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Response of Fungi toxic Properties of Aframonum Melegueta (*Alligator Pepper*) against Pythium Aphanidermatum in Cowpea in Umuagwo, Imo State, Nigeria

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ABSTRACT

This experiment was designed to investigate the effects of Aframonum melegueta in the control of Phythium aphanidermatum in cowpea. This study was conducted in the green house of agricultural laboratory of Imo State Polytechnic Umuagwo, Nigeria from August to October, 2007 using completely Randomized Design (CRD). This experiment was replicated four times. The results of the experiment on cum appearance at different time intervals of 24, 36, 48, 60 and 72 hours (3 days) revealed that the extract (liquid) LiAm 0.0 – LiAm 0.4) changed the colour from curtney brown colour to whitish colour. This result was more pronounced in L10g0.4mls> 0.3 > 0.2 > 0.1mls and 0.0mls which was the control. It was also noticed that after the medium was inoculated, there existed different forms of Phythium aphanidermatumring, circular and dotted forms which never appeared in the control. On application of different concentrations of the liquid extract, the different forms of growth were cleared. The extent of control was more on 0.4mls > 0.3mls > 0.2mls >0.1mls respectively. On colony diameter after test of significance at 5%, it was noticed that 0.4mls reduced the colony diameter more than the other levels (0.3, 0.2 and 9.1mls respectively). The ability of the extracts to exert these effects may be linked to the presence of phytochemicals which were extracted from the plant materials.

Keywords: *Fungitoxic Properties, Aframonum melegueta (Alligator pepper), Pythium aphanidermatum, Cowpea, Colony.*

INTRODUCTION

The cowpea *(Vigna unguiculata)* is an important grain legume in tropical and sub tropical regions where a shortage of animal protein sources is often experienced (Falade *et al.*, 2017). They are heat- and drought- tolerant crops (D'Mello, 1995), requiring low input costs and are well adapted to the arid agronomic areas of Nigeria (Ukaegbu *et al.*, 2007). Suleiman (2010) reported that 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* respectively. 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 and Linder man, 1993) from soils collected from the banks of a river and from a forest area.

Cowpea are susceptible to a wide range of pests and pathogens that attack the crop at all stages; rust caused by *Uromyces appendiculatum* (Onuh *et al.*, 2005), softstem and root rots caused by *Pythium aphanidermatum* (Dutta, 2005), blotch caused by *Colletotrichum capsici* (Croft, 2007) and blight caused by *Ascochytaphaseolorum* (Adeyeye and Olufolaji, 2004). Pythium root rot, sometimes called damping off, or wilt, may be caused by several species of *Pythium* (Agrios, 2005). The author reported that those most commonly found on beans, especially cowpea, are *P. ythium ultimum, P. debaryanum, P. mytiotylum, P. helicoids, P. aphanidermatum, P. oligandrum, P. rostratum, P. pulchrum, P.*

vexans, *P. anandrum* and *P. acanthicum*. *Pythium* is a soil pathogenic fungus belonging to the family pythiaceae, it usually attack seedlings at the base and root under condition of overcrowding and over-watering (Amadioha, 2003). In Nigeria, fungi constitute the major limiting factor to the production of cowpea. Losses caused by fungi attack vary from 20 to almost 30% (Wokocha and Okereke, 2005).

Due to identifiable problems (for example, chemical residues, biodegradation, phyto-toxicity and pollution) associated with chemical control strategies; alternative control methods are being attempted. The aim of this research is to provide useful information on cheaper, affordable, natural and environmentally friendly pesticide in the control of root rot disease of *V. unguiculata*. *Aframonum melegu et al of* the family *zingiberaceae* is a spice in the ginger family with the common name "Alligator pepper" (Obike *et al*., 2014). The spice is used in West Africa for the purpose of alleviating stomachache anddiarrhea1 as well as hypertension (Ilic *et al*., 2010) with some limited reports on it being used for tuberculosis (Gbolade, 2012) and aremedy for snakebites and scorpion stings (Ogbole and Ajaiyeoba, 2009). The seeds are used for culinary reasons (due to the pungency of the seeds, it is common used as seasoning on food products (Lans *et al*., 2001).The seeds also tend to have general anti-microbial properties similar to many spices (Van And el *et al*., 2012) and has somemolluscidal 7 and repellant (Konning *et al*., 2004) properties as well. It is one of many pungent herbs said to aid in sexuality and aphrodisiac (Ukel, 2009, 2010). *Aframonum melegueta* appears to have apolyphenolic content of 2.28 ± 0.02mg/g (0.2% dry weight) with 0.55mg/g (0.06%) flavonoids which is comparatively high to other Africa spices tested although low relative to other herbs (Kamtchouing *et al.*, 2001).

Fungal diseases constitute the major limiting factor to the production of cowpea in Nigeria. Losses caused by fungi vary from 20 to almost 40 percent. *Pythium* sp. is the major source of root rot disease of cowpea in Nigeria (Suleiman *et al.*, 2011). *Pythium* is a pathogenic fungus belonging to the family *Pythiaceae*. It usually attack seedlings at the base and root under conditions of overcrowding and over-watering (Juliet, 2003). They are members of omycetesor water molds. Some require specific temperature optima: *P. ultimum* relatively low, 20^{o}C; *P. aphanidermatum* up to 35^{o}C. *Pythium* fungus cause soft, watery rots of seeds, roots, storage organs and crown tissues. Their hyphae are hyaline and aseptate (not containing cross walls) (Klemsdal *et al.*, 2007). Nwanguma *et al.*, (2005) reported *P. debaryanum*as the most common species and when attacked, the seedlings become weakened at the base and soon fall off or die off. Ogundana (1971) reported *Pythiumaphanidermatum* as one of the major pathogen of cowpea, which is widely distributed in Nigeria where cowpea is extensively grown.

Table1. *Chemical Composition of cowpea*

Component	Percent (%)
Dry matter	93.3
Crude protein	20.91
Ether extract	2.0
Crude fiber	3.4
Ash	4.1
Nitrogen free extracts	62.89
Metabolisable energy	(ME) (MJ/Kg)13.4

Table2. *Frequency of Fungal Pathogens Isolated from infected Cowpea (Vigna unguiculata) plants in Imo State*

Fungi	Total No of times Isolated	Isolation Frequency
Sclerotiumrolfsii	98	22.02
Fusarium oxysporum	87	19.55
Curvularialunata	6	1.35
Alternairatenius	8	1.87
Penicillumnotatum	22	4.94
Cercosporacruenta	25	5.61
Collectrichum	80	17.98
lindemuthianum		
Pythium aphanidermatum	10	2.25

Aspergillus niger	30	6.74
Botrodiplodiatheobromae	15	3.37
Phytophthoravigne	13	2.92
Microphominaphaseoline	17	3.82
Cladosporiumvignae	15	3.37
Rhizoctonia solani	19	4.27

Field work, (2007) and Bosah, 2013

MATERIALS AND METHOD

The study was designed to investigate the efficacy of liquid extract of the seeds of *Aframonum melegueta* in the control of *Phythium aphanidermatum* in cowpea. Seeds of *Aframonum melegueta* were procured from a local market in Imo State, Nigeria.

Field Experiments

The experiment was carried out at the Imo State Polytechnic green house and agricultural laboratory (longitudes 7o 01 0611E and 7o 031 0011 and latitudes 5o 281 0011N and 5o 301 0011N) (IMLS, 2009) from August through October, 2007.

Experimental Materials

Experimental materials used are: petri-dishes, electron microscope, polythene bags, slides, cover slips, ethanol, mortal, and petroleum spirit, and potato dextrose, agar-agar, inoculating loop, syringes, streptomycin powder, dilution water, benzoyl and Soxhlet extractor respectively.

Soil mixture was made in the ratio of 3:2:1 of humus mature poultry droppings, top soil and sharps and. The top soil provided the needed water retention capacity in the mixture and mineral content while the poultry droppings provided the nutrients and also helped in moisture conservation. The sharp sand aided in sufficient aeration.

The polythene bags served as source of (inoculum) Phythium aphanidermatum. After 3 months of growth of cowpea, the affected leaves (suffering from leaf rot) were collected from each replicate, crushed and used to inoculate the PDA medium.

PHYTOCHEMICAL DETERMINATION

Serial Dilution Method

The method was used to isolate pathogen from the contaminated soil as described by Amoo et al., (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 10-1to 10-4were 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.

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 Suleiman (2010). 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 – 30oC) under natural daylight (10 hours) 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 insterile 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.

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 Forestry Department, Imo State Polytechnic Umuagwo, and Nigeria. The soil mixture used was autoclaved at 180oC and pressure for 8 hours 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 hours 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 3×104 sporangia per cm3of 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 et al., (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.

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.

RESULTS

The survey to determine disease occurrence and severity in cowpea showed that local variety is most susceptible to the disease while the improved variety is the least susceptible

DISCUSSION

Pythium aphanidermatum was shown to be the causal agent of root rot of cowpea in Umuagwo, Imo State, Nigeria. The success in isolating P. aphanidermatum from cowpea field soils in Imo 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 un-inoculated control plants showed nosymptoms of rot. The causal agent of the root rot was re- isolated and identified as Pythium aphanidermatum

CONCLUSION

This research work therefore provides alternative method for the management of cowpea diseases without necessarily relying on the use of synthetic fungicides and can be easily recommended to small scale farmers in Nigeria.

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