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Occurrence of foliar fungal diseases of cowpea and their management with garlic (*Allium sativum* L.) extracts in Abuja Nigeria

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Abstract
This study assessed the incidence and severity of foliar fungal diseases in selected cowpea varieties and the efficacy of garlic (*Allium sativum* L) extract in the management of the major disease in Abuja, Nigeria. Four cowpea varieties namely, Sampea 20T, Sampea 14, Abuja local white, and Abuja local Brown obtained from the National Biotechnology Development Agency (NABDA), Abuja were used. A Randomized Complete Block Design was adopted in the study. The experiment covered two growing seasons - 2021 and 2022. Three levels of garlic extract treatment, one level of mancozeb (synthetic fungicide) as the positive control, and de-chlorinated water application as the control. Each treatment was replicated thrice. Garlic extract at (100%, 50%, and 25%) and synthetic fungicide (mancozeb 3.33g/L) were used for field application. *Cercospora* leaf spot (CLS) and brown blotch cowpea diseases were obviously identified. The results indicated a significantly higher incidence ($p \leq 0.05$) of *Cercospora* leaf spot diseases on Sampea 20T at 8 and 10 WAS, with 52.47% and 55.20%, respectively. The incidence of BB disease at 8 and 10 WAS was also significantly higher ($p \leq 0.05$) in Sampea 20T at 50.80% and 63.90%, respectively, compared to other varieties. Abuja local (white) had significantly higher ($p \leq 0.05$) days to 50% flowering in the two seasons**.** The 100% garlic extract and mancozeb treatments significantly lowered ($p \leq 0.05$) CLS disease incidence and severity at 10 WAS on the field compared to other treatments and the control. This study highlights the effectiveness of environment-friendly garlic extract for managing leaf spot and brown blotch diseases in cowpeas, the possibility of including it in an integrated approach to cowpea disease management.

Keywords: Cowpea varieties, foliar fungal disease, management, plant extract and yield

1. Introduction
Cowpea (*Vigna unguiculata* [L.] Walp) stands as a crucial food legume, playing a vital role in the lives of millions in West and Central Africa. Cowpea is cultivated for sustenance, animal feed, and income generation (Mhango *et al*., 2013) [13]. Nigeria, with an estimated consumption exceeding 2.14 million metric tons, is the leading cowpea producer in Africa, supporting numerous subsistence farmers (FAOStat, 2017; Neya *et al*., 2015) [10, 15]. Often referred to as the "poor man's meat" (Boukar *et al*., 2010) [6], cowpea faces challenges from various fungal, viral, and bacterial diseases, resulting in significant yield reduction and seed deterioration during storage (Boukar *et al*., 2017) [7]. The impact of diseases on cowpea production is substantial, affecting different developmental stages and leading to diminished yields. Leaf spot diseases, particularly *Cercospora* leaf spot, curtail available leaves for photosynthesis, contributing to decreased productivity. Reported losses range between 20% and 40% due to leaf spot disease (Boa *et al*., 2014) [5]. Cowpea diseases also contribute to the global food production losses from plant diseases, estimated at 10% (Strange and Scott, 2015) [22]. Farmers commonly resort to informal seed acquisition methods, such as reusing seeds from previous seasons or exchanging seeds locally, which may inadvertently encourage the buildup of pathogens in seeds and soils, hindering the interruption of disease cycles (Rubyogo *et al*., 2007) [20]. This study assessed the incidence and severity of *Cercospora* leaf spot and brown blotch on cowpeas in four selected varieties and evaluated the effectiveness of garlic extracts against these diseases compared with a synthetic fungicide.

2. Materials and Methods

2.1 Study Area: The field experiment was conducted at the Teaching and Research Farm, Faculty of Agriculture, University of Abuja, situated along Airport Road, Nigeria, Federal Capital Territory, with coordinates of approximately 8.9817° N, 7.1811° E, and an elevation of 273 m asl. The experiment took place during both the dry and rainy seasons.

2.2 Source of Cowpea Seeds and Varieties: Cowpea seeds were obtained from the National Biotechnology Development Agency (NABDA). Four distinct varieties, each weighing 2 kg (two improved varieties, SAMPEA 14 IT199K-573-1-1, and SAMPEA 20T, as well as two local Abuja varieties in white and brown), were utilized for the experiment. The cowpea seeds exhibited characteristics such as an ovoid shape, brown to white coloration, a rough seed coat with brown helium, medium seed size, and maturity occurring at 70-75 days after planting.

2.3 Experimental Site and Design: The experimental site underwent clearing, removal of stumps, plowing, and harrowing. Experimental plots were demarcated using measuring tapes and pegs (60 cm high). The Randomized Complete Block Design (RCBD) was employed for two factorial experiments, with a total of 12 plots in the 2021 trial and 60 plots in the 2022 trial. Each plot measured 2 m by 3m, with inter-row and intra-row spacings of 0.75 m and 0.3m, respectively. A 1-meter gap was maintained between plots to prevent spray drift to adjacent areas, covering a total plot size of 40 m^2. The experiment involved five treatments and four cowpea varieties (SAMPEA 20T, SAMPEA 14, and two Abuja Local varieties in white and brown), randomly assigned. Two factorial experiments incorporated three levels of garlic extract treatment, one level of mancozeb (Synthetic fungicide) as the positive control, and de-chlorinated water application as the control. Each treatment had three replicates. Two weeks after planting, cowpea seedlings were thinned to two seedlings per hill, resulting in a plant population of 28 stands per plot. Manual weeding was performed at two, five, and nine weeks after planting.

2.4 Soil Sampling

An auger was used to collect soil samples at a depth of 0-30 cm from both the 12 main plots and the subplots of the 60 plots within the study area. The samples were thoroughly mixed to ensure homogeneity, and from these, sub-samples were taken for physicochemical analyses. The samples were sieved using a 2 mm mesh sieve to eliminate debris before conducting the analyses.

2.5 Sowing: The cowpea seeds were sown at spacing of 75 cm by 25 cm, about 2 cm deep and two per stand.

2.6 Laboratory Experiment

2.6.1 Isolation and Identification of *Cercospora* Leaf Spot Pathogen

For isolation and identification of the *Cercospora* leaf spot pathogen, leaf samples with necrotic spots were collected from the field trial. Sterilized sections were prepared and placed on Potato Dextrose Agar (PDA) for incubation. The resulting pure cultures were examined microscopically for fungal structures and identified using a compound microscope. Further confirmation was carried out at the Germplasm Health Unit of IITA in Ibadan.

2.6.2 Isolation and Identification of Brown Blotch Pathogen

Brown blotch isolates were obtained from infected cowpea pods, stems, and tissues. The collected pod materials underwent a sterilization process, and small pieces from infected pods were transferred to Petri dishes. The identification of *Colletotrichum* spp. was based on morphological characters observed under stereomicroscopes and compound microscopes. Pure isolates were obtained through sub culturing and stored for future use.

2.6.3 Purification of Colletotrichum Single Spore Isolates

To obtain fresh single-spore pure cultures of Colletotrichum isolates, a suspension was prepared and spread on new PDA plates. After incubation, single cells from each isolate were transferred onto new PDA plates, and after seven days of growth, pure cultures were stored at 20 °C until needed.

2.7 Source of Garlic Bulb and Preparation of Garlic Extract Formulation and Fungicide

White garlic was procured from a market, and an aqueous extract of garlic clove was prepared to inhibit *Cercospora* leaf spot and brown blotch pathogens. Fungicidal solutions, following recommended doses, were prepared using Mancozeb 80% WP. Spraying with these solutions occurred at 20-day intervals in the morning, while a control group without treatment received normal water spray.

2.7.1 The treatment layout was as follows

T_0 = Control.
T_1 =100% concentration (w/v) of garlic Extract.
T_2 =50% concentration (w/ v) of garlic extract.
T_3 = 25% concentration (w/v) of garlic extract.
T_4 = Mancozeb (synthetic fungicide) at 3.3g per ltr. of H_20
The treatments were applied at 4, 6, 8, and 10 weeks after sowing (WAS).

2.8 Parameter measured

- **Days to 50% flowering:** The number of days required for 50% of the plants to produce flowers was recorded per plot through regular field visits and visual observations.
- **Identification of Foliar Diseases of Cowpea:** *Cercospora* leaf spots and brown blotches of cowpea were identified using a manual method and confirmed at the Germplasm Health Unit of the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria.

2.8.1 Disease Incidence

Observations on the disease incidence were recorded three weeks after sowing. The count involved tallying the number of stands exhibiting disease symptoms in each plot. The data were then expressed as a percentage of the total number of stands in each plot, calculated using the following formula:

$$\text{Disease Incidence} = \frac{\text{Number of infected stands}}{\text{Number of total stands in the plot}} \text{ X } 100$$

2.8.2 Disease Severity

The degree of disease severity was assessed utilizing a scale ranging from 1 to 5. Nine randomly chosen plants were

observed and evaluated for the extent of disease manifestation on each. Scores were assigned based on the established scale, as outlined by Shock *et al*. (2008).

2.8.3 Disease Severity Scale

- 1 = no disease.
- 2 = 25% slightly infected.
- 3 = 50% moderate.
- 4 = 75% severe.
- 5= 90% very severe.

$$\text{Disease Severity} = \frac{\text{area of tissue affected}}{\text{Total area}}$$

$$\text{Severity Index} = \frac{0(x0)+1(x1)+2(x2)+\cdots+n(xn)}{x0+x1+x2\ldots.+xn} \text{ x } 100 \text{ (Maximum grade)}$$

Incidence and Severity of Brown Blotch in Cowpea: The assessment of brown blotch severity in cowpeas will be conducted using a scale ranging from 1 to 5, with corresponding descriptions as follows.

- 1 = No symptoms.
- 2 = Small spots of brown blotch on the stem.
- 3 = Coalescent spots on the stem.
- 4 = Coalescent spots with the presence of acervuli but the plant survives.
- 5 = Withered stem and plant death (Sereme, 1999) [21].
- The disease severity index will be determined utilizing the formula proposed by Allen *et al*. (1998) [8], expressed as.

Where
Xi = the note of disease for each plant.
ni = individual number of category Xi.
N = total number of the observed plant.
E (Xi) = scale range.

2.9 Yield Parameters

Number of Pods per Plant: The count of pods per plant was determined by enumerating the pods on ten randomly selected cowpea plants.

2.9.1 Hundred Seed Weight (g): Using an electronic scale, the weight of a hundred cowpea seeds from each plot was measured. This involved counting and weighing 100 seeds after the study for each treatment.

2.9.2 Yield per Plant: The yield per plant was determined by weighing the harvested cowpea from an individual plant strand using an electronic scale.

2.9.3 Yield per Hectare: The yield per hectare was calculated by extrapolating the pod yield from a plot to a hectare.

2.9.4 Seed Yield: After the study, seed yield per plot was obtained by weighing the seeds from each treatment and projecting the values per hectare.

2.10 Data Analysis: The collected data were subjected to descriptive statistics, including graphs, and some data were subjected to one way Analysis of Variance (ANOVA). Means were separated using Duncan's Multiple Range Test (DMRT) at a 5% level of significance. This was performed using the generalized model of Statistical Package for the Social Sciences (SPSS) version 26.

3. Results and Discussion

The varietal impact on the mean incidence of *Cercospora* leaf spot disease among four selected cowpea varieties in the 2021 and 2022 cropping seasons is illustrated in Table 1. There was a gradual increase in disease incidence among the four cowpea varieties from 6 Weeks after Sowing (WAS). Significantly higher incidence ($p \leq 0.05$) was observed on Sampea 20T at 8 and 10 WAS, with 52.47% and 55.20%, respectively, compared to 11.06% and 13.57% observed at 4 and 6 WAS. However, there was no significant difference ($p \geq 0.05$) in the incidence of *Cercospora* leaf spot disease among the four selected cowpea varieties at 6 WAS during the 2021 cropping season. In 2022, foliar diseases were reaffirmed with a 40% reduction in severity of CLS. This observation aligns with Nutsugah *et al*. (2007) [18], who highlighted that disease incidence levels vary based on geographical locations, districts, and ecological differences attributed to environmental conditions. Also Binagwa *et al*. (2023) [4] submitted that the influence of environment in mungbean development cannot be underrated. Baysah (2013) [3] observed that the interaction of the quality of cowpea seeds with varied varieties of cowpea significantly affected the growth and yield.

Table 1: Mean Disease Incidence of *Cercospora* Leaf Spot on Four Selected Cowpea Varieties during the 2021 and 2022 Cropping Seasons

Incidence CLS (%)				
Cowpea variety	**4WAS**	**6WAS**	**8WAS**	**10WAS**
Sampea 20T	11.06^{a}	13.57^{a}	52.47^{a}	55.20^{a}
Abuja local (white)	18.63^{ab}	20.83^{a}	28.37^{b}	31.23^{b}
Sampea 14	30.43^{b}	33.33^{a}	36.70^{b}	41.23^{b}
Abuja local (brown)	31.63^{b}	36.93^{a}	42.27^{b}	47.97^{b}

Means followed by the same letter(s) within a varietal column are not significantly different using Duncan Multiple Range Test at 5% level of probability. WAS = Weeks after Sowing

Figure 1 depicts the mean severity of *Cercospora* leaf spot disease on four chosen varieties of cowpea in the 2021 and 2022 cropping season. Results revealed a gradual increase in disease severity among the four cowpea varieties 6 WAS. Significantly higher severity percentages ($p \leq 0.05$) were noted for Sampea 20T at 8 and 10 WAS, recording 53.33% and 71.00%, respectively. However, Abuja local (white) exhibited the least reduction in disease severity at 8 WAS (39.00%) but later increased to 50.70% at 10 WAS. Notably, at 10 WAS, there was a significant difference in disease severity among the four cowpea varieties. This variance could be attributed to varietal differences. Narayana (2021) [14] emphasized that disease severity levels vary based on geographical locations, districts, and ecological differences due to variations in environmental conditions.

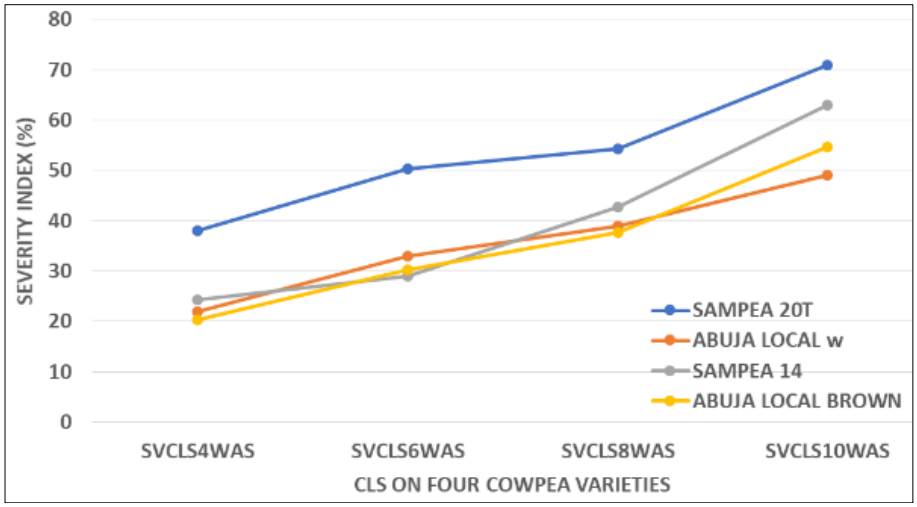

Fig 1: Mean severity of *Cercospora* leaf spot on four cowpea varieties during the 2021 and 2022 cropping seasons

4. The Disease Incidences and Severity of Brown Blotch (BB) on Four Selected Cowpea Varieties during the 2021 and Cropping Seasons: The mean incidence and severity of Brown Blotch (BB) disease on four selected cowpea varieties in the 2021and 2022 cropping seasons are detailed in Table 2. Throughout the two cropping seasons, there was a gradual increase in the disease incidence among the four selected cowpea varieties. The disease incidence of BB in Sampea 20T at 8 and 10 WAS significantly higher ($p \leq 0.05$) at 50.80% and 63.90%, respectively, compared to other varieties. However, Abuja local (brown) and Sampea 14 exhibited a reduction, recording 20.63% at 10 WAS.

The disease severity of BB in Sampea 20T and Abuja local (brown) at 8 WAS was significantly higher ($p \leq 0.05$) at 48.33% and 26.00%, respectively, compared to other varieties. At 10 WAS, Sampea 20T had the highest disease severity at 51.00% compared to other varieties. This observation aligns with the report of Nwofia (2015) [16] that cowpea genotypes differs in their attributes especially during flowering and developmental stages.

Table 2: Disease incidence and severity of Brown Blotch on Four Selected Varieties of Cowpea during the 2021 Cropping Season

Cowpea Varieties	Inc BB 8 WAS	Inc BB 10 WAS	SV BB 8 WAS	SV BB 10 WAS
Sampea 20T	50.80^{a}	63.90^{a}	48.33^{a}	51.00^{a}
Abuja Local (white)	17.67^{b}	47.93^{b}	19.00^{a}	27.00^{b}
Sampea 14	19.57^{b}	20.63^{c}	26.00^{b}	30.67^{b}
Abuja local (brown)	20.23^{b}	23.43^{c}	18.00^{b}	22.00^{b}

Means followed by the same letter(s) within a varietal column are not significantly different using Duncan Multiple Range Test at a 5% level of probability. WAS = Weeks after sowing

5. Mean Days to 50% flowering and Yield of Four Varieties of Cowpea during the 2021 and 2022 Cropping Seasons

Abuja local (white) had significantly higher mean Days to 50% flowering in the 2021 and 2022 growing seasons (Table 3). The data on the number of pods per plant indicated that Sampea 14 had the highest pod per plant (37.62 kg) while Abuja local (Brown) recorded the lowest pod per plant (30.33 kg) during the two cropping seasons.

In terms of 100-seed weight, Sampea 20T yielded the highest (30.31 kg), while Abuja local white produced the lowest (19.00%) in 2021. The seed yield analysis over the two cropping seasons showed that in 2021, Abuja local (Brown) achieved the highest yield (700.63 kg), whereas Sampea 20T had the lowest yield (500.33 kg/ha). Varietal differences observed in this study were in line with the report of Narayana (2021) [14] who reported that cowpea genotypes differs in their attributes especially during flowering and developmental stages.

Table 3: Days to 50% flowering and yield of selected varieties of cowpea during the 2021 cropping

Cowpea variety	Days to 50% flowering	No of pod/plant	100 seed weight (g)	Seed yield (Kg/ha)
Sampea 20T	86.20^{b}	32.41^{a}	30.31^{a}	500.33^{b}
Abuja local (White)	90.67^{a}	23.40^{b}	25.00^{a}	690.23^{a}
Sampea 14	88.08^{b}	37.62^{a}	27.20^{a}	511.33^{b}
Abuja local (Brown)	76.90^{bc}	30.33^{a}	19.00^{b}	700.63^{a}

Means followed by the same letter(s) within a varietal column are not significantly different using Duncan Multiple Range Test at a 5% level of probability. WAS = Weeks after sowing

6. Effects of Garlic Extract and Mancozeb on Incidence and Severity of *Cercospora* Leaf Spot of Cowpea on Sampea 20T during the 2022 Cropping Season

The impact of garlic extract on the incidence and severity of *Cercospora* leaf spot on Sampea 20T during the 2022 cropping season is detailed in Table 4. In the 2022 cropping season, the 100% garlic extract and mancozeb treatments significantly lowered ($p \leq 0.05$) CLS disease incidence at 4 and 10 WAS compared to treatments and the control. Also, 100% garlic extract and mancozeb significantly reduced ($p \leq 0.05$) the disease severity at 8 and 10 WAS, with values of 22.94 and 23.94, respectively. In a contrast, both

incidence in untreated plants (control) at 8 and 10 WAS were significantly higher ($p \leq 0.05$) at 34.80% and 39.94%, respectively, compared to other treatments. Such variation) n observed in the efficacy of the treatments agrees with the reports of Golakiya *et al*. (2018) [11] and Dania and Omidiora (2019) [9] that that different fungicidal treatments can result in an overall reduction in disease index and an increase in yield among cowpea plants.

Table 4: Effects of Garlic extract and mancozeb on incidence and severity of *Cercospora* leaf spot of cowpea on Sampea 20T during the 2022 cropping season

	Incidence (weeks)				Severity (weeks)		
Treatment Garlic conc. %	**4WAS**	**6WAS**	**8WAS**	**10WAS**	**6WAS**	**8WAS**	**10WAS**
100	11.30^{c}	16.13^{b}	21.27^{bc}	17.94^{c}	27.37^{c}	22.94^{b}	22.94^{b}
50	21.04^{a}	16.14^{ab}	27.40^{ab}	24.84^{b}	35.00^{bc}	32.53^{ab}	32.64^{a}
25	16.24^{ab}	17.27^{b}	27.64^{ab}	27.37^{ab}	39.94^{ab}	38.47^{a}	33.64^{a}
Mancozeb (3.33g/L)	5.94^{c}	13.60^{b}	18.57^{c}	18.37^{c}	29.20^{bc}	23.94^{b}	23.94^{b}
Control	18.20^{a}	25.01^{a}	34.80^{a}	32.40^{a}	41.04^{a}	39.23^{a}	39.24^{a}

Means followed by the same letter(s) within a varietal column are not significantly different using Duncan Multiple Range Test at a 5% level of probability. WAS = Weeks after sowing

7. Days to 50% Flowering and Yield of Cowpea Varieties Affected by Different Concentrations of Garlic Extract during the 2022 Cropping Season

Table 5 presents the impact of different concentrations of garlic extract on days to 50% flowering and the yield of cowpea varieties during the 2022 cropping season. There was no significant difference ($p \geq 0.05$) in the days to 50% of the cowpeas treated with the garlic extracts, mancozeb and the control. The 100% garlic extracts and Mancozeb exhibited relatively higher number of pods per plant (62.57 and 64.44, respectively). The 100 seed/weight (kg and seed/yield/ha (kg) of the cowpeas treated with the 100% garlic extracts and mancozeb were significantly higher ($p \leq 0.05$) than other treatments. Nwagboso *et al*. (2024) [17] and Kukreja *et al*. (2018) [12] confirmed and submitted that seed treatments and field application of pesticides can significantly improve the growth and yield of cowpea. Awurum and Enyiukwu (2013) [2] specifically confirmed and reported the efficacy of the seed-dressing of *Carica papaya* and *Piper guineense* extracts on the germination and against the seed-borne fungi associated with cowpea seeds. The success of efficacy of *Jatropha curcas* leaf extract in the control of brown blotch disease of cowpea was also reported by Onuh *et al*., 2008 [19].

Table 5: Days to 50% flowering and yield of sampea 20T cowpea variety as affected by different concentrations of Garlic extract and mancozeb during the 2022 cropping season

Treatment Garlic conc.%	**Days to 50% flowering**	**No pod/plant**	**100 seed/weight(kg)**	**Seed/yield/ha(kg)**
100	66.64	62.57	20.67^{a}	1341.00^{a}
50	64.24	52.20	18.00^{b}	1041.00^{a}
25	66.64	53.44	18.43^{b}	940.67^{b}
Mancozeb (3.33 mg/L)	69.04	64.44	20.34^{a}	1138.33^{a}
Control Significant	74.24 NS	51.60 NS	16.67^{b*}	804.00^{b*}

Means followed by the same letter(s) within a varietal column are not significantly different using Duncan Multiple Range Test at a 5% level of probability. NS = Not significant. * = Significant WAS = Weeks after sowing

8. Conclusion

Significantly higher incidence ($p \leq 0.05$) of *Cercospora* leaf spot diseases was observed on Sampea 20T at 8 and 10 WAS, with 52.47% and 55.20%, respectively, compared to 11.06% and 13.57% observed at 4 and 6 WAS. The incidence of BB disease at 8 and 10 WAS significantly higher ($p \leq 0.05$) in Sampea 20T at 50.80% and 63.90%, respectively, compared to other varieties. Abuja local (white) had significantly higher days to 50% flowering in the two seasons**.** The 100% garlic extract and mancozeb treatments significantly lowered ($p \leq 0.05$) the CLS disease incidence and severity at 10 WAS compared to treatments and the control.

This study highlights the effectiveness of environment-friendly garlic extract for managing leaf spot and brown blotch diseases in cowpeas. The 100% concentrations of garlic extract, demonstrated a significant control over the *Cercospora* leaf spot and brown blotch of cowpea indicating its possibility in including it into an integrated approach to cowpea disease management.

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