated pest and disease management (IPDM) be deployed in the cowpea growing areas of northern Ghana.

In this study, the incidence and severity of *Macrophomina* root rot disease of cowpea varied across the locations surveyed. Incidence and severity were, however, not significantly different for irrigated and rain fed conditions. Incidence and severity associated with *Macrophomina* root rot is strongly influenced by the prevailing environment conditions (Olaya and Abawi, 1996). There is a strong correlation between moisture and heat stress conditions on the occurrence; incidence and severity of *Macrophomina* root rot infection (Dhingra and Sinclair 1975; Gangopadhyay et al., 1982; Kaur et al., 2012). Generally, northern Ghana experiences a high temperature range of 32 °C and 38 °C with a short rainy season (May-October) and long dry season (November to April) (Ghana Meteorological Agency-GMet, 2019). These conditions are suitable for the development of *Macrophomina* root rot infection in cowpea (Kaur et al., 2012). These conditions were observed in the locations surveyed along the Black Volta River and areas near dams and dugouts during the dry season. Incidence and severity of the disease may be managed by ensuring adequate irrigation of fields as a short-term management strategy (Dhingra and Sinclair 1975; Kendig et al., 2000). Incidence and severity was also high during the rainy season for the areas surveyed and this observation contradict the assertion of Dhingra and Sinclair, (1975), who reported that, high moisture conditions inactivates and adversely affects the survival of *Macrophomina* root rot sclerotia (propagules). In northern Ghana, cowpea is normally, planted after all crops have been planted, thus exposing it to dry spells, which occur in July–August. The high temperature conditions in northern Ghana even in the rainy season combined with the short dry spells predispose cowpea seedlings and plants on the field to *Macrophomina* root rot infection (Olaya and Abawi, 1996; Mayek-Perez et al., 2002; Muchero et al., 2011; Kaur et al., 2012).

The levels in disease incidence and severity observed across the locations surveyed may also be attributed to other factors including the presence of sclerotia on decomposing plant materials in the area. Its been suggested that, the presence of sclerotia in an area has a strong influence on *Macrophomina* root rot infection and disease incidence and severity (Mohanapriya et al., 2017). The cropping culture may have contributed to the high incidence and severity observed across the locations surveyed. In northern Ghana, mixed cropping and crop rotation is a common practice, and observed in all the locations surveyed. In most of the locations, cereal-legume intercrop was a common practice, where cereals such a maize (*Zea mays*), sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*) are intercropped with soybean (*Glycine max*), cowpea (*Vigna unguiculata*) or both. The partially decomposed plant material from previous cropping may therefore be contributing to the high prevalence of *Macrophomina* root rot by serving as inoculum for reinfection (Mohanapriya et al., 2017).

In all the locations surveyed, decaying crop residue including cereal and legume were observed with some showing sign of sclerotia formation consistent with *M. phaseolina* (Dhingra and Sinclair 1973; Barnett and Hunter 2006). The pathogen survives in plant tissue in the form of sclerotia becoming a source of primary inoculum under favourable conditions (Anderson et al., 2004; Gupta et al., 2012; Naseri 2014). Decomposed plants may also release the sclerotia into soils which have been reported to survive for long durations and still remain pathogenic for several years (Islam et al., 2012). Studies have suggested that, the pathogen can survive and remain infectious for up to 39 months in infested seeds and 37 months in plant debris as microsclerotia (Wagan et al., 2019). The pathogen may also survive as microsclerotia in the soil for up to 15 years under favourable conditions (Gupta et al., 2012).

Other factors that may have contributed to the *Macrophomina* root rot infection may have been contaminated seed. *M. phaseolina* has been established to be seed borne and infected seed has been suggested to be the first point of infection and a source of spread to new locations (Rao et al., 2015; Abbas et al., 2020). The pathogen also has the ability to withstand high thermal range of up to 48–62 ^{o}C (Loda et al., 2002). These attributes makes it difficult to control the disease using the commonly recommended methods such as crop rotation or heat treatment by soil solarisation (Pandey et al., 2018). Due to the high persistence of the pathogen and its ability to tolerate adverse conditions, a successful management of the root rot disease should be based on an IPDM approach that will encourage farmers to use resistant cultivars, and the burning of crop residue that will help reduce the inoculum load on farmers' fields.

Conclusion

This study revealed 100% prevalence of the cowpea root rot disease in major cowpea growing locations in northern Ghana. Incidence and severity of the cowpea root rot disease varied significantly among the locations under rainfed and irrigated cowpea production in northern Ghana for 2016 and 2017 cropping seasons. A comparison of the two production conditions, namely rain-fed and irrigated was however not significant.

The root rot pathogen of the cowpea root rot disease was identified and confirmed to be *M. phaseolina.*

Recommendations

Molecular analysis of the pathogen will help determine variation among the pathogen isolates. This will facilitate the screening and identification of resistant cowpea varieties. This study was limited to northern Ghana and did not cover other cowpea production locations in the other agro-ecological zones in Ghana including the transitional and coastal zones. A study should therefore be undertaken to determine the prevalence of the disease in these locations.

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