



Identification and Characterization of Fungi Associated with Spots Symptoms On Cowpea Pod in Zaria, Kaduna State, Nigeria

Ibrahim H¹, Dangora D. B², Tahir, S. M¹., Aminu, A. M³., Haruna, M⁴ and Yakubu, S¹

¹Department of Biological Sciences, Kaduna State University, Kaduna State, Nigeria

²Department Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

³Department of Science Laboratory Technology, Nuhu Bamalli Polytechnic, Zaria. Kaduna State.

⁴Department of Biology, Umaru Musa Yar' adua University Katsina, Katsina State. Nigeria

Corresponding Author's Email: halima.ibrahim@kasu.edu.ng. Phone No: 07034502167

Received on: September 2019

Revised and Accepted on: November 2019

Published on: December 2019

ABSTRACT

Fungi associated with spot symptoms on cowpea pods (*Vigna unguiculata* L Walp) were studied. Commercially prepared Potato Dextrose Agar (PDA) it was prepared according to the manufacturer's instructions; streptomycin sulphate was added to the media. Pieces of spots from cowpea pods were surface sterilized in 0.1% mercury chloride for 3 minute after which they were rinsed 3 times in sterile distilled water and plated aseptically in petri dishes containing PDA. They were incubated at room temperature and observed for the growth of pathogens after every 24 hours for 7 days. The isolated pathogens were then sub cultured on fresh PDA and used for identification. A total of ten different types of spot symptoms reveal eight different fungi. Fungi isolated include; *Rhizoctonia solani*, *Fusarium equisetum*, *Macrophomina*, *Sclerotium*, *Septoria*, *Cladosporium*, *Bispora* and *Mycogone* spp. Out of the eight fungi isolated *Rhizoctonia solani* has the highest percentage occurrence of 38.5% followed by *Sclerotium* sp which has a percentage occurrence of 15.4%, the remaining six fungi isolated have the same percentage occurrence of 7.7%.

Key Words; Fungi, Cowpea, Potato Dextrose Agar (PDA) and Spot Symptoms

1.0 INTRODUCTION

Cowpea (*Vigna unguiculata*) L. Walp belonging to the family Fabaceae is an annual legume; it is commonly referred as southern pea, black-eye pea, Crowder pea. The history of cowpea dates back to ancient West Africa cereal farming, five to six thousand years ago where it was closely associated with the cultivation of sorghum and pearl millet (Davis *et al.*, 1991).

Fungi Pod diseases of cowpea include; brown blotch caused by *Colletotrichum capsici* and *C. truncatum* (Emechebe and Florin, 1997, Singh and Allen, 1979, Alabi, 1994), lambs tail pod rot induced by *Choanephora curcubitarum* (Adegbite and Amusa, 2008, Singh and Allen, 1979), *Phomopsis* pod spot induced by *Phomopsis longicola* (Roy and Ratnayake, 1997)

and scab induced by *Sphaceloma* sp (Singh and Allen, 1979, Allen *et al.*, 1996).

The aim of this study is to isolate and identify fungi associated with leaf spot symptoms on cowpea pods. The information obtained will enhance the understanding of diversity and epidemiology of fungi inducing leaf spot on cowpea in Zaria area. The information can be used by plant breeders and Quarantine stations.

2.0 MATERIALS AND METHODS

Cowpea pods showing leaf spot symptoms were collected from the farmer's field in samaru study area Latitude 11° 11 N and 07° 38 E. Potato Dextrose Agar was prepared as described by the manufacturer's instructions (Oxoid, England) and used throughout the study.

Lesions were cut off from infected plants with sterile surgical blades. All lesions were surface sterilized in 0.1% mercury chloride (HgCl) for three minutes and rinsed three times with sterile distilled water. Lesions were plated on Petri dishes (four lesions per plate) containing Potato Dextrose Agar (PDA) amended with streptomycin sulphate (1.5g/l) to inhibit bacterial growth. Each sample was in duplicate. The Petri dishes were incubated at room temperature. Observation was made daily for seven days, for the presence of colonies of fungi. The fungi colonies were sub cultured on fresh PDA for subsequent identification (Mungo, 1996). Identification was carried out from pure cultures. Fungi were identified by macroscopic characters such as colony texture, color, and its cultural characteristics on PDA. Fungi were also identified by microscopic characters such as conidiophores morphology and shape. Confirmation of the fungal identification was done using identification key (Barnett and Hunter, 1999), they were also compared with already described isolates in the department of crop protection diagnostic unit Institute of Agricultural Research, ABU Zaria. Isolates were send to Commonwealth Agricultural Bureaux international (CABI), UK for further confirmation.

Percentage occurrence of fungi isolated was determined using the formula below (Gonzalez, 1995).

$$\text{Percentage occurrence} = \frac{\text{frequency of occurrence of fungi}}{\text{Total number of fungi}} \times 100$$

Total number of fungi

3.0 RESULTS

Ten different types of spot symptoms were as shown in Table 1 and Plates 1 and 2. Circular Dark brown spot on cowpea pod that leads to isolation of *Rhizoctonia solani*. Spot with grey center brown margin on cowpea pod, *Sclerotium* sp. was isolated from it. Mixed infection: *Septoria* sp. was isolated from the white center brown margin and *Cladosporium* sp. was isolated from brown spots also at the sutures. Oval white center brown margin on cowpea pod. *Rhizoctonia solani* was isolated. Raised circular dark brown lesions which lead to isolation of *Rhizoctonia solani*. Circular brown lesions on cowpea pod. *Bispora* sp. was isolated. Coalesced dark brown lesion, *Macrophomina* sp. was isolated. Lesions along sutures having grey center dark brown margin, *Rhizoctonia solani* and *Mycogone* sp. were isolated. Lesions on cowpea peduncles with grey centre brown margin, *Rhizoctonia solani*, *Sclerotium* sp. and *Fusarium equiseti* were isolated. Purplish brown lesions on cowpea pod, no fungus was isolated from it.

Table 1: Fungi Isolated from Different Leaf Spot Symptoms on (*V. unguiculata*) Pods

Description of symptoms	Fungi isolated
Circular dark brown spot	<i>Rhizoctonia solani</i>
Grey center brown margin	<i>Sclerotium</i> sp
Mixed infection;	
Grey center brown margin.	<i>Septoria</i> sp
Brown coalesced lesion	<i>Cladosporium</i> sp
Oval spot with white center and brown margin	<i>Rhizoctonia solani</i>
Raised circular dark brown spot	<i>Rhizoctonia solani</i>
Circular brown spot	<i>Bispora</i> sp
Coalesced dark brown lesions	<i>Macrophomina</i> sp
Lesions along sutures having grey center brown margin	<i>Rhizoctonia solani</i> <i>Mycogone</i> sp
Brown lesions with grey center on peduncle	<i>Rhizoctonia solani</i> <i>Sclerotium</i> sp <i>Fusarium equisetum</i>

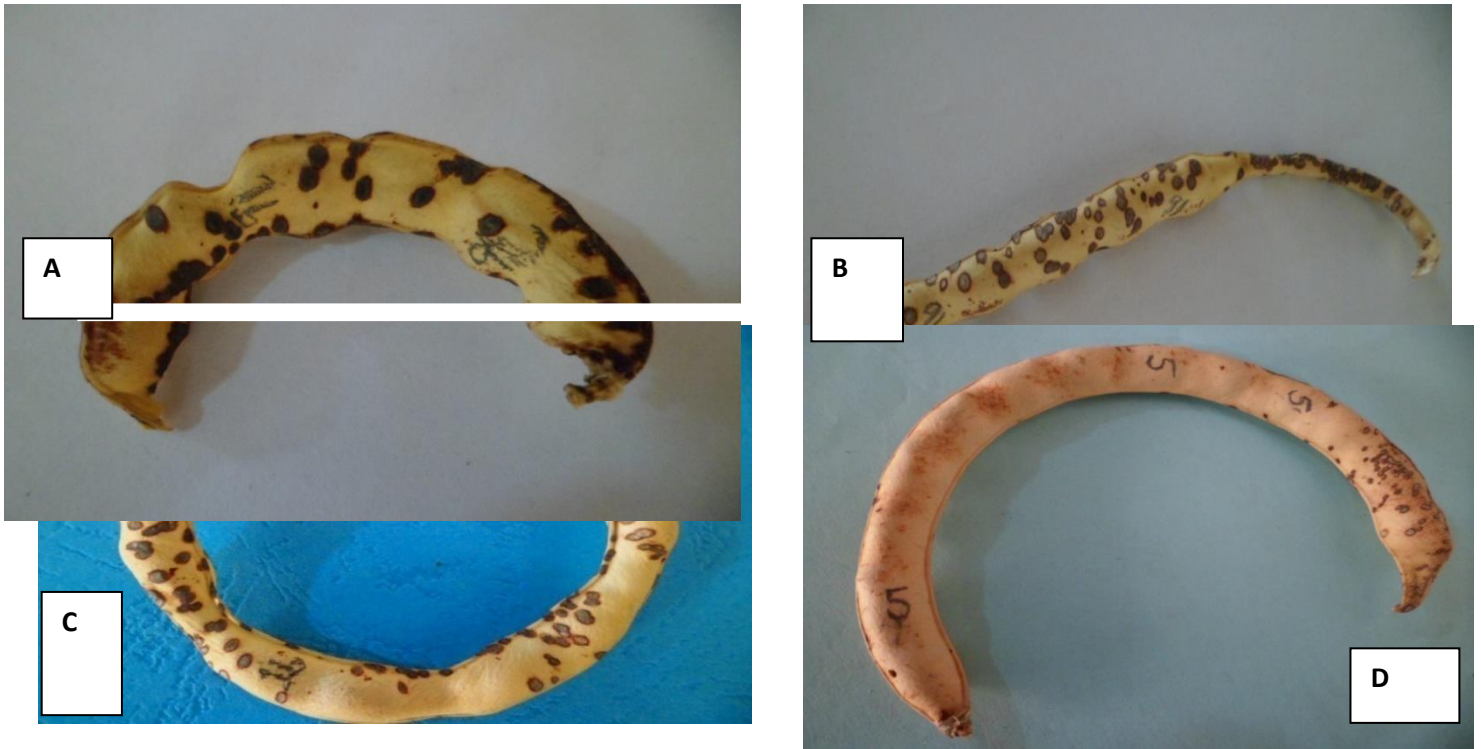


Plate 1: Cowpea pods showing different spots symptoms I. A= Circular Dark brown spots on cowpea pod. *Rhizoctonia solani* was isolated from this spot. B= Grey center brown margin lesions on cowpea pod. *Sclerotium* sp. was isolated from it. C: mixed infection, Ca= *Septoria* sp. was isolated from the white center brown margin Cb= *Cladosporium* sp. was isolated from brown spots also at the sutures. D= Oval white center brown margin spots on cowpea pod. *Rhizoctonia solani* was isolated.





Plate 2: Cowpea pods showing different spots symptoms II.A: Raised circular dark brown lesions which lead to isolation of *Rhizoctonia solani*. B: Circular brown lesions on cowpea pod. *Bispora* sp. was isolated. C: Coalesced dark brown lesion. *Macrophomina* sp. was isolated. D: Lesions along sutures having grey center dark brown margin. *Rhizoctonia solani* and *Mycogone* sp. were isolated. E: Lesions on peduncles with grey centre brown margin. *Rhizoctonia solani*, *Sclerotium* sp. and *Fusarium equiseti* were isolated.

From cowpea pods, eight different fungi were isolate. *Fusarium equiseti* macroscopic character show upper surface white, reverse pink, microscopic character shows macroconidia with septation. *Sclerotium* sp. macroscopic character shows upper surface white with sclerotia, reverse white, microscopic character shows non septate hyphae with clamp connection. *Rhizoctonia solani* macroscopic character shows upper surface grey with pink wave edge, reverse dark brown, microscopic character shows branched hyphae with point of septation. *Septoria* sp.

macroscopic character upper surface white, reverse white. Microscopic character shows conidia hyaline elongated with several cells. *Cladosporium* sp. macroscopic character shows upper surface white reverse black, microscopic character shows conidia ovoid dark two celled. *Mycogone* sp. macroscopic character shows upper surface grey, microscopic character shows branched conidiophores with globose conidia. *Bispora* sp. macroscopic character shows upper surface grey, reverse brown, microscopic character shows conidiophores dark and short. *Macrophomina* sp. macroscopic character shows upper surface grey, reverse black. Microscopic character shows pycnidia with hyphae. These are shown in Table 2 and Plates 3 to 10.

Percentage occurrence of fungi isolated from cowpea pods shows *R. solani* have the highest percentage occurrence of 38.5 % followed by *Sclerotium* sp. with 15.4%. *Cladosporium* sp. with *Macrophomina*, *Septoria*, *Fusarium equiseti*, *Bispora* and *Mycogone* spp. have the lowest percentage occurrence of 7.7 %. These are shown in Fig 1.

Table 2: Identification of Fungi Isolated from Cowpea

Fungi	Macroscopic character	Microscopic character
<i>Fusarium equisetum</i>	Upper surface white cottony, Reverse pink.	Macroconidia with septation and Microconidia.
<i>Sclerotium</i> sp	Upper surface white with sclerotia, Reverse white.	Non septate hyphae with clamp connection.
<i>Rhizoctonia solani</i>	Upper surface grey with pink wave edge, Reverse dark brown.	Branched hyphae with point of septation.

<i>Septoria</i> sp	Upper surface white, Reverse white.	Conidia hyaline elongated with several cells.
<i>Cladosporium</i> sp	Upper surface white cottony, Reverse black.	Conidia ovoid dark two celled, with dark conidiophores.
<i>Mycogone</i> sp	Upper surface grey cottony, lower surface grey.	Branched conidiophores with globose conidia.
<i>Bispora</i> sp	Upper surface grey. Reverse brown.	Conidiophores dark and short.
<i>Macrophomina</i> sp	Upper surface grey, cottony, reverse black.	Pycnidia

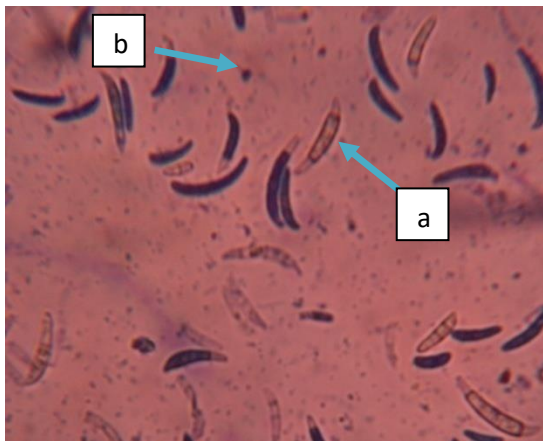


Plate 3: *Fusarium equisetum*
Macroconidia with septation,

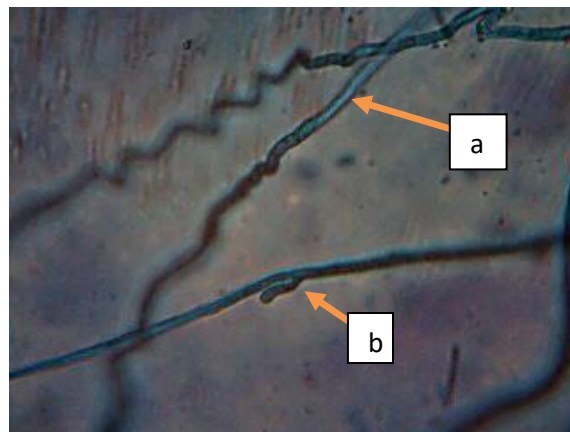


Plate 4: *Sclerotium* sp.
a= Non septate hyphae, b= Clamp connection. b=Microconidia. a=

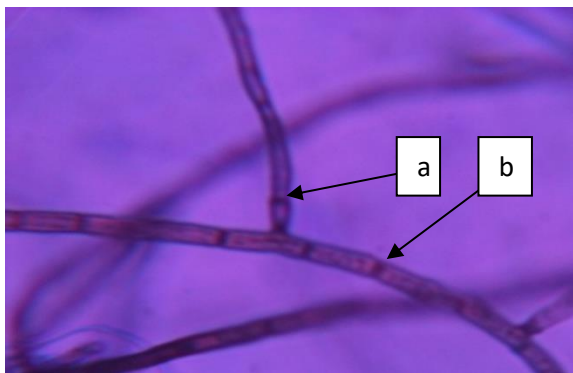


Plate 5: *Rhizoctonia solani*.
a=Branched hyphae, b= point of septation.



Plate 6: *Septoria* sp.
Conidia hyaline elongated with several cells.



Plate 7: *Cladosporium* sp.
 a= Conidia ovoid dark two celled
 b= dark conidiophores

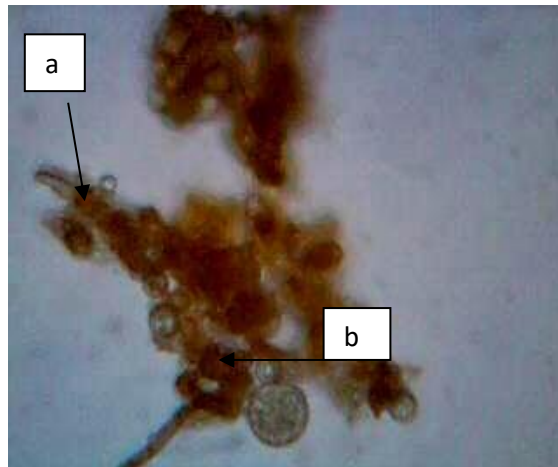


Plate 8: *Mycogone* sp.
 a= Branched conidiophores
 b= globose conidia

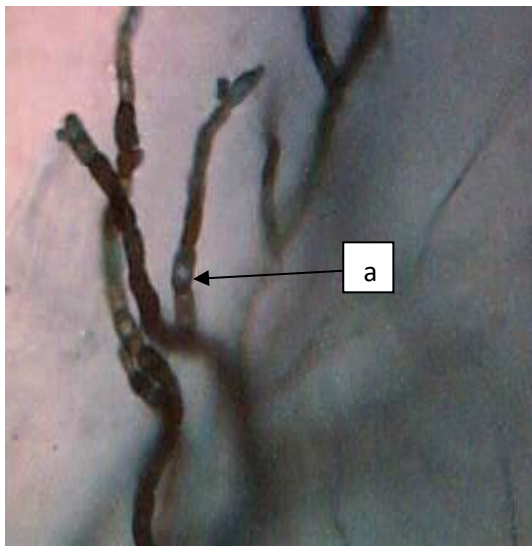


Plate 9: *Bispora* sp.
 a= conidiophores dark and short

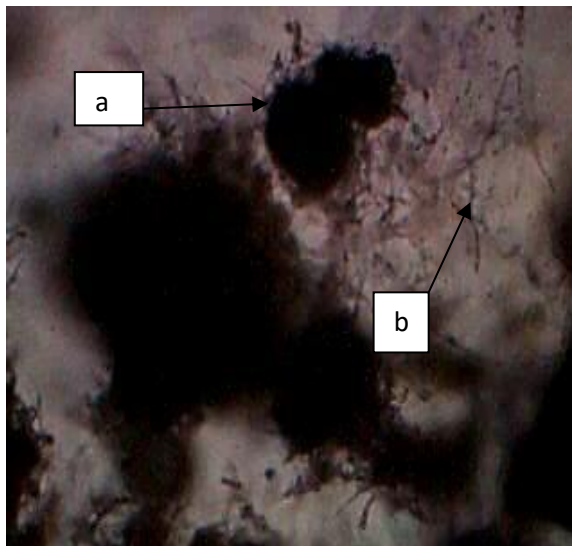


Plate 10: *Macrophomina* sp.
 a= Pycnidia, b=Hyphae

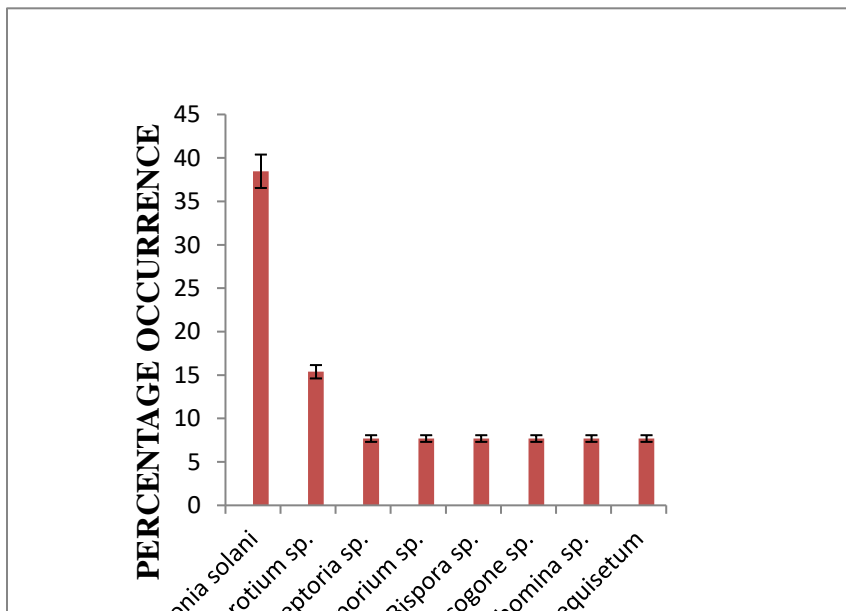


Fig 1: Percentage Occurrence of Fungi Isolated**4.0 DISCUSSION**

A total of fourteen different types of leaf spot symptoms were observed in this study. *Rhizoctonia solani* produced five different types of symptoms on cowpea pods and peduncle. Adebite and Amusa, 2008 also reported similar symptoms on cowpea pods. *R. solani* also produce lesions along sutures having grey Centre, oval spots with white centre with brown margin and spots on peduncle with grey center brown margin. These three types are being reported for the first time in Northern Nigeria. The difference in the symptoms may be attributed to the causal organisms and stage of development. The difference may also be due to varietal differences. In this study *R. solani* was not found to infect any other part other than the pod and peduncle, though young stems and leaves have been reported (Emechebe *et al*, 1980; Allen and Lenne, 1998; Adebite and Amusa, 2008). *Sclerotium* sp., *Macrophomina* sp., and *Fusarium equisetum* were isolated from cowpea pod and peduncle, the difference in symptoms might be due to varietal difference that is only restricted to pod and peduncle, although it has been reported to cause diseases which infect cowpea (Singh and Allen, 1979; Emechebe *et al*, 1980; Singh *et al*, 1990, Abdon *et al*, 1980; Adejumo and Ikotun, 2003; Emechebe and Lagoke, 2002; Amusa *et al*; 2007). Isolation of *Septoria* sp. is in line with the report of (Singh and Allen, 1979; RPD (1999) and Ojiambo *et al* (2007) which reported *Septoria* leaf spot to produce circular to angular grayish white center with dark margin. *Cladosporium* sp was isolated for the first time on cowpea pods in Zaria. (Emechebe *et al*, 1980; Howard and David, 2007) reported symptoms of *Cladosporium* leaf spot on eggplant and pepper.

5.0 CONCLUSION

Rhizoctonia solani, *Cladosporium* sp, *Septoria* sp, *Sclerotium* sp., *Macrophomina* sp and *Fusarium equisetum* were found to induce spot symptoms on cowpea pod and peduncle in Zaria, Nigeria.

REFERENCES

- Abdon, Y. A. Hassan S. A. Abbas H. K. (1980). Seed transmission and pycnidial formation in sesame wilt disease cause by *Macrophomina phaseolina* Maubi. *Ashby. Agricultural Research Rev.* 52: 63-69.
- Adebite, A. A. and Amusa, N. A. (2008). The major economic field diseases of cowpea in the humid agro-ecologies of south-western Nigeria. *African Journal of Biotechnology.* 7 (255): 57-64.
- Adejumo T. O. Ikotun, T. (2003). Effect of planting date on incidence and severity of leaf smut of cowpea in northern Nigeria. *Moor. Journal of Agricultural Research.* 4: 106-110.
- Akram, W. Ajum, T. Ahmad, A. and Moeen, R. (2014). First report of *Curvularia lunata* causing leaf spots on *Sorghum bicolor* from Pakistan. *Abstract plant diseases.* 98(7).
- Amusa, N. A. Oechukwu, R. U. and Akinfenwa, B. (2007). Reactions of cowpea to infection of *Macrophomina phaseolina* isolates from leguminous plants in Nigeria. *African Journal of Agricultural research.* 2(3):073-075.
- Alabi, O. (1994). *Epidemiology of cowpea brown blotch induced by Colletotrichum capsici and assessment of crop losses due to the diseases.* Ph.D Thesis. Ahmadu Bello University, Zaria, Nigeria. P. 95
- Allen, D. J. Ampofo, J. K. O. and Wortmann. C. S. (1996). *Pest, Diseases, and Nutritional Disorders of the common Bean in Africa.* A Field Guide. International center for Tropical Agriculture. Publication no. 260. Pp
- Allen, D. J. and Lenne, J. M. (1998). Diseases as constraints to production of legumes in agriculture. Pages 40–61 In: *The pathology of food and pasture legumes*, edited Allen, D.J. and Lenne, J.M. CAB International, Wallingford, UK.
- Badifu, G. I. O and Ogunsina, A. O. (1991). Chemical composition of kernels from some species of Curcubitaceae grown

- in Nigeria. *Plant food Human nutrition*. 4135-4144.
- Barnett, H.L. and B.B. Hunter, (1999). *Illustrated Genera of Imperfect fungi*. 4th edition. The American phytopathological Society St. Paul, Minnesota, U.S.A. Pp 218.
- Davis, D. W. Oelke, E. A, Oplinger, E. S. Doll, J. D. Hanson, C. V. and Putnam, D. H. (1991). *Cowpea*. University of Wisconsin-Madison. United States. Pp 1-10.
- Emechebe, A. M, Erinle, I. D, Bos, W. S Tyagi P. D, Sundaram, N. V, Manzo, S. K and Subbarayudu, S. (1980). A check list of diseases of crops in the savanna and semi-arid areas of Nigeria. Edited by Emechebe, A. M. *Samaru Miscellaneous paper* 100. Institute Agricultural Research Samaru. Ahmadu Bello University Zaria Nigeria. Pp 1-35.
- Emechebe A. M. and Lagoke S. T. O. (2002). Recent advances in research on cowpea diseases. Pp94-123 In: *Challenges and Opportunities for enhancing sustainable cowpea production*. edited by Fatokun, C. A Tarawali, S.A Kormawa, P. M.
- Emechebe, A. M. and Florini, D. A. (1997). Shoot and pod diseases of cowpea induced by fungi and bacteria. In: *Advances cowpea Research center*. Singh.B.B and Mohan. D. R (Eds), pp 176-192.
- Fajemisin, J. M. and Okuyemi, O. (1976). Fungicidal control of *Curvularia* leaf spot of maize. Abstract. Vol. 22(2)
- Gonzalez, M., Puol, M., Metraux, J. P., Gonzalez-Garcia, V., Bolton M. D. and Borrás- Hidalgo O. (2011). Tobacco leaf spot and root rot caused by *Rhizoctonia solani*. *Molecular plant pathology*. 12(3): 209-16.
- Gruben, G. J. H. and Benton, O. A. (2004). Plant resources of tropical Africa 2: vegetables protection foundation, wagen inen. <http://en.m.wikipedia.org/wiki/pant.resourcsoftropicalafrica>. 27-10-2014.
- Howard, F. S. and David, H. G. (2007). *Cladosporium leaf mold, egg plant, pepper and Tomato*. High plains IPM guide, a cooperative effort of the university and Montana State University. <http://wiki.bugwood.org/uploads/cladosporiumleafmoldeggplantpeppertomato.pdf> 11-11-2014.
- Iken, J. E. and Amusa, N. A. (2004). Maize research and production in Nigeria. *African Journal of Biotechnology*. 3(6): 302.
- Kayode, A. A. A. and Kayode, O. T. (2011). Some medicinal values of *Telfairia occidentalis* ; A review of American journal of Biochemistry and molecular biology, 1: 30-38.
- Marley, S. P. (2013). *Mycology and Fungal Diseases*. Diligent publishers limited, Kauri Road Kawo Kaduna, Nigeria. Pp 108-287.
- Mungo, C.M. (1996). Biology and Epidemiology of *Sphaceloma* sp. The pathogen of cowpea scab. Ph.D Thesis. Ahmadu Bello University, Zaria, Nigeria.
- Nwufu, M. I. and Atu, U. G. (1987). Survey of the diseases and pests of ugu (*T. occidentalis*) in south eastern Nigeria. *Proceeding of the 9th annual conference of Horticulture society of Nigeria*. Pp 43-45.
- Odiaka, N. I. Akoroda, M. O. and Odiaka, E. C. (2008). Diversity and Production methods of fluted pumpkin (*T. occidentalis* Hool F). Experience with vegetable farmers in Makurdi, Nigeria. www.ajol.info/index.php/ajb/article/view/10-10-2014.
- Ojiambo, P.S. Scherm, H. and Brannen, P. M. (2007). Temporal dynamics of *Septoria* leaf spot of blueberry and its relationship to defoliation and yield. *Plant management Network*. www.plantmanagementnetwork.org/pub/php/symposium/methus/septoria 13-09-2014.
- Olaoye, G. (2009). Evaluation of new generations of maize streak virus (MSV) Resistant varieties for grain yield, Agronomic potential and adaptation tom a southern guinea savanna. *Ecology of Nigeria journal of tropic Agriculture, Environment and Extension*. 8:104-109.
- Olson, J. (1978). Anthracnose and other common leaf diseases of deciduous shade trees.

- Oklahoma cooperative extension service. EPP-7634.
<http://pods.dasnr.okstate.edu/docushare/dsweb/get/document.10-11-2014>.
- Report on Plant Disease (RPD) (1999). Early blight, *Septoria* leaf spot and anthracnose Ripe rot of Tomato. (NO. 908). Department of Crop Science University of illinois at Urbana-Champaign. <http://illinois.edu/diseases/rpds/908.pdf>. 15-08-2014.
- Roy, K.W. and Ratnayake, S. (1997). First report of *Phomopsis longicolla* infection of cowpea pods and seeds in Mississippi. *Plant Disease*. 81(6): 693.
- Singh, S. R. And Allen, D. J. (1979). *Cowpea pest and Diseases*. International institute of Tropical agriculture, Ibadan, Nigeria. Manual series no. 2. Pp 40-61.
- Singh, B. B. and Singh, S. R. (1990). Breeding for bruchid resistance in cowpea. Pages 212-228. In: *Bruchids and Legumes: Economics, Ecology, and Co-evolution*. K. Fujii, A. M. R. Gatehouse, C. D. Jonson, R. Mitchel, and Yoshida, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.
- Singh, B. B. Mai kodomi, Y. and Terao, T. (1990). A Simple screening method for drought tolerance in cowpea. *Indian Journal of Agronomy* 59 (2):211 – 220.
- Staff, W. (2011). CN food fact sheet fluted pumkin leaf (*Telfairia occidentalis*). <http://connectnigeria.com/articles/foodfactflutedpumin-leaf>. 15-10-2014.
- Tindal, H. D. (1975). Commercial- vegetable growing. Oxford university press, London. <http://acsedu.co.uk/courses/crops/commercial-organic-vegetable-growing>. 28-09-2014.
- Toit, L. and Vernon W. M. (2006). Spinach diseases: field identification, implication and management practices. Organic seed alliance spinach seed field day, sequim, WA. *International spinach conference*.
- Udo, S. E. Madunagu, B. E. Umana, E. J. and Markson, A. A. (2008). Effect of *Colletotrichum* leaf spot disease on growth parameters of *Colocynthis citrillus* and *Cucurbita pepo*. *International Journal of Natural and Applied science* 2(2):40-44.
- Williams, C. N. Uzo, J. O. and Peregrine, W. T. H. (1991). Vegetable promotion in the tropics; *international Tropical Agriculture series*. Longman scientific and Technical Essex.