



# An Investigation on the Initial Source of Inoculum of *Cercospora* Leaf Spot Pathogens of Groundnut (*Arachis hypogaea* L.) in Nigerian Southern Guinea Savannah Ecology

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## **Authors' contributions**

*This work was carried out in collaboration between all authors. Author BIR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AON and MUU managed the analyses of the study. Author MUU managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

**Aim:** An investigation was conducted in a greenhouse to determine the possible source of initial inoculum of leaf spot of pathogens of groundnut.

**Place and Duration of Study:** The experiment was conducted at the Teaching and Research Farm of the University of Agriculture Makurdi, Benue State of Nigeria in 2012/2013 cropping seasons:

**Methodology:** Ex-Daker and Shar-nya were the two groundnut varieties used, while the treatments include: T1- (sterilized soil + sterilized seeds + unsterilized groundnut shells), T2 - (sterilized soil + unsterilized seeds), T3 - (sterilized soil + sterilized seeds + unsterilized debris), T4

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- (unsterilized soil + sterilized seeds) and T5 – (sterilized soil + sterilized seeds). The establishment count, disease incidence and severity were the parameters recorded.

**Study Design:** The 2/5 factorial combination treatments was arranged in Complete Randomized Design (CRD) and replicated 3 times in an area measuring 2.4 / 6.60 m (15.84 m<sup>2</sup>).

**Results:** In 2012 and 2013, results show that there was no significant difference ( $P > 0.05$ ) among varieties, and the interactive effect between treatments and varieties on percentage of plant establishment. The treatments significantly ( $P < 0.05$ ) influenced percentage plant establishment in both years. In 2012, higher percentage plant establishment were recorded among T1 (80.56), T2 (94.44), T3 (77.78) and T5 (80.55) compared to T4 (58.33). Similarly in 2013, higher percentage plant establishment were recorded from T5 (97.22) and T2 (94.44) compared to the rest treatments. Results show that the incidence and severity of early leaf spot at 37 and 44 DAS, and combined incidence; severity of both early late leaf spots were not significantly ( $P > 0.05$ ) influenced by varieties and the interactive effect between treatments and varieties at 51 and 58 DAS in 2012 and 2013. Results revealed that the effect of treatments on incidence and severity of early leaf spot at 37 and 44 DAS; and combined incidence and severity of both early and late leaf spots at 51 and 58 DAS were significantly different ( $P < 0.05$ ) in 2012 and 2013. Among the treatments, plants grown from T3, T4, and T1 recorded higher and differential levels of early leaf spot incidence and severity at 37 and 44 DAS, and also combined incidence and severity of both early and late leaf spots at 51 and 58 DAS in both years. The results revealed that there was no evidence of disease development on the plants grown to T2 and T5 which affirmatively confirmed that these treatments could not have been the potential sources of the leaf spot pathogens.

**Conclusion:** The results of this investigation have proved that soil, debris and groundnut shells are the potential initial sources of leaf spot disease inoculums as these pathogens thrives in these sources from one season to another.

*Keywords: Incidence; severity; disease early; late leaf spot.*

## 1. INTRODUCTION

*Cercospora* leaf spot of groundnut (*Arachis hypogaea* L.) caused mainly by *Cercospora arachidicola* Hori and *Phaeoisariopsis* (Berk and Curt) are fungal diseases of economic importance which constitute a big constraint in groundnut production Worldwide. Groundnut is the principal source of dietary, oil and vitamins such as thiamine, riboflavin and niacin, its paste is an important source of calories for small children particularly those being weaned (Kamara et al. [1]). The severe attack of the disease very often resulted to unattainable expected yield wherever the crop is cultivated in Nigerian Sudan Savanna. The loss of photosynthetic ability of the plant tissue and rapid premature defoliation of the leaves is an obvious effect of leaf spot on a susceptible groundnut variety [2]. The pathogen can infect almost every parts of the crop causing different levels of yield losses [3]. In West Africa, yield loss of 50 to 70% has been reported [4] and in USA yield losses of 50% due to groundnut leaf spots has also been reported ([3]; Hagan et al. [5]). Groundnut yield is still low 0.96 t/ha, compared to its yield potential of 2 t/ha [6]. The destructive nature leaf spots merited the disease early recognition Worldwide long time ago (Subrahmanyam et al. [7]).

The incidence and severity of the disease varies with groundnut varieties, location, season, initial inoculums and environmental factors [8] have reported leaf spot incidence of 84.1% and 82.3% and severity of 44.6% and 58.3% on uncontrolled plot at 63DAS in 2002 and 2003 cropping seasons respectively in Nigerian Sudan Savanna. Many researchers in the developed countries have reported the over-wintering ability of the pathogen. [3] reported that mycelium of *Cercospora* leaf spot pathogen on stems, petioles, and pegs are likely to over-season than on leaflets. In Nigeria and other parts of the developing countries, the majority of the rural farmers if not all who constitute the major producers of groundnut lack adequate knowledge about the etiology and epidemiology of the disease. For example, in Nigeria most of the local groundnut farmers erroneously attached the symptom appearance of leaf spot lesions on groundnut leaves as an obvious indication of pod maturity hence they do not employ any disease control measures from the ongoing. There is dearth of enough information on initial source of the disease pathogen and its seasonal perpetuation to enable the poor-source farmers to manage the disease effectively.

Therefore, this paper reports the results of greenhouse investigation on the possible source

of initial inoculums of leaf spot pathogen of groundnut in Nigerian Southern Guinea Savanna.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Soil Samples and Sterilization

Top soil samples were collected randomly from different parts of the farm which were previously under intensive groundnut cultivation at the Teaching and Research Farm of College of Agronomy, University of Agriculture Makurdi. These soil samples were thoroughly heated for 35-40 minutes at the temperature of 82°C according to (Stapleton et al. [9]; [10]) using metal pot and allowed to cool. Some quantity of soil samples were left unheated.

### 2.2 Sterilization and Incorporating of Plant Materials in the Soil

About 100 g plant debris and groundnut shells were collected from previous farm grown to groundnut crops. These plant materials and seeds were surface sterilized separately with 10 ml of sodium hypochlorite (11.0%) in 90 ml of sterile distilled water, rinsed thrice with sterile distilled water, while some quantities of the plant debris, groundnut shells and seeds were left unsterilized. Furthermore, the groundnut shells and plant debris were separately crushed with an electronic blending machine. The crushed plant debris and groundnut shells were each dissolved in 200 ml of sterile water in a conical flask and autoclaved at 121°C for 15 minutes, while some of the crushed plant debris and groundnut shells were left without being autoclaved. The solutions of these materials were autoclaved separately. Those solutions that were autoclaved and those that were not autoclaved were mixed separately with the soil thoroughly. The incorporated soil mixtures were thereafter filled into various greenhouse pots leaving about 5 cm depth to allow room for watering.

### 2.3 Seed Sterilization

The seeds were surface sterilized in a similar way like the groundnut debris and shells and some quantity was left unsterilized.

T1 = sterilized soil + sterilized seeds + unsterilized shells  
T2 = Sterilized soil + unsterilized seeds  
T3 = sterilized soil + sterilized seeds + unsterilized debris

T4 = unsterilized soil + sterilized seeds and  
T5 = sterilized soil + sterilized seeds (control), were laid in the sub-plots.

### 2.4 Preparation and Arrangement of Experimental Area Pots

The experimental area used in the greenhouse measured 15.84 m<sup>2</sup> (2.4 x 6.60). The two groundnut varieties used include: Shar-nya and Ex-Daker. The treatment combinations were replicated three times and laid in a Complete Randomized Design (CRD) Three greenhouse pots each measuring 14 x 15.4 cm in length and diameter respectively were arranged leaving about 30 cm spacing in-between them representing a plot. About 40 cm and 50 cm were left in-between the plots and replicates as passing alley. Two seeds were sown per pot and the pots were kept weed free regularly by hand-pulling. The plants were watered twice per day. The seeds were sown on the 27<sup>th</sup> of October in 2012 and on the 20<sup>th</sup> of November in 2013.

### 2.5 Data Collection

#### 2.5.1 Establishment count (%)

The establishment count in percentage was recorded at 2 weeks after sowing (WAS) by counting the total number of plant stands that germinated in the greenhouse pots of each plot and then divided by the number of plant stands expected in the plot and was multiplied by one hundred.

#### 2.5.2 Disease incidence (%)

The incidence of the early leaf spot was recorded at 37 and 44 DAS, while the combined incidence of early and late leaf spots was recorded at 51 and 58 DAS. The disease incidence in percentage was recorded by visually to counting the number of plant stands infected in the sub-plots, then divided by the total number of plant stands (infected and healthy) and multiply by one hundred.

#### 2.5.3 Disease severity (%)

The severity of the early leaf spot was scored at 37 and 44 DAS, while the combined severity of early and late were recorded at 51 and 58 DAS using the disease severity scale of 0 = No disease, 2 = 1 – 14% of leaves with few small spots, 4 = 15 – 28% of leaves with many spot, 6 = 29 – 42% of leaves with few large spots, 8 = 43 – 56% of leaves with few large and small spots,

10 = 57 – 70% of leaves with many large spots and 12:>70% leaves with many and small spots according to Alabi et al. [11].

The percentages of disease severity were computed using the formular:

- Formular

$$\text{Disease severity: } \frac{\sum n \times 100}{N \times 12}$$

Where:

$\sum n$  = summation of individual assessments

N = Total No. of plant assessed

12 = Highest score of the severity scale

### 3. RESULTS

The results in Table 1 show the establishment count in percentage at two weeks after sowing (2WAS) as influenced by varieties, treatments and their interaction in 2012 and 2013 cropping seasons. In 2012 and 2013, the results show that

the establishment count (%) was not significantly different ( $P > 0.05$ ) among the two groundnut varieties, and likewise the interaction between the treatments and varieties. The treatments significantly influenced plant establishment count in both years. In the 2012, among the treatments, higher and statistically similar establishment count of 86.56, 94.44, 77.78 and 80.55% were recorded from plants grown on an unsterilized shell (T1), unsterilized seeds (T2), unsterilized debris (T3) and sterilized soil/seeds (T5) respectively. The unsterilized soil (T4) gave the lowest establishment count of 58.33%. In 2013, similar trend was observed where plants grown on unsterilized shell (T1) and unsterilized seeds (T2) recorded higher plant establishment count of 80.55 and 94.4% which did not vary significantly from each other. Those plants grown on unsterilized debris (T3) and sterilized soil/seed (T5) establishment count of 63.89% each, while 58.33% was recorded from those grown on unsterilized soil recorded (T4) which did not vary significantly from each other respectively.

**Table 1. Effect of varieties, treatments and their interactions on plant establishment count (%) at (2WAS) in 2012 and 2013 cropping seasons**

Variety/Treatments/Interactions	Cropping season	
	2012	2013
<b>Variety(A)</b>		
Ex-Daker	75.55±4.26	80.00±5.68
Shar-nya	81.11±5.11	77.78±4.79
<i>P-value</i>	0.41 <sup>ns</sup>	0.76 <sup>ns</sup>
CV	23.30	25.80
<b>Treatments(B)</b>		
Unsterilized shell (T <sub>1</sub> )	80.56±6.69 <sup>a</sup>	80.55±7.95 <sup>b</sup>
Sterilized Seeds (T <sub>2</sub> )	94.44±3.51 <sup>a</sup>	94.44±3.51 <sup>ab</sup>
Unsterilized Debris (T <sub>3</sub> )	77.78±7.03 <sup>a</sup>	63.89±5.12 <sup>c</sup>
Unsterilized Soil (T <sub>4</sub> )	58.33±5.69 <sup>b</sup>	58.33±5.69 <sup>c</sup>
Sterilized Soil & Seeds (T <sub>5</sub> )	80.55±6.69 <sup>a</sup>	63.89±5.12 <sup>c</sup>
<i>P-value</i>	<0.01	<0.01
CV	19.00	16.50
<b>Interactions(AXB)</b>		
Ex-Daker X Unsterilized shell (T <sub>1</sub> )	72.20	83.30
Ex-Daker X Sterilized Seeds (T <sub>2</sub> )	88.90	94.40
Ex-Daker X Unsterilized Debris (T <sub>3</sub> )	72.20	66.70
Ex-Daker X Unsterilized Soil (T <sub>4</sub> )	55.60	55.60
Ex-Daker X Sterilized Soil & Seeds (T <sub>5</sub> )	88.90	100.00
Shar-nya X Unsterilized shell (T <sub>1</sub> )	88.90	77.80
Shar-nya X Sterilized Seeds (T <sub>2</sub> )	100.00	94.40
Shar-nya X Unsterilized Debris (T <sub>3</sub> )	83.30	61.10
Shar-nya X Unsterilized Soil (T <sub>4</sub> )	61.10	61.10
Shar-nya X Sterilized Soil & Seeds (T <sub>5</sub> )	72.20	94.20
<i>P-value</i>	0.34 <sup>ns</sup>	0.94 <sup>ns</sup>
CV	18.60	18.10

Mean values within the same alphabet in a row are not significantly different from each other according to Fisher-Least significant different (F-LSD) at 95% CL ( $P < 0.05$ ). CV = coefficient of variation, ns = not significant

Table 2 present the results on incidence of early leaf spot at 37 and 44DAS and combined incidence of early and late leaf spot at 51 and 58 DAS as influenced by varieties, treatments and their interaction in 2012 cropping seasons. In 2012, the results show that the incidence of early leaf spot at 37 and 44 DAS and combined incidence of early and late leaf spot at 51 and 58 DAS were not significantly different ( $p > 0.05$ ) among the varieties, and also the interaction between treatments and varieties. But the treatments significantly influenced the percentage incidence of the leaf spot disease from 37 to 58 DAS in 2012. In 2012 results show that among the treatments, higher early leaf spot incidence of 24.17, 28.61 and 29.72% which are statistically similar were recorded from plants grown on an unsterilized shell ( $T_1$ ), unsterilized debris ( $T_3$ ) and unsterilized soil at 37 DAS respectively. Similarly, higher leaf spot incidence of 40.0, 45.56 and 46.11% which did not significantly vary from each other was recorded on those plants grown on an unsterilized shell ( $T_1$ ), unsterilized debris ( $T_3$ ) and unsterilized soil ( $T_4$ ) at 44 DAS respectively. The lowest early spot incidence of 0.0% was observed from those plants grown on unsterilized seeds ( $T_2$ ) and sterilized soil/sterilized seeds ( $T_5$ ) from 37 to 44 DAS. Results at 51 DAS show that among the treatments, higher and statistically indifferent disease incidence of 75.0 and 75.84% were recorded from plants grown on unsterilized debris( $T_3$ ) and unsterilized soil ( $T_4$ ), followed by 60.56% obtained from plants grown on unsterilized shell ( $T_1$ ) as a result of combined incidence of early and late leaf spot. Similarly at 58 DAS, higher disease incidence of 90.28, 88.33 and 79.44% which did not significantly differ from each other were recorded from plants grown on unsterilized soil ( $T_4$ ), unsterilized debris ( $T_3$ ) and unsterilized shell ( $T_1$ ) respectively as a result of combined incidence of early and late leaf spot disease. The results also revealed that from 51 to 58 DAS, plant grown from the unsterilized seeds ( $T_2$ ) and sterilized soil/sterilized seeds exhibited no disease symptom similar to those from 37 to 44 DAS.

In 2013, results in (Table 3) show that at 37 DAS among the treatments, higher but statistically indifferent early leaf spot incidence of 28.33 and 24.72% were recorded from plants grown on unsterilized soil ( $T_4$ ) and unsterilized debris ( $T_3$ ), followed by those plants grown on unsterilized shell ( $T_1$ ). Similarly at 44 DAS, higher early leaf spot incidence of 35.83 and 32.22% which did not vary significantly from each other were

recorded from plants grown on unsterilized soil ( $T_4$ ) and unsterilized debris ( $T_3$ ) respectively; this was followed by those plants grown from the unsterilized shell ( $T_1$ ) which had disease incidence of 26.11%. The results revealed that those plants grown on unsterilized seeds ( $T_2$ ) and sterilized soil/sterilized seeds did not show incidence of the disease from 37 to 44 DAS. The results at 51DAS revealed that among the treatments higher but statistically similar disease incidence of 63.89 and 64.17% were recorded from plants grown on unsterilized debris ( $T_3$ ) and unsterilized soil respectively, followed by 44.17% disease incidence recorded from those plants grown on unsterilized shell ( $T_1$ ) as a results of the combined incidence effect of early and late leaf spots. At 58 DAS, results also indicate that among the treatments, higher disease incidence of 90.0 and 86.11% which did not differ significantly from each other were recorded from those plants grown on unsterilized debris ( $T_3$ ) and unsterilized soil ( $T_4$ ) respectively, followed by disease incidence of 70.56% recorded from those plants grown on unsterilized shell ( $T_1$ ) due to combined incidence effect of early and late leaf spots. The results show that those plants grown on unsterilized seeds ( $T_2$ ) and sterilized soil/sterilized seeds ( $T_5$ ) did not show leaf spot symptom and hence recorded the lowest disease incidence with 0.0% each from 51 to 58DAS.

The severity of early leaf spot at 37 and 44 DAS and combined severity of early and late leaf spots as influenced by varieties, treatments and their interaction in 2012 cropping seasons are presented in (Table 4). In 2012, the effect of varieties and interaction between treatments and varieties on severity of early leaf spot at 37 and 44 DAS and combined severity of early and late leaf spots at 51 and 58DAS were not significant difference ( $P > 0.05$ ) The effect of treatments on severity of early leaf spot at 37 and 44 DAS, and combined severity of early and late leaf spots at 51 and 58 DAS were significantly different ( $P < 0.05$ ).

In 2012, the results at 37 DAS show that among the treatments, those plants grown on unsterilized debris ( $T_3$ ) recorded significantly the highest early leaf spot severity of 16.58% followed by those grown on an unsterilized soil ( $T_4$ ) and unsterilized shell ( $T_1$ ) which scored statistically similar disease severity of 13.89 and 13.19% respectively. At 44 DAS, results indicate that those plants grown on an unsterilized soil ( $T_4$ ) recorded significantly the highest early leaf

spot of 29.86%; followed by those grown on an unsterilized debris (T<sub>3</sub>) and unsterilized shell (T<sub>1</sub>) which had disease severity of 24.30 and 18.05% respectively. The results revealed that those plants grown on an unsterilized seeds (T<sub>2</sub>) and sterilized soil/sterilized seeds (T<sub>5</sub>) recorded the lowest early leaf spot severity of 0.0% each at 37 and 44 DAS. In 2012, results on combined severity of early and late leaf spots at 51 DAS show that those plants grown on an unsterilized soil (T<sub>4</sub>) and unsterilized debris (T<sub>3</sub>) recorded higher disease severity of 50.69 and 48.61% respectively but which did not vary significantly from each other; this followed by those plants

grown on an unsterilized shell (T<sub>1</sub>) which scored leaf spots severity of 25.69%. At 58 DAS, results show that higher combined leaf spots severity of 57.63 and 59.03% which did not differ significantly from each other was respectively recorded from those plants grown on an unsterilized soil (T<sub>4</sub>) and unsterilized debris (T<sub>3</sub>). This was followed by combined disease severity of 37.94% recorded from those plants grown on an unsterilized shell (T<sub>1</sub>). Results at 51 and 58 DAS revealed that those plants grown on an unsterilized seeds (T<sub>2</sub>) and sterilized soil/sterilized seeds (T<sub>5</sub>) recorded the lowest leaf spots severity of 0.0% each.

**Table 2. Effect of variety, treatment and their interaction on incidence of early leaf spot at 37 and 44 DAS and combined incidence of early/late leaf spots of groundnut at 51 and 58 DAS in 2012 cropping season**

Variety/Treatments/Interactions	Early leaf spot		Early & late leaf spot (Combined)	
	37DAS	44DAS	51DAS	58DAS
<b>Variety (A)</b>				
Ex-Daker	15.67±3.60	25.56±6.37	41.89±9.66	50.40±11.40
Shar-nya	17.33±4.06	26.11±6.10	42.67±9.70	52.80±11.80
<i>P</i> -value	0.76 <sup>ns</sup>	0.96 <sup>ns</sup>	0.95 <sup>ns</sup>	0.88 <sup>ns</sup>
CV	30.10	31.70	38.70	37.10
<b>Treatments (B)</b>				
Unsterilized shell (T <sub>1</sub> )	24.17±2.31 <sup>a</sup>	40.00±5.00 <sup>a</sup>	60.56±6.69 <sup>b</sup>	79.44±5.39 <sup>a</sup>
Sterilized Seeds (T <sub>2</sub> )	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>
Unsterilized Debris (T <sub>3</sub> )	28.61±3.34 <sup>a</sup>	45.56±3.91 <sup>a</sup>	75.00±4.17 <sup>a</sup>	88.33±5.69 <sup>a</sup>
Unsterilized Soil (T <sub>4</sub> )	29.72±2.37 <sup>a</sup>	46.11±6.11 <sup>a</sup>	75.84±5.34 <sup>a</sup>	90.28±6.24 <sup>a</sup>
Sterilized Soil & Seeds (T <sub>5</sub> )	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>
<i>P</i> -value	<0.01	<0.01	<0.01	<0.01
CV	31.20	36.70	24.70	21.50
<b>Interactions (A X B)</b>				
Ex-Daker X Unsterilized shell (T <sub>1</sub> )	23.33	38.30	60.00	78.30
Ex-Daker X Sterilized Seeds (T <sub>2</sub> )	0.00	0.00	0.00	0.00
Ex-Daker X Unsterilized Debris (T <sub>3</sub> )	24.44	44.40	68.90	82.20
Ex-Daker X Unsterilized Soil (T <sub>4</sub> )	30.55	50.00	80.60	91.70
Ex-Daker X Sterilized Soil & Seeds (T <sub>5</sub> )	0.00	0.00	0.00	0.00
Shar-nya X Unsterilized shell (T <sub>1</sub> )	25.00	41.70	61.10	80.60
Shar-nya X Sterilized Seeds (T <sub>2</sub> )	0.00	0.00	0.00	0.00
Shar-nya X Unsterilized Debris (T <sub>3</sub> )	32.78	46.70	81.10	94.40
Shar-nya X Unsterilized Soil (T <sub>4</sub> )	28.89	42.20	71.10	88.90
Shar-nya X Sterilized Soil & Seeds (T <sub>5</sub> )	0.00	0.00	0.00	0.00
<i>P</i> -value	0.52 <sup>ns</sup>	0.90 <sup>ns</sup>	0.56 <sup>ns</sup>	0.83 <sup>ns</sup>
CV	31.80	30.00	25.70	22.80

Mean values within the same alphabet in a row are not significantly different from each other according to Fisher-Least significant different (F-LSD) at 95% CL (*P* < 0.05). CV = coefficient of variation, ns = not significant  
T<sub>1</sub> = sterilized soil + sterilized seeds + unsterilized shells T<sub>2</sub> = sterilized soil + unsterilized seeds, T<sub>3</sub> = sterilized soil + sterilized seeds + unsterilized debris. T<sub>4</sub> = unsterilized soil + sterilized seeds and. T<sub>5</sub> = sterilized soil + sterilized seeds (control)

**Table 3. Effect of variety, treatment and their interaction on incidence of early leaf spot at 37 and 44 DAS and combined incidence of early/late leaf spots of groundnut at 51 and 58 DAS in 2013 cropping season**

Variety/Treatments/Interactions	Early leaf spot		Early & late leaf spot (Combined)	
	37DAS	44DAS	51DAS	58DAS
<b>Variety (A)</b>				
Ex-Daker	14.67±3.51	18.78±4.60	35.67±8.01	50.90±11.50
Shar-nya	14.56±3.28	18.89±4.56	33.22±7.92	47.80±10.70
<i>P-value</i>	0.98 <sup>ns</sup>	0.98 <sup>ns</sup>	0.83 <sup>ns</sup>	0.84 <sup>ns</sup>
<i>CV</i>	30.10	34.10	39.50	37.30
<b>Treatments (B)</b>				
Unsterilized shell (T <sub>1</sub> )	20.00±1.67 <sup>b</sup>	26.11±3.77 <sup>b</sup>	44.17±4.17 <sup>b</sup>	70.56±3.00 <sup>b</sup>
Sterilized Seeds (T <sub>2</sub> )	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>
Unsterilized Debris (T <sub>3</sub> )	24.72±1.99 <sup>a</sup>	32.22±4.59 <sup>ab</sup>	63.89±4.08 <sup>a</sup>	90.00±4.47 <sup>a</sup>
Unsterilized Soil (T <sub>4</sub> )	28.33±2.36 <sup>a</sup>	35.83±3.44 <sup>a</sup>	64.17±3.44 <sup>a</sup>	86.11±6.33 <sup>a</sup>
Sterilized Soil & Seeds (T <sub>5</sub> )	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>
<i>P-value</i>	<0.01	<0.01	<0.01	<0.01
<i>CV</i>	26.30	39.90	21.30	18.50
<b>Interactions (A X B)</b>				
Ex-Daker X Unsterilized shell (T <sub>1</sub> )	16.67	22.20	78.20	69.40
Ex-Daker X Sterilized Seeds (T <sub>2</sub> )	0.00	0.00	0.00	0.00
Ex-Daker X Unsterilized Debris (T <sub>3</sub> )	26.11	32.80	82.20	93.30
Ex-Daker X Unsterilized Soil (T <sub>4</sub> )	30.55	22.20	91.70	91.70
Ex-Daker X Sterilized Soil & Seeds (T <sub>5</sub> )	0.00	0.00	0.00	0.00
Shar-nya X Unsterilized shell (T <sub>1</sub> )	23.33	30.00	80.60	71.70
Shar-nya X Sterilized Seeds (T <sub>2</sub> )	0.00	0.00	0.00	0.00
Shar-nya X Unsterilized Debris (T <sub>3</sub> )	23.33	31.70	94.40	86.70
Shar-nya X Unsterilized Soil (T <sub>4</sub> )	26.11	30.00	88.90	80.60
Shar-nya X Sterilized Soil & Seeds (T <sub>5</sub> )	0.00	0.00	0.00	0.00
<i>P-value</i>	0.12 <sup>ns</sup>	0.67 <sup>ns</sup>	0.83 <sup>ns</sup>	0.72 <sup>ns</sup>
<i>CV</i>	24.70	32.20	22.80	19.30

Mean values within the same alphabet in a column are not significantly different from each other according to Fisher-Least significant different (F-LSD) at 95% CL ( $P < 0.05$ ). CV = coefficient of variation, NS = Not significant T<sub>1</sub> = sterilized soil + sterilized seeds + unsterilized shells, T<sub>2</sub> = sterilized soil + unsterilized seeds, T<sub>3</sub> = sterilized soil + sterilized seeds + unsterilized debris. T<sub>4</sub> = unsterilized soil + sterilized seeds and. T<sub>5</sub> = sterilized soil + sterilized seeds (control)

In 2013 (Table 5), the effect of variety, and the interaction between treatments and varieties on severity of early leaf spot at 37 and 44 DAS and combined severity of early/late leaf spots of groundnut at 51 and 58 DAS were not significantly ( $P > 0.05$ ). The severity of early leaf spot at 37 and 44 DAS and combined severity of early and late leaf spot at 51 and 58 DAS were significantly influenced by the treatments. The results at 37 DAS indicate that among the treatments, higher but statistically indifferent early leaf spot severity of 15.97 and 13.89%

were recorded from those plants grown on unsterilized debris (T<sub>3</sub>) and unsterilized soil (T<sub>4</sub>) respectively; followed by those plants grown on an unsterilized shell (T<sub>1</sub>) which scored disease severity of 11.81%. Similarly at 44 DAS, results show that among the treatments higher early leaf spot severity of 32.72 and 34.72% which did not significantly vary from each other was recorded from those plants grown on unsterilized debris (T<sub>3</sub>) and unsterilized soil (T<sub>4</sub>) respectively. This was also followed by disease severity of 21.70% recorded from those plants grown on an

unsterilized shell (T<sub>1</sub>). Results at 37 and 44 DAS revealed that the lowest early leaf spot severity of 0.0% was recorded from those plants grown on an unsterilized seeds (T<sub>2</sub>) and sterilized soil/sterilized seeds (T<sub>5</sub>). The results on combined severity of early and late leaf spots at 51 DAS show that among the treatments, those plants grown on an unsterilized soil (T<sub>4</sub>) and unsterilized debris (T<sub>3</sub>) scored higher combined disease severity of 52.08 and 49.91% which did not differ statistically from each other. The next was those plants grown on an unsterilized shell (T<sub>1</sub>) which recorded combined disease severity of 31.91%. Similarly, at 58 DAS results also revealed that among the treatments, higher but statistically similar combined leaf spots severity of 59.02 and 56.94% were recorded from those plants grown on an unsterilized soil (T<sub>4</sub>) and unsterilized debris (T<sub>3</sub>) respectively. This was followed by the combined disease severity of 40.60 recorded from those plants grown on an unsterilized shell (T<sub>1</sub>). Results at 51 and 58 DAS show that those plants grown on an unsterilized seeds (T<sub>2</sub>) and sterilized soil/sterilized seeds (T<sub>5</sub>) consistently recorded the lowest combined leaf spots of 0.0% which equally indicate no disease infection.

#### 4. DISCUSSION

*Cercospora* leaf spot of groundnut is significantly important wherever the crop is grown in the developing and developed Countries. Generally, disease is a major economic setback in several countries including Nigeria where effective disease management is not practice and where genetic improvement is very tedious, slow and take several years before improved varieties are introduced (Richard et al. [12]). Disease pathogens are responsible for the continuous seasonal perpetuation, increase in incidence and severity of disease which eventually result to low yield attainment. The basic idea and knowledge on where the potential pathogen usually over-winter during the off-season would provides a sense of direction for administering disease management strategy option.

In 2012 and 2013, the establishment count of Ex-Daker and Shar-nya were not significantly (P>0.05) from each other, but the treatments significantly influenced the plant establishment in the two cropping seasons. Results indicates that unsterilized shell (T<sub>1</sub>), unsterilized seeds (T<sub>2</sub>), unsterilized debris (T<sub>3</sub>) and sterilized soil/sterilized seeds (T<sub>5</sub>) influenced higher plant establishment compared to unsterilized soil (T<sub>4</sub>)

in 2012, while the unsterilized shell (T<sub>1</sub>) and unsterilized seeds (T<sub>2</sub>) influenced higher plant establishment compared to unsterilized debris (T<sub>3</sub>), unsterilized soil (T<sub>4</sub>) and sterilized soil/sterilized seeds (T<sub>5</sub>) in 2013. Generally, unsterilized soil (T<sub>4</sub>) had lower plant establishment compared to other treatments in both seasons. In 2012 and 2013 cropping seasons, results revealed that the groundnut varieties; Ex-Daker and Shan-ya did not show significant difference in the incidence and severity of early leaf spot from 47 and 44DAS, and also combined incidence and combined severity of both early and late leaf spots at 51 and 58 DAS. [8] reported that the combined effect of the two leaf spots is more devastating than their individual effects. Results of the greenhouse experiment show significant difference in the incidence and severity of leaf spots with respect to the source of initial inoculums of the pathogen (Treatments) in 2011 and 2012. The results of the investigation revealed that the unsterilized soil (T<sub>4</sub>), unsterilized groundnut debris (T<sub>3</sub>) and unsterilized shells (T<sub>1</sub>) were the three possible sources of leaf spots inoculums. The results established that the appearance of Early leaf spot (*Cercospora arachidicola*) at the upper leaf surface and Late leaf spot (*Phaeosariopsis personata*) the lower leaf surface and the associated necrotic lesions on the leaves of the groundnut varieties grown to these treatments makes the three treatments potential source of the initial inoculums of these pathogens. There were no evidence of both diseases on plants grown from unsterilized seeds/sterilized soil (T<sub>2</sub>) and those grown from sterilized seeds/sterilized soil (T<sub>5</sub>). This implies that seeds could not have been the possible source of the initial inoculums of groundnut leaf spot disease. This finding is in agreement with McDonald et al. [13] who reported that no evidence of either pathogen being internally seed-borne and that the pathogens may survive from season to season on volunteer groundnut plants and infected crop debris. [3] also reported that mycelium of the pathogen on stems, petioles and pegs are likely to over-season than that on leaflets. With this first hand information, the reduction of the initial inoculums becomes the crux of the issue. Therefore, effective disease management approach at all levels becomes very necessary since Agricultural sector is still of economic importance especially as it relate to employment generation and contribution to National Gross Domestic Product (GDP) and foreign exchange earnings (Manyong et al. [14]).



**Table 4. Effect of variety, treatment and their interaction on severity of early leaf spot at 37 and 44 DAS and combined severity of early/late leaf spots of groundnut at 51 and 58 DAS in 2012 cropping season**

Variety/Treatments/Interactions	Early leaf spot				Early & late leaf spot (Combined)			
	37DAS	Scale	44DAS	Scale	51DAS	Scale	58DAS	Scale
<b>Variety (A)</b>								
Ex-Daker	8.89±2.03	(2)	15.00±3.49	(4)	25.83±6.20	(4)	32.15±7.32	(6)
Shar-nya	8.58±1.92	(2)	13.89±3.27	(2)	24.16±5.85	(4)	29.69±6.82	(6)
<i>P-value</i>	0.91 <sup>ns</sup>		0.81 <sup>ns</sup>		0.84 <sup>ns</sup>		0.80 <sup>ns</sup>	
CV	37.50		30.70		33.40		38.60	
<b>Treatment (B)</b>								
Unsterilized shell (T <sub>1</sub> )	13.19±0.69 <sup>b</sup>	(2)	18.05±0.87 <sup>c</sup>	(4)	25.69±2.50 <sup>b</sup>	(4)	37.94±1.7 <sup>b</sup>	(6)
Sterilized Seeds (T <sub>2</sub> )	0.00±0.00 <sup>c</sup>	(0)	0.00±0.00 <sup>d</sup>	(0)	0.00±0.00 <sup>c</sup>	(0)	0.00±0.00 <sup>c</sup>	(0)
Unsterilized Debris (T <sub>3</sub> )	16.58±1.08 <sup>a</sup>	(4)	24.30±1.28 <sup>b</sup>	(4)	48.61±2.56 <sup>a</sup>	(8)	59.03±1.99 <sup>a</sup>	(10)
Unsterilized Soil (T <sub>4</sub> )	13.89±0.87 <sup>b</sup>	(2)	29.86±2.26 <sup>a</sup>	(6)	50.69±1.67 <sup>a</sup>	(8)	57.63±1.67 <sup>a</sup>	(10)
Sterilized Soil & Seeds (T <sub>5</sub> )	0.00±0.00 <sup>c</sup>	(0)	0.00±0.00 <sup>d</sup>	(0)	0.00±0.00 <sup>c</sup>	(0)	0.00±0.00 <sup>c</sup>	(0)
<i>P-value</i>	<0.01		<0.01		<0.01		<0.01	
CV	19.50		20.80		17.30		10.80	
<b>Interactions (A X B)</b>								
Ex-Daker X Unsterilized shell (T <sub>1</sub> )	12.50	(2)	18.06	(4)	26.39	(4)	39.94	(6)
Ex-Daker X Sterilized Seeds (T <sub>2</sub> )	0.00	(0)	0.00	(0)	0.00	(0)	0.00	(0)
Ex-Daker X Unsterilized Debris (T <sub>3</sub> )	18.06	(4)	26.39	(4)	50.00	(8)	61.11	(10)
Ex-Daker X Unsterilized Soil (T <sub>4</sub> )	13.89	(2)	30.55	(6)	52.77	(8)	59.72	(10)
Ex-Daker X Sterilized Soil & Seeds (T <sub>5</sub> )	0.00	(0)	0.00	(0)	0.00	(0)	0.00	(0)
Shar-nya X Unsterilized shell (T <sub>1</sub> )	13.89	(2)	18.06	(4)	25.00	(4)	35.94	(6)
Shar-nya X Sterilized Seeds (T <sub>2</sub> )	0.00	(0)	0.00	(0)	0.00	(0)	0.00	(0)
Shar-nya X Unsterilized Debris (T <sub>3</sub> )	15.11	(4)	22.22	(4)	47.22	(8)	56.94	(10)
Shar-nya X Unsterilized Soil (T <sub>4</sub> )	13.89	(2)	29.17	(6)	48.61	(8)	55.55	(8)
Shar-nya X Sterilized Soil & Seeds (T <sub>5</sub> )	0.00	(0)	0.00	(0)	0.00	(0)	0.00	(0)
<i>P-value</i>	0.28 <sup>ns</sup>		0.73 <sup>ns</sup>		0.91 <sup>ns</sup>		0.56 <sup>ns</sup>	
CV	19.30		21.70		18.50		10.20	

Mean values within the same alphabet in a row are not significantly different from each other according to Fisher-Least significant different (F-LSD) at 95% CL ( $P < 0.05$ ). CV = coefficient of variation, ns = not significant

T<sub>1</sub> = sterilized soil + sterilized seeds + unsterilized shells, T<sub>2</sub> = sterilized soil + unsterilized seeds, T<sub>3</sub> = sterilized soil + sterilized seeds + unsterilized debris, T<sub>4</sub> = unsterilized soil + sterilized seeds and. T<sub>5</sub> = sterilized soil + sterilized seeds (control)

**Table 5. Effect of variety, treatment and their interaction on severity of early leaf spot at 37 and 44 DAS and combined severity of early/late leaf spots of groundnut at 51 and 58 DAS in 2013 cropping season**

Variety/Treatments/Interactions	Early leaf spot				Early & late leaf spot (Combined)			
	37DAS	Scale	44DAS	Scale	51DAS	Scale	58DAS	Scale
<b>Variety (A)</b>								
Ex-Daker	9.17±2.04	(2)	16.94±4.06	(4)	26.66±6.28	(4)	32.71±7.36	(6)
Shar-nya	7.50±1.92	(2)	18.71±4.41	(4)	26.90±6.10	(4)	29.94±6.76	(6)
<i>P-value</i>	0.55 <sup>ns</sup>		0.77 <sup>ns</sup>		0.97 <sup>ns</sup>		0.78 <sup>ns</sup>	
<i>CV</i>	32.00		30.70		39.60		37.40	
<b>Treatments (B)</b>								
Unsterilized shell (T <sub>1</sub> )	11.81±2.50 <sup>b</sup>	(2)	21.70±1.74 <sup>b</sup>	(4)	31.91±2.31 <sup>b</sup>	(6)	40.66±1.29 <sup>b</sup>	(6)
Sterilized Seeds (T <sub>2</sub> )	0.00±0.00 <sup>c</sup>	(0)	0.00±0.00 <sup>c</sup>	(0)	0.00±0.00 <sup>c</sup>	(0)	0.00±0.00 <sup>c</sup>	(0)
Unsterilized Debris (T <sub>3</sub> )	15.97±0.69 <sup>a</sup>	(4)	32.72±1.60 <sup>a</sup>	(6)	49.91±1.94 <sup>a</sup>	(8)	56.94±1.76 <sup>a</sup>	(10)
Unsterilized Soil (T <sub>4</sub> )	13.89±0.87 <sup>ab</sup>	(2)	34.72±3.82 <sup>a</sup>	(6)	52.08±0.93 <sup>a</sup>	(8)	59.02±1.28 <sup>a</sup>	(10)
Sterilized Soil & Seeds (T <sub>5</sub> )	0.00±0.00 <sup>c</sup>	(0)	0.00±0.00 <sup>c</sup>	(0)	0.00±0.00 <sup>c</sup>	(0)	0.00±0.00 <sup>c</sup>	(0)
<i>P-value</i>	<0.01		<0.01		<0.01		<0.01	
<i>CV</i>	36.10		27.60		12.90		8.80	
<b>Interactions (A X B)</b>								
Ex-Daker X Unsterilized shell (T <sub>1</sub> )	13.89	(2)	20.83	(4)	29.16	(6)	42.72	(8)
Ex-Daker X Sterilized Seeds (T <sub>2</sub> )	0.00	(0)	0.00	(0)	0.00	(0)	0.00	(0)
Ex-Daker X Unsterilized Debris (T <sub>3</sub> )	16.67	(4)	34.72	(6)	52.77	(8)	59.72	(10)
Ex-Daker X Unsterilized Soil (T <sub>4</sub> )	15.28	(4)	29.17	(4)	51.39	(8)	61.11	(10)
Ex-Daker X Sterilized Soil & Seeds (T <sub>5</sub> )	0.00	(0)	0.00	(0)	0.00	(0)	0.00	(0)
Shar-nya X Unsterilized shell (T <sub>1</sub> )	9.72	(2)	22.56	(4)	34.66	(6)	38.61	(6)
Shar-nya X Sterilized Seeds (T <sub>2</sub> )	0.00	(0)	0.00	(0)	0.00	(0)	0.00	(0)
Shar-nya X Unsterilized Debris (T <sub>3</sub> )	15.28	(4)	30.72	(6)	47.05	(8)	54.16	(8)
Shar-nya X Unsterilized Soil (T <sub>4</sub> )	12.50	(2)	40.28	(6)	52.77	(8)	56.94	(10)
Shar-nya X Sterilized Soil & Seeds (T <sub>5</sub> )	0.00	(0)	0.00	(0)	0.00	(0)	0.00	(0)
<i>P-value</i>	0.71 <sup>ns</sup>		0.08 <sup>ns</sup>		0.08		0.11	
<i>CV</i>	36.50		24.90		11.90		6.90	

Mean values within the same alphabet in a row are not significantly different from each other according to Fisher-Least significant different (F-LSD) at 95% CL ( $P < 0.05$ ). CV = coefficient of variation, ns = not significant

T<sub>1</sub> = sterilized soil + sterilized seeds + unsterilized shells, T<sub>2</sub> = sterilized soil + unsterilized seeds, T<sub>3</sub> = sterilized soil + sterilized seeds + unsterilized debris, T<sub>4</sub> = unsterilized soil + sterilized seeds and. T<sub>5</sub> = sterilized soil + sterilized seeds (control)

## 5. CONCLUSION

In conclusion, the finding of this investigation can be very useful to the Agricultural extension personnel's who can further disseminate this viable informative package to farmers in areas where *Cercospora* leaf spot is endemic and posed a big threat in groundnut production. By and large, it will give the farmers a good mind set on how and where to initiate the disease management strategies within their reach in order to mitigate the menace of the disease to a minimum level and subsequently increase their groundnut production.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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