*Niger. J. Mycol. Vol.7 , 85-92*

# MICROORGANISMS OF *VIGNA UNGUICULATA* (L.) Walp (COWPEA) SEEDS AND THE EFFECT ON GERMINATION AND SEEDLING GROWTH.

**[1]N. G. Iyanyi, [2]A. E. Ataga and [3]I. A. Nwaukwu**

[1]Department of Plant Science and Biotechnology,University of Port Harcourt, Rivers State. Nigeria.

[2]Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State. Nigeria.

[3]Department of Plant Science and Technology, University of Jos, Plateau State . Nigeria.

e-mail: iyanyiinkechi@yahoo.com, aeataga@yahoo.com and nwaukwui@yahoo.com

## ABSTRACT

A study was conducted to assess the effect of pathogenic fungi and bacteria on seed germination and seedling growth of *Vigna unguiculata*. The fungi used in the study are: *Fusarium oxysporum* Schlecht, *Aspergillus niger* Van Tieghem, *Botryodiplodia theobromae* (Pat) Novel, and *Rhizopus stolonifer* Ehrenb ex Link and the bacteria were: *Pseudomonas* sp., *Xanthomonas* sp., *Corynobacterium* sp. and *Micrococcus* sp. all isolated from diseased cowpea seeds. The individual microorganisms inhibited germination and seedling growth in varying degrees. *Fusarium oxysporum* had the least germination percentage (20%) followed by *Aspergillus niger* (24%), *Botryodiplodia theobromae* (36%), *Xanthomonas* sp. and *Pseudomonas* sp. (40%), *Rhizopus stolonifer* (44%), *Corynobacterium* sp. (48%) and lastly *Micrococcus* sp. (60%). *Fusarium oxysporum* and *Aspergillus niger* are the most pathogenic organisms in the study as they greatly affected the germination and seedling growth of cowpea.

**Keywords**: Pathogenic, microorganisms, germination, cowpea.

## INTRODUCTION

Cowpea belongs to the legume family leguminoseae and sub-family Papillonaseae (Enwere, 1998). Cowpea is a warm-season, annual, herbaceous legume. Plant types are often categorized as erect, semi-erect, prostrate (trailing), or climbing. Cowpea is a valuable component of farming systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Sanginga *et al*., 2003).

Cowpea is susceptible to a wide range of pests and pathogens, which can cause damage to the crop at all stages of growth (Summerfield and Roberts, 1985). It has been reported by Emechebe and Shoyinka (1985) that 16 major diseases (fungal and bacterial) affect cowpea in the four ecological zones of cowpea production in Africa. It is very likely that

some of these field diseases are carry-over diseases from storage. In countries such as Nigeria, seedling diseases caused by *Rhizoctonia* sp., *Pythium* sp. and *Fusarium* sp. are of economic importance and they can cause great losses in the low attitude rain forests because of seed decay and seedling damping-off (Singh and Rachie, 1985). *Fusarium oxysporum* is responsible for wilt and cortical rot diseases of more than 100 economically important plants (Swift *et al*., 2002). Fusarium wilt usually causes the lower leaves on one side of the plant to turn yellow. Infected plants usually are stunted and wilted as the organism develops in the food and water conducting tissues.Cowpea plays a critical role in the lives of millions of people in Africa and other parts of the developing world, where it is a major source of dietary protein that nutritionally complements staple low-protein cereal and tuber crops, and is a valuable and dependable commodity that produces income for farmers and traders (Langyintuo *et al*., 2003). So there is a pressing need to control the incidence of fungal diseases that reduce the crop yield so as to ensure a steady and constant food supply to the ever increasing world population.

Potential means of pathogen control and disease management are: regulation of production and transfer of propagation material, seed disinfestation, crop rotation, biocontrol agents, and fungicides (Gamliel and Yarden,1998).
This study is aimed at gathering more information on the effect of pathogenic microorganism on cowpea seed germination and seedling growth in Nigeria.

## MATERIALS AND METHODS

### Sources of cowpea seeds

The cowpea seeds, *Vigna unguiculata* (L.) Walp used for this study were obtained from International Institute of Tropical Agriculture, Ibadan (IITA). The seeds were stored in an airtight black polythene bags and kept in a refrigerator for adequate preservation until when needed.

### Inoculation of cowpea seeds

The fungi, *Fusarium oxysporum* Schlecht, *Aspergillus niger* Van Tieghem, *Botryodiplodia theobromae* (Pat) Novel, and *Rhizopus stolonifer* Ehrenb ex Link and the bacteria; *Pseudomonas* sp., *Xanthomonas* sp., *Corynobacterium* sp. and *Micrococcus* sp. were isolated from diseased cowpea seeds (Iyanyi and Ataga, 2014). Ten grams of healthy surface-sterilized seeds in sterile conical flasks were inoculated with the organisms.
For each fungus, 1.5 cm diameter cork borer was used to pick the test fungus, transferred aseptically into the sterile conical flasks and 10ml sterile distilled water. The conical flasks were shaken vigorously to obtain a homogeneous mixture and then kept in a dark place for 24hours. For the bacterial isolates, the inoculums were prepared from 24-hr nutrient agar culture plates. The bacteria were washed off the cultures with sterile normal

saline (0.85 g of NaCl in 1litre of sterile distilled water), each test bacterium was used to make a serial dilution with physiological saline and $10^{-3}$ dilution was used. Exactly 0.1ml of each of the isolated bacteria were inoculated into the conical flasks and shaken for homogeneous mixture. This was kept in the dark room for 24 hours. The same procedure was applied to the control but the seeds were not inoculated.

Germination test was carried out. Each black polythene bag was filled with sterile sandy loam soil obtained from University of Port Harcourt. Five infected cowpea seeds were sown per bag. A total of eight test organisms were used and the control for each organism. The treatments were replicated 5times. A total of 45 polythene bags were used for the experiment. The bags were kept in the greenhouse in the Department of Plant Science and Biotechnology, University of Port Harcourt and were irrigated at 24 hours interval. Completely Randomized Design (CRD) was used as the experimental layout. The observation of each treatment was recorded on germination by the appearance of the cotyledon above the ground between 2 to 7 days after planting. Symptom growth of disease such as leaf spots, wilt, blight were checked and recorded. The growth parameters measured were leaf number, shoot length, root length and total seedling height. The number of emerged leaves were recorded daily for three weeks. The length of the shoot was measured from the root-collar to the terminal bud with a meter rule. The length of the longest root was measured with a meter rule. Total seedling height was obtained by measuring from the tip of the longest root to the shoot terminal bud with a meter rule.

The experiment was observed for 3 weeks and leaves of samples were taken to the laboratory for re-isolation to confirm the identity of the isolates.

## RESULTS

The effect of microorganisms on the germination of cowpea seeds are presented in Figure 1. There was significant reduction (P= 0.05) in germination of cowpea seeds treated with the microorganisms when compared with control. *Fusarium oxysporum* caused the highest reduction in the germination of cowpea seeds.

Different symptoms manifested on the seedlings as a result of the effect of the microorganisms. Cowpea seedlings infected with *Aspergillus niger* and those infected with *Fusarium oxysporum* all showed stunted growth, yellowing, wilting and drying of leaves. *Rhizopus stolonifer* showed darkening of veins and wilting on seedlings. *Pseudomonas* infected seedlings resulted in wilting and yellow to light brown spots on leaves. *Xanthomonas* caused defoliation with pale yellowish green spots and leaf blight on seedlings. Defoliation also occurred with *Corynobacterium* infected seedlings with yellow necrotic leaf lesions and small dark brown lesion. *Micrococcus* showed drying of leaves on seedlings.

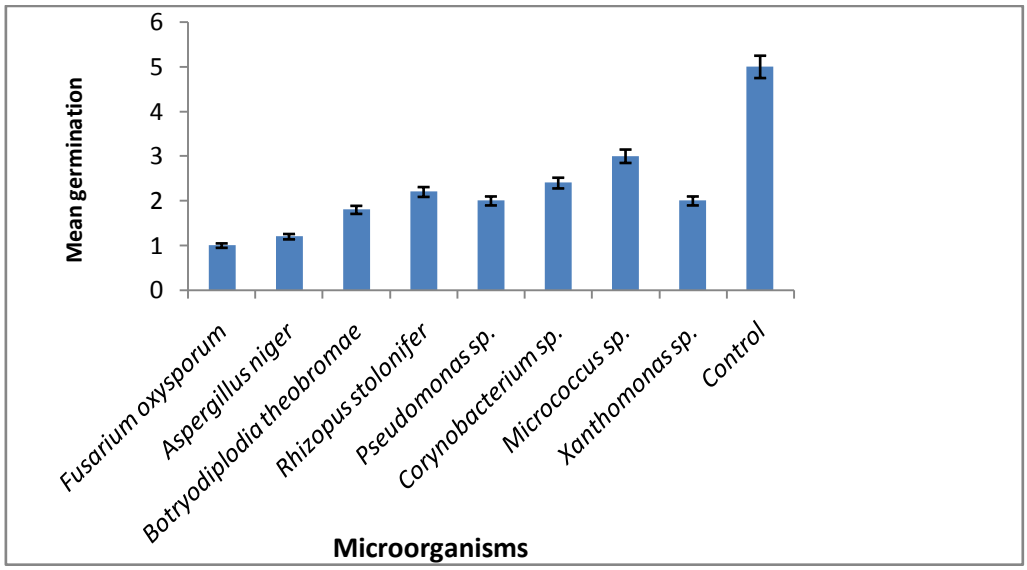


**Figure 1: Effect of microorganisms on mean germination of cowpea (*Vigna unguiculata*) seed.**

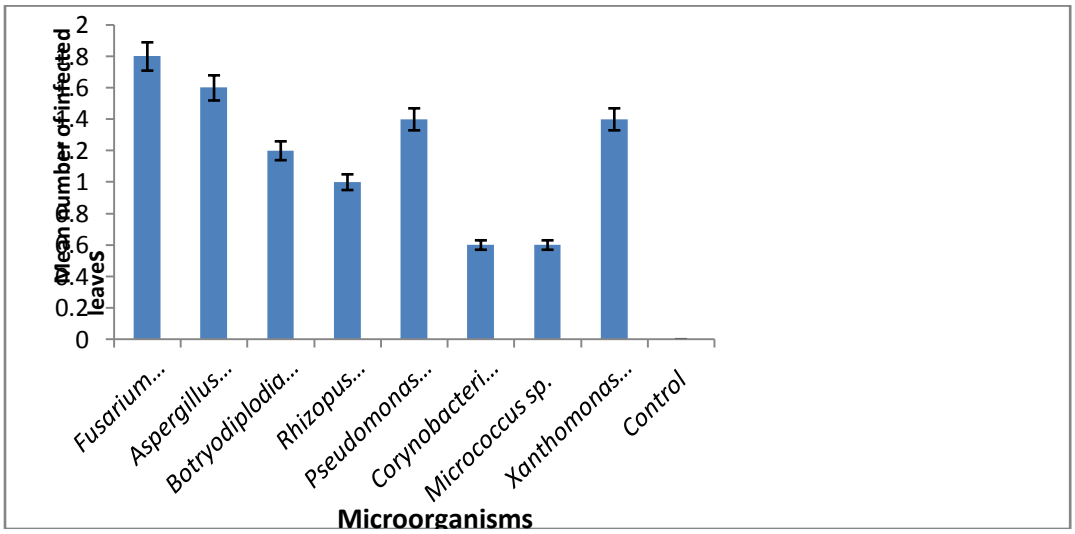


**Figure 2: Number of infected leaves (disease incidence) 2 weeks after germination**

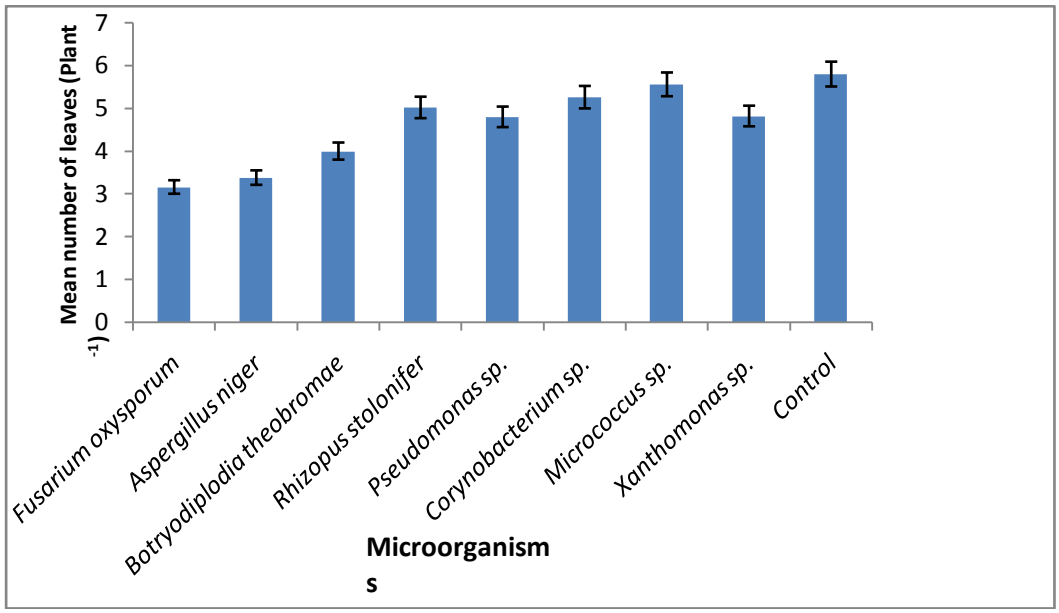


**Figure 3: Effect of microorganisms on number of leaves of cowpea (*Vigna unguiculata*) three weeks after planting.**

I= Standard error (P= 0.05)

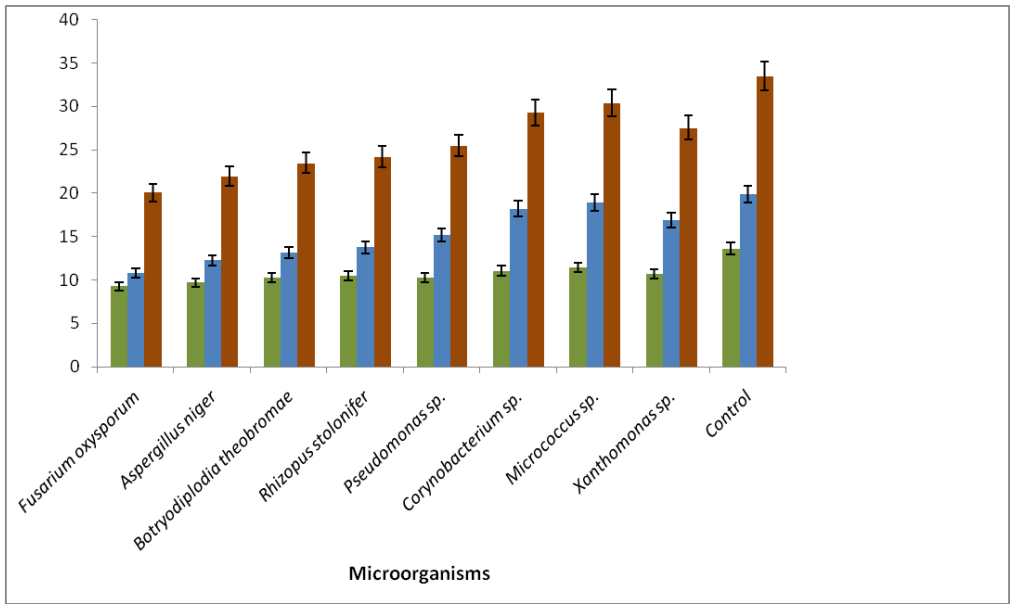


**Figure 4: Effect of microorganisms on root length, shoot length and total seedling height of cowpea (*Vigna unguiculata*) three weeks after planting.** I= Standard error (P= 0.05)

The control experiment has no symptoms on the leaves of the plant.
There was significant reduction (P= 0.05) in the seedling growth of cowpea caused by the effect of the inoculated micro-organisms as presented in Figures 2 to 4. The mean number of infected leaves (disease incidence) 2weeks after germination showed that *Fusarium oxysporum* had the highest mean number of infected leaves followed by *Aspergillus niger*, *Pseudomonas* sp. and *Xantho-monas* sp., *Botryodiplodia theobromae*, *Rhizopus stolonifer*, *Corynobacterium* sp. and *Micrococcus* sp. The control had zero number of infected leaves (Figure 2). *Fusarium oxysporum* (44.8%) caused the highest reduction in mean number of leaves of cowpea seedlings, followed by *Aspergillus niger* (41.4%), *Botryo-diplodia theobromae* (31.0%), *Pseudom-onas* sp. and *Xanthomonas* sp. (17.2% respectively), *Rhizopus stolonifer* (13.8%), *Corynobacterium* sp. (8.6%) and *Micrococcus* sp. (3.4%) when compared with the control (Figure 3).
The microorganisms significantly reduced the root length of cowpea seedlings. *Fusarium oxysporum* and *Aspergillus niger* (27.2%) caused the highest reduction in mean root length of cowpea seedlings, followed by *Botryodiplodia theobromae*, *Rhizopus stolonifer* and *Pseudomonas* sp. (25.8% respectively), *Xanthomonas* sp. (24.2%), *Corynobacterium* sp. (22.7%) and *Micrococcus* sp. (21.2%) when compared with the control (Figure 4). The shoot length of the treated cowpea seedlings was significantly (P= 0.05) reduced by the microorganisms. *Fusarium oxysporum* (34.0%) caused the highest reduction in the mean shoot length, followed by *Aspergillus niger* (30.9%), *Botryodip-lodia theobromae* (29.9%), *Rhizopus stolonifer* (28.9%), *Pseudomonas* sp. (25.8%), *Xanthomonas* sp. (23.7%), *Corynobacterium* sp. (16.5%) and *Micrococcus* sp. (15.5%) when compared with the control (Figure 4). The Total seedling height of the treated cowpea seedlings was significantly (P= 0.05) reduced by the microorganisms. *Fusarium oxysporum* (31.3%) caused the highest reduction, followed by *Aspergillus niger* (29.4%), *Botryodiplodia theobromae* (27.6%), *Rhizopus stolonifer* (27%), *Pseudomonas* sp. (24.5%), *Xanthomo-nas* sp. (24%), *Corynobacterium* sp. (18.4%) and *Micrococcus* sp. (17.8%) when compared with the control (Figure 4).

## DISCUSSION

The individual microorganisms had varying degrees of inhibition on the germination and seedling growth of *Vigna unguiculata*. *Fusarium oxysporum* had the least germination (20%) when compared with the control plant which showed 100% germination followed by *Aspergillus niger* (24%), *Botryodiplodia theobromae* (36%), *Xanthomonas* sp. and *Pseudomo-nas* sp. (40%), *Rhizopus stolonifer* (44), *Corynobacterium* sp. (48%) and lastly *Micrococcus* sp. (60%).
Amadi and Oso (1996) reported that seedling emergence and development in cowpea seeds inoculated with *Cercospora cruenta* and *Corynespora cassiicola* were both delayed and reduced. They asserted that the pathogens could have produced some toxic substances which inhibited seed germination. Reduction and inhibition in germination of

cowpea caused by culture filtrates of cyanobacteria has been reported by Sengar *et al*. (2010). Damage to the cell was caused by the release of enzymes and toxic metabolites by the infecting fungus.

*Fusarium oxysporum*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Rhizopus stolonifer*, *Pseudomonas* sp., *Xanthomonas* sp., *Corynobacterium* sp. and *Micrococcus* sp. induced various symptoms on cowpea seedlings. *Aspergillus niger* and *Fusarium oxysporum* caused stunted growth, yellowing, wilting and drying of leaves. *Rhizopus stolonifer* induced darkening of leaf veins and wilting on seedlings. *Pseudomonas* caused wilting and yellow to light brown spots on leaves. *Xanthomonas* caused defoliation with pale yellowish green spots and leaf blight on seedlings. Defoliation also occurred with *Corynobacterium* infected seedlings with yellow necrotic leaf lesions and small dark brown lesion. *Micrococcus* induced drying of leaves on seedlings. The control experiment has no symptoms on the leaves of the plant.

Manyangarirwa *et al*. (2009) reported that cowpea seedlings grown from seeds infected with *Fusarium* sp. resulted in wilting of seedlings. Oluyemisi *et al*. (2006) reported that cowpea seeds infected with *Fusarium oxysporum, F. solani, F. semitectum* and *Penicillium* sp. all developed into stunted seedlings. Gulya *et al*.(1982) reported that *Pseudomonas syringae* induced chlorosis in sunflower (*Helianthus annus*). Mew *et al*. (1993) also reported that *Xanthomonas oryzae* pv. *oryzae* caused bacteria blight on rice seedlings. Seedling diseases in cowpea result in low yields, especially in the rural areas where no control measures are taken against the diseases.

## REFERENCES

**Amadi, J. E. and Oso, B. A. (1996)**. Mycoflora of Cowpea Seeds (*Vigna unguiculata* L.) and their Effects on Seed Nutrient Content and Germination.*Nigerian Journal of Science*, 30: 63 -69.

**Emechebe, A. M. and Shoyinka, S. A. (1985)**. Fungal and Bacteria Diseases of Cowpea in Africa.In *Cowpea Research, Production and Utilization*. Singh, S. R., Rachie, K. O. (Eds.), John Wiley and Sons, Chichester, UK. pp. 173-192.

**Enwere, J. N. (1998)**. *Plant Origin*. Afro-Orbis Publisher, Nsukka, pp: 24, 25, 240.

**Gamliel, A. and Yarden, O. (1998**). Diversification of Diseases Affecting Herb Crops in Israel Accompanies the Increase in Herb Crop Production. *Phytoparasitica*, 26: 1-6.

**Gulya, T. J., Urs, R. and Banttari, E. E. (1982)**. Apical Chlorosis of Sunflower caused by *Pseudomonas syringae pv. Tagetis, Plant Disease*, 66: 598-600.

**Iyanyi, N. G. and Ataga, A. E. (2014**). Fungal Species Associated with *Vigna unguiculata* (L.) Walp (Cowpea) Seeds from Parts of Enugu State, Nigeria. *Scientia Africana*, B(1): 103 –108.

**Manyangarirwa, W., Bwerazuva, T. and Mortensen, C. N. (2009**). Seed-borne Fungal and Bacterial Pathogens on Farm-retained Cowpea Seeds from Zimbabwe. *African Crop Science Society*, **9:**595-599.

**Mew, T. W., Alvarez, A. M., Leach, J.E. and Swings, J. (1993**). Focus on bacterial Blight of Rice. *Plant Dis*ease, **77**: 5-12.

**Langyintuo, A. S., Lowenberg-DeBoer, J., Faye, M., Lambert, D., Ibro, G., Moussa, B., Kergna, A., Kushwaha, S., Musa, S., Ntoukam, G. (2003**). Cowpea Supply and Demand in West Africa. *Field Crops Research*, **82**:215–231.

**Oluyemisi, B. F., Oladimeji A. and Olusegun, S. B. (2006**). Pathogenicity and Cellwall-Degrading-Enzyme Activities of some Fungal Isolates from Cowpea (*Vigna unguiculata* [L.] Walp). *Biokemistri*,**18**(1):45- 51

**Sanginga, N., Dashiell, K. E., Diels, J., Vanlauwe, B., Lyasse, O., Carsky, R. J., Tarawali, S., Asafo- Adjei, B., Menkir, A., Schulz, S., Singh, B. B., Chikoye, D., Keatinge, D., Ortiz, R. (2003**). Sustainable Resource Management Coupled to Resilient Germplasm to Provide New Intensive Cereal–Grain-Legume–Livestock Systems in the DrySavanna. *Agric*ulture *Ecosystem and Environ*. **100**: 305–314.

**Sengar, R. M., Bhadauria, S. and Sharma, P. (2010**). The Effect of Cyanobacterial Toxin on Seed Germination. *Indian Journal of Science and Research*, **1**(2): 4 –45.

**Singh, J. E. and Rachie, K. O. (1985**). *Cowpea Reaearch, Production and Utilization*. John Wiley & Sons, Chichester, New York. Pp. 1 – 7.

**Summerfield, R. J. and Roberts, E. H. (1985)**.*Grain Legume Crops*. Collins, London. Pp. 1 -6.

**Swift, C. E., Wickiffe, E. R., and Schwartz, H. F. (2002)**.Vegetative Compatibility Groups of *Fusarium oxysporum f.sp cepae*from Onion in Colorado. *Plant Disease*, **86:**606 -610.