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Full Length Research

Component Assessment of Pathogenic Fungi Organisms Associated with Egusi Melon (*Citrillus coloncythis* L) Seeds and Control Using Some Plant Extracts and Synthetic Fungicide

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This study was carried out to investigate the effects of plant extracts:*Monodora myristica* and *Allium sativum* and synthetic fungicide on seed borne fungal pathogens of Egusi melon (*Citrillus colocynthis*). Melon seeds were incubated for seed health test using blotter paper method. A total of ten seeds were plated in each 9cm Petri dish. The anti-fungal effects of methanol extracts of test plants as well as synthetic fungicide were studied in an in-vitro experiment against the seed borne fungal pathogen at 5%, 15% and 25% concentrations. The design used was a Factorial in a Completely Randomized Design with three replications. Means were separated using Least Significant Difference (LSD) at 5% Probability level. The result showed that up to 84% germination was obtained in the seed health/ viability tests of Egusi melon seeds. The result of isolation and identification of fungi pathogen from the different components of melon seeds showed that there were more incidence of fungi pathogen in the whole Melon seeds followed by the germ cell while the least was that of the shell. The only fungi pathogen identified was *Aspergillus flavus* which was common in all the components of melon seeds incubated and assessed. The results also showed that the plant extracts and synthetic fungicides inhibited the growth of the fungus in culture. The effect of synthetic fungicide (75.00%) and that of *Monodora myristica* extract (75.00%) were statistically same in days 2 and 3 but the effect of the synthetic fungicide (75.00%) was significantly higher (p>0.05) than *Monodora myristica*extract on day 4 and 5 where *Monodora myristica* had 64.42% and 63.02% respectively The result also showed that the higher the concentration of plant extracts, the higher the percentage growth inhibition of the fungus. The result as well revealed that there was a depreciation in the effects of plant extracts on radial growth inhibition of test fungus with time. From the result of this investigation, it is recommend that plant extracts used in this research could be an alternative to synthetic fungicides since they showed similar effectiveness in inhibiting growth of fungus responsible for spoilage of melon seeds.

Keywords: Melon seeds, component assessment, fungi organisms, plant extracts, synthetic fungicide**.**

INTRODUCTION

Egusi melon (*Citrullus colocynthis)*belongs to the family Cucurbitaceae. This plant is commonly known as bitter apple or bitter cucumber (Abdel-Hassan *et al,* 2000). It is also commonly known in the Eastern region of Nigeria in West-Africa as 'Egusi'. The plant which is native to dry areas of North Africa, is scattered throughout the Sahara areas of Morocco, Egypt and Sudan and eastward through Iran to India and other parts of tropical Asia. It has been known since Biblical times and cultivated in the Mediterranean region, especially in Cyprus and in India for many centuries (Duke, 1983).

Egusi seeds are small and flat. They grow in gourds which are mainly cultivated for their seeds, as the flesh is neither sweet nor edible. One end of the seed is rounded while the other is tapered; Egusi melon is an annual herbaceous, monoecious plant which is creeping but non-climbing (Ng, 1993). Pollination is by insects and 'Egusi' melon fruits which are indehiscent smooth berries, often large and seedy, are ready for harvest 3-4 months after planting (Ng, 1993).

After harvest, the gourds are left to ferment and the fermented flesh is then washed off the seeds. The seeds are then dried and the light brown husks removed by hand or mechanically. When ready to be used in food recipes, the white/cream seeds are ground into powder and used as soup thickener. The food plant Citrullus colocynthis could be rightly described as a desert plant of the Cucurbitaceae, naturally adapted to arid environments. Although Ng, (1993) reported that they thrive in temperate locations in addition to tropical, subtropical and arid deserts.

Egusi seeds are available all year round. This is because it is dried after harvest and in this state, it can be stored for a very long time. Cultivation is at the beginning of the rainy season, in the months of April through June, either on ridges or on flat ground in holes about 75-90 cm apart. It is harvested at the onset of the dry season, in the months of October through December.

'Egusi' melon are known for their high protein and oil content. Seeds of 'egusi'are sources of oils and protein with about 50% oil and up to 35% protein (Achu, 2005). Specifically for these reasons they are cultivated and consumed world over. Previous studies have shown that most "melons" have therapeutic and nutritional value and these have been well documented.

The edible family member, *Momordica charantia*, also called bitter melon or bitter gourd, is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit. This has been reported to have quite a number of medicinal uses ranging from antiviral, anti-diabetic, anti-ulcerogenic, antioxidant and hepato-protective (Semiz and Sen.,2007) to anti-helmintic (Beloin et al., 2005), anti-malarial (Waako et al., 2005), anticancer (Kohno *et al.*, 2004; Kobori *et al.,* 2008) to cardio-protective properties (Gadang *et al.,* 2011).

During storage, melon seeds are attacked by several different fungi which both reduce the food nutrients as well as the market value of infected seeds One major challenge melon seeds face in storage is that of deterioration and several fungi have been implicated, (Bankole et al., 2005). Fungi of the genera Aspergillus and Penicillium are widely distributed storage fungi of melon seeds, causing seed discoloration, decrease in nutritional value, increase in free fatty acid and peroxide values, decreased seed germination and production of a number of toxic metabolites, including aflatoxin., (Basra et al.,2001).

Adeleke *et al*., (2012) have reported Aspergillus flavus, A.niger, Rhizopus stolonifer, Burgoa nigra and Fusarium spp. in stored melon seeds. Seed deterioration constitutes major constraint to all year round availability of melon seeds in Nigeria and other parts of the world.

However, there is need for an efficient and economic means of controlling the activities of melon seed fungi. The constraints associated with the use of chemical fungicides gave rise to the exploitation of botanicals, also known as plant extracts, as a safer, cheaper and effective plant disease control measure (Amadi, 2011). The use of plant extract in the control of plant disease is gaining importance because of the increase in the awareness of the hazardous effects of chemical fungicides to human health and environment, Babu et al. (2008) and Yasmin et al..(2008).

MATERIALS AND METHODS

Experimental Site

The study was carried out in the laboratory of the Department of Crop Science and Horticulture and at the Biotechnology Research Laboratory, all located at Nnamdi Azikiwe University, Awka.

Sources of Materials

Seeds from one variety of Citrillus coloncynthiswere obtained from Ogbeogonogo modem market in Oshimili South Local Government of Delta State. The calabash Nutmeg seeds (Monodora myristica) and some bulbs of garlic as well as the synthetic fungicide (Aldrex T) were also obtained from the Eke-Awka market in Awka, Anambra State.
There were a total of 120 seeds randomly selected from the variety of Egusi melon which were used.

Germination Test

Germination test was carried out to investigate the viability of the seeds. Four components of the melon seeds were used which included the whole seeds, the cotyledons,the germ cells and the shells The percentage germination of the Citrillus colocynthis seedswas thus determined;

$$\frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times \frac{100}{1}$$

Blotter Paper Method

Here, the seeds were washed in a mixture of 5% Sodium hypochloride and distilled water and rinsed twice with sterile distilled water, after which they were spread to dry in- between two layers of blotter papers. Then three layers of blotter paper were placed in each petri dish of 9cm diameter and incubated at 25±2°C. One hundred and twenty healthy melon seeds were used. Ten seeds were placed in each petri dish and twelve replicates were made after which a masking tape was used to seal each Petri dish to prevent any form of contamination. Percentage germination counts from the first day to the seventh day of incubation were taken and recovered.

Preparation of PDA

Twenty grams of PDA powder was weighed with the electronic weighing balance and was mixed in 500ml of distilled water in a conical flask. The mixtures were stirred vigorously until it became homogeneous. It was then corked using cotton wool wrapped with aluminium foil before being placed into the autoclave. The conical flask containing PDA was placed into the autoclave and was properly sealed. The autoclave boiled to a temperature of 120°C and pressure of 15±1 Psi for 20-25 minutes after which it was ready for use.

Isolation of Fungal Pathogen

The working bench was surface sterilized with methylated spirit in cotton wool so as to prevent contamination. A sterile inoculating loop was used to place the isolated fungi into sterile Petri dishes containing the l0mls of PDA with two drops of lactic acid. This was done to inhibit the growth of bacteria, after which it was properly sealed and labelled. The plates with 3 replicates were incubated at temperature of 28±2°C and left for seven days and closely observed daily for fungal growth.

Sub-Culturing of Fungal Pathogen

The initial culture was sub-cultured twice to obtain a pure culture. The method used in sub-culture was the spot method where an inoculating loop was used to collect the fungi and put at the centre of the fresh plate of the prepared PDA after which it was sealed to prevent contamination. The sub-culture was left for three days and observed daily for fungal growth.

The resulting pure cultures were used for characterization and subsequent identification of the fungi with the aid of a compound microscope and identification guides (Sulton, 1980).

Identification of Isolated Pathogen

A compound microscope of the model (Olympus-XN 50) was used to view the organisms. Sterilized slides were also used. A drop of distilled water was placed on the slide, and a small portion of the culture from the seven-day culture was collected from the growth using a sterile needle, it was then covered with the slide cover and placed under the microscope for viewing.

The identification of the fungal inoculum was based on the morphology of the culture and the fruiting bodies. An illustrated Manual on the identification of fungi by (Barnet and Hunters, 1994) and (Alexopolus *et al*., 2002) were used for identification.

Preparation of Plant Extracts

The plant .materials which were *Monodora myristica* seeds and *Allium sativa*bulbs were ground and put in sterile plastic containers and were taken to the Biotechnology Laboratory at the Nnamdi Azikiwe University for extraction.

Extraction Methods

Extraction method used was hot continuous extraction (Soxhlet). Other extraction methods include; maceration, microwave assisted extraction, ultrasound assisted extraction and super critical fluid extraction. (Azwanida et al., 2015).

Hot Continuous Extraction (Soxhlet)

Thirty-nine (39g) grams and twenty-five (25g) of the Monodora myristica and Garlic respectively were weighed out and placed in a thimble. Paper thimble was used in the Soxhlet apparatus, using Methanol as the solvent separately for their extraction. After extraction, the Methanol used for the plant extracts was evaporated to dryness by placing the filtrates in a rotary evaporator and at a temperature of 300°C.

After extraction, 5g, 15g and 25g portion of each plant extract were mixed with 100ml of distilled water into a measuring cylinder to produce percentage concentrations of the extracts.

***In-Vitro* Experiment**

The plant extracts and synthetic fungicide which were prepared at different concentrations 5% 15% and 25% were mixed separately with molten 10 mls of the PDA with two drops of lactic acid into the Petri dishes. This was done to inhibit the growth of bacteria. After gelling a sterile cork border of diameter 0.4cm was used to cut the pure culture of isolated pathogen and the discs containing the test fungi were placed in the centre of each Petri dish containing 10mls of PDA and different concentrations of the plant extracts and the synthetic fungicide after which it was properly sealed and labelled. Water (distilled sterile) was used as control. Twelve Petri dishes were used for each plant extract against the test fungus at a time. Another twelve dishes were used for the synthetic fungicides. The Petri dishes were incubated at 27^{o}C with 12 hours of alternating light and darkness.

Daily observation and measurement of the radial growth were carried out. The diameter of the fungal colony was determined by measuring from the back sides of the plates with the use of meter rule. The colony growth was measured taking the average of the largest and shortest diameter of the same colony. This is because the colony growth of fungus is not always a regular circle. The fungitoxicity of the extracts was calculated in terms of percentage colony inhibition using the formula (Amadioha, 2003).

$$\% \text{ growth inhibition} = \frac{dc - dt}{dc} \times \frac{100}{1}$$

Where dc is the average diameter of fungal colony with control, dt is average diameter of fungal colony with treatment.

Data Analysis

The experimental design used was Factorial in a Complete Randomized Design (CRD). The data collected were subjected to analysis of variance (ANOVA) and means were separated using least significant difference (LSD) at 0.05 probability level. The Genstat Release 10.3 version was used for all the statistical analysis.

RESULTS

Figure 1, shows the germination percentage of one variety of melon seeds observed for seven days. The highest germination was observed on day seven where 84% germination was obtained. There was no germination on first day and second day, but on third day, 18% percentage germination was recorded.Further germination was recorded on fourth day (48%), while 70%, and78% percentage germination were obtained on fifth dayand sixth day respectively as illustration by the graph below. Figure 1 also showed that germination of melon was continuous until the seventh day when 84% germination was obtained. The rate of increase in germination of melon seeds kept on decreasing after the fourth day but was a little stable from the fifth day until the seventh day

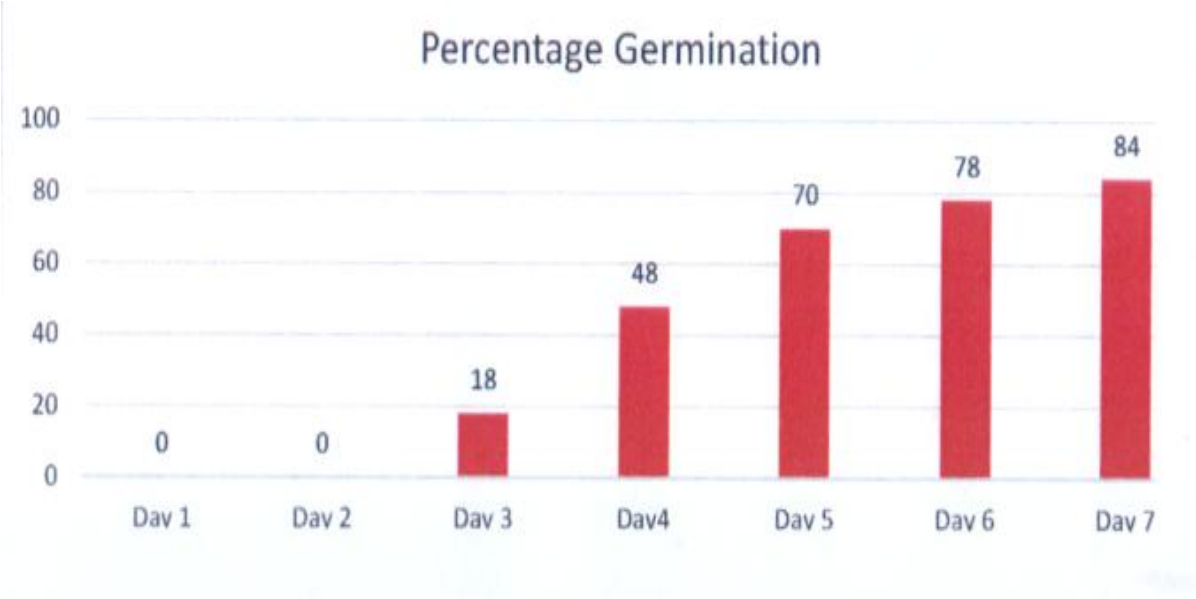
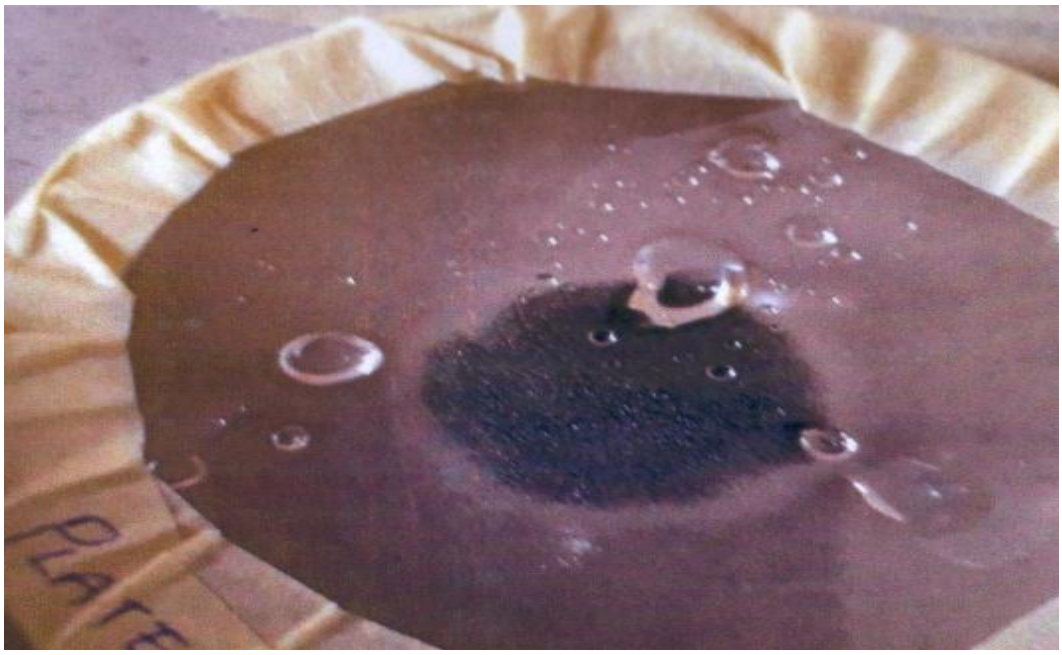


Figure 1: Bar chart representation of percentage germination of melon seeds.

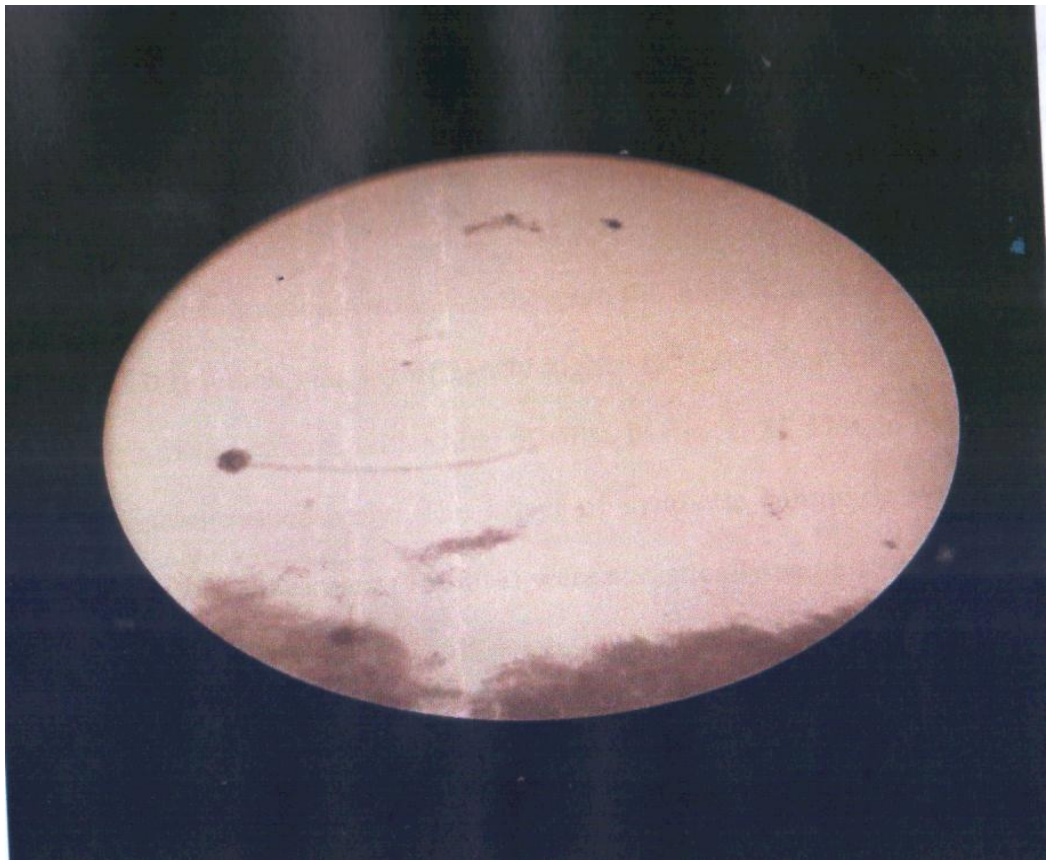
Result on Isolation and Identification of Fungi Pathogens from the Different Components of Melon Seeds

The result of isolation and identification of fungi pathogens from the different components of melon seeds showed that there were more infections in the whole - melon seed, followed by the shell while the least was that of the germ cell. The only fungi pathogen identified and confirmed using the illustrated fungi pathogen by Barnet and Hunters 1994, was *Aspergillus flavus* which was common in all the components of Melon seeds incubated and assessed



Mag. x75%

Plate 1: Pure culture of *Aspergillus flavus* isolated from different components of egusi melon seeds



Mag. x100

Plate 2: Micrograph of *Aspergillus flavus* isolated from different components of egusi melon seeds

Table 1: shows that there was significant difference in the effect of plant extract and synthetic fungicide and their concentration on the radial growth inhibition of *Aspergilus flavus* in culture.

Synthetic fungicide consistently gave the highest growth inhibition in all the days of culture (75.00%) which was significantly higher ($p>0.05$) than the effect of garlic in methanol extract in all the days of the culture with value of 60.40% in first day, 55.57% in day 2 and 53.79%, 50.40% in day 4 and 5 respectively. The effect of synthetic fungicide (75.00%) andthat ofMonodora myristica extract (75.00%) were statistically same in days 2 and 3 but the effect of the synthetic fungicide were significantly higher ($p>0.05$) (75.00%) than Monodora myristica extract on day 4 and 5 where Monodora myristica had 64.42% and 63.02% respectively. It could be observed that while the synthetic fungicide maintained its fungitoxicity throughout the days in culture the Monodora myristica started depreciating after the third day. That was also seen with garlic extract which started depreciating after the second day. The result also showed that Monodora myristica extract consistently had significantly higher inhibition percentage than garlic in all the days in culture except day 5.

The result also showed that there was significant difference in the effect of the different concentrations of the plant extracts and synthetic fungicide. The concentration level of 25g/ml had consistently the highest inhibition values than the rest with 100% inhibition in all the days in culture except in the third day (99.38%) for Synthetic Fungicide though still higher than 5g/m concentration but same with 15g/ml concentration with the value of 97.31%. The concentration level with second highest inhibition value was 15g/m in all the days in culture while the least was 5g/ml. All the concentration tested did better than the control. Also from the result it could be observed that the fungitoxic effect of the plant extract as well as the synthetic fungicide increased with an increase in concentration.Moreso, the concentration effect was observed to be decreasing with time in days of the culture especially in the concentration level of 15g/ml.

Table 1: Main effects of *Monodora myristica* seeds and Garlic bulb extracts and synthetic fungicide and their concentrations on percentage (%) growth inhibition of*Asperillus flavus in culture*

	Incubation period (days) and Growth inhibition (5)			
Treatments	Day 2	Day 3	Day 4	Day 5
MME	75.00	75.00	65.42	63.02
GME	60.40	55.57	53.79	60.48
SF	75.0	75.00	75.00	75.00
LSD $_{(5\%)}$	**9.50**	**1.86**	**4.61**	**3.79**
Concentration(g/ml)				
0	0.00	0.00	0.00	0.00
5	88.00	77.41	61.94	69.22
15	92.60	87.31	97.00	95.48
25	100.00	99.38	100.0	100.0
LSD $_{(0.05)}$	**2.72**	**2.516**	**1.103**	**1.510**

Note: MME= Monodora myristica and methanol, GME= Garlic and methanol,SF = Synthetic Fungicide, TRT= Treatment, Conc = Concentration,NS = Not Significant

Table 2 showed that there was significant interaction effects of Monodora myristica, garlic plant extract and synthetic fungicide and their different concentrations on the growth inhibition of Aspergillus flavus in culture, where the Monodora myristica plant extract had significantly same effect with synthetic fungicide with inhibition values of 100.00% respectively, which was significantly different from the value of garlic with inhibition value of 64.0. The trend was observed in day 3, day 4 and day 5 where garlic extract consistently had the least inhibition value. It was observed that the effect of the plant extract was

Table 2: Interaction effects of *Monodora myristica* seeds and *Allium sativa* plant extracts and synthetic fungicide and their different concentrations on the percentage growth inhibition of *Aspergillus flavus* of melon seeds in culture

Day 2						Day 3				Day 4				Day 5			
CONC (g/ml)	0	5	15	25	0	5	15	25	0	5	15	25	0	5	15	25
MME	0.00	100.0	100.0	100.0	0.00	100.0	100.0	100.0	0.00	100.0	100.0	100.0	0.00	100.0	100.0	100.0
GME	0.00	64.0	77.8	100.0	0.00	32.23	91.93	98.3	0.00	24.17	91.0	100.0	0.00	21.57	86.37	100.0
SF	0.00	100.0	100.0	100.0	0.00	100.0	100.0	100.0	0.00	100.0	100.0	100.0	0.00	100.0	100.0	100.0
LSD(0.05)	19.00					3.721				9.222				7.575		

NOTE: MME= Monodora myristica and methanol, GME= Garlic and methanol, SF = Synthetic Fungicide. NS = Not Significant

DISCUSSIONS

Isolation of Fungal Pathogen

The result of the isolation and identification of spoilage fungi that caused post-harvest spoilage of melon seed showed that the only fungi pathogen identified was *Aspergillus flavus*. This result corroborates with the works of (Bankole et al., 2005, Adeleke et al., 2012) who reported that fungi of the genera Aspergillus spp and Penicillium spp are widely distributed storage fungi of melon seeds, causing seed discoloration, decrease in nutritional value, increase in free fatty acid and peroxide values, decreased seed germination and thus confirmed as the causal organism of post-harvest spoilage of melon seed.

The result also showed that there was more spoilage microorganism from whole melon seed followed by those isolated from the shells and the least being that from the germ cells. This is also similar to the work of Mahmood et al., (2014) who reported micro pathogens of components of melon seeds that included:*Aspergillus niger, Aspergillus flavus* and *Penicillium spp*

Effects of Plant Extract, their Concentrations on Radial Growth of *Aspergillus flavus* in culture.

The result showed that Monodora myristica,*Allium sativum* plant extracts and synthetic fungicide hadsignificant effect on radial growth inhibition ofAspergillus flavus in culture. This is in agreement with Amadi *et al.,*(2014), who reported that the plant extracts used in their investigation were effective in reducing the radial growth of Aspergillus flavus that cause post-harvest spoilage of melon seeds. The result also showed that Monodora myristica had similar inhibition effect with synthetic fungicide in day 2 and 3 of this investigation. This is in agreement with the findings of Firempong*et a*l.,(2016), who reported that the phytochemicals and antifungal activities of the seeds of *Monodora myristica* and root parts were the most effective in inhibiting the growth of Candida *albican* in an *in-vitro* experiment. It was also observed that the effect of the plant extract were depreciating in days in culture. This is similar to the findings of Nweke et al.,(2015), who reported that the efficacy of the plant extracts used decreased after two days indicating that the active ingredients of the plant species were not persistent enough as compared with the synthetic fungicides.

Also the effectiveness of the plant extracts was observed to increase with an increase in concentration. This result corroborates with the work of Tijjani et al.,(2014), who reported that the effectiveness of plant extracts depend on the nature and amount of biologically active ingredients it contains therefore increase in concentration of these extracts implies an increase in active ingredient and significant decrease in the growth of fungus.

CONCLUSION

From the investigations carried out on the isolation and identification of post-harvest spoilage organisms of melon seeds and their control using Monodora myristica, Allium sativa and Aldrex-T, it was found that the only microorganism isolated from the various components of melon which include the whole seed, the germ cell, the cotyledon and the shell used in this research was *Aspergillus flavus*. The study also shows that the plant extract and synthetic fungicide were very effective as the synthetic fungicide in reducing radial growth of *Aspergillus flavus* in culture

RECOMMENDATIONS

From the results of this investigation, I therefore recommend that plant extract could be used in alternative to synthetic fungicide since they showed similar effectiveness in reducing spoilage of melon seeds in storage in Oshimili South Local Government Area of Delta State. This is because the plant extract are abundantly available than the synthetic fungicide which is not always available and affordable to farmers coupled with its adverse health effects on humans.

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