

**OCCURRENCE OF *RHIZOCTONIA SOLANI* INDUCED STEM ROT ON COWPEA {*VIGNA UNGUICULATA* (L.) WALP} IN NORTH - WEST NIGERIA**

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**SUMMARY**

Cowpea stem rot was reported to cause significant yield loss which varies with the associated organism. Observance of typical symptoms of stem rot in some parts of North West Nigeria prompted this study with the objective of assessing and identifying the causal organism of this disease. The incidence and severity of cowpea stem rot in farmers' fields were assessed during 2013 and 2014 rainy seasons. Four cowpea producing states of North - West Nigeria: Kano, Kaduna, Katsina and Zamfara were selected and three Local Government Areas from each were surveyed. The results showed that the incidence and severity recorded in these states significantly varied ( $p \leq 0.05$ ). Fields in Kaduna State had significantly higher incidence (29.09 %) and severity (21.14 %) than those in Kano, Katsina and Zamfara States for the two seasons. Katsina State had the lowest incidence (9.24 %) and severity (7.31 %) of the disease. The fungi isolated from diseased cowpea stem on Potato Dextrose Agar (PDA) were identified as *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotium* sp., *Colletotrichum capsici*, *Fusarium solani* and *Curvularia lunata* with *R. solani* recording the highest occurrence. Pathogenicity of the isolated fungi on susceptible cowpea variety SAMPEA 10 was conducted and only *Rhizoctonia solani* produced the typical stem rot symptoms observed on farmers' fields while the other isolated fungi induced other symptoms. It is thus concluded that *R. solani* induced stem rot is wide spread in North - West Nigeria with varying incidence and severity influenced by weather condition.

**Keywords:** Cowpea; stem rot; Incidence; severity; fungi

**COWPEA** (*Vigna unguiculata* L. Walp) is a leguminous plant, whose center of origin and domestication is reported to be in Africa (7). Cowpea has been promoted as high-quality protein constituent among economically depressed communities in developing countries with the aim of reducing high prevalence of protein malnutrition (8). About 6.5million metric tons of cowpea are produced annually on about 14.5 million hectares worldwide (6). Nigeria is the world largest producer and consumer of cowpea, producing 2.95 million tonnes in 2013. The annual production is high, but the average grain yield per hectare in Nigeria is only 776.31 Kg/ha which though above the average yield worldwide of 521.91 Kg/ha in 2013, but is far lower than yield of 5333.33 Kg/ha and 5132.35 Kg/ha obtained in USA and Occupied Palestinian Territory (11). The low productivity of cowpea is attributable to numerous abiotic and biotic constraints. The abiotic factors comprised drought, low soil fertility, and heat while biotic constraints include insects, diseases, parasitic weeds, and nematodes. Cowpea farmers also have limited access to quality seeds of improved varieties for planting (14). Stem rot disease constitute a constraint to cowpea production in Nigeria. The disease has been reported in USA,

Brazil, Tanzania, Benin, South Africa and Nigeria (1) and caused by a complex of fungi including species of *Pythium*, *Phytophthora*, *Sclerotium*, *Rhizoctonia*, *Macrophomina* and *Fusarium* (2). Yield loss of 11 – 40 % caused by stem rot induced by *Pythium aphanidermatum*, *Sclerotium rolfsii* and *Fusarium solani* was reported at Moor plantation Ibadan, Nigeria (16). A study conducted in Oman revealed 20 fungal species belonging to 9 genera which included *Rhizoctonia*, *Curvularia*, *Alternaria* and *Fusarium* associated with cowpea root and stem rot (4).

In 2011 cropping season, there were reports of cowpea stem rot leading to severe yield loss up to total crop failure by farmers in Sabuwa Local Government Area of Katsina State which was followed by observance of the disease symptoms in research farm of Institute for Agricultural Research (IAR), Samaru, Zaria. This calls for need of assessing the disease in North West Nigeria as no research on cowpea stem rot was conducted in the Northern part of Nigeria where most of the cowpea is produced. This study was therefore conducted to determine the occurrence of cowpea stem rot in North – West Nigeria.

## **MATERIALS AND METHODS**

### **Survey and Collection of Diseased Samples**

Survey was conducted in Kaduna, Kano, Katsina and Zamfara States in September - October of 2013 and 2014. Three Local Government Areas (LGAs) chosen as advised by Agricultural Development Project (ADP) staff were surveyed per State and in each LGA, 3 farm communities were selected. Two

fields were chosen at random at each farm community and a 2 × 2 m<sup>2</sup> quadrat was placed at four corners of each field and at the centre. All cowpea stands in the quadrats were assessed for disease incidence and severity. Disease incidence was determined by expressing disease plants as percentage of total plants in each quadrat. Individual plants were scored for disease severity based on a modified scale of 0 - 6 by Fernando and Linderman (12), where:

0 = No visible symptoms.

1 = Lesions at base stem (≤ 15 % stem length); leaves pale green

2 = Lesions advanced upward (16 - 30 % stem length); leaves wilting

3 = Lesions advanced upward (31 - 45 % stem length); leaves wilted

4 = Lesions advanced upward (46 - 60 % stem length); stem rotting at base

5 = Lesions advanced upward (> 60 % stem length); stem rotten at base

6 = Plant rotten, wilted and death

Disease severity percentage was determined using the following formula:

Disease severity

$$= \frac{\text{sum of all disease ratings}}{\text{Total no. of plants assessed} \times \text{Maximum disease score}} \times 100$$

Diseased plants were collected and taken to the laboratory for isolation and identification of associated organisms.

### **Isolation and Identification of Associated Organisms**

Infected cowpea stem 5 cm long was cut from uprooted diseased plants collected during field survey, and adhering soil washed off using tap

water. The stem tissue was cut into pieces of about 2 - 3 mm long, surface sterilized by immersion in 0.1 % Sodium Hypochlorite (NaOCl) for three minutes, rinsed in three successive changes of sterile distilled

water (SDW) and blotted dry with a sterile filter paper. The disinfected stem tissue was plated onto freshly prepared Potato Dextrose Agar amended with streptomycin sulphate (PDAs), incubated at  $27 \pm 2^\circ \text{C}$  and observed daily for fungal growth. Fungal growths were sub-cultured into freshly prepared PDAs to obtain pure cultures. Fungi were identified based on cultural and microscopic characteristics using a manual by Watanabe (22) and the identities of the isolated organisms confirmed by the Centre for Biosciences and Agriculture International (CABI), United Kingdom. The number of fungi isolated was recorded and frequency of isolation was expressed per state.

#### **Pathogenicity Test of Isolated Fungi**

The fungi were multiplied on millet grain according to the method described by Weideman and Wehner, 1993 (23). Millet grains (250 g) contained in 2 L conical flask were soaked in water for 24 hours before draining and autoclaving at  $121^\circ \text{C}$  under 15 psi for 30 min. After cooling, each fungus was transferred into the autoclaved millet by aseptically placing six, 5 mm discs cut out from a 5 - day old pure culture of the fungus growing on PDAs using sterile cork borer. The grains were

incubated for 15 days on a laboratory bench at  $27 \pm 2^\circ \text{C}$  with daily shaking and air-dried. The prepared millet grain inoculum (50 g) was broadcasted on top of 1000 g of heat sterilized soil contained in plastic pot seven days after sowing with surface sterilized seeds of cowpea variety SAMPEA 10 at the rate of three seeds per pot. Pots with soil incorporated with 50 g sterilized millet grains served as control. The pots were arranged in Completely Randomized Design (CRD) with three repetitions. Plants showing stem rot symptoms were recorded. Three weeks old seedlings showing symptoms of stem rot were removed and disease stem plated onto PDAs media. The re-isolated fungi were cultured on PDAs, colony and microscopic characteristics were recorded and compared with the inoculated isolate.

#### *Statistical Analysis*

The data generated from survey were subjected to analysis of variance (ANOVA) procedure of SAS and means were separated using the Duncan Multiple Range Test (DMRT) at 5 % level of significance.

## RESULTS

### **Incidence and Severity of Cowpea Stem Rot in the Surveyed Locations**

In the survey conducted in 2013 and 2014, the symptoms observed were typical of stem rot. The symptoms were reddish brown lesions on the stem at soil level which sometimes girdle the stem. Heavily infected plants wilt and die or break at the base and fall over. The observed symptom occur widely in the surveyed area. The disease incidence and severity for

2013 were significantly ( $p \leq 0.05$ ) higher than those of 2014 (Table 1). The overall incidence and severity of the disease in the two seasons for the four states showed that Kaduna State had the highest disease incidence and severity while Katsina State had the lowest incidence which was statistically similar to that of Zamfara State. Katsina also had the lowest severity which did not significantly differ from that of Zamfara State (Table 1).

**Table 1:** Cowpea stem rot incidence and severity in four surveyed states during

Treatment	Disease incidence (%)	Disease severity (%)
<u>Year</u>		
2013	21.55 <sup>a</sup>	15.90 <sup>a</sup>
2014	14.49 <sup>b</sup>	11.97 <sup>b</sup>
SE±	1.82	1.36
<u>State</u>		
Kaduna	29.09 <sup>a</sup>	21.14 <sup>a</sup>
Kano	20.94 <sup>b</sup>	16.32 <sup>ab</sup>
Katsina	9.24 <sup>c</sup>	7.31 <sup>c</sup>
Zamfara	12.81 <sup>c</sup>	10.97 <sup>bc</sup>
SE±	2.58	1.93
<u>Interaction</u>		
State x Year	NS	NS

Means followed by similar superscript in a column are not significantly different at 5 % level of probability ( $P \leq 0.05$ ) using Duncan Multiple Range Test (DMRT).

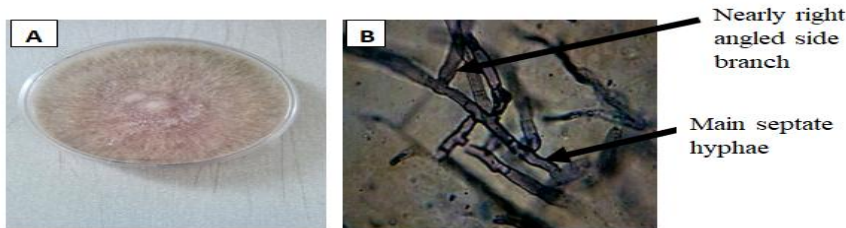
NS = Not significant at  $P \leq 5 \%$

### Isolation and Identification of Fungi

Fungi isolated from diseased plant stems were identified as *Rhizoctonia solani* Kuhn, *Macrophomina phaseolina* (Tassi) Goidanich, *Sclerotium* sp., *Colletotrichum capsici* (H. Sydow) E. Butler and Bisby, *Fusarium solani* (Mart) Sacc and *Curvularia lunata* (Wakker) Boedjn. The cultural and

morphological characteristics of the different isolates were studied.

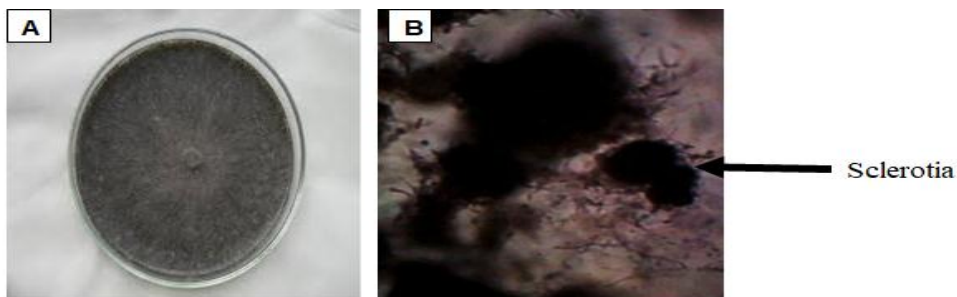
Cultures of *Rhizoctonia solani* on (PDAs) were brown in colour and covered 90 mm petri dish in four to five days at room temperature. Microscopic examination shows pale brown hyphae with nearly right - angled side branches constricted basally and no conidia were observed (Plates I A and B).



**Plate I:** A = Culture of *Rhizoctonia solani* on plate.  
B = *Rhizoctonia solani* hyphae.

*Macrophomina phaseolina* on PDAs produced black mycelia that covered 90 mm petri dish in 16 - 18 days at room temperature (Plates II A and B).

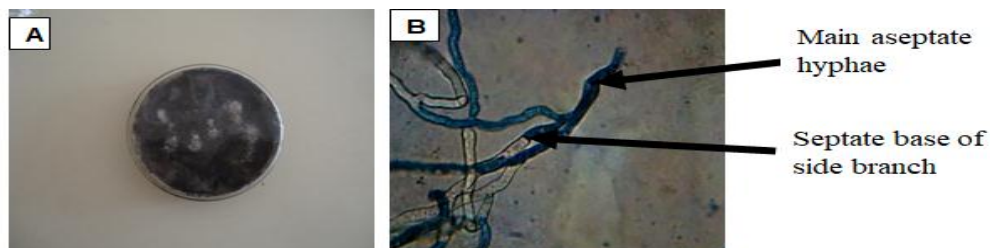
Sclerotia when viewed were black and neither conidia nor pycnidia were observed.



**Plate II:** A = *Macrophomina phaseolina* culture on plate.  
B = *Macrophomina phaseolina* sclerotia

Culture of *Sclerotium* sp. was grey in colour which covered 90 mm petri dish in four to five days at room temperature ( $27 \pm 2^{\circ}$  C). Their appearance was dense cottony with

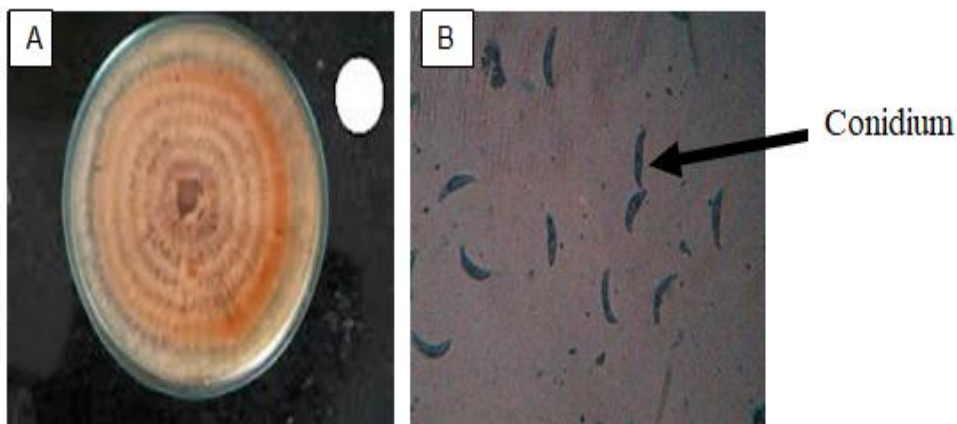
profuse hyphae. No conidia were observed. Hyphae were hyaline, branched and constricted basally with side branches septate near the main hyphae (Plates III A and B).



**Plate III:** A = *Sclerotium* sp. culture on plate.  
B = *Sclerotium* sp. hyphae

The culture of *Colletotrichum capsici* was orange coloured with concentric zonations which covered 90 mm petri dish in 18 – 20 days when grown on

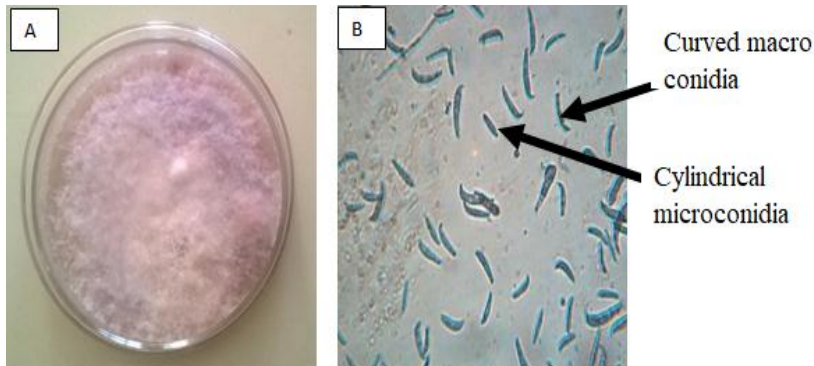
PDA. Conidia were hyaline one-celled and cylindrical to sickle in shape (Plates IV A and B).



**Plate IV:** A = *Colletotrichum capsici* culture on plate.  
B = *Colletotrichum capsici* conidia

Culture of *Fusarium solani* on PDAs was pinkish and covered 90 mm petri dish in 10 - 14 days. When microscopically examined, *F. solani* produced two types of conidia:

Macroconidia which were slightly curved, three to five celled; and microconidia that were cylindrical, one to two celled (Plates V A and B).

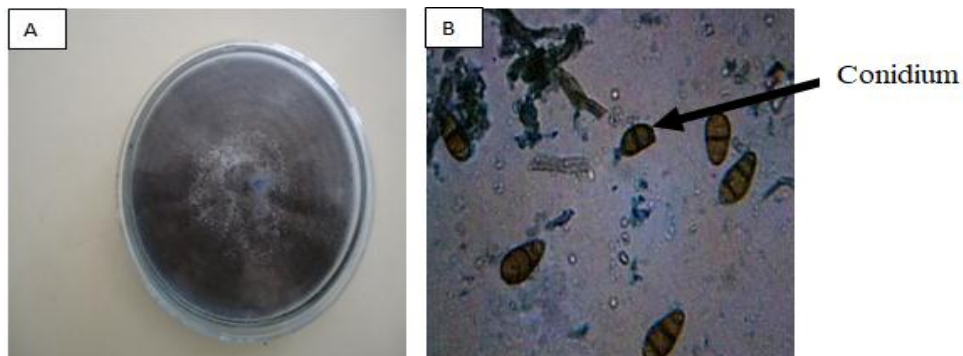


**Plate V:** A = *Fusarium solani* culture on plate.  
B = *Fusarium solani* conidia

When grown on PDAs, *Curvularia lunata* produced black coloured, hairy and rapidly growing mass of hyphae (covering 90 mm petri dish in 4 - 5

days). Conidia were dark brown, curved, four celled and larger in penultimate cells (Plates VI A and B).

Frequency of isolation of the different



**Plate VI:** A = *Curvularia lunata* culture on plate.  
B = *Curvularia lunata* conidia

State was highly significant ( $p \leq 0.01$ ). *R. solani* had the highest frequency in Kaduna, Kano and Zamfara States (40.00 %, 50.00 % and 52.94 % respectively) while *M. phaseolina* had the highest frequency

in Katsina State (38.89 %). *Sclerotium* sp. was only isolated from Kano State, *C. capsici* from Kaduna and Kano only with higher frequency (10.00 %) in Kaduna State.

**Table 2:** Frequency of fungi isolated from diseased cowpea stem in 2013 and 2014

Treatment	Frequency of isolation (%)
<u>Year</u>	
2013	19.10
2014	17.55
SE $\pm$	2.49
<u>Fungi</u>	
<i>Rhizoctonia solani</i>	45.90 <sup>a</sup>
<i>Macrophomina phaseolina</i>	29.55 <sup>b</sup>
<i>Sclerotium</i> sp.	2.22 <sup>c</sup>
<i>Colletotrichum capsici</i> .	2.92 <sup>c</sup>
<i>Fusarium solani</i>	21.04 <sup>b</sup>
<i>Curvularia lunata</i>	8.30 <sup>c</sup>
SE $\pm$	4.31
<u>State</u>	
Kaduna	16.11
Kano	16.67
Katsina	16.67
Zamfara	23.84
SE $\pm$	3.52
<u>Interactions</u>	

Year x Fungi	NS
Fungi x State	**
Year x State	NS
Year x Fungi x State	NS

Means with the same superscript in a column are not significantly different at 5 % level of significance ( $P \leq 0.05$ ) using Duncan Multiple Range Test (DMRT).

\*\*= significant at 1 %; NS = Not significant at 5 %

Zamfara had the highest frequency (35.29 %) of *F. solani* followed by Kano (25.00 %). Katsina State had the lowest frequency of *F. solani* (11.11 %) which did not significantly

differ from that of Kaduna State (10.00 %). *C. lunata* was only isolated from Kaduna and Katsina States with Kaduna State having the highest frequency (Table 3).

**Table 3:** Interaction between frequency of fungi isolated from diseased cowpea and the four States surveyed

State	Frequency of isolation (%) of:					
	<i>Rhizoctonia solani</i>	<i>Macrophomin a phaseolina</i>	<i>Sclerotiu m sp.</i>	<i>Colletotrichu m capsici</i>	<i>Fusariu m solani</i>	<i>Curvulari a lunata</i>
Zamfar a	52.94 <sup>a</sup>	11.56 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	35.49 <sup>b</sup>	0.00 <sup>d</sup>
Kano	50.00 <sup>a</sup>	10.00 <sup>cd</sup>	15.00 <sup>bc</sup>	5.00 <sup>de</sup>	20.00 <sup>b</sup>	0.00 <sup>e</sup>
Kaduna	40.00 <sup>a</sup>	10.00 <sup>c</sup>	0.00 <sup>d</sup>	10.00 <sup>c</sup>	13.33 <sup>c</sup>	26.67 <sup>b</sup>
Katsina	33.33 <sup>b</sup>	43.78 <sup>a</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	11.44 <sup>c</sup>	11.44 <sup>c</sup>
LSD	6.27					

Means with the same superscript in each row are not significantly different at 5 % level of significance ( $P \leq 0.05$ ) using Least Significant Difference (LSD)

## DISCUSSION

The two season's survey showed that stem rot of cowpea widely occurs in

North West Nigeria but at different intensities. Highest disease incidence and severity were obtained from Kaduna State, while the lowest

disease incidence and severity were obtained from Katsina State. The uneven distribution of the disease indicates that the environmental conditions favourable for the disease varied over the surveyed areas. The weather data (Appendix 1) showed that Kaduna state receives more rain and have high humidity while Katsina state had the least among the surveyed states. This conforms to the report by Dorrance *et al.*, (2003), that soil moisture and relative humidity favour development of disease induced by *R. solani* (10). According to a work by Adandonon *et al.*, (2004), disease incidence of cowpea stem rot was higher in the South and Central zones of Benin Republic than its Northern zone. The researchers attributed the variation to different amount of rainfall received by each zone, varied cultural practices and different ways that farmers prepare soil before sowing (2). *Rhizoctonia solani* was the organism most frequently isolated from the naturally infected plants obtained from the surveyed locations. In earlier works, of all the isolated organisms from this study, only *C. capsici* and *C. lunata* were not implicated as causal organisms of cowpea stem rot but causes Cowpea brown blotch (3) and *Curvularia* leaf spots of maize (18) respectively. *Fusarium* induced stem rot on cowpea was reported in

Tennessee in 2015 (Shrestha). *S. rolfsii*, *Fusarium* spp., *Pythium ultimum*, *R. solani*, *Phoma* spp., and *Trichoderma harzianum* were reported as causal organisms of cowpea stem rot across the ecological zones of Benin (3). But contrary to Adandonon findings that consider *S. rolfsii* as the main causal agent of stem rot, *R. solani* based on this study is considered the main causal agent of stem rot in North - West Nigeria having produced typical disease symptoms on artificially inoculated plants and also being the most frequently isolated. Though this is contrary to Onuorah (16) and Suleiman (20) who reported *Pythium aphanidermatum* as causal agent of cowpea stem rot in Oyo and Kogi State of Nigeria respectively. The isolation of *C. lunata* only from Kaduna and Katsina states is attributed to cowpea maize relay that predominates in the State, because *C. lunata* is a major maize foliar fungal pathogen that is found widely in maize growing areas (18). This is the first report of *C. lunata* causing disease on cowpea in Nigeria. Pathogenicity test showed that only *Rhizoctonia solani* induced typical symptom observed on the field which were reddish brown discoloration of the plant stem at base even though all the isolated organisms produced disease symptoms. This agreed with

findings of Adandonon *et al.* (2003) that damping off and stem rot of cowpea in South Africa is caused by complex of *Pythium ultimum* and *Rhizoctonia solani* (1). He also reported that root and stem rot of cowpea may be caused by various fungi most notable of which are *Rhizoctonia solani*, *Fusarium solani*, *Pythium* spp and *Sclerotium rolfsii*. (21) have found that *S. rolfsii* induce disease on different cowpea varieties at Badeggi, Nigeria. Isolates of *Macrophomina phaseolina*, from different host were found to induce stem necrotic lesions on stem of two cowpea species (5), this also agrees with findings of this study. Kankam (13) also reported *R. solani* to induce brown discoloration on seven cowpea varieties in Ghana.

### CONCLUSION

This study has found wide occurrence of cowpea stem rot in North West Nigeria having observed the symptoms of the disease in all the surveyed states. The incidence and severity of the disease was found to be influenced by weather conditions with higher severity in locations with high rainfall and relative humidity. *Rhizoctonia solani* is the main causal organism of cowpea stem rot in North - West Nigeria base on the findings of this study. *Macrophomina phaseolina*, *Sclerotium* sp,

*Colletotrichum capsici*, *Curvularia lunata* and *Fusarium solani* cause diseases on cowpea. This is the first report of *Curvularia lunata* associated with cowpea in Nigeria; further research should therefore be conducted to ascertain its pathogenicity on cowpea.

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**Appendix 1:** Total Annual Rainfall and Mean Temperature of the Surveyed States, 2013 and 2014.

State	Rainfall (mm)		Mean Temperature (°C)	
	2013	2014	2013	2014
Kaduna	1739.80	1623.60	32.30	32.10
Kano	1376.00	1009.30	34.40	33.40
Katsina	648.00	481.50	34.40	34.20
Zamfara	761.80	705.60	34.80	34.50

Source: NAERLS and FDAE (15)