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BIOCIDAL EFFECTS OF DENNETTIA *TRIPETALA, ZINGIBER OFFICINALE* AND BENLATE ON SEEDBORNE FUNGAL PATHOGEN (*FUSARIUM MONILIFORME*) OF WATERMELON (*CITRULLUS LANATUS*) VARIETIES.

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ABSTRACT

This study was carried out to investigate the effect of plant extracts, African pepper fruit (*Dennettia tripetala)* and Ginger (*Zingiber officinale)* on seed borne fungal pathogens of Watermelon *(Citrullus lanatas)* seeds. Watermelon seeds were extracted for seed health test using blotter paper method. The antifungal effects of ethanol and acetone extracts of the two plant extracts and synthetic fungicide were studied under in- vitro experiment against the seed borne fungal pathogen of Watermelon at 0%, 50% and 75%. It was a 3x3 factorial experiment at 5% Probability level laid out in a Completely Randomized Design experiment with three replications Ninety percent germination was obtained in the germination and seed health test of Watermelon seeds. Seed borne fungal pathogen (*Aspergillus spp.* and *Fusarium spp*.) were identified. The potential of these organisms for pathogenicity were tested using Kock's postulate. The result of the pathogenicity test showed that *Fusarium spp*. was pathogenic. All plant extracts and Benlate inhibited the fungus (*Fusarium moniliforme*) in culture. The inhibition was also greater as concentration increased from 50% to 75%. It was also observed that ethanol extraction solvent did better than acetone. Generally, *Dennettia tripetala* extract performed better than *Zingiber officinale.* It could therefore be recommended that the two plant extracts used in this investigation which were very effective in the control of *Fusarium moniliforme* of watermelon could be an alternative to the synthetic fungicide. It could also be suggested that further studies be carried out to isolate, identify, characterize and standardize the bioactive components of these phytochemicals in a bid to commercializing their production.

KEYWORDS: Biocidal, fungicide, plant extract, seeds, synthetic.

INTRODUCTION

Watermelon (*Citrullus lanatus*) is one of the most widely cultivated crops in the world (Mayberry *et al* 2000). According to FAO (2011) statistics, China is the world's leading producer of watermelon. The top twenty leading producers of watermelon produced a collective quantity of approximately 92.7 million metric tons in 2011, of which China produced 75% while Turkey, Iran and Brazil have a production share of 4.7%, 3.5% and 2.4% respectively in 2011.

Nigeria produced more watermelons in 2011 than other leading fresh producers in African. Other exporters include: Kenya, which produced 66,196 Metric tons and South Africa that produced 77,993 Metric tons (This Day Live, 2014). There are over 1,200 varieties of watermelon worldwide and quite a number of these varieties are also cultivated in Africa (Zohary and Hopf, 2000). The global consumption of the crop is greater than that of any other cucurbit.

Watermelon is a tender, warm season vegetable belonging to the family *Cucurbitaceae*. It is enjoyed by

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many people across the world as fresh fruit. It is highly nutritious and thirst-quenching and also contains vitamins C and A in the form of disease-fighting beta-carotene. Watermelon is rich in carotenoids, some of the carotenoids of which include lycopene, phytofluene, phytoene, beta-carotene, lutein and neurosporene. Lycopene and beta-carotene work in conjunction with other plant chemicals not found in vitamin/mineral supplements. Potassium is also available in it which is believed to help in the control of blood pressure and possibly prevention of stroke (De Lannoy, 2001). Despite how important this crop is, it has some setbacks in terms of its production. Fungal seed borne pathogens are the important constraints to watermelon production.

Fungi are the most important and prevalent pathogens of a wide range of host plant and causing destructive and economically important diseases of most fresh fruits and vegetables during production, storage and transportation (Sommer, 1985*)*. The main fungal diseases of watermelon produce in the world are: fusarium wilt, Alternaria leaf blight, Alternaria leaf spot and Anthracnose, infected plants may develop, fruit rot, or stem canker which often leads to wilting and plant death in severe cases (Buzi *et.al*., 2002). Other fungal species responsible for seed borne diseases of watermelon include; *Alternaria porri, A. alternata, Aspergillus amstelodami, A. flavus, A. fumigatus, A. nidulans, A. niger, A. sydowi*, *A.wentii, Fusarium equiseti, F. moniliforme, F. oxysporum, F. semitectum, Penicillium notatum, Rhizoctonia* sp., and *Rhizopus arrhizus which has* been isolated from watermelon (Amadi *et al*., 2009). Within the fungi group, *Colletotrichum* is one of the most important plant pathogens worldwide causing the economically important disease anthracnose in a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruits (Bailey and Jeger, 1992). The diseases are mainly problematic on mature Watermelon fruits, causing severe losses due to both pre and post-harvest fruit decay (Bosland and Votava, 2003). Various control strategies have been employed to reduce losses caused by anthracnose and fruit rot. These include; crop rotation; removal and destruction of infected fruits; and spraying with recommended fungicides (Obeng-Ofori, 2007); however, despite these control measures, there has not been significant improvement in the elimination of the diseases because the pathogens responsible are seed borne. Seed is an important input for crop production. About 90% of the world food crops including watermelon are propagated by seed (Maude, 1996). Seeds are the passive carriers of some important seed borne diseases caused by microorganisms which usually result in considerable yield losses. Fungi, bacteria, viruses and nematodes can be carried with, on or in seeds. The use of healthy seeds is important for crop establishment, yield and productivity (Balogun *et al*., 2005). Seed testing is needed to achieve this (Balogun *et al*., 2005). Although several workers have reported isolation of various fungi from vegetable seeds (Al-kassim and Monawar, 2000; Balogun *et al*., 2005; Maleko, 2010). Evidently, there is a need to increase the yield and improve the seed health and quality of the crop by controlling seed-borne fungal pathogens. Among the control practices used, seed treatment is one of the effective techniques to eliminate seed-borne inoculants. Treatments of seed should be done as a routine practice as it is a cheap insurance against possible disasters at a later stage (Assadi and Behronzi, 1987). Various methods have been practiced to control these pathogens. Use of plant extracts against plant disease is however, a recent approach to plant disease control. It helps to avoid environmental pollution by chemicals. Plant extracts have been used Successful in controlling seed-borne infection in certain crops (Amadioha and Markson, 2007; Okigbo *et al*, 2009). Extracts obtained from many plants have recently gained popularity and scientific interest for their antifungal activities (Sownumi and Akinusi, 1983). Laboratory studies of extracts of plants species have revealed powerful fungi toxicities in relation to many fungal pathogens (Hay and Waterman1993). Allicin a volatile antimicrobial substance synthesized in garlic when tissues are damaged, is effective in controlling seed borne *Alternaria* spp. of carrot, *phytophthora* leaf blight of tomato and tuber blight of potato as well as *Magnaporthe spp* on rice (Slusarenko *et al*., 2008). Previous reports by (Akpomedaye and Ejechi,1998, Ejechi and Ilondu,1999 and Ejechi *et,al*.,1999) showed that spices, herbs and other plant materials possess antifungal activity. The inhibitory properties of the methanolic extracts of *Dennentia tripetela* and *Zingiber officinale* were also reported by Chiejina and Ukeh (2012) Salah and Fhami, (2017) on *Helminthosporium solani, Mucor piriformis, Penicillium digitatum* and *Aspergillus niger* pathogens isolated from tomato and watermelon .The *in- vitro* fungitoxic activity of crude extracts of ginger (*Zingiber officinale)*, on *Pythium aphanidermatum* isolated from root rot of tomato, showed that the extract did well in the mean percentage inhibition of mycelia growth (Suleiman, 2011).Ejechi *et al.*., (1999) reported that some of the fruits extract of Dennettia have been shown to be active as antifungal agent against, *Candida tropcalis, Aspergillus* and *Fusarium spp* . Due to increased awareness about the risks involved in the use of synthetic pesticides, much attention is now being focused on the alternative method of pathogen control. The use of pesticides and fungicides of botanical origin has been pin-pointed by many researchers as an option to synthetic fungicides (Amadioha and Obi, 1999, Amadioha, 2000). Most synthetic fungicides used in controlling some of these phyto- pathogens are costly and therefore, not economically viable. The excessive use and misuse of chemical fungicides have raised serious concern about health and environments. These have significant draw back including increase in cost of fungicides, application hazard, and concern about fungicides residue in food (Kiran *et,al.,* 2006). Synthetic fungicides are not environmentally friendly as they pollute the soil, water and air (Kiran *et,al.,* 2006). And by the accumulation of obnoxious chemicals residues as a result of continuous use, leads to the development of resistant race to these chemicals at in the field and at storage (Kiran *et,al.,* 2006) **.** Watermelon fruits is being affected by a large numbers of fungi diseases associated to seed borne pathogens both in the field and during storage and transportation which has become detrimental leading to substantial yield loss worldwide. This has become a

source of serious concern (Ijato *et, al.,* 2010). At present the use of synthetic fungicides in controlling these diseases has posed a serious threat to human health and non-target organisms (Chao *et, al.,* 2011). Therefore, searching for alternative sources for diseases control is of great important in sustainable agriculture. (Sallam *et, al.,* 2012).

MATERIALS AND METHODS

Location of research

This research was carried out at Botany Laboratory of Botany Department, Nnamdi Azikiwe University Awka campus. Awka is located at the latitude (6°5' 10.1" N) and longitude (7°0.8' 39.1" E).

Materials Used: The materials used include: Autoclave machine, Sterilized filter paper, Conical flasks, Sterilized Petri dishes, Pipettes, Potato dextrose agar (PDA) powder, Lactic acid, Cottonwool, Distilled water, Metlyted spirit, Spirit lamp, Absolute Ethanol, Acetone, Analytical Weighing balance, blender (Sonik SB-1212) and Microscope (Olympus-XN50), Test tubes, Aluminum foil, Spatula, Inoculating -loop, Wire gauze, funnel, Slides and Slid cover, Whatman Blotter paper.

Sources of seeds and plant materials: The watermelon fruits Kaolak and Crimson sweet were purchased from Eke Awka market. The fruits were washed, seeds extracted and air dried at room temperature. The two plant samples used for this work, African pepper fruit *(Dennettia tripetala*) and rhizomes of Ginger *(Zingiber officinale*) plant were purchased from Eke Awka market in Anambra State.

Sterilization of materials: All glass wares, paper bags, towels, inoculating needles were wrapped with aluminum foil and sterilized in a high-pressure Steam Sterilizer Autoclave at a temperature of 121°C for 5 minutes (Jawetz*, et.al*., 2004)

Seed health test: Seed health test for seed borne fungi was carried out following the international seed testing association (ISTA 1996). Standard blotter method was used for the study. Two hundred and sixteen seeds were randomly taken from seed-lot of each variety for seed health test. The seeds were treated with 1% Sodium- hypochloric for 5 minutes and rinsed two times with distilled water in order to effectively remove surface contamination without affecting the percentage germination of the watermelon seeds (Pemezny *et al*.., 2002)

Blotter method: Twelve seeds of each watermelon variety were placed in 9cm petri dishes and wetted with sterile distilled water containing three layers of Whatman filter paper. The arrangement of the seeds was according to International Seed Testing Association (ISTA 2003). The seeds were incubated at a temperature of 28 °C±1°C under 12hours of light and darkness cycle for 7 days (ISTA,1984). Germination count was observed for seven days and recorded. Fungal growth was observed on the plates based on the mycelial colour and hyphal growth.

Preparations of potato dextrose agar (PDA) media: Twenty grams of PDA powder was weighed using analytical weighing balance and was poured in a beaker. Five-hundred-millimeter of distilled water was added and stirred and later corked with cotton wool wrapped with a foil. It was autoclaved at 121^{o} C at 15psi for 15 minutes.

Fungal isolation: Fifteen millimeters of molten PDA medium was poured into Petri dishes and the medium was acidified by adding two or three drops of lactic acid and mixed thoroughly by shaking gently and allowed to gel. Three Petri dishes were used each for the two varieties of watermelon. Lactic acid was added to prevent bacterial contamination. The Petri dishes containing the acidified PDA were then inoculated with fungi from infected watermelon seeds. The media plates were incubated at 28^{o} C ±2°C for 7 days and then observed for microbial growth. The fungal isolates were sub-culture three times to obtain pure cultures, which were properly preserved in agar slant kept at 4°C to be used subsequently for microscopic identification.

Microscopic Identification and characterization of fungi isolates: Temporary slides were prepared by collecting small portion of each **f**ungus from the active growth region of the pure cultures and placed on clean glass slides each containing few drops of lactophenol in cotton blue and viewed under a compound microscope (Olympus-XN50)**.** Identification of fungal organisms was based on the morphological characteristics of the mycelia and the nature of the fruiting bodies guided by identification key of illustrated diagrams of imperfect fungi by Barnett and Hunter (1999). Digital microphotography of each fungi species was taken and recorded.

Pathogenicity Test: Pathogenicity test was carried at screenhouse of Crop Science and Horticulture, Nnamdi Azikiwe University Awka.

Materials Used: Materials used include; hand sprayer, light polythene materials, corn flour, perforated buckets, Sodium hydrochloride sterilized top soil.

Preparation of soil media: The top soil was heat treated at 120^{o}C for three hours and allow to cool overnight. Five kilograms of the soil media was weighed and poured into each perforated bucket of 30cm top diameter. Healthy seeds of the two varieties of water melon were surface sterilized in water containing five percent Sodium hydrochloride for three minutes, rinsed two times in sterile distilled and were then sown into the buckets containing sterilized top soil. Germination of the seedlings started after six days. The seedlings of the two varieties of Watermelon were thinned down to three seedlings per bucket. Ten buckets were used for each variety. The seedlings were sowed at a spacing of 5cm apart.

Inoculation of Watermelon plants: After two weeks when the watermelon seedlings were at pencil height, spore suspension was applied using spraying method of inoculation. Pure culture of *fusarium moniliforme* obtained from the culture maintained on agar slant. Full grown mycelia were scraped using a scalpel into 100ml of distilled water and stirred thoroughly. 10ml of the solution was added to 100ml of water, this was done six times. The suspension used was 5x10^{4} spore ml^{-1} of distilled water. Corn starch was made in a slurry form and sterilized at 100◦C in an autoclave. The corn starch helped to bind the suspension on the surface of the watermelon seedlings. The conidial suspension was poured into a sterilized hand sprayer which was rinsed twice with distilled water before use. Fifteen milliliters of the inoculum were hand-sprayed on the leaves of the watermelon seedlings. After the introduction of the isolates, each bucket was covered with waterproof of polyethene material which served as a moist chamber to

keep the plant moist and prevent contamination. The polyethene material was left for 24 hours after which it was removed to enable the germination of the spores. Signs and symptoms of fusarium wilt were observed after six weeks in the screen house.

Preparation of plant extracts: The plant samples *Dennettia tripetala* and *Zingiber officinale* were blended separately using Sonic blender model (SB-1212). The method used for extraction was cold maceration (Manousi,2019). Fifty grams and seventy-five-gram powder of the plant samples was weighed. The extraction process used was the soaking or cold extraction method (Doherty *et al*., 2010). This involved soaking fifty grams and seventy- five grams of the powdered plant sample in two hundred (200) millimeters of ethanol and acetone for 24hrs at room temperature for maximum extraction of the components (Wokocha and Okereke, 2005). The extracts were filtered through blotter paper in a funnel. The residue was discarded and the filtrate was put in flask and covered using foil paper. The filtrate was autoclaved briefly to sterilize it and remove the solvent so that it is only the active ingredient that remained. The filtrate was poured into sterile bottles, corked and kept in the refrigerator to be used subsequently

***In- vitro* test: Evaluation of *Fusarium moniliforme* radial inhibition using plant extract:** Effect of plant extract on fungal pathogen was assessed using five millimeters from each of the various concentrations of the plant extracts. Fifty percent, seventy- five percent and zero percent concentration of the extract were dispensed into 9cm petri-dishes and agitated thoroughly with 20ml of potato dextrose agar medium forming potato dextrose-extract-agar medium (PDEAM) following poison food Z mixture was allowed to solidify and then inoculated with a 12mm diameter mycelial disc obtained from a seven-day-old pure culture of the *Fusarium moniliforme* with a sterilized cork borer and placed on the center of each petri-dish. The zero percent concentration of the extract serve as the control (no plant extract). The position of the disc was marked on the base of the dish with a marker pen and two perpendicular lines passing through the center of the petri-dish were marked to serve as reference for measuring the radial growth. All the culture plates were incubated on the laboratory bench covered with sterile Aluminums foil paper and at temperature of 28±2^{o}C. Radial growth along each line was measured at exactly 24hours interval using linear measurements by the aid of a meter rule to determine the radial growth. The radial growth inhibition in each of the plate was measured for three days period. Each treatment was replicated three times. Percentage radial inhibition was determined according to Sundar *et al.* (1995). Using the formula below

$$\frac{dc - dt}{dc} \times \frac{100}{1}$$

Where: dc – average diameter of fungal radial growth of *Fusarium moniliforme,* in the control plates, dt - average diameter of fungal radial growth of *Fusarium moniliforme,* in the treated plates. The experiment was laid out in completely randomized block design with three replications. The measurements of the radial growth were made and recorded.

Plate 1: Inoculated plates for *in-vitro* test for evaluation of *Fusarium moniliforme* radial growth inhibition using *Dennettia tripetala* plant extract

Plate 2: Inoculated plates for *in-vitro* test for evaluation of *Fusarium moniliforme* radial growth inhibition using *Zingiber officinale* plant extract

Evaluation of inhibition of *Fusarium moniliforme* growth using synthetic fungicide

Benlate was used to evaluate the radial growth inhibition of *Fusarium moniliform* following poison food technique (Begum and Bhuiyan, 2006). Fifty grams, seventy-five gram and zero gram of Benlate were added into 100ml of distilled sterile water separately. Five millimeters of the suspension was dispensed into 9cm petri-dishes and agitated thoroughly with 20ml of potato dextrose agar medium which was autoclaved. The zero percent concentration of the Benlate serve as the control. After solidifying, the plates were inoculated with 12mm diameter mycelial disc obtained from the seven-day old culture of the *Fusarium moniliform* with a sterilized cork borer and placed in the center of each petri-dish. The position of the disc was marked on the base of the dish with a marker pen and two perpendicular lines passing through the center of the Petri dishes were marked to serve as reference line for measuring the growth. All plates were placed on laboratory bench covered with sterile aluminum foil paper and at temperature of 28±2^{o}C. Radial growth along each line was measured at exactly 24hours interval using linear measurements by the aid of a meter rule to determine the radial growth. The radial growth inhibition in each plate was measured for three days period. Each treatment was replicated three times. Percentage radial inhibition was determined according to Sundar *et al. (*1995).

$$\text{Percentage radial Inhibition} = \frac{dc - dt}{dc} \times \frac{100}{1}$$

Where: dc – average diameter of fungal radial growth average *Fusarium moniliform,* in the control plates. dt - average diameter of fungal radial growth *Fusarium moniliforme,* in the treated plates.

Plate 3: Inhibition of linear growth of *Fusarium moniliforme* at different concentration of Benlate

Data Analysis: Data collected were subjected to Analysis of Variance (ANOVA) for a 3x3 Factorial experiment in Complete Randomized Design (CRD). Using Gen-Stat. The means were separated using Least Significant Difference (LSD) at 5% probability level.

RESULTS

Isolation and identification of micro-organisms associated with Watermelon (*Citrullus lanatas L.*) seeds.

The result of isolation and identification of seedborne fungi organisms of Watermelon showed that *Fusarium moniliforme* and *Aspergillus niger* were implicated

Plate 4: Pure culture of *Fusarium moniliform* isolated from infected seeds of Watermelon

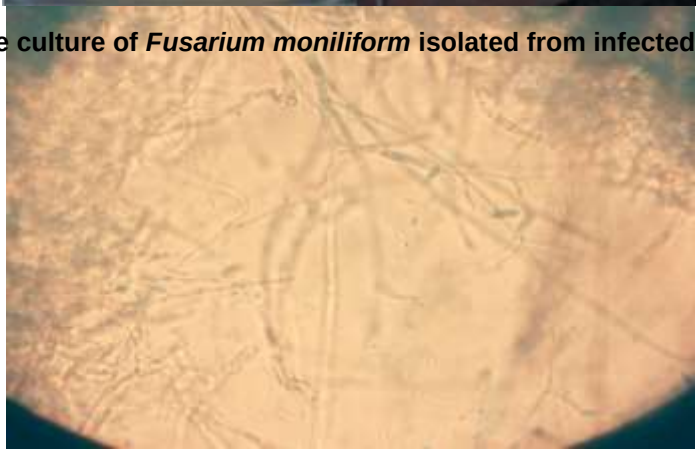

Plate 5: Micrograph *of Fusarium moniliforme* isolated from infected seeds of Watermelon

Plate 6: Pure culture of *Aspergillus niger* isolated from infected seeds of Watermelon

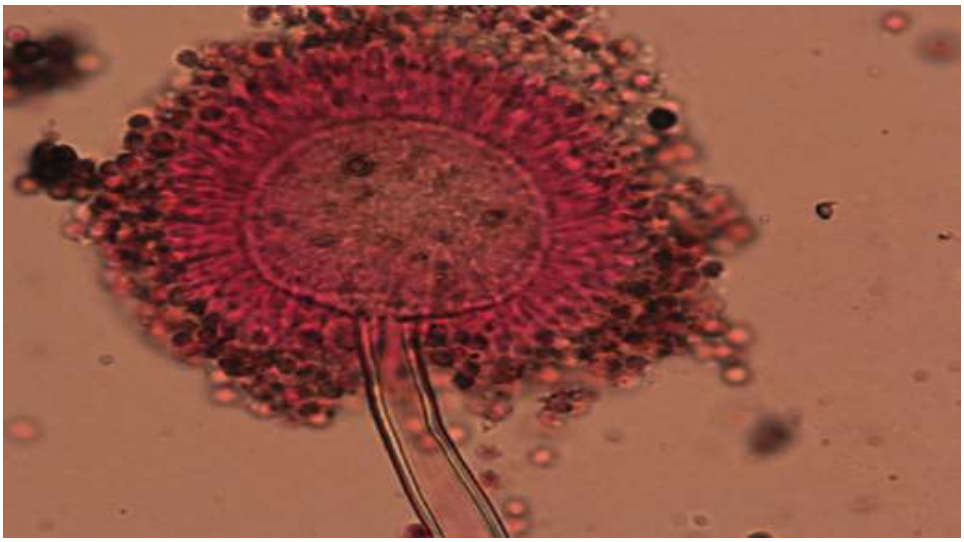

Plate 7: Micrograph of *Aspergillus niger* isolated from infected seeds of Watermelon

Effects of plant extract, their concentration and synthetic fungicide on percentage inhibition of radial growth of *fusarium moniliforme* in culture

Effects of plant extract by ethanol extraction, their concentration and synthetic fungicide on percentage inhibition on the radial growth of *Fusarium moniliforme* at day 1 in culture.
Table 1 shows that there was a significant effect of plant extracts and synthetic fungicide on percentage germination on the radial growth of *Fusarium moniliforme* where Benlate with inhibition mean value of 34.80%) performed significantly (<0.05) better than *Z. officinale* with value of 16.20% and *D. tripetala* wit*h* the inhibition value of 10.40%. Concentration level of 75% did better than 50%. Table 1 also showed that there was no significant interaction effect between the plant extracts.

Table 1: Effect of plant extracts by ethanol extraction, their concentration and synthetic fungicide on percentage inhibition on the radial growth of *Fusarium moniliforme* at Day1 in culture.

	Plant extracts		**Synthetic fungicide**	
Concentration	***D. tripatela***	***Z. officinale***	**Benlate**	**Mean Conc.**
50%	13.6000	26.100	51.300	30.400
75%	17.600	22.400	53.100	31.000
0%	00.000	00.000	00.000	00.000
Mean fungicide	10.400	16.200	34.800	20.500

$LSD_{0.05}$ (fungicide) = 7.280
$LSD_{0.05}$ (Conc.) = 7.280
$LSD_{0.05}$(Plant extract*Conc.) =12.61

Effects of plant extract by ethanol extraction, their concentration and synthetic fungicide on percentage inhibition of the radial growth of *Fusarium moniliforme* at day 2 in culture.
Table 2 shows that there was significant difference among the effects of the two plant extracts and synthetic fungicide in inhibiting radial growth of *Fusarium moniliforme* at day two in culture.
The results shows that Benlate had the highest mean inhibition value of 40.68%, which is significantly different from the two plant extracts. This was followed by *D. tripetala* with mean inhibition value of 17.48% while the least (13.41%) was from *Z. officinal,* but all the fungicides did better than control. Concentration level of 75% did better than 50% though they are statistically the same. The result also showed that interaction level at 75% did better than interaction level at 50% in all the plant extracts and synthetic fungicides.

Table 2: Effect of plant extracts by ethanol extraction, their concentration and synthetic fungicide on percentage inhibition on the radial growth of *Fusarium moniliforme* at Day 2 in culture.

	Plant extracts		Synthetic fungicide	
Concentration	***D. tripatale***	***Z. officinale***	**Benlate**	**Mean Conc.**
50%	22.730	17.790	59.040	33.190
75%	29.700	22.420	62.980	38.370
0%	00.000	00.000	00.000	00.000
Mean fungicide	17.480	13.410	40.680	23.850

$LSD_{0.05}$ (fungicide) = 3.875
$LSD_{0.05}$ (Conc.) = 3.875
$LSD_{0.05}$(Plant extract*Conc.) =6.711

Effects of plant extracts by ethanol extraction, their concentrations and synthetic fungicide on percentage inhibition on the radial growth of *Fusarium moniliforme* at day 3 in culture.

Table 3, shows that Benlate had the highest mean inhibition value of 47.52% which is significantly different from pepper fruit and ginger. This was followed by pepper fruit with mean inhibition value of 29.04% which also is significantly different from ginger with value of 20.82% also being the least. All the plant extracts and synthetic fungicide did better than control. The results also shows that concentration level of 75% performed significantly better than concentration level of 50% but both were better than control.

Table 3: Effect of plant extracts by ethanol extraction, their concentration and synthetic fungicide on percentage inhibition on the radial growth of *Fusarium moniliforme* at Day 3 in culture.

	Plant extracts		Synthetic fungicide	
Concentration	***D. tripatale***	***Z. officinale***	**Benlate**	**Mean Conc.**
50%	41.470	30.310	68.920	46.900
75%	45.650	32.150	73.640	50.480
0%	00.000	00.000	00.000	00.000
Mean fungicide	29.040	20.820	47.520	32.460

$LSD_{0.05}$ (fungicide) = 3.156
$LSD_{0.05}$ (Conc.) = 3.156
$LSD_{0.05}$(Plant extract*Conc.) =5.467

Effects of plant extracts by acetone extraction, their concentrations and synthetic fungicide on percentage inhibition of radial growth of *Fusarium moniliforme* at Day1 in culture.

Table 4: Shows that Benlate with mean inhibition value of 34.78% which performed significantly better than the two-plant extract. This was followed by *Zingiber officinale* with mean value of 17.28% while the least (8.10%) was obtained in *D. tripetala*. The effects of *Zingiber officinale* and *D. tripatela* were also statically different from each other but there was no significant difference among the interaction levels in the various concentration levels. The results also shows that concentration level of 75% performed higher than concentration level of 50%, but both concentrations did better than control.

Table 4: Effect of plant extracts by acetone extraction, their concentration and synthetic fungicide on percentage inhibition of radial growth of *Fusarium moniliforme* at Day 1 in culture.

	Plant extracts		Synthetic fungicide	
Concentration	***D. tripetala***	***Z. officinale***	**Benlate**	**Mean Conc.**
50%	11.140	23.950	51.280	28.790
75%	13.150	27.890	53.060	31.360
0%	00.000	00.000	00.000	00.000
Mean fungicide	8.100	17.280	34.780	20.250

$LSD_{0.05}$ (fungicide) = 3.061
$LSD_{0.05}$ (Conc.) = 3.061
$LSD_{0.05}$(Plant extract*Conc.) =5.302

Effects of plant extracts by acetone extraction, their concentrations and synthetic fungicide on percentage inhibition of radial growth of *Fusarium moniliforme* at Day 2 in culture.

Table 5 shows that there was a significant difference in the effect of plant extracts and synthetic fungicide on radial growth of *Fusarium moniliforme* where Benlate had the highest mean inhibition effect (40.70%), followed by 23.50% obtained in *Z. officinale* while the least (17.50%) occurred in *D. tripatela* . The three fungicides did better than control. Table 5 also shows that concentration level of 75% performed significantly better than concentration level of 50% but the two concentration levels did better than control.

Table 5: Effect of plant extracts by acetone extraction, their concentration and synthetic fungicide on percentage inhibition of radial growth of *Fusarium moniliforme* at Day 2

	Plant extracts		Synthetic fungiicide	
Concentration	***D. tripatela***	***Z. officinale***	**Benlate**	**Mean Conc.**
50%	22.700	30.900	59.000	37.500
75%	29.700	39.600	63.000	44.100
0%	00.000	00.000	00.000	00.000
Mean fungicide	17.500	23.500	40.700	27.200
$LSD_{0.05}$ (fungicide) = 6.930				
$LSD_{0.05}$ (Conc.) = 6.930				
$LSD_{0.05}$(Plant extract*Conc.) =12.01				

Effects of plant extracts by acetone extraction, their concentrations and synthetic fungicide on percentage inhibition of radial growth of *Fusarium moniliforme* at Day 3 in culture.

Table 6 shows that there was a significant difference in the effects of plant extracts and synthetic fungicide on radial growth of *Fusarium moniliforme* at Day 3 in culture, where Benlate did significantly better than plant extracts with a mean inhibition value of 47.65% followed by *D. tripetala* with mean value of 29.04% while the least (17.51%) occurred in *Z. officinale*.

Table 6 also shows that there was a significant difference between the effects of the plant extract in all the treatments and at all concentration but all the interaction levels did better than control.

Table 6: Effect of plant extracts by acetone extraction, their concentration and synthetic fungicide on percentage inhibition of radial growth of *Fusarium moniliforme* at Day 3 in culture.

	Plant extracts		Synthetic fung.	
Concentration	***D. tripetala***	***Z. officinale***	**Benlate**	**Mean Conc.**
50%	41.460	24.220	69.310	45.000
75%	45.650	28.300	73.630	49.190
0%	00.000	00.000	00.000	00.000
Mean fungicide	29.040	17.510	47.650	31.400
$LSD_{0.05}$ (fungicide) =3.119				
$LSD_{0.05}$ (Conc.) =3.119				
$LSD_{0.05}$(Plant extract*Conc.) =5.402				

DISCUSSION

Isolation of seed borne fungal organisms

The result of the isolation of seed borne fungal organisms from *Citrullus lanatus* showed that some fungi pathogens were isolated from the two watermelon varieties. Three different species of fungal pathogens were recorded these includes *Aspergillus niger, Fusarium monliliforme* and *Rhizopus stolonifer*. However, *Fusarium monliliforme* was isolated from all the varieties, while *Rhizopus stolonifer and Aspergillus niger* were isolated from kaolak variety. This is in agreement with several works like Bruton and Damicone *(*1999) who reported that watermelon is liable to attack by many pathogens but fungal pathogens attack watermelon more. The result is also similar to the report of Iwuagwu et.al., (2014), who isolated *Aspergillus niger, Fusarium monliliforme* and *Rhizopus stolonifer*. From three leafy vegetables and one fruit vegetable stored in evaporative coolant system called 'Vegetable Basket'

Pathogenicity

The result of the comparison of the symptoms observed in the field and those that manifested after artificial inoculation in the screen house showed a great similarity in terms of lesions seen on the vegetative parts of C*itrullus lanatus* crop. The result also showed that the re-isolated fungal organism confirmed that the fungus inoculated was actually the cause of the disease symptoms observed in the field according to Kock's postulate. This is in agreement with several workers like

Iwuagwu *et al* (2018) and Akhatre *et al* (2009). All these workers have used artificial inoculation of fungi spores isolated from infected tissues of various plant species into healthy ones and later re-isolated the same fungi inoculated with the confirmatory characteristics as were seen on those isolated from the infected ones before the test

Effect of Plant Extract their Concentration and Synthetic Fungicide on Radial Growth of *Fusarium monliliforme*

The result of the effect of plant extracts, their concentration and synthetic fungicides (Benlate) on radial growth of *Fusarium moniliforme* isolated from the seeds of the two varieties of *Citrullus lanatUs* showed that Benlate had the highest inhibition effect which was significantly higher than *Dennettia tripetala* and *Zingiber officinale.* This is in agreement with the finding of Ibaim *et al* (2000) and Ibaim *et al* (2006) who reported that Benlate is very effective in the control of *Fusaruim moniliforme* in rice, they also reported that the fungicide improve *in- vitro* seed germination, *in- vivo* seedling emergence and crop yield. The result of the research showed that the antifungal activity of the phytochemicals increased with increase in concentration. There was as a result of increase in effectiveness of the bioactive compounds as the concentration increased from 50% active ingredient to 75%. This is in consonance with Iwuagwu *et al* (2018), who reported in their experiment the efficacy and safety of some plant extracts in the control of cocoyam leaf necrotic fungi in Aguata Local Government Area of Anambra State, Nigeria. This is also in agreement with the works of Amadioha and Obi (1999) and Udo (2001). The result also revealed that Benlate was very effective in reducing mycelia growth of *Fusarium moniliforme* isolated from *Citrullus lanatus seeds*. This result is similar to the works of Iwuagwu *et al* (2018), who observed that some synthetic fungicide such as Apron- Plus was very effective in controlling of cocoyam leaf necrotic fungi in Aguata Local Government Area of Anambra State, Nigeria. This result also agrees with the report of Mtisi (1996), who have demonstrated the effectiveness of systemic fungicide and positive return per hectare from the use of the fungicides. According to Ibiam *et al* (2006), Benomyl, Bavistin, Fernasen D and Apron- plus 50DS being systemic fungicides would have inactivated or killed the pathogens in the seeds or seedlings as the seeds germinated. They also stated that these fungicides could have increased the resistance of seeds or seedlings or must have interfered with pathogenic processes, thus blocking the development of the symptoms in the seeds or seedlings.

CONCLUSION

From the research, it was discovered that the major organisms isolated were fungal organism; consequently, it could be concluded that fungal organisms are the major cause of seed deterioration, leading to poor viability, loss of seedling vigor and poor field establishment of Watermelon. All plant extracts and Benlate inhibited the fungus (*Fusarium moniliforme*) in culture. The inhibition was also greater as concentration increased from 50% to 75%. It was also observed that ethanol extraction solvent did better than acetone. Generally, *Dennetia tripatala* extract performed better than *Zingiber officinale.*

RECOMMENDATIONS

It could therefore be recommended that the two plant extracts used in this investigation which were very effective in the control of *Fusarium moniliforme* of watermelon could be an alternative to the synthetic fungicide. It could also be suggested that further studies be carried out to isolate, identify, characterize and standardize the bioactive components of these phytochemicals in a bid to commercializing their production.

CONFLICT OF INTEREST: All the authors declare that there is no conflict of interest in the conduct of this research neither in the results made from it.

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