ISSN No:-2456-2165

Isolation and Identification of Fungi Associated with the Spoilage of Cucumber Fruit Sold in Igbariam, Anambra State and Possible Control Method Using Lime Juice Extract

Ejimofor Chiamaka Frances[1], Oledibe Odira Johnson[2*]
[1]Department of Biological Sciences, Chukwuemeka Odumegwu University, Uli, Anambra State, Nigeria.
[2] *Corresponding author, Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Abstract:- Fruits and vegetables are generally very vital to the human system due to their various minerals and vitamin contents. Some of them are more nutritious than others and are in higher demands. This study was conducted to isolate and identify fungi associated with the spoilages of cucumber fruit sold in Igbariam. The pesticidal control method using lime juice extract was also examined.The samples (cucumber) were analyzed and examined using standard laboratory procedures. Ethanol was used in extraction of the lime juice while chloramphenicol was used as control. The result of the analysis showed fungual pathogen such as mold, *Aspergilius spp, Fusarium spp* and *Alternaeria spp* were found to be associated with spoilage in cucumber. The result also showed that ethanol extract of lime juice tend to have good inhibitory effect on these organism, but a better inhibition level is noted at 6mg/l which is significant at p<0.05%. Lime juice extract showed the highest good inhibitory effect on mold (12mm) and lowest on *Aspergillus spp* (6 mm) at 6mg/ml while chloramphenicol showed the highest good inhibitory effect on *Alternaeria spp* (3.2mm) and lowest on *Fusarium spp* (1mm). This showed that the lime juice extract have antifungal properties that can be helpful in the control and management of scab fungi of apple fruit.Therefore, the study recommended that farmer should use lime juice for the control of fungi rot organisms associated with cucumber and has the potentials of causing damage loss of cucumber.

Keywords:- *Cucumber, Lime Juice, Fungi.*

I. INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an important vegetable used to be cultivated once annually during the rainy season in Nigeria. It is an important vegetable cultivated in Northern central, Nigeria both in rainy and dry season using irrigation. The fruits serves as refreshments during farming activities [1].

Cucumber is a creeping vine that bears cylindrical fruits. It is known as *Cucumis sativus* L, it belongs to the gourd family cucubitacea Other vegetables which belong to this family include Melon, squash, Watermelon and Pumpkins [2]. It originated from the Asia continent. Cucumber plant can be cultivated in both temperate and tropical environment hence it is said to be a native of many regions of the world [3,4].

The fruit is normally consumed raw alone or eaten with other vegetables such as salad in Nigeria [5]. Cucumber is majorly cultivated because of its nutritional and medicinal relevance. Seed kernels are occasionally eaten [6].

Cucumber (*Cucumis sativus* L.) is one of the most important economic vegetable crops all over the world. It is cultivated in open fields and protected houses in Egypt for both local consumption and export. The occurrence of fungal spoilage of fruits is recognized as a potential health hazard to man due to their production of mycotoxins [7].

Cucumber plants are subject to attack by several fungal diseases that affect the yield quantity and quality including *Alternaria tenuis*, *A. alternata*, *Botrytis cinerea*, *Choanephora cucurbitarum*, *Didymella bryoniae*, *Fusarium oxysporum*, *Geotrichum candidum*, *Penicillium oxalicum*, *Phytophthora capsici*, *Rhizopus nigricans* and *Sphaerotheca fuliginea* [8,9,10,11].

The ability of public health agencies to identify through enhanced epidemiological and surveillance techniques, raw vegetables as probable sources of infectious microorganisms has undoubtedly resulted in increased numbers of documented outbreaks. The risk of illness associated with raw vegetable products can be reduced by removing or killing pathogenic microorganisms by washing or treating them with sanitizers. However, the hydrophobic cutin, diverse surface morphologies and abrasions in the epidermis of fruits and vegetables limits the efficacy of this treatment [12].

Vegetables are frequently consumed raw without being exposed to the processes that reliably eliminates pathogens. Washing fruits and vegetables in chlorinated water can reduce bacterial levels but cannot be relied upon to eliminate pathogens. Eating or drinking contaminated foods or drinks

ISSN No:-2456-2165

can cause foodborne disease. Many different types of bacteria, viruses and parasites can contaminate food, so there is numerous different food borne infections. The consumption of carrot, cucumber, onions and cabbage in Nigeria has increased tremendously in the recent years properly due to increased awareness on their health important. Carrot is known to contain an important biologically active compound, carotenoid [13].

Cucumber fruit rot is not only a challenge to the farmers but also the vegetable and fruit vendors who will have to shelf it for sometimes in the course of selling. Rot occurring on shelf reduces shelf life and market value and altogether renders fruits unfit for human consumption. There is paucity of information on fungi associated with or responsible for spoilage of cucumber fruits in the Nigeria, especially in Igbariam, Awka Anambra state. It as result of this that this study focus on isolation and identification of fungi associated with the spoilage of cucumber fruit sold in Igbariam and the pesticidial control method using lime juice

II. MATERIALS AND METHOD

The samples lime and cucumber were all purchased from Igbariam and transported in a sterile bag to Maeve academic research laboratory where it was identified before being analysed in the laboratory.

2.1 Experimental Equipment

The equipment's used in the experiment includes, autoclave, knife, binocular microscope, microscopic slide, slid cover, conical flask, 250ml and 500ml beaker, inoculating loop, cotton wool, cork borer, foil, burnson burner petri dish and masking tape.

2.2 Experimental Reagents

The reagents used includes Distil water, Ethanol, lactophenol, Potato Dextrox agar (PDA).

2.3 Samples Preparation Procedure

2.3.1 Preparation of the samples

The surface of the sample was first surface sterilized with 70% alcohol and the infected portion cut off into small pieces of size 3cm by 3cm then kept in beaker ready for use. The cucumber fruit was grounded into paste under room temperature at 25± 1^0c and kept in 150ml beaker ready for inoculation into culture media. The lime juice was extracted manually from the lime orange fruit through cold press method and well kept in 150ml beaker.

2.3.2 Preparation Of Culture Media

The method was employed and used in the preparation of the culture media. One gram of potato dextros agar (PDA) was dissolved in 45ml of distil water, then autoclaved for 15min under the pressure of 15 pounds pressure(psi) at a temperature of 121^0_C. The media was poured into petri dish and allowed to cool into cake form ready for inoculation. The prepared apple sample that was cut from off from the infected surface was inoculated culture media and incubated for 72hours for growth. After the incubation period, the observed growth was subculture to get a pure culture.

2.4 Colony Count

The direct colony count method was used. In this method, the colonies of fungi were counted directly from the cultured plate.

2.5 Identification of Fungi Isolate

Both morphological and anatomical characteristics of the fungi were used in the identification process of the fungi isolate. This was done as follows.

A: Morphological identification Methods of as described by Cheesebrough [14]: The growth pattern and pigments produced by the fungi was observed, match against those in fungi identification kit and recorded accordingly.

B: Anatomical Identification: Here, guides according to Alexopoulos [15] were used for the various tests and examinations. A smear of the fungi growth was fix on two different slide and stained with distil water and lacto phenol. Both of the smears were viewed under binocular microscope and the anatomical characteristic recorded and as well match against those on fungi identification kit then identified accordingly.

2.6 Pathogenicity Test

The pathogenicity test was carried out to establish which of the fungal isolates caused the rot and to determine whether they could induce similar symptoms on inoculation and be re-isolated, thus fulfilling Koch's postulates. The method of Okafor [16], Okigbo and Ikediugwu [17] were adopted for the pathogenicity study.

Pure culture of the fungi was isolated using inoculation loop of length 5cm while a healthy apple fruit was surface sterilized using 100% ethanol. With the use of cork borer, a smooth deep cut was made on the healthy yam tuber to a depth of 2cm with a diameter of 10mm wide. The pure culture of the fungi was inoculated and the 10mm cover of the initial cut healthy apple fruit part was used to cover it. The sample was kept in an incubation chamber for 72hr then being observed every 3 days to ascertain the pathogenicity of the organism.

2.7 Anti-microbial Assay

Effect of plant extract on mycelia growth of the test fungi was studied using the food poisoning techniques [18]. 4mg/l, 6mg/l and 8mg/l of the plant extract were pipetted into labelled petri dishes containing the pure culture of the microbial organism respectively. The Inhibition zones of the microorganism was evaluated and recorded in terms of radial growth of the microbes on the medium with and without extracts and results were analysed on the basis of percentage growth Zone inhibitions of microorganisms. The inhibition of microbial growth on PDA medium was used to quantify the toxicity of extracts. Percentage growth inhibition for 5 days was calculated.

$$\% \text{ growth inhibition} = R_1 - R_2 \times \frac{100}{R_2}$$

Where R_1 = is the furthest radial distance of pathogens in control plates
Where R_2 = is the furthest radial distance of pathogens in extract (treated) plates. The inhibition percentage was determined as a guide in selecting the minimum inhibition concentration that will be effective in controlling the microbial organisms.

2.8 Statistical Analysis

Data collected were subjected to two-way analysis of variance (ANOVA) with the use of Sigmaplot version 12 statistical software to ascertain the level of significance of the treatment given to at $LSD_{0.05\%}$.

III. RESULTS

Table 4.1 Anti-Microbial Inhibition zone of lime juice

Microorganism	4mg/ml	6mg/ml	Control (Chloramphenicol)
Aspergilius spp	3mm	6 mm	3.1mm
Fusarrium spp	5mm	8mm	1mm
Mold	3.mm	12mm	3mm
Alternaeria spp	7mm	9mm	3.2mm

Table 4.1 shows that the fungi rot organism isolated from cucumber included *Aspergilius spp, Fusarrium spp, mold* and *Alternateria spp*. This fungi were noted to be at different dosage with corresponding inhibition zone. *Aspergilius spp* was seen to be inhibited up 3mm at 4mg/l, fusarium is 5mg/l, mold fungi, 3mm and *Alternaeria spp* 7mm. at 6mg/l *Aspergilius spp* was inhibited up to 6mm *Fusarium spp* 8mm, mold fungi was highly inhibited as indicated by 12mm zone of inhibition while *Aternaeria spp* is 9mm. the control experiment inhibition is less than the extract inhibition level. This indicates that lime juice has a significant effect on the fungi as a control method.

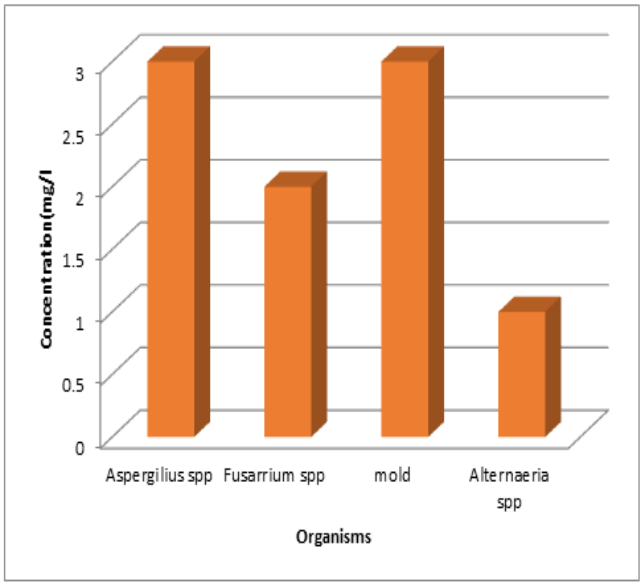

Fig 1: Zone of inhibition fungi organism treated with lime juice extract at 4mg/l

Fig 1, shows that *Alternaeria spp* is highly inhibited by the extract followed by *Fusarium spp*, while *Aspergilius spp* and mold fungi are not really inhibited.

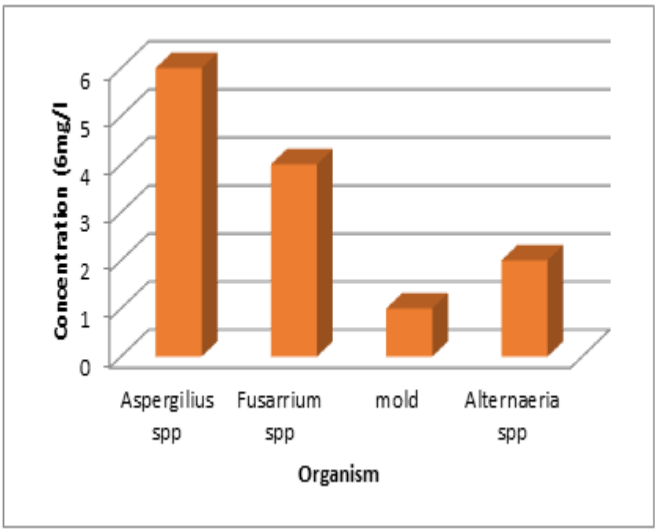

Fig 2: Zone of inhibition fungi organism treated with lime juice extract at 6mg/l

Fig 2 shows that at 6mg/l mold fungi is highly inhibited followed by *Alternaeria spp* and *Fusarium spp* and least in *Aspergilius spp*. This mean that at concentration of 6mg/l there will be more inhibition of the mold and *alternaeria spp*

Table 4.2:Two-Way ANOVA

Source of Variation	DF	SS	MS	F	P
ORGANISM	3	5.677	1.892	0.549	0.670
TREATMENT DOSAGE	2	78.807	39.404	11.442	0.014
Residual	5	17.219	3.444		
Total	10	112.800	11.280		

Tested at 0.05% level of significance

Table 4.2 shows that the treatment dosages of 4mg/l and 6mg/l are all significant at $p<0.05\%$. This means that at both dosages, the fungi organisms of cucumber is inhibited.

Table 4.3: Least significant mean differences for treatment Dosage

Group	X±SD
FOUR	4.500±0.928
SIX	9.492±1.136

Result are in mean± standard deviation ($LSD_{0.05\%}$)

Table 4.3 shows that treatment dosage of 6mg/l has the highest mean value which as compared to 4mg/l. this infers that 6mg/l is a better treatment dosage for the control of cucumber rot fungi.

ISSN No:-2456-2165

Plate 1: Colonies of Cultured Fungi

Plate 4: Pure culture of *Alternaeria spp*

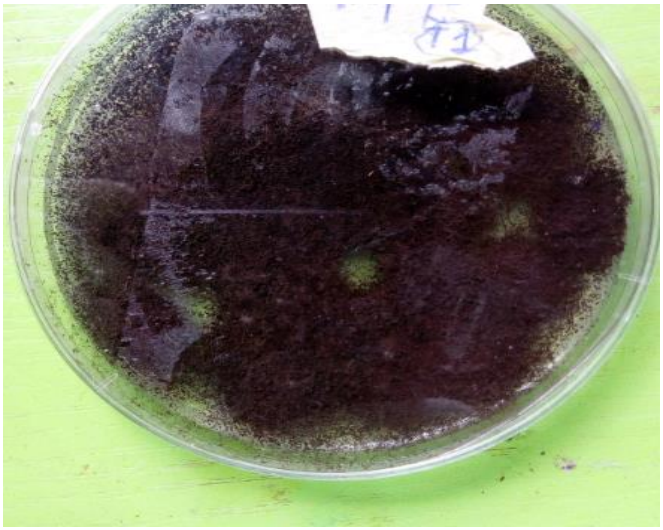

Plate 2: Pure culture of *Aspergilius spp*

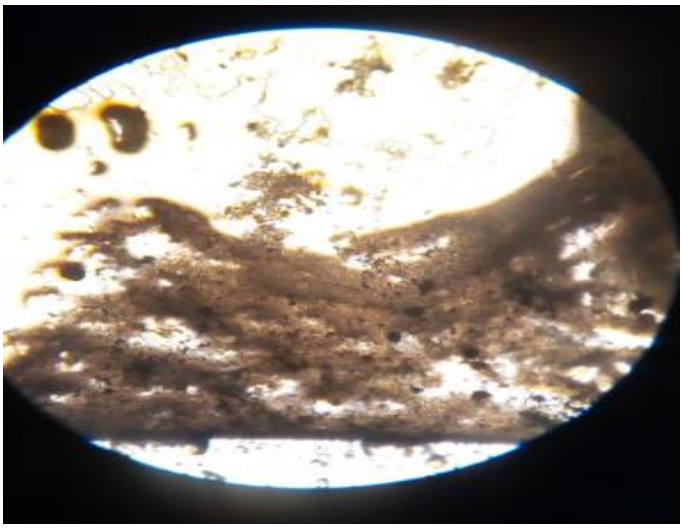

Plate 5: Microscopic view of *Alternaeria spp*(x10)

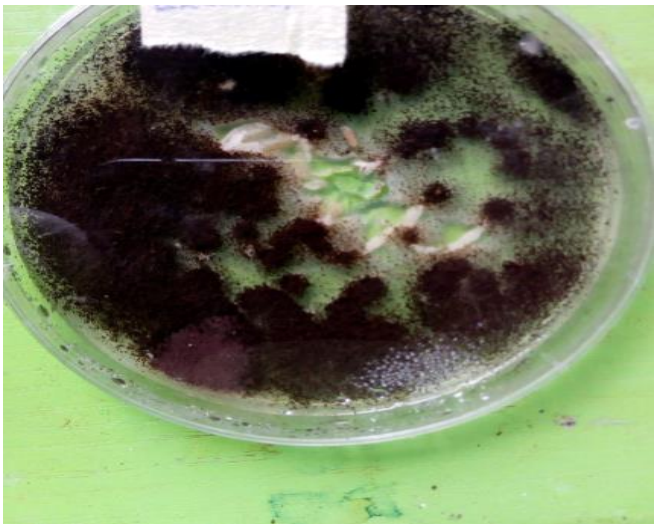

Plate 3: Pure Culture of mold fungi

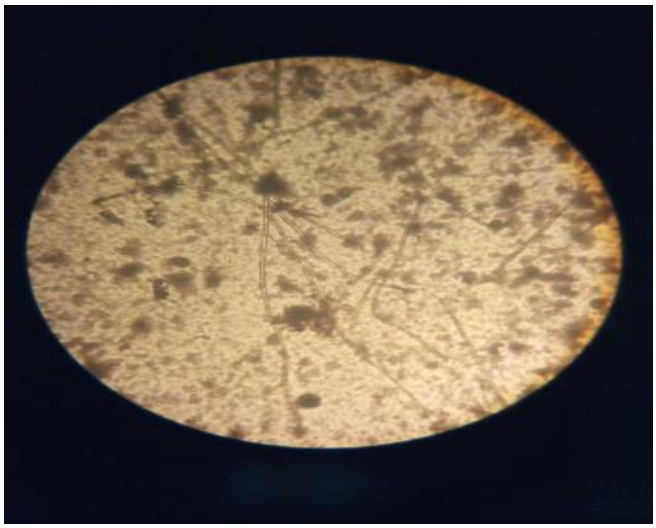

Plate 6: Microscopic view of *mold* fungi(x10)

ISSN No:-2456-2165

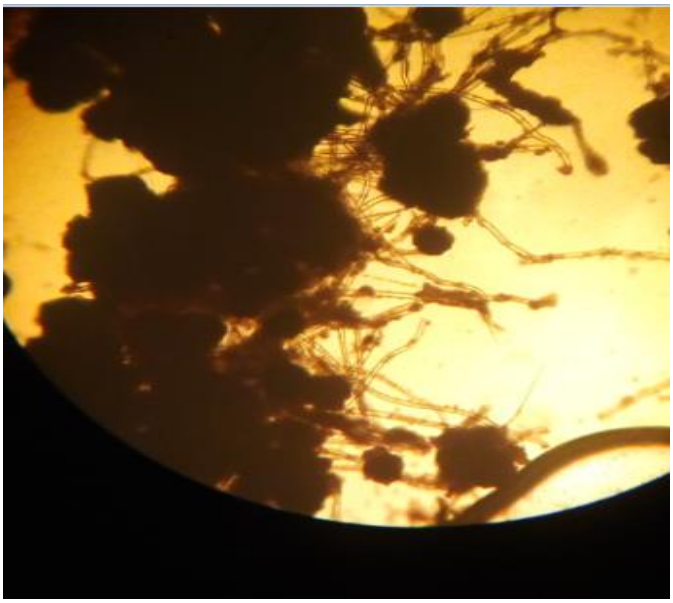

Plate 7: Microscopic view of *Aspergilius spp*(x10)

IV. DISCUSSION

The findings of the study showed that *Aspergilus spp*, *Fusarium spp*, mold fungi and *Alternaeria spp* are the fungi organisms that are found associated with cucumber fruits. These fungi were all noted to be inhibited at treatment dosage of lime extract at 4mg/l and 6mg. This inhibition level were also noted to be significant at P<0.05%. Finding from the least significant difference (LSD $_{0.05\%}$) shows that treatment dosage of the lime extract at6mg/l is far better than that of 4mg/l. This effect may be due to the medicinal properties of the lime juice extract. This finding agree with the finding of Chinen-chun and Chukunda [19],who reported that plant extracts like lime and Lemongrass oil is used to control turtle borne pathogen and can also change the activities of drug metabolizing enzymes and reduce oxidative stress in the liver . The findings also agree with the findings of Verma and Onyike [20] who also said that alcoholic extract of ripe dried plants like lime, coconut shell have action against *Microsporum canis, M. gypseum, M. audouinii, Trichophyton mentagrophytes, T. rubrum, T. tonsurans*, and *T. violaceum.*

V. CONCLUSION

It's evident from the study that, there are various fungi organisms found associated with cucumber fruit which causes damage to the fruit vegetable. This fungi can as well be controlled effectively using lime juice at a higher concentration. Which means that to effectively control fungi rot organism of cucumber, there is a need to use high concentration of lime juice.

REFERENCES

[1]. Jidda M.B, Muhammad M.M (2017). Assessment of fungal pathogens associated with spoilage of cucumber (Cucumis sativu L.) fruits. *International Journal of Current Micro-biology Applied Science* 6(3):510-516.

[2]. Bello M.O, Owoeye G, Abdulhammed M, Yekeen T.A (2014). Characterization of gourd fruit (Cucubitaceae) for dietary values and anti-nutrition constituent. Journal of Pharmaceutical Biology Chemistry Science.6:7575-7585.

[3]. Abulude O A, Adeleke K O (2010). Comparative studies on nutritional composition offourmelonseedsvarieties.PakistanJournalofNutrition. 9:905-908

[4]. Mortimore M (2015). Dry land developer's success stories from West Africa environment. Journal of Biological Science. 45:10-21.

[5]. Ibrahim M, Sada MD (2015). Yeasts associated with spoilage of some selected fruits in Sokoto Metropolis.Available: http://scienceq.org/Journals/JALS.php (Accessed 10th September, 2019)

[6]. Aboloma RI, Onifade AK, Adetuyi FC (2009). Fungi associated with the deterioration of some fruits of the family Cucurbitaceae. Nigerian Journal of Mycolog. 2:229-236.

[7]. Effiuvwevwere B.J.O. (2000). Microbial Spoilage Agents of Tropical and Assorted fruits and Vegetables (An Illustrated References Book). Paragraphics Publishing Company, Port Harcourt, Nigeria, 39 pp.

[8]. Blancard D., Lecoq H., Pitrat M. 2005. A color atlas of cucurbit diseases observation, identification and control Manson Publishing Ltd. London, United Kingdom, 304 pp.

[9]. Farrag E.S.H., Ziedan E.H., Mahmoud S.Y.M. 2007. Systemic acquired resistance induced in cucumber plants against powdery mildew disease by pre-inoculation with tobacco necrosis virus. Plant Pathology Journal 6 (1): 44−50. DOI:10.3923/ppj.2007.44.50

[10]. Sani M.A., Usman N., Kabir F., Kutama A.S. 2015. The effect of three natural preservatives on the growth of some predominant fungi associated with the spoilage of fruits (Mango, Pineapple and Cucumber) Global Advanced Results Journal of Agricultural Sciences 4 (12): 923−928

[11]. Ziedan E.H.E., Saad M.M. 2016. Efficacy of nanoparticles on seed borne fungi and their pathological potential of cucumber. International Journal of PharmTech Research 9 (10): 16−24.

[12]. Burnett and Beuchat. Emerging infectious Diseases. Produce Handling and Processing Practices. 2001; 5:6

[13]. Asagbra A and Oyewole OB. Fermentation studies on carrot juice processed to table wine. Nigeria's food Journal, 2002; 20:74-77.

[14]. Cheesbrough M (1984), District laboratory practice in tropical countries. Part 2. London: Cambridge University Press; p. 134–242.
[15]. Alexopoulos CJ (1962). Introductory mycology. 2nd ed. Sydney: Wiley; p. 119–59
[16]. Okafor N. Microbial rotting of stored yam (*Dioscorea* spp) in Nigeria. Exp Agric. 1966;2:179–82.
[17]. Okigbo RN, Ikediugwu FEO. Studies on biological control of post-harvest rot of yam (*Dioscorea* sp.) with *Trichoderma viride*. J Phytopathol. 2000;148:351–5.
[18]. Sangoyomi TE, Ekpo EJA, Asiedu R. Fungitoxic effects and attributes of *Allium sativum* and *Occimum gratissimum* extracts on *Rhizoctonia solani*, the causal organism of yam *Dioscorea rotundata* (Poir) rot disease. Niger J Mycol. 2009;2(1):166–7.
[19]. Chinen-chun, B.A. and Chukunda, F.A. (2018). Influence of some macro-elements, moisture and oxalic acid on rot of Cocoyam (Colocasia and Xanthosoma species). *Int'l J. Crop Science* 3(1): 10 – 14.
[20]. Verma , J.N.C. and Onyike R.C.I, (2012). *Fungal rotting of cocoyams in storage in Nigeria. Tropical root crops*: Research strategies for the 1980's (Terry E.R; K. AOduro and F. Cavenesseds) pp 235-238.