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Occurrence of *Pythium aphanidermatum* on cowpea (*Vigna unguiculata* (L.) Walp) in Nigeria

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ABSTRACT

Objective: To investigate the occurrence, distribution, and pathogenicity of *Pythium aphanidermatum* (Edson) Fitzp.) on cowpea (*Vigna unguiculata* (L.) Walp) in Kogi State, Nigeria.

Methodology and results: Four Local Government Areas were surveyed for the disease incidence with emphasis on Dekina, Abejukolo, Ajaaka and Ankpa towns between 2005 and 2007. Three cowpea cultivars i.e. Local variety (gwana), Ife-brown and IAR-16 were used for the study. These were cultivated on ridges or in potting bags and kept in a screen house for pathogenicity studies. *P. aphanidermatum* was isolated from root lesions of cowpea and contaminated soils and was identified based on morphological characters and pathogenicity tests. Disease incidence was highest in Dekina followed by Ajaaka and Abejukolo. Among the cowpea cultivars, Ife-brown was the most resistant (with the least infection).

Conclusion and application of findings: The study provides information serving as a base line in establishing *P. aphanidermatum* root rot of cowpea in Kogi State, Nigeria. The study also showed that susceptibility varies among the different cowpea cultivars tested with IAR-16 and local variety being more susceptible. Ife-brown can be used to improve cowpea production in the study area. Further research is suggested on control measures using either plant extracts or commercial fungicides.

Key words: Pathogenicity, cowpea, disease, incidence, *Pythium aphanidermatum*.

INTRODUCTION

In Nigeria, cowpea is the most important indigenous grain and is found in most areas north of the confluence of Rivers Niger and Benue. Nigeria is the largest producer of cowpea with one-third of the total world production as reported by Agricultural Products Monitoring and Evaluation Unit of the Federal Ministry of Agriculture (2005). However, according to William (1975), about 30% of the nation's cowpea yield is lost due to fungal attacks which include seedling mortality and root rot caused by *P. aphanidermatum* and *Corticum solani* (Prill. & Delact.).

A root rot and stem rot of cowpea, *Vigna unguiculata*, was first reported by Purss (1953) in Queesland, Australia. . The disease caused by *P. vignae* was distinct from a root rot of cowpeas caused by *Pythium myriotylum* and *P. aphanidermatum* (Croft, 1988). The severity and pathogenicity of the cowpea root and stem rot was also reported in Sri Lanka (Fernando & Linderman,

1993) from soils collected from the banks of a river and from a forest area.

The objectives of this study were to (i) isolate the pathogen causing cowpea root rot and describe the symptoms; (2) evaluate the distribution of the pathogen in field soils; (3) determine any alternative hosts, and (4) determine the pathogen's disease potential and virulence to cowpea cultivars grown in Kogi State, Nigeria.

MATERIALS AND METHODS

Disease survey and collection of samples: The survey of root rot disease of cowpea was carried out during the growing seasons (August - November) between 2005 and 2007 in privately owned farms at five main cowpea growing locations (Dekina, Abejukolo, Ajaaka and Ankpa). These locations were known for cowpea productions and hence high rate of disease incidence. The climatic data, monitored by the Metrological Station, Geography Department, Kogi State University, where the research was conducted indicated that the daily temperature during the cowpea growing season was between 35^{o}C and 35^{o}C with optimum at 30^{o}C. Disease incidence was assessed by expressing the numbers of affected cowpea plants as a percentage of the total number of cowpea plants. Disease Severity Index (DSI) was expressed in infection indices (Table 1), similar to those of Alasoadura and Fajola (1970). Soil samples and infected roots were collected from the rhizosphere of apparently diseased plants (15-20 cm deep within rows and near plant roots at about 10-m intervals) and randomly over the entire area of each field sampled. Composite samples were then made by mixing together the samples from the same farm site according to Amoo (2007). One hundred soil samples (100 g) were taken within a sampling site, bulked and mixed in large buckets, and then placed in polyethylene bags. Infected plants were carefully uprooted and the roots placed in polyethylene bags as soon as symptoms of rot were noticed. The samples were transported to the research station just immediately after collection.

Pathogen isolation and identification using plant trap method: This involves the use of plants of susceptible cowpea cultivars to trap the pathogen from soil, techniques adopted by Tsao (1983) and Ribeiro (1978). Soils were transferred to 10-cm-long pots within 24 hr after collection. Three seeds of each cultivar were planted in each pot. There were fifteen replicate pots for each cultivar. The control for each field was autoclaved soil. The experimental pots were placed in the screen house (27 – 30^{o}C) under natural daylight (10 hr) conditions. The plants were watered daily and kept until disease symptoms developed. If disease occurred, small pieces of root from the advancing margin of lesions were cut and immersed in 0.1 % mercuric chloride for 30 sec, washed three times in sterile distilled water, and blotted dry before being placed on PDA. Mycelia growing out of root tissue were incubated for 1 week under the same condition before examination and identification.

Serial dilution method: The method was used to isolate pathogen from the contaminated soil as described by Amoo (2007). Ten grams from the composite soil sample was added to 100ml sterile distilled water in a conical flask and thoroughly shaken by a mechanical shaker for 30min. One milliliter of the mixture was added to 9ml sterile distilled water in a test tube (now containing 10ml) and from this mixture, subsequent dilution rates to give 10^{-1} to 10^{-4} were prepared. One milliliter was taken from each test tube at different dilution levels (using syringe and needle) and inoculated on each of three replicate plates.

Pathogenicity tests: This was carried out by inoculating potted seedlings of three cowpea cultivars with sporangia and mycelial suspensions according to the method of Agrios (2005). The cultivars were obtained from the Ahmadu Bello University Institute of Agricultural Research (IAR). The soil mixture used was autoclaved at 180^{o}C and pressure for 8 hrs before use following the method of Fernando and Linderman (1993). This was to eliminate soil inhabiting pathogenic micro-organisms. The sterile soil was left for two days to cool, before packing into sterile pots, which were used in the screen house experiments. The potted soil was watered and left for 24 hr before planting three disease-free seeds of cowpea in each pot. There were fifteen replicates per treatment, and the experiment was repeated once. At the emergence of the seedlings, mycelial and sporangial suspension of a seven day-old culture with an average density of about 3×10^{4} sporangia per cm^{3} of *Pythium* was applied by drenching. The seedlings were covered with a large

polythene bag to provide a humid environment and to prevent entry of other pathogens following the methods of Amoo (2007).

Pathogen identification: Plates containing the mycelial plugs transferred from the different isolation methods were observed under a Leica microscope for sporangia and/or oogonia and antheridia. The cultured isolate was maintained in test-tube slants and sub cultured every month.

Alternative hosts: While cowpea fields in all the four locations were being sampled, legumes and vegetables growing close to the fields were surveyed for root lesions and wilt symptoms. Any plants showing such symptoms were collected in moistened bags, and isolation attempted as described above.

RESULTS

The survey to determine disease occurrence and severity at the four locations showed that local variety is most susceptible to the disease while Ife-brown is the least susceptible Disease incidence was highest in Dekina followed by Ajaaka and least in Abejukolo (Table 2).

Table 1: Infection indices used for rating Pythium root rot on cowpea in Kogi state, Nigeria.

Infection Index	Number Of Infected roots	Description
0	0	No infection
1	1-30	Very light infection
2	31-55	Moderate infection
3	56-75	Severe infection
4	> 75	Very severe infection

Table 2: Incidence and severity of Pythium root rot on cowpea in Kogi state, Nigeria.

Cowpea cultivar	Dekina		Abejukolo		Ankpa		Ajaaka	
	% I	S.I	% I	S.I	% I	S.I	% I	S.I
Local	66	3	72	3	69	3	60	3
Ife-Brown	30	1	20	1	19	1	22	1
IAR - 16	50	2	21	1	30	1	41	2

I = % incidence; S.I = severity index

Disease symptoms and pathogen identification: Symptoms of cowpea root rot consisted of wilting when leaves were still green and shrinking of the stem at or near the soil surface or slightly above. Advanced infection caused stunting of the affected plant, chlorosis, drooping, premature shedding and withering of leaves and death of the plant. A slow-growing fungus with aseptate hyphae was isolated from surface-sterilized root lesions placed on PDA. The fungus has coenocytic (aseptate) hyphae with whitish vegetative mycelium that is richly branched, slender, and cylindrical profusely branching, hyaline and rapidly growing mycelium. The mycelium gives rise to terminal, or intercalary sporangia visible at ×400 magnification. The sporangia, which are usually produced in vesicles during sexual reproduction, are globose to oval or irregular in shape and germinate directly by producing one to several germ tubes. The identity of the fungus, which had all the morphological features of *P. aphanidermatum* was confirmed at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Pathogenicity tests: All the three cowpea varieties tested, i.e. local variety, Ife-brown and IAR-16 showed varying levels of susceptibility to the disease. Of the 27 seeds of each variety planted, eight were infected in local variety (34.8%), while Ife-brown and IAR-16 had three (20%) and five (27.8%) infected seedlings, respectively, at six weeks after planting (Table 3).

Table 3: Pathogenicity of *Pythium aphanidermatum* on the three cowpea cultivars grown in Kogi State, Nigeria.

Cowpea cultivar	No. seeds planted	Germinated seeds	% Germination	Infected seedlings	% of seedlings infected
Local	27	23	85.2	8	34.8
Ife-Brown	27	15	55.6	3	20
IAR-16	27	18	66.7	5	27.8

All the four methods of isolation showed similar results. Symptoms developed as described above and Koch's postulates were fulfilled with isolation of the pathogen from the inoculated plants and pathogen identified as *Pythium aphanidermatum*.

Alternative hosts: Repeated attempts were made to isolate *P. aphanidermatum* from leguminous and vegetable plants, other than cowpea, growing in the area and exhibiting some possible root rot symptoms. *P. aphanidermatum* was recovered from root of sorrel (*Corchorus olitorius*) and tomato (*Lycopersicon esculentum*), with similar symptoms noticed as in cowpea plants. These plants were confirmed as alternate hosts to the pathogen.

DISCUSSION

Pythium aphanidermatum was shown to be the causal agent of root rot of cowpea in Kogi State, Nigeria. The success in isolating *P. aphanidermatum* from cowpea field soils in Kogi State, Nigeria can be attributed largely to the use of the susceptible cowpea cultivars. Growing the susceptible cowpea cultivars allowed the pathogen to cause lesions on which the pathogen could multiply. Heavy watering also created environmental condition conducive for the disease to occur in the greenhouse.

The result of pathogenicity test showed that 28.6% of the tested plants showed typical root rots symptoms while all the uninoculated control plants showed no symptoms of rot. The causal agent of the root rot was re- isolated and identified as *Pythium aphanidermatum*, thus fulfilling Koch's postulates. Of the cowpea cultivars grown, Ife-brown was the most resistant to the disease compared to the local variety and IAR-16. It was also observed in the present study that maximum infection can occur in cowpea plants anytime from planting till flowering. The decline in incidence from flowering stage may probably be due to increased lignifications as the plant matures since lignin has been noted to increase resistance to fungal rot (Ogundana, 1971). This may have accounted for the local variety being more susceptible to the disease and therefore its very soft and easier for the pathogen to penetrate. Tomato (*Lycopersicon esculentum*) and sorrel plants (*Corchorus olitorius*) exhibited symptoms of root rot, from which the pathogen was consequently isolated.

CONCLUSION AND RECOMMENDATION

In view of the level of severity of the fungal attacks and disease incidence observed in the study area, it has become incumbent to call on the Local, State and Federal governments, through the agricultural agencies saddled with this responsibility and other concerned stake holders, to as a matter of urgency. To embark on a wide scale control measure, aimed at putting the continuous spread of this fungus and disease under control. This will have the effect of enhancing the development and productivity of grain crop such as cowpea, and indeed other crops sustainably.

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