MANAGEMENT OF PANAMA DISEASE *(Fusarium oxysporum f.sp. cubense*) USING ORGANIC AMENDMENTS: A CASE STUDY OF KISII COUNTY.

STUDENT NAME: MOMANYI, KENNEDY MOSETI
ADM. NO. A56/80823/12

SUPERVISORS:

1) **Dr. NARLA RAMA D.**
2) **Prof. J. KIMENJU.**
3) **Dr. J.MUTHOMI**

BROAD OBJECTIVE:

To evaluate the level of Fusaium wilts occurrence in Kisii County, the affected bananas and the appropriate soil amendments that can manage the disease thereby boosting yields.

SPECIFIC OBJECTIVES:

1) To determine the occurrence level of Fusarium oxysporum f.sp. Cubense in Kisii County.

2) To evaluate the organic and inorganic soil amendments in the management of Fusarium oxysporum f.sp. Cubense**.**

ISOLATION AND MULTIPLICATION OF *Fusarium oxysporum f. sp cubense* FROM PLANT MATERIALS:

a) Fusarium oxysporum f.sp cubense From Source Samples:

•Samples will consist of a section from the pseudostem of the wilted banana plant where typical continuous discolored vascular strands are evident.

•Samples will be taken from as low as possible but not from areas where decay is advanced.

•Similarly samples will be taken from as close to the centre of the pseudostem as is possible, as opposed to the outermost leaf bases.

•NB.

Since banana tissue is very wet, the risk of bacterial contamination of samples is high, particularly in warm weather and samples can deteriorate rapidly. Therefore samples should be kept in heavy paper bags or wrapped in papers (e.g. newspapers) until the strands van be excised. Care will be taken to avoid using plastic bags as this will cause samples to sweat and promote growth of bacteria.

b) Dissecting discoloured vascular strands from samples:

•Discoloured vascular strands will be dissected on the same day of collection or as soon as possible after collection.

•Sterile blotting papers and aseptic techniques will be applied to the dissection of the strands i.e.

Surface sterilizes samples by wiping with 70% alcohol or surgical spirit.

•A fresh piece of blotting paper will be used for each sample and scapel blades be flamed or at least wiped with 70% alcohol between samples.

•Excised strands, with as little as possible of the adjacent tissue, should be placed between sterile blotting papers in a paper envelope to dry naturally.

c) Isolating the fungus from discoloured vascular strands:

•Isolation will be attempted when the strands have dried (possibly as earlier as the next day).

•Small sections of three to six (3-6mm) long of dry discoloured vascular strands are submerged into plates of quarter strength PDA medium amended with an antibacterial agent (e.g. Streptomycin @ 1.2ml/240ml PDA).

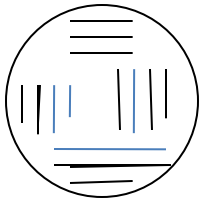
•If Fusarium is present, growth is expected to appear from the strands in two –three days.

d) **Generating monoconidal cultures:**

Monoconidal cultures are single spores prepared from an isolate of each specimen.

Procedure:

•Two pieces of culture approximately six millimeters square from healthy culture isolated on quarter strength PDA will be cut under aseptic conditions.
•The two pieces will then be transferred to a small nine milliliter McCartney bottle containing sterile distilled water.
•The bottle will then be swirled gently to wash the spores into solution, then a loop will be flamed and one or two loops of spore suspension transferred onto one side of agar plate.
•The flamed loop will then be dragged through the deposit of spore suspension several times to create streaks of spore suspension across the plate either in parallel lines or in sixteen-streak method used in microbiology i.e.



•This will result in separating conidia from each other to enable single, separated, germinated spore to be easily identified and excised 18-24 hours later using a stereo microscope.
•Germinated spores are observed by looking for a germ tube growing from under the stereo microscopy.
•Once a single germinated spore is located, a flamed scalpel will be used to transfer it to an individual plate of quarter strength PDA. This will be repeated four times for each isolated culture to ensure a pure monoconidal culture is obtained.

•Once a single germinated spore is located, a flamed scalpel will be used to transfer it to an individual plate of quarter strength PDA.

• This will be repeated four times for each isolated culture to ensure a pure monoconidal culture is obtained.

•After three days, mycelia growth should be observed.

•A culture that will be healthy and showing typical growth will be chosen as the representative isolate of the culture and the rest will be discarded.

•This culture will then be transferred immediately to carnation leaf agar (CLA) for short term storage or filter paper for long term storage

Maintenance of healthy cultures.

•Healthy (sporodochial-type) monoconidal cultures of *Fusarium oxysporum f. sp cubense (*Foc) should be maintained on carnation leaf agar (CLA) to prevent mutation.

•Cultures can be initiated on weak quarter strength PDA medium to check the morphology of cultures for taxonomic purposes or for **spore production**.

•Healthy (sporodochial-type) cultures of Foc growing on PDA medium will exhibit abundant fluffy aerial mycelium after **two days** and produce abundant microconidia.

•Some Macroconidia may also be produced on PDA, although this type of spore is more commonly produced on CLA medium.

•Cultures of Foc should not be kept on PDA medium for more than **four to five days** as mutations can rapidly occur and these cannot be reversed.

•Cultures are normally maintained in an incubator at 25^0c

SOIL INOCULATION WITH FUSARIUM PATHOGEN:

The soil will be sterilized twice at 120^0c in the autoclave for 30 minutes at 24 hour interval. Each pot will be filled up with 100 grams of sterilized soils. The pots will then be inoculated with the wilt pathogen at inoculums concentration of $1x10^3$ cfu per gram of soil.

ASSESSMENT OF THE INCIDENT AND SEVERITY OF FUSARIUM WILT.

a) Incident:

The total number of seedlings affected will be counted and then related to the total number of seedlings planted i.e.

% Incident= affected seedling x100/total number of seedlings planted.

b) Severity:

The extent of the stem damage/brown discoloration of the stem in length will be measured. This will then be calculated out of the total length in percentage. This will be

in a tabular form as below:

Quantification of the growth parameters:

TREATMENT / PARAMETERS	Wood ash	Tithonia	DAP	Urea	Methyl bromide	Poultry manure	No treatment
Height of banana (cm)							
Number of leaves							
Width of the pseudostem (cm)							
Length of crack (cm)							
Length of discoloration/lesion(cm)							
TOTAL							

DATA ANALYSIS:

The data which will be collected in this experiment will be analyzed to make decisions and inferences. Analysis of variance
(ANOVA)will be used. Also statistical techniques like graphical representations will be used for data presentation.

EXPECTED OUTPUTS:

1. Manage panama disease and boost Apple banana (Silk banana); “Kisukari ndizi” banana yield and hence generate increased
income for farmers in Kisii County.
2. MSC. Research Thesis.
3. Publish in a journal.

WORK PLAN:

	2013												2014											
Activity	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
Course Work	X	X	X	X	X	X	X	X	X															
Development of thesis proposal	X	X	X	X	X	X	X	X	X	X	X	X	X											
Present research proposal at departmental seminar	X	X	X	X	X	X	X	X	X	X	X	X	X	X										
Initiate research – setup of experiments														X	X	X								
Data collection entry and analysis																X	X	X	X					
Data collection entry and analysis																		X	X	X				
Compile first 3 chapters of thesis (introduction literature review and methodology)	X	X	X	X	X	X	X	X	X	X	X	X	X	X										
Complete experiments																	X	X	X	X				
Data interpretation – write results chapter																		X	X	X	X			
Compile and complete thesis and thesis evaluation by supervisors																				X	X	X		
Thesis submission to BPS																						X	X	
Thesis examination																								
Thesis oral defense																								