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*RESEARCH ARTICLE*

# POTENTIAL OF SOME BACTERIA ISOLATED FROM COMPOST ORGANIC MANURE IN THE CONTROL OF COCOYAM (*Colocasia* spp) LEAF BLIGHT DISEASE.

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## *Abstract*

An investigation of bacteria isolates from composted manure and their potential in the control of *Phytophtora colocasiae* was carried out in the Teaching and Research Farm of Imo State University, Nigeria. Bacteria organisms isolated from composted organic materials (manure) were tested against the *Phytophtora colocasiae* which is the pathogen that causes leaf blight disease in cocoyam. Results revealed four bacterial isolates- *Pseudomonas spp.*, *Bacillus spp.*, *Escherichia coli*, and *Staphylococcus aureus* from the composted manure. The results also showed that *Pseudomonas spp. Bacillus spp.*, *Escherichia coli* and *Staphylococcus aureus* gave mean diameter of growth inhibition of 43.167mm, 40.353mm, 19.667mm and 17.333mm, respectively. There was no significant difference ($P \leq 0.05$) in mean diameter of growth inhibition between *Pseudmonas spp.* and *Bacillus spp*. but the two were significantly different from that of *Escherichia coli* and *Staphylococcus aureus.* Observations showed that the bacterial isolates from the composted manure were more effective in their antagonism activity when the disease pathogen concentration was lower. Based on the antagonism properties of *Pseudomonas spp.* and *Bacillus spp.*, they serve as potential agents in the program involving biological control of cocoyam leaf blight diseases.



## Introduction:-

The production and yield of Taro is faced with a lot of problems as a result of damage by Taro leaf blight disease caused by *Phytophtora colocasiae*. According to Helen and Graham, (2000), taro leaf blight reduces the life of leaves, thus instead of lasting for 40 days, they last for only 20 days resulting in plants with few leaves and small corms. The disease reduces yield by about 40%-70% (Jugumauth *et al*., 2002). Taro leaf blight disease also causes a firm, brown corm rot after harvest and it takes 7-10 days before the corm is completely decayed, (Sid *et al.*, 2003). The disease is mainly foliar oriented, although post- harvest storage rots occur. Initially symptoms of the disease are small brown water-soaked lesions, often with a yellow margin (Wilson, 2007). Secondary infections lead to rapid destruction of the leaf, which may occur in 10-20 days after infection or less in susceptible varieties. The normal longevity of a healthy leaf is 40 days but the disease can significantly reduce the number of functional leaves which can lead to yield reduction in the magnitude of 50% (Gadre and Joshi, 2003). Leaf blight disease causes yield losses of 30-50%, including post-harvest decay of taro corms, shortages of planting materials and high production costs.

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*Phytophtora colocasiae* belongs to the kingdom *chromista* and phylum *Oomycota* (Ooka 1994). It is known to infect taro (*Colocasia esculenta*) while *Xanthosoma* is resistant to it (Brooks, 2000). It has been found in all taro growing areas of Hawaii, and it is also present in Papua New Guinea, Solomon Islands, Philippines, Palua, Gaum, the Northern Marianas Island, West Samoa, American Samoa, Ponape, Yap, Truk, India, Taiwan and Trinidad (Trujillo, *et al.,* 1993). The disease was first reported in Nigeria in 2009, and by 2010 it became an epidemic in Cameroon (Mbong, *et al.*, 2013). It is believed to have originated in South East Asia and is widely distributed throughout the tropical regions of the world (CMI, 1997). Investigation carried out in Nsukka, Eastern Nigeria in 2011 revealed that the taro leaf blight diseases has become epidemic and has drastically reduced cocoyam production by 60% (Chiejina and Ugwuja, 2011)

Other fungal organisms associated in the storage rotting of cocoyam include, *Aspergillus niger, Fusaruim solani, Botryodiplodia theobroma, Fusarium oxysporum, Cortium rolfsii; Geotrichum candida and Sclerotium* rolfsii (Ugwuanyi and Obeta, 1996, Ohazurike *et al*., 2003). The early stages of leaf blight disease are characterized by the formation of small, frequently circular brown to olive green spots (Ooka, 1994). Helen *et al.,* (1996) reported that the fungus is active in wet weather; spores produced on the leaves and spread in winds and rain to nearby plants or longer distance to new gardens. If they fall on a wet leaf they germinate and continue attack (Ugwuanyi and Obeta, 1996; Trujillo, 1996).The spores can germinate like a seed and infect leaves or they produce smaller spores, which burst out and swim short distances over the leaf before they germinate and cause infection,(Helen and Graham, 2000).In both cases, the fungus kills the cell of the leaf and brown spots occur.

The spots expand very fast and produce yellow margins with red-brown droplets developing on the under surface where the droplets dry as dark pellets, (Ngoka, 1997). Infection can occur anywhere on the leaf surface, but often starts at the edges where rain dew collects. After few days of infection, a white ring can be seen near the margin of the spot; this is the area where spores are produced. However, the spores dry out quickly in the sun and by mid-morning they will shrivel and die,(Chiejina and Ugwuja, 2013). They only stay alive if it is cloudy or raining. Apart from wind, spread of the disease can occur in other ways, such as suckers planted with infected leaves attached or stalks of planting material probably on the cut ends in wet weather when stalks are trimmed for planting.

Jackson, (1996) reported good control of the disease by spraying copper oxychloride applied at the rate of 4.5kg per 100 litres of water per hectare. Maheswari *et al.,* (2001) again reported the use of copper fungicide (especially copper oxychloride), to be effective in the control of the disease but have to be used often and are only recommended where taro are grown at commercial scale.

Chukwu, (2012) also reported the effective control of phytophotora leaf blight disease of taro by spraying of Ridomil, Nordox and Kocide forthnightly on the leaves of affected plants; (One sachet of 100grams in 15 litres of water). Similar result was also reported by Scot *et al.,* (2011) in Samoa when sprays of Ridomil; Manzate; and phosphorous acid product were used in the control of taro leaf blight disease. Misra, (1999)reported that Metalaxyl- and Mancozeb based fungicides have proved effective in controlling taro leaf blight disease but the waxy leaf surface and the occurrence of the disease during rainy season make fungicidal sprays ineffective.

Over the years, the protection of Agricultural crops and products was achieved almost entirely through the use of synthetic chemicals (Adams, 1991; Jacobson, 2001; Alabouvette *et al.,* 2006). These chemicals though valued for their effectiveness are costly and constitute health hazard to farm households and the environment. This observation was reported by Ohazurike and Obi, *(*2000) who emphasized that, the ubiquitous use of these chemical pesticides pose numerous problems which include; development of resistance to the chemical pesticides by the target pest or pathogen species, outbreak of secondary pests or pathogen, extermination of beneficial organisms in the environment, environmental pollution and accidental poisoning of farmers and handlers. In view of the associated problems of synthetic chemicals, the U.S. Department of Agriculture specially recommended a general re-orientation of research and extension to develop new technologies to reduce cost, increase efficiency and facilitate the economic viability of small to medium sized farms.

In the light of the above, biological control using microbial agents in diseases control cannot be over-emphasized. Alavi *et al.,* (2013) described biological control as the purposeful utilization of introduced or resident living organisms, other than disease-resistant host plant, to suppress the activities and populations of one or more plant pathogens. Several works have been carried out using biological agents extracted from animal farm refuses. Compost tea, an extract from compost mixed with water was found to suppress certain plant diseases, such as

*Botrytis* on green beans, straw berries, grapes and geraniums, powdery mildew on apples and others (Dianez *et al.,* 2006). According to EL. Jahil and Zinedine, (2008), some microbial and chemical properties of poultry wastes manure after lactic acid fermentation at $30^0$C for 7-10 days using 10% molasses, humidified and inoculated with *Lactobacillus planarum* and *Pediococcus acidilactici* removed hazardous microorganisms like *Enterobacteria, Enterococci, Clostridum* and *Salmonella.*

Microbial fertilization from poultry manure were processed into Omug, Ecud and Pudret using hot air ($60^0$C); fermentation at $40^0$C for 7-8 days and treatment with infra radiation at $48^0$C, respectively. Omug which has 55% dry matter and 15% water contained heterotrophic aerobic microorganisms (microflora) that improved the quality of fertilizer, promoted bacterial growth and suppressed phytopathogenic micro-organisms (Fisinin and Arkhipchenko, 2001). Jager *et al.,* (1991), also confirmed that biological control of *Rhizoctonia solani* in potato by *Verticillum biguttatum* increased the percentage of harvests to 56%, compared to 24% in non-inoculated. Also gram-positive *Bacillius spp.* and Gram negative *Pseudomonas spp* were effectively used in the control of soil borne pathogens that affected Brassicas vegetables (Wulff *et al.,* 2003).

*Pseudomonas fluorescens* and *Bacillus pumulis* significantly reduced the incidence, severity and vascular discolouration of *Venticillium* wilt in tested potato cultivars in growth room trials and field testing (Uppal *et al.,* 2008). Okamoto *et al.,* (2000) recorded that, three bacterial strains isolated from rhizosphere of angelica trees strongly inhibited mycelia growth of *Cactorum.* This is in conformity with work of the Rajkuma *et al.,* (2005) in which 12 isolated *Pseudomonas spp.* from rhizosphere of pepper significantly inhibited *Phytophtora* blight disease. Hoopen *et al.* (2003) reported that mycoparasitic bacteria collected from aerial parts of the cocoa plant have shown great effect in the control of black pod disease, caused by *Phytophtora palmivora.*

Foliar application of biological control agents has some potential to protect taro plants from infection of taro leaf blight disease. Considering the importance and enormous potential of cocoyam in the human food chain and livestock feed in Nigeria, it is necessary that any factor militating against continued and increased production of the crop should be avoided or checkmated. Efforts have been made in the control of this disease especially, in the use of chemicals. But some of these chemicals are froth with challenges.

Therefore, efforts should be made in fashioning control measures that will guarantee the safety of animals, man and the environment. In view of this, the study investigated potential of some bacteria isolated from compost organic manure in the control of cocoyam (*Colocasia* spp) leaf blight disease.

## Materials and method:-

**Study Area:-**

The study was carried out at the Teaching and Research Farm of Faculty of Agriculture and Veterinary Medicine, Imo State University, Owerri Nigeria. The samples used in this study were; organic manure and cocoyam leaves (*Colocasia esculenta*) (L) Schott) infected with *Phytophtora colocasiae*. Nutrient Agar incorporated with 1% Nystatin which inhibits fungi but promotes the growth of bacteria.

**Making Compost:-**

Organic manure comprising of animal dungs saw dust, grasses, legumes and dry plantain leaves were collected from Alex farms, Ubomiri, Somachi cattle market Egbu road; Umuonyeali-Ugo timber market, Imo ADP zonal office and the Teaching and Research Farm of Faculty of Agriculture and Veterinary Medicine, Imo State University, Owerri. All the areas of material sources are located in Owerri. Two pits were dug to 60cm depth and the organic materials were piled in each of the pits. Water was sprinkled on the materials to facilitate decay. An iron peg was driven at the Centre of the organic materials in the pit to create space for aeration. The pit was then covered with perforated black polythene sheet. The compost materials were checked after one week for decomposition, by removing and feeling the hotness of the iron peg thereafter turning started after four weeks. Turning continued fortnightly till 16 weeks when the manure became fully decomposed

**Collection of Samples and Sterilization of Materials:-**

Five (5) grams of the composted manure was aseptically collected using sterile rubber container with stopper. The container was wrapped with foil and taken immediately to the Department of Microbiology, Imo State University, Owerri laboratory for microbiological analysis. Also, leaves of cocoyam infected with *Phytophtora colocasiae* were

collected and cleaned with disposable alcohol swab pad and the alcohol allowed to evaporate. A sterile knife was used to cut off the infected leaves and packed in a sterile paper packet ensuring that there was no delay, to retain the viability of the organisms. The samples were taken to the same laboratory for microbiological analysis and the study was done under aseptic conditions. The materials used were sterilized by standard laboratory methods as described by Chesbrough, (2002); Obiajulu and Ozumba, (2009).

**Media Preparation and Dilution of Composted Manure:-**
Twenty-eight (28) grams of the powder was dissolved in 1 litre of de-ionized water and allowed to stand for 10 minutes for complete dissolution. It was swirled to homogenize and sterilized by autoclaving at $121^0$C for 15 minutes. It was allowed to cool to $40^0$C and 1% Nystatin suspension was added to it to inhibit fungal growth. It was then poured; 20ml into each sterile disposable petridishes and allowed to solidify at room temperature. The petridishes were then stored in the refrigerator until required for use.

One (1) gram of the composted manure was weighed out and mixed with 10ml of distilled water and swirled to make a good solution. Five test tubes were arranged in the test tube rack, each was filled with 9ml of distilled water, and 1ml of the composted manure solution was collected with pipette and added to the first test tube containing 9ml of distilled water, thereby making it 10ml. From the first test tube, 1ml was also collected and added to the second test tube. This process was carried out in successive manner up to the fifth test tube where 1ml was discarded, leaving it as 9ml. With the five-fold serial dilution, different concentrations of the manure were achieved in each of the test tube. From each test tube, 0.1ml of the diluted sample was cultured directly on duplicate plates (petridishes) of nutrient agar with Nystatin (Uwaezuoke *et al.,* 2008). The inoculated plates (petridishes) were labeled properly with different concentrations and dilutions of the corresponding test tubes. Plates containing nutrient agar were incubated at $37^0$C for 24 hours and were further examined for bacterial growth.

**Isolation and Identification of Bacteria from Composted Manure:-**
The bacterial isolates were sub-cultured on nutrient agar (NA) to obtain pure cultures and their characteristics and morphologies were identified. They were further subjected to series of test, which include:- gram staining reaction and biochemical tests such as catalase, coagulase, citrate, indole methyl red, oxidase and motility.

**Isolation and Identification of Cocoyam Leaf Blight Pathogen (*Phytophtora colocasiae*):-**
One (1) gram of sections of the leaves which was infected with cocoyam leaf blight disease was cut off with a sterile razor blade. This was mashed to make a paste, and 10ml normal saline was added to it. Five test tubes were arranged in a test tube rack, and each was filled with 9ml distilled water. 1ml of the infected cocoyam leaf solution was taken with pipette and added to the first test tube containing 9ml of distilled water, making it 10ml.

From the first test tube, 1ml was also collected and added to the second test tube. This process was carried out in successive manner up to the fifth test tube. From the fifth test tube, 1ml was discarded, leaving it as 9ml; with the five-fold serial dilution, different concentrations of the cocoyam leaf blight was achieved in each of the test tubes.

**Antagonism of Composted Manure Microbial Isolates to Cocoyam Leaf Blight Pathogen Using Well In Agar Method:-**
From each test tube containing different concentrations of the diseased cocoyam leaf solution, 0.1ml was collected and inoculated on NA agar plate and spread evenly on the surface of the media with a glass rod spreader. For each concentration four agar plates were prepared to test the four microbial isolates of composted manure.

Control plate of only cocoyam leaf solution on agar was also prepared for the different concentrations. Hole of 8.0mm in diameter was created on each of the agar plates using a sterile core borer by removing circular plugs of agar. For each of the four agar plates, the four microbial isolates were introduced into the hole using sterile wire loop.

Plates containing the isolates were incubated at $37^0$C for 24-48 hours. At the end of incubation, plates were checked for areas of antagonism or diameter of clearance. The arrangement was laid out in a completely randomized design, while the experiment was replicated three times.

**Data Collection:-**
Data were collected on the following parameters: Morphological appearance of bacterial culture, gram staining and biochemical qualities of bacteria isolates. Types and number of bacterial isolates from the composted culture, level

of antagonism of cocoyam leaf blight pathogen by isolates of bacteria from composted cultures. The level of antagonism was expressed as the diameter of zone of growth inhibition in the culture, measured in millimeters (mm).

**Data Analysis:-**

Data on the diameter of zone of clearance (antagonism) was subjected to analysis of variance of the completely Randomized Design procedure, under the following model; $X_{ij} = \mu+t_i+\sum_j$ .However, the above analysis was achieved with the use of Statistical Analytical System (SAS) package 10.1 editions (2007). Means were separated with Least Significance Difference (LSD) test and interpreted according to methods of Onuh and Igwemma (2007).

## Results:-

Tables 1 and 2 below showed the appearance of the bacterial culture for 24 and 48 hours in terms of colour, elevation, number of colonies, capacity, surface, shape and type of colony. It was observed that higher concentrations of the manure produced higher colonies of bacteria.

**Table 1:-**Morphological Appearance of Bacterial Culture after Twenty Four (24) Hours in Nutrients Agar

| Concentration | Colour | Elevation | N0. Of Colonies | Capacity | Surface | Shape | Types of colonies | Suspected Organisms |
|---|---|---|---|---|---|---|---|---|
| $10^{-1}$ | Blue-green | Low convex | 102 | Opaque | Rough | Oval | 1 | *Pseudomonas spp.* |
| $10^{-2}$ | Yellow-green | Low convex | 84 | Opaque | Rough | Oval | 1 | *Pseudomonas spp.* |
| $10^{-3}$ | Colourless Gray | Low convex | 76 | Translucent | Smooth | Entire | 1 | *Escherichia coli* |
| $10^{-4}$ | Gray | Raised | 60 | Transparent | Rough | Ground-glass | 1 | *Bacillus spp.* |
| $10^{-5}$ | Orange yellow | Raised | 51 | Opaque | Smooth glistering | *Cocci* | 1 | *Staplylococcus aureus* |
| $10^{-6}$ | Blue – green | Low convex | 42 | Opaque | Rough | Oval | 1 | *Pseudomonas specie* |
| $10^{-7}$ | Colourless gray | Low convex | 34 | Translucent | Smooth | Entire | 1 | *Escherichia coli* |
| $10^{-8}$ | Orange yellow | Raised | 22 | Opaque | Smooth glistering | Cocci | 1 | *Staplylococcus aureus* |
| $10^{-9}$ | Colourless gray | Low convex | 14 | Translucent | Smooth | Entire | 1 | *Escherichia coli* |
| $10^{-10}$ | Blue – green | Low convex | 5 | Opaque | Rough | Oval | 1 | *Pseudomonas specie* |

**Table 2:-** Morphological Appearance of Bacterial Culture after Forty Eight (48) Hours in Nutrients Agar

| Concentration | Colour | Elevation | N0. Of Colonies | Capacity | Surface | Shape | Types of colonies | Suspected Organisms |
|---|---|---|---|---|---|---|---|---|
| $10^{-1}$ | Blue-green | Low convex | 110 | Opaque | Rough | Oval | 1 | *Pseudomonas spp.* |
| $10^{-2}$ | Yellow-green | Low convex | 92 | Opaque | Rough | Oval | 1 | *Pseudomonas spp.* |
| $10^{-3}$ | Colourless Gray | Low convex | 81 | Translucent | Smooth | Entire | 1 | *Escherichia coli* |
| $10^{-4}$ | Gray | Raised | 73 | Transparent | Rough | Ground-glass | 1 | *Bacillus spp.* |
| $10^{-5}$ | Orange yellow | Raised | 62 | Opaque | Smooth glistering | *Cocci* | 1 | *Staplylococcus aureus* |
| $10^{-6}$ | Blue – green | Low convex | 50 | Opaque | Rough | Oval | 1 | *Pseudomonas specie* |
| $10^{-7}$ | Colourless gray | Low convex | 44 | Translucent | Smooth | Entire | 1 | *Escherichia coli* |
| $10^{-8}$ | Orange yellow | Raised | 32 | Opaque | Smooth glistering | Cocci | 1 | *Staplylococcus aureus* |
| $10^{-9}$ | Colourless gray | Low convex | 21 | Translucent | Smooth | Entire | 1 | *Escherichia coli* |
| $10^{-10}$ | Blue – green | Low convex | 12 | Opaque | Rough | Oval | 1 | *Pseudomonas specie* |

Table 3 summarized gram staining reactions and biochemical tests of samples from pure culture of manure. The tests were used to identify the particular species of the bacteria organism.

NA is nutrient Agar while T is the treatment. In the course of the study the treatments were repeated to get pure cultures in order to avoid doubt.

**Table 3:-**Gram Staining and Biochemical Characteristics of Bacteria Organisms Isolated from the Manure Sample

| Samples | Gram Reactions | Catalase | Coagulase | Citrate | Indole | Methyl red | Oxidase | Motility | Suspected organism |
|---|---|---|---|---|---|---|---|---|---|
| $NAT_2$ | Gram negative (-ve) rod in clusters | + | - | - | - | - | + | + | *Pseudomonas spp.* |
| $NAT_1$ | Gram negative (-ve) red in clusters | + | - | - | - | - | + | + | *Pseudomonas spp.* |
| $NAT_4$ | Gram negative (-ve) in pairs | - | - | - | + | + | + | + | *Escherichia coli* |
| $NAT_1$ | Rod positive (+ve) in clusters | + | - | + | - | + | - | - | *Bacillus spp* |
| $NAT_2$ | Rod positive in clusters | + | - | + | - | + | - | - | *Bacillus spp* |
| $NAT_5$ | Gram positive cocci in cluster | + | + | + | + | - | - | - | *Staphylococcus aureus* |
| $NAT_2$ | Rod positive Rod in clusters | + | - | + | - | + | - | - | *Bacillus spp* |

**Table 4:-**Bacteria Organisms Isolated From Composted Farmyard Manure Cultured In Nutrients Agar.

| Bacteria Type | Number of Colonies |
|---|---|
| *Pseudomonas spp.* | 14 |
| *Escherichia coli* | 6 |
| *Bacillus spp* | 8 |
| *Staphylococcus aureus* | 3 |
| **Total** | **31** |

Four bacteria species were isolated from composted manure cultured in nutrient Agar medium. Out of the 4 bacteria organisms isolated, *Pseudomonas spp* occurred highest (14 in number) followed by *Bacillus spp* (8), *Escherichia coli* (6), and *Staphylococcus aureus* (3) giving a total of 31(Table 4)

The result presented in the Table 5 showed that *Pseudomonas spp* had the highest antagonism to cocoyam leaf blight pathogen with a clearance diameter of 43.167mm. This was followed by *Bacillus spp* that produced diameter of clearance of 40.333mm, but it was not significantly different ($P \leq 0.05$) from the diameter of clearance produced by *Pseudomonas spp*(Table 5). However, there was no significant difference ($P \leq 0.05$) in the diameter of clearance produced by (*Eucherichia coli* (19.667mm) and that of *Staphylococcus aureus*(17.333mm) as presented in the Table 6. But the performance *E. coli and S. aureus* was significantly different from that of *Pseudomonas spp* and *Bacillus spp.* (Table 5).

There was no significant difference between *pseudomonas spp* and *Bacillus spp* while significant difference exists between them and *Escherichia coli* and *staphylococcus*. Also there was no significant difference between *Escherichia coli* and *staphylococcus aureus*.

**Table 5:-**Mean Diameter of Clearance (Antagonism) of Leaf Blight Disease Pathogen (*Phytophtora colocasiae*) by Bacteria Isolates from Composted Farmyard Manure.

| Bacteria Antagonist | Mean* Diameter of growth inhibition |
|---|---|
| *Pseudomonas spp.* | 43.167[a] |
| *Bacillus spp .* | 40.333[a] |
| *Escherichia coli* | 19.667[b] |
| *Staphilococcus aureus* | 17.333[b] |
| LSD 0.05 | 3.06 |

*Means having the same letter are not significantly different at $P \leq 0.05$*

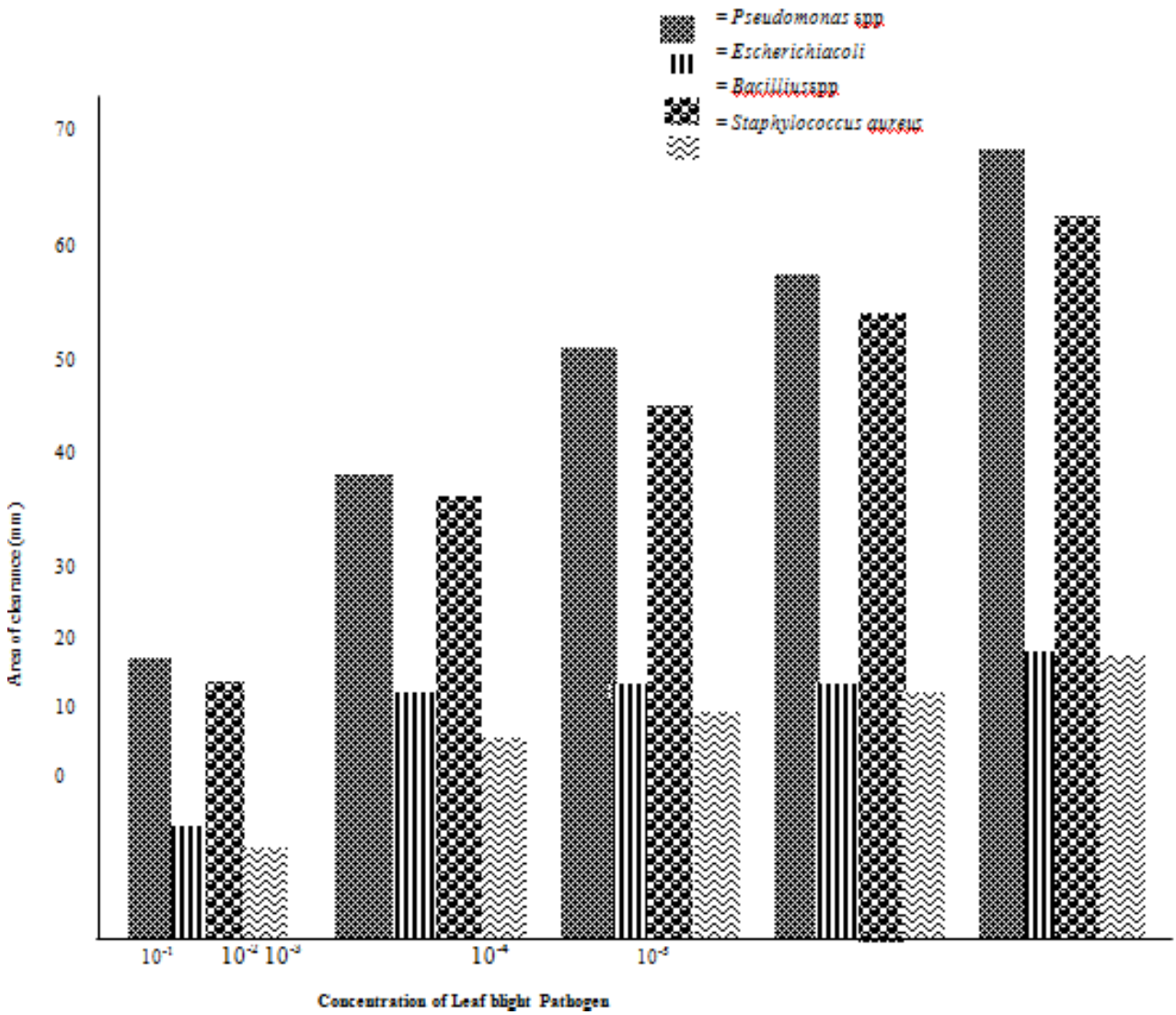


**Figure 1:-**Level of antagonism of the Leaf blight disease pathogen by the bacteria organisms isolated from composted farmyard manure.

## Discussion:-

This study was carried out to investigate the potentiality of bacteria isolated from composted manure that can be effective in providing biological control to the leaf blight disease (*Phytophtora colocasiae*) of cocoyam. Results of the morphological appearance of bacterial culture after twenty four hours and forty eight hours showed steady increase in the number of colonies. After 24 hours the highest number of colonies was 102 while at 48 hours it recorded 110 showing an increase of 8 colonies in the $10^{-1}$ manure concentration.

The bacteria colonies were investigated by adopting the procedure of Aryamtha *et al.,* (2006) on the basis of colour, elevation, number of colony, capacity, surface, shape and type of colony. It was observed that the colonies coloured blueyish green, yellowish green, colourless to grey. Some colonies have opaque capacity, while others have transparent capacity. Majority of the colonies have smooth and rough surface. These observations are similar to the

findings of Aryamtha *et al.,* (2006) who reported that bacteria colonies appear in different colours, capacity, shape, surface and elevation.

It was observed that number of bacteria colonies decreased as the concentration of the composted manure samples decreased. Thus, the $10^{-1}$ manure concentration recorded the highest number of bacteria colonies while $10^{-10}$ manure concentration recorded the lowest colonies. This observation may have been as a result of concentration of more manure samples in the $10^{-1}$ dilution, which gradually decreased in manure concentration as the dilution factor increased; given rise to lower bacterial colonies in the higher diluted manure samples.

Gram staining reaction and some biochemical tests such as catalase, coagulase, citrate indole, methyl red, oxidase, and motility were done based on the characteristics of the bacterial organisms. Four species of bacteria were isolated and they are: *Pseudomonas spp*, *Bacillus spp, Escherichia coli* and *Staphilococcus aureus*. It was observed that *Pseudomonas spp* was the most frequent organism isolated from the composted manure, followed by *Bacillus spp, Escherichia coli* and *staphylococcus aureus, respectively.*

Studies made on the mean diameter of growth inhibition (antagonism) of leaf-blight disease pathogen (*Phytophtora colocasiae*) by bacteria isolates from composted manure recorded *Pseudomonas spp* as having the highest antagonistic properties followed by *Bacillus spp*, even though there was no significant difference between them. This efficacy of *Pseudomonas spp* was also reported by Maryem *et al.,* (2013) in biological control of root-knot nematode. Also Alavi, *et al.,* (2013) confirmed this in his work; biological control of take-all disease by isolates of *Pseudomonas* and biosynthesis of silver nanoparticles by the culture supernatant of *Pseudomonas*. Pal and Gardener, (2006) in their study, reported the efficacy of *Bacillus spp* in the biological control of plant fungal pathogens such as *Alternaria, Pythium, Aspergillus, Fusarium, Rhizoctonia, Phytophtora* etc. *Escherichia Coli* and *Staphylococcus aureus* also showed some level of antagonistic properties to cocoyam leaf blight disease but they were not as effective as *Pseudomonas* and *Bacillius spp.*

It was observed from the results that the level of antagonism of the bacteria isolates from the composted manure increased with decrease in the concentration of the leaf blight disease pathogen samples. Thus, the antagonistic bacteria were effective in antagonizing the pathogen in the lower concentrations, perhaps as a result of reduction in the density of the pathogens in the lower concentrations to be attacked. Hence, it was easier for the level of antagonist bacteria used to attack the pathogen under scanty density. The results generally indicated that composted manure can yield bacterial isolates such as *Pseudomonas spp* and that can be harnessed in the biological control of cocoyam leaf blight disease.

## Conclusion:-

The potential of bacteria in the control of cocoyam leaf blight disease was evaluated. Responses from the different group of micro-organisms, viz-bacteria showed antibiotic and parasitic properties to the disease pathogen .It was also observed that the microbial isolates from the composted manure were more effective in the antagonism activity when the disease pathogen concentration is lower. It is therefore, recommended that *Pseudomonas spp* and *Bacillus spp.* should be explored further in their possible use as biological agents in the control of leaf blight disease of cocoyam.

## Reference:-

1. Adams, J.A. (1991) Weight loss caused by development of *Sitophilus zeamais (Motsch) in maize. Jour. Stor. Res 12: 269-272*In: Ugwuoke *et al*. (2008). The efficacy of botanical Protectants in the storage of Cocoyam (*Colocacia esculenta (L) Schott). Journal of Tropical Agriculture, Food and Environment and Extension Volume 7 Number 93-98.*
2. Alabouvette C, Olivain C, Stem berg C, (2006) Biological control of plant diseases: the European situation Eur. J *plant Pathol 114: 329-341*.
3. Alavi, N. R. Saberi R, P. Khodayam and R. Rhanjbar, K. (2013)Archives of *Phytopathology* and Plant Protection; Biological control of take all disease by isolates of *Pseudomonas fluorescens* and *biosynthesis of silver nano particles* by the culture supernant of *Pseudomonas fluoresces* CHAO; DOI:10.1080/0323 5408. 2013. 85 6550.
4. Anryantha, I.P., Cross, R and Guest, D. I. (2006) School of life sciences and technology and center for life sciences, Institute Tekcnologi Bandung, JalanGanesha 10, Bandung 40132, Indonesia. Plant Pathology Journal 5 (3): 291-298.

5. Brooks, F.E. (2000). Cultivar Resistant to Taro leaf Blight Disease in American Samoa, America Samoa Technical Report, 34, 49-58.
6. Chesbrough, M. (2002) Medical Laboratory Manual for Tropical Countries Volume II. Microbiological Tropical health Technology Buteroworths and Co Ltd. Cambridge/Sevanaks.
7. Chiejina, N.V, Ugwuja, F.N. (2013). Incidence of *Phytophtora* leaf – Blight Disease of Cocoyam in Nsukka area of South-Eastern Nigeria. *Journal of Botanical Research, Volume 4, Issue 1, Pp 21-*24.
8. Chukwu, G.O (2012) Edible species of Cocoyam, and Diseases. Paper Presented at ADP Monthly Technology Meeting (MTRM) 1-4p.
9. CMI, (1997). Commonwealth Mycology Institute, Distribution Maps of Plant Diseases, Map No. 466, Edition 3. *Phytophtora colocasiae.* Commonwealth Agricultural Bureau, Wallingford, Oxfordshire, Uk.
10. Dianez, F., Santos, M., Boix, A., De Cara, M., Trillas, I., Aviles, M and Tello J.C, (2006). Grape Marc compost tea suppressiveness to plant pathogenic fungi: role of siderophores. Compost Sci: util. 14: 48-53.
11. El-Jahil, M.H. and Zinedine, A. (2008). International Journal of Agriculture and Biology http://www.fspublishers .org.
12. Fisinin, V.I. and Arkhipchenko I.A. (2001). Microbial fertilizers from Poultry manure. All Russian Scientific Research and Technological Poultry Institute and All-Russian Research Institute of Agricultural Microbiology volume 17.No. 2.
13. Gadre, U.A and Joshi, M.S. (2003). Influence of weather factors on incidence of leaf blight of *Colocasia.* Annual Plant Protection Science, 11: 168-170.
14. Helen, T. (1996).Taro breeding programme for disease resistance. *In Annual Report 1994, Solomon Islands Government, Agriculture Division, Ministry of Agriculture and Lands, Research Department* (Pp, 30-32). Honiara, Solomon Islands: Dodo Creek Research Station.
15. Helen, T. and Graham, J. (2000).Improved plant protection in Solomon Islands. Extension factsheet 14: taro leaf blight 1-2p.
16. Hoopen, G.M.T, Reese, P, Aisa, T. Stirrup and U. Kuanss, (2003) Population dynamics of epiphytic mycoparasites of the biocontrol of black pod (*Phtophtora palmivora)* and *moniliasis (Moniliophtora roreri)* on cocoa (*Theobroma cacao).* Mycol. Res, 107:587-596.
17. Jackson, G.V.H, (1996). A Bibliography of Taro leaf Blight with special reference to the Pacific Islands. Pp. 7.
18. Jacobsen, B. J, (2001) Biological Control of potato pathogen. In: Biological control of plant diseases, New York (NY); Marcel Dekker; P. 179-189.
19. Jager, G., Velvis, H., Lamers, J. G, Mulder, A and Roosjen, J. S, (1991) Control of *Rhizoctonia solani* in potato by Biological, Chemical and Integrated measures. Potato resources, 34:269-284.
20. Jugurnauth S., Soomary S. and Hanoomanjee P. (2002) Fifth Annual Meeting of Agricultural Scientists, 37.
21. Maheshwari, S.K, Salu, A.K. Misra, R.S. and Sriram, S. (2001). Field Evaluation of fungicides against *Phylophtora* leaf blight. Integrated management of *Phytophtora* leaf blight disease of taro (*Colocasia esculenta (L.) Schoott*).*Journal of Root Crops, 2007 Vol. 33 No. 2144-146.* Indian Society for Root Crops.
22. Manyem, T.N; Navazollah, S; Hassan, R.E. (2013). Archieves of *Phytopathology* and Plant protection: Biological Control of root knot nematode (Meloidogyne javanica) disease by *Pseudomonas flurescens (Chao). Pp:2.*
23. Mbong, G.A, Fokunang, C.N, Lum A. F., Bombot M. B, Tembe, E.A. (2013). An over view of *Phytophtora colocasiae* of cocoyam. *Discourse Journal of Agriculture and Food Sciences.*
24. Misra, R.S. (1999). Management of *Phytophtora* leaf blight disease of taro. Tropical Tuber Crops: Food Security and Nutrition. Oxford and IBH. New Delhi, India.
25. Ngoka, D.A. (1997) Guide to crop production: Alphabet Nigeria Publishers, Owerri 11p.
26. Obiajuru, I.O.C and Ozumba, U.C. (2009).Laboratory Methods for Medical Microbiology and Parasitology Lifeway Amalgamations. Owerri. ISBN: 25799. 107. P. 183.
27. Ohazurike, N.C., Obi, V.I. (2000). Basic Principles of plant pathology; Tetan Publisher Ltd; Owerri.
28. Ohazurike, N.C; Arinze, A.E; Onuh, M.O; Ozurumba, C.I. (2003).The Nature of Plant Pests and Disease. Supreme Publishers, 47 Okigwe Road Owerri, Nigeria pp. 2.
29. Okamoto, H.M. Sato, Y. Miyata, M. Yoshikawa and M. Isaka (2000) Biocontrol of *Phytophtora* root rot of Angelica trees by *Enterobacter cloacae and serratiaficaria*s trains J. General plant Pathol. 66: 86-94.
30. Onuh, M.O. and Igwemmaa, A.A. (2007).Applied Statistical Techniques for Business and Basic Sciences, Skillmark Media Limited 18 Kagha street Owerri.
31. Ooka, J.J. (1994) Taro disease. *A guide for field identification HITARH Res. Extension series No. 148.*CTAHR Publication University of Hawaii Honolulu, H.I.

32. Pal, K. K.and Gardener, B. M. (2006).*Biological Control of plant pathogens. The plant Health Instructor.*1-25.
33. Rajkumar, M. Lee, W. H. and Lee, K.J. (2005).Screening of bacterial antagonist for biological control of *Phytophtora* blight of pepper.*J, Basic microbial*, 45:155-63.
34. Scot, N., Fred, B. and Glenn T. (2011) college of Tropical Agriculture and Human Resources, University of Hawaii at Manoa Plant Disease Pp -71.
35. Sid, A.M., Ezziyyani, C., Egea-Gilabert and Candela M. E. (2003).Selecting bacterial strains for use in the bio control of diseases caused by *Phytophtora capsici* and *Alternaria alternata* in sweet pepper plants. Biol. Plant. 47:569-574.
36. Trujillo, E.E. (1996). Tara leaf blight research in the American pacific *ADAP Bulletin 1, 1-3.*
37. Trujillo; E.E (1993).Status of *Phytophtora* leaf blight of taro in Western Samoa and recommendations for its control. Washington DC, USA: USDA/OICD/DRD/AAE.
38. Ugwuanyi J.O. and Obeta J.A. (1996). Fungi associated with storage rots of cocoyams (*Colocasia* spp.) in Nsukka, Nigeria. Mycopathologia, 134(1), 21-25.doi:10.1007/BF00437048.
39. Ugwuja F.N. and Chiejina N.V. (2011) Nigerian Journal of Plant Protection, 25, 203-213.
40. Uppal, A.K, El-Hadrami, A., Adam, L.R., Tenuta, M. and Daayf, F. (2008). Biological Control of Potato verticillium wilt under controlled and field conditions using selected bacterial antagonists and plant extracts Biol Control, 44:90-100.
41. Uwaezuoke, J.N, Obiukwu, C.E, Nwachukwu M.I. (2008). *Basic Microbiology Laboratory Manual.* Megasoft *Publisher. Owerri,* Pp.7.
42. Wilson, W. and Lynnette, T. (2007).Green culture *www.greenculturesg.com* 1-4p.
43. Wulff, E.G., Van Vuurde J.W. L; Hockenhull J. (2003). The ability of the biological control agent *Bacillus subtilis*, strain BB, to colonize vegetable brassicas endophytically following seed inoculation. *Plant soil.* 225: 463-474.