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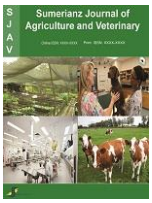

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Antifungal In-Vitro Effects of Ethanol and Acetone Extracts of *Aframomum Melequeta* Lin. and (*Azadirachta Indica* Lin.) on Seedborne Fungal Pathogen (*Fusarium Solani*) of Sweet Pepper (*Capsicum Annum* Lin.) in Awka, Anambra State Nigeria

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Abstract

The use of plant extract in the control of seed borne fungal pathogen has been found to be economically and environmentally friendly and as well as easily biodegradable. This study was carried out to investigate the effect of plant extracts, Alligator pepper (*Aframomum melegueta*) and Neem (*Azadirachta indica*) on the seed borne fungal pathogen of sweet pepper *Capsicum annum* (Otuocha and Nsukka). *Capsicum annum* seeds were extracted for seed health test using blotter paper method. Seed borne fungal pathogen (*Aspergillus spp. Fusarium spp*.) were identified. The potential for these organisms for pathogenicity were tested using Kock's postulate. The antifungal effects of ethanol and acetone extracts of the test plants were studied under in- vitro experiment against the seed borne fungal pathogen (*Fusarium solani*) of *Capsicum annum* at 0%, 50% and 75% concentration using synthetic fungicide (Benlate) as a standard control. The design was a 3x3 factorial laid out in a Completely Randomized Design (CRD) with three replications. Data collected were subjected to analysis of variance (ANOVA) and means were separated with Fishers' Least Significant Difference (FLSD) at 5% probability level. Ninety percent germination was obtained in the viability test of Nsukka pepper. The result of the pathogenicity test showed that the organisms isolated were pathogenic. All plant extracts (*Aframomum melegueta*) and Neem (*Azadirachta indica*) and Benlate inhibited radial growth of the test fungus. *Azadirachta indica* and Benlate inhibited the growth of *Fusarium solani* for three days in culture, though *Azadirachta indica* performed better than Benlate. The inhibition was also greater as concentration increased from 50% to 75% concentration level. The result of this study showed that the plant extract *Azadirachta indica*, which is naturally more abundant and readily available could be explored as an eco-friendly alternative to synthetic fungicide (Benlate) in the control of *Fusarium solani* on sweet pepper varieties since it performed as much as the synthetic fungicide.

Keywords: Seedborne pathogens; Plant extracts; Antifungal effects; In-vitro and Sweet pepper.

1. Introduction

Sweet pepper is one of the five most important vegetable crops used in Nigeria as condiment and food flavour. Sweet pepper cultivation has existed for several hundred years as a sustainable form of agriculture [1]. Sweet pepper exists as an annual herbaceous vegetable or shrub of the *Solanaceae* family, genus Capsicum and species *annuum, frutescens* or *chinense* [2]. It is a spice grown in both tropical and sub-tropical regions [3].

Sweet pepper is suitable for the diets of the Obese and is useful in the control of cancer of the stomach and colon [4]. They are low in sodium, cholesterol free, rich in vitamins A and C, and are a good source of Potassium, Folic acid and Vitamin E [3]. Fresh green sweet peppers contain more vitamin C than citrus fruits and fresh red sweet pepper has more vitamin A than carrots (Than *et al*, 2008). *Capsicum* fruits are used in sauces, soups, stews and generally as a flavouring agent [5] The different varieties of sweet pepper provide income for women and children who cultivate it in large quantities [2].

The Ministry of Food and Agriculture, MOFA (2010) reported that the average yield of sweet pepper under rain fed conditions was 6.5MT ha^{-1} with achievable yields standing at 32.3 MT ha^{-1} depending on the variety. The decline in yields of the harvested produce with the subsequent decline in the total export has been attributed to many factors, paramount among them are diseases.

Fungi are one of the major causes of diseases of sweet pepper. Many fungal species include; *Alternaria porri, A. alternata, Aspergillus amstelodami, A. flavus, A. fumigatus, A. nidulans, A. niger, A. sydowi*, *A.wentii, Botrytis cinerea, Cladosporium macrocarpum, Curvularia lunata, Fusarium equiseti, F. moniliforme, F. oxysporum, F. semitectum, Macrophomina phaseolina*, *Myrothecium roridum*, *Penicillium notatum, Rhizoctonia* sp., and *Rhizopus arrhizus* been isolated from sweet pepper [6]. 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 [7]. The diseases are mainly problematic on mature sweet pepper fruits, causing severe losses due to both pre and post-harvest fruit decay [8]. 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, *et al.* [9] and Iwuagwu, *et al.* [10]; 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 sweet peppers are propagated by seed [11]. 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 [12]. Seed testing is needed to achieve this [12]. Although several workers have reported isolation of various fungi from vegetable seeds [12-14].

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 [15]. Among the control practices used, seed treatment is one of the effective techniques to eliminate seed-borne inocula. Treatments of seed should be done as a routine practice as it is a cheap insurance against possible disasters at a later stage [16].

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. Successful use of plant extracts in controlling fungal pathogens through plant extracts have been used in controlling seed-borne infection in certain crops [10, 17, 18].

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* on rice [19].

Azadirachtin a chemical substance produced in *Azardirachta indica* is effective in the control of Anthracnose of sweet pepper [20], the seed and leaf is effective against early blight of tomato, sheath blight of rice, Fusarium oxysporum [21].

Extracts from the seeds of *Aframomum melequeta* have shown potent antiseptic, fungicidal and bactericidal properties [22]. Plant extract of *Aframomum melequeta* contains high doses of tannins, saponins, glycosides and polyphenols and is effective in the control of crop diseases [23].

2. Materials and Methods

The experiment was carried out at Botany Laboratory of Botany Department, Nnamdi Azikiwe University Awka campus. The Laboratory is situated at Latitude (6^o 5′ 10.1″ N) and Longitude (7^o 0.8′ 39.1″ E).

The materials used include; Beakers, Test tubes, Foil paper, Cotton wool, Petri dishes, Distilled water, Automated electric oven, Blender, Conical flask, Autoclave machine, Incubator, Whatman 9cm filter paper, Spatula, Inoculating loop, Wire gauze, Wire loop, Funnel, Slides and Slide cover, Sonic blender. Fruits of Nsukka pepper (*Capsicum annum*) and Otuocha pepper (*Capsicum annum*) were bought from Eke Awka market. The fruits were washed, seeds were extracted and air dried at room temperature. The two plant samples used for this work, Alligator pepper (*Aframomum melequeta)* was bought from the Eke Akwa market while *Azardirachta indica* seeds were plucked from the *Azardirachta indica* tree in Nnamdi Azikiwe University and were washed using sterile water and oven dried at 70^{o}C for 48 hours.

2.1. 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 121^{o}C for 15 minutes.

2.2. Seed Health Test

Seed health test for seed borne fungi was carried out following the rules of International Seed Testing Association [24]. Standard blotter method was used for the study. Two hundred (200) seeds were randomly taken from each sample for seed health test. The seeds were treated with 1% sodium hypochlorite for 5 minutes and rinsed two times with sterile distilled water inorder to effectively remove surface contamination without affecting the germination percentage of sweet pepper seeds [25-27].

2.3. Blotter Method

Twenty (20) seeds of each sweet pepper variety were plated in 9cm petri-dish containing 3 layers of whatman filter paper separately. The arrangement of the seeds was according to International Seed Testing Association [24]. Ten (10) petri dishes were used for each sample and a total of four hundred (400) seeds were plated for the two samples. Twenty (20) seeds of sweet pepper in a plate were arranged in thirteen (13) seeds at the outer ring, six (6) seeds at the middle ring and one (1) seed at the inner ring. After plating the sweet pepper seeds, distilled sterile water was used in wetting the blotter paper before plating and more water was added to re-wet the paper after initial wetting. The petri-dishes were sealed up using masking tape and labeled properly. The plates were replicated ten times and were placed on a working bench which was surface sterilized with methylated spirit and cotton wool to ensure an aseptic condition. The temperature of the room was 28^{o}C±2or1. Germination count was observed seven (7) days from the first day of germination and was recorded. Fungal growth was observed on the plates based on the mycelial colour and hyphal growth.

2.4. Preparations of Potato Dextrose Agar (PDA) Media

Twenty (20) gram of PDA powder was weighed using analytical weighing balance and was poured in a beaker. Five-hundred-millimeter (500) ml 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.

3. Fungal Isolation and Inoculation

Fungi infected sweet pepper seeds were isolated from the plated seeds and transferred into a PDA medium. One millimeter (1ml) of lactic acid was poured in to the petri- dishes containing 15ml of PDA mixed thoroughly by shaking gently and allowed to gel. Lactic acid was added to prevent bacterial contamination. The media plates were incubated at 28^o C±2or1 for 7 days and then observed for microbial growth from the 3rd day. Pure cultures were obtained by sub-culturing the fungal inoculum from the plates using PDA media. Fungal growth was observed based on mycelial growth, colour and spore shape and size.

3.1. Identification of the Fungal Pathogen

. Fungal inoculum was placed on a slide and stained using Lactophenol in cotton blue and covered with a slide cover. The prepared slide was viewed under a compound microscope model (Olympus -XN 50). Identification of the fungal inoculum was based on observations of the culture growth patterns, colour of mycelia and microscopic examinations of vegetative and reproductive structures. An illustrated Manual on identification fungi by Barnett and Hunter [28], Alexopoulois, *et al.* [29] were used for identification.

3.2. Pathogenicity Test

Pathogenicity test was carried out in an improvised greenhouse close to Crop science research farm.

3.3. Materials

Materials used include; hand sprayer, light polythene, corn flour, perforated buckets, sodium hydrochloride (bleach), top soil (sterilised).

3.4. Preparation of Soil Media

Soil media used for this experiment consist of top soil. The top soil was heat treated for 120^{o}C for three hours and allow to cool overnight. Five (5) kilogram of the soil media was weighed for each perforated bucket. The top diameter of the bucket was 25cm. Healthy seeds of the two varieties of sweet pepper were surface sterilised in water containing five millimeter of sodium hydrochloride (bleach) and were sown into the buckets containing sterilized top soil. After 5-7 days the sweet pepper seeds emerged. The seedlings of the two varieties of sweet pepper were thinned down to ten seedlings per bucket. Five set of plastic 7cm diameter bucket were used that gave a total of fifty pepper seedlings.

3.5. Inoculation of Fungal Spores

After one month the sweet pepper plant grew to seedling stage at about a pencil height. Spore suspension spray method of inoculation was used. Pure culture of *Fusarium solani* obtained from the culture maintained on PDA. Fully grown mycelial was scraped using a sterile scalpel into 100ml of distilled water and stirred thoroughly.10ml of the solution was added to 100ml of water, it was done for about 6 times. Corn starch was made in a slurry form and was sterilize at 100^{o}C in an autoclave. Conidial suspension was added into the serial dilution into the corn starch. Sterile hand sprayer was rinsed with bleach and rinsed twice with distilled water. 15ml of the inoculum was sprayed on the leaves of the sweet pepper seedlings. Light polythene bag was used to cover the sprayed sweet pepper seedlings for 24 hours to serve as a humid chamber providing an appropriate environment for infection. The light polythene bag was removed and observations on symptoms initiation were taken.

3.6. Preparation of the Plant Extracts

The plant samples Alligator pepper (*Aflamomum melequeta)* and Neem (*Azardirachta indica)* seeds were grounded separately using Sonik blender model (SB-1212). Fifty (50) gram and seventy-five (75) gram powder of the plant samples was weighed. The extraction process used was the soaking or cold extraction method [30]. This involved soaking fifty (50) gram and seventy-five (75) gram 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 [31]. The extracts were filtered through funnel filtered paper, 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 should remain. The filtrate was poured into the bottles and kept in the refrigerator until it was used.

4. An In- Vitro Test of Plant Extracts on the Growth of *Fusarium Solani*

Effect of plant extract on fungal pathogen was assessed using five millimeters (5ml) from each of the concentration of the extract. Fifty (50) percent, seventy-five (75) percent and zero (0) percent concentration of the extract was dispensed into 9cm petri-dishes and agitated thoroughly with 20ml of potato dextrose agar medium forming potato dextrose seed extract agar medium (PDSA) following poison food technique [32]. The agar extract mixture was allowed to solidify and then inoculated centrally with a 12mm diameter mycelial disc obtained from a seven (7) day old pure culture of the *Fusarium solani* with a sterilized cork borer and placed in the center of each petri-dish. The zero (0) percent concentration of the extract serve as the control (without the addition of 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 growth. All plates were placed on a laboratory bench covered with sterile Aluminum foil paper and at a room 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 inhibition. The radial growth inhibition in each plate was measured for 3 days. Each treatment was replicated three times. Percentage radial inhibition was determined according to Sundar, *et al.* [33].

Percentage (%) radial Inhibition = $\frac{dc-dt}{dc}$ x $\frac{100}{1}$

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

The experiment was laid out in completely randomized block design with three replications. The measurement of the radial growth of were made and recorded.

Plate-1. Inhibition of radial growth of *Fusarium solani* at different concentration of ethanol and acetone extracts of the test plants

5. Evaluation of Standard Fungicide on the Radial Growth of *Fusarium Solani* in Culture

Benlate was used to evaluate the radial growth of *Fusarium solani* following poison food technique [32]. Fifty (50) gram, seventy five (75) gram and zero (0) gram of Benlate were added in 100ml of distilled sterile water separately. Five (5) millimeter of the suspension was dispensed into 9cm petri-dishes and agitated thoroughly with 20ml of potato dextrose agar medium which was autoclaved. The zero (0) percent concentration of the Benlate serve as the control (without the addition of Benlate). After solidifying, the plates were inoculated centrally with a 12mm diameter mycelial disc obtained from the seven-day old culture of the *Fusarium solani* with a sterilized 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 for measuring the growth. All plates were placed on a laboratory bench covered with sterile Aluminum foil paper and at a room 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 inhibition. The radial growth inhibition in each plate was measured for 3 days. Each treatment was replicated three times. Percentage radial inhibition was determined according to Sundar, *et al.* [33].

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

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

The in vitro experiment was laid out in 3x3 factorial in completely randomized block design with three replicates. For statistical analysis, data were subjected to the analysis of variance (ANOVA) using Genstat at 5% level of significance.

6. Results

6.1. Germination Percentage of Nsukka and Otuocha Pepper for Three Days

The germination percentage of Nsukka and Otuocha pepper for three days; the highest germination percentage was seen at day three, Nsukka pepper had 75% and Otuocha pepper had 50% germination respectively. Followed by day two, Nsukka pepper had 35% and Otuocha pepper had 25% germination respectively and the least germination was seen at day one where Nsukka pepper had 15% and Otuocha pepper had 10% germination respectively (Figure 1).

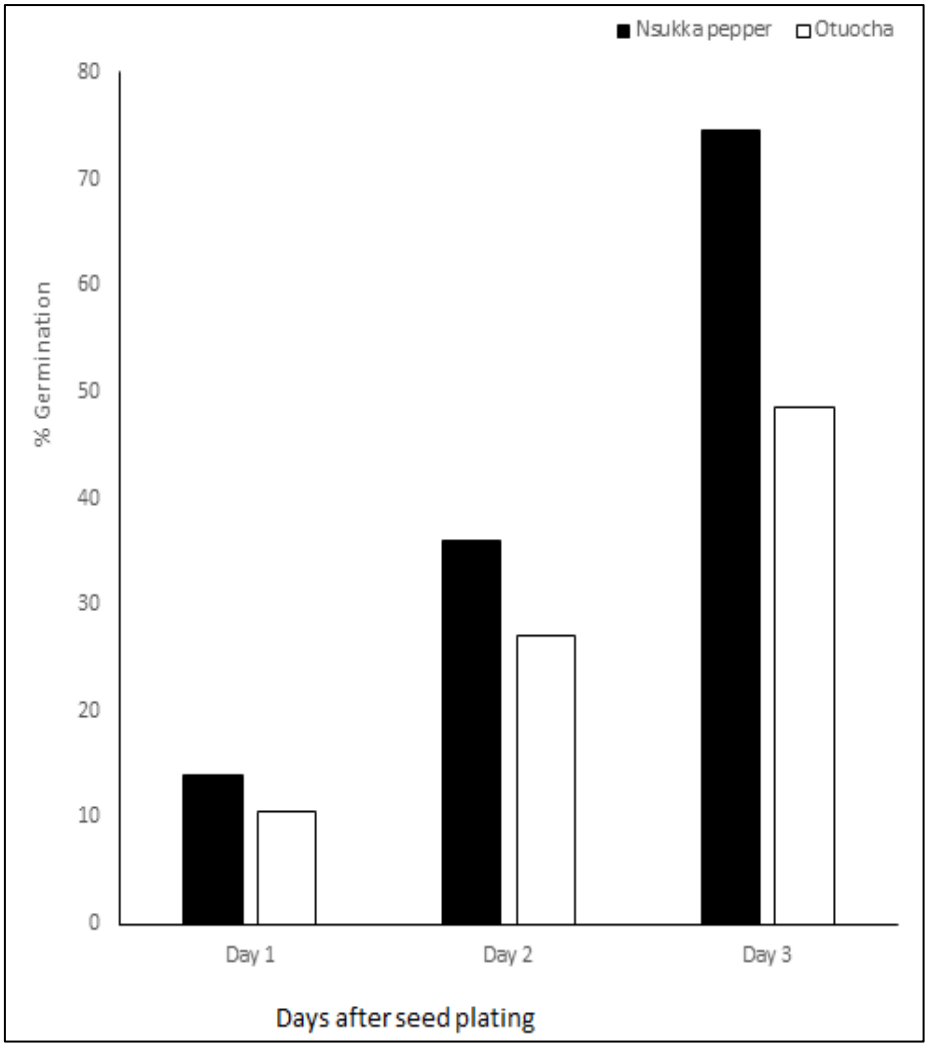

Figure-1. Percentage germination of Nsukka and Otuocha for three days

7. Seed Borne Fungal Isolated from Nsukka and Otuocha Pepper

Fungal organisms were isolated in the course of seed health test carried out on seeds of Nsukka and Otuocha pepper. The organisms include: *Aspergillus niger* (plate 2), *Aspergillus nidulans* (Plate 3), *Fusarium solani* (Plate 4) and *Aspergillus flavus* (plate 5).

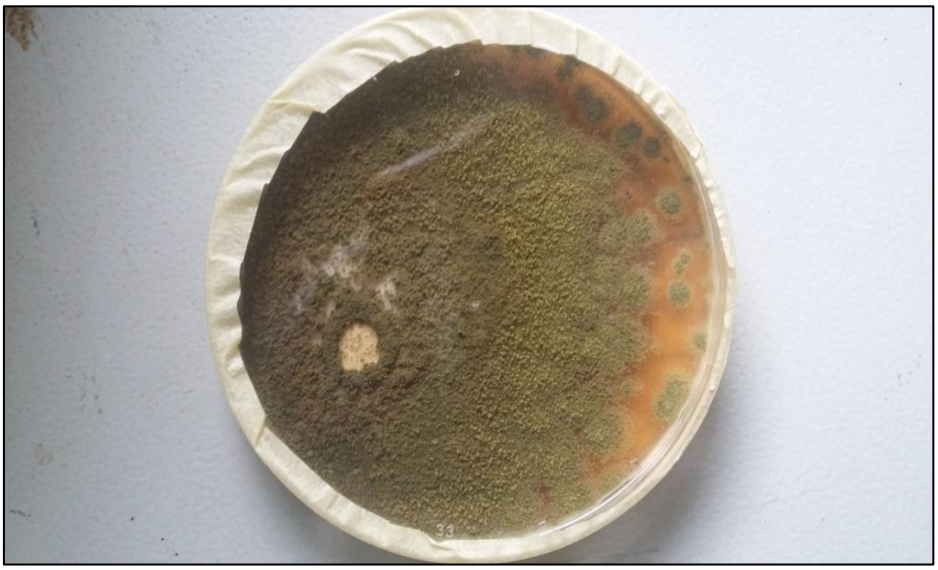

Plate-2. Pure culture of *Aspergillus flavus* isolated from seeds of Nsukka and Otuocha pepper

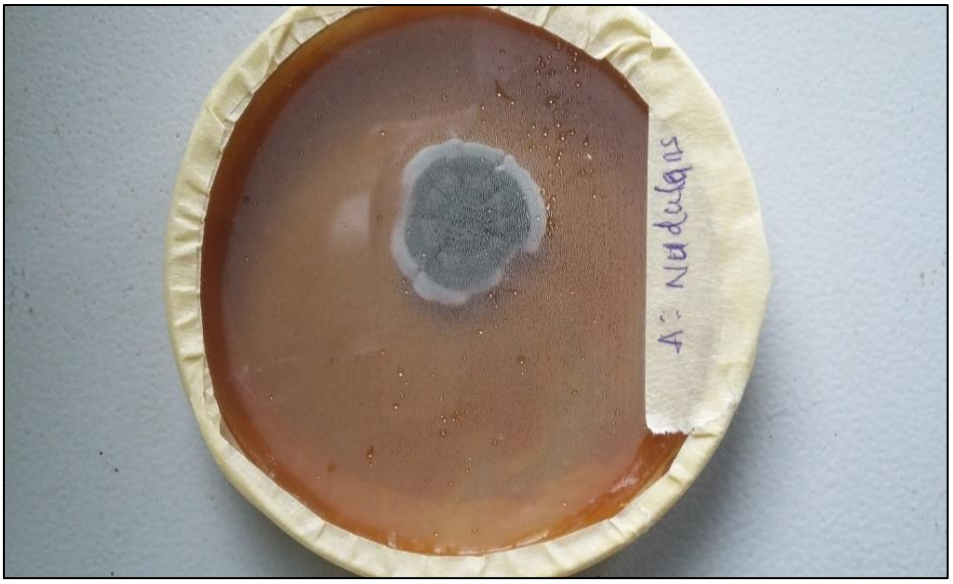

Plate-3. Pure culture of *Aspergillus nidulans* isolated from seeds of Nsukka and Otuocha pepper

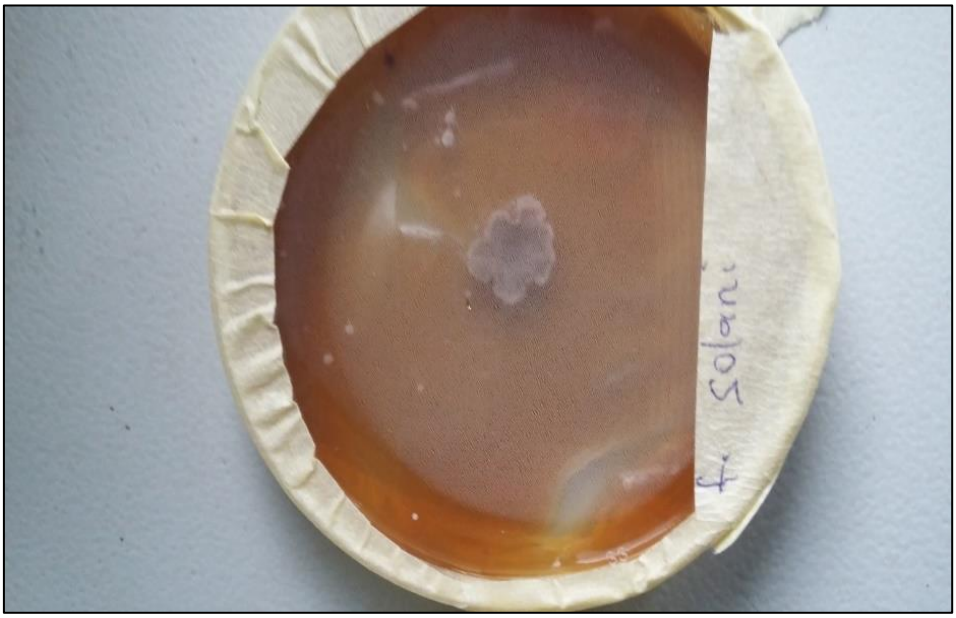

Plate-4. Pure culture of *Fusarium solani* isolated from seeds of Nsukka and Otuocha pepper

Plate-5. Pure culture of *Aspergillus niger* isolated from seeds of Nsukka and Otuocha pepper

Plate-6. Sweet pepper seedlings before inoculation

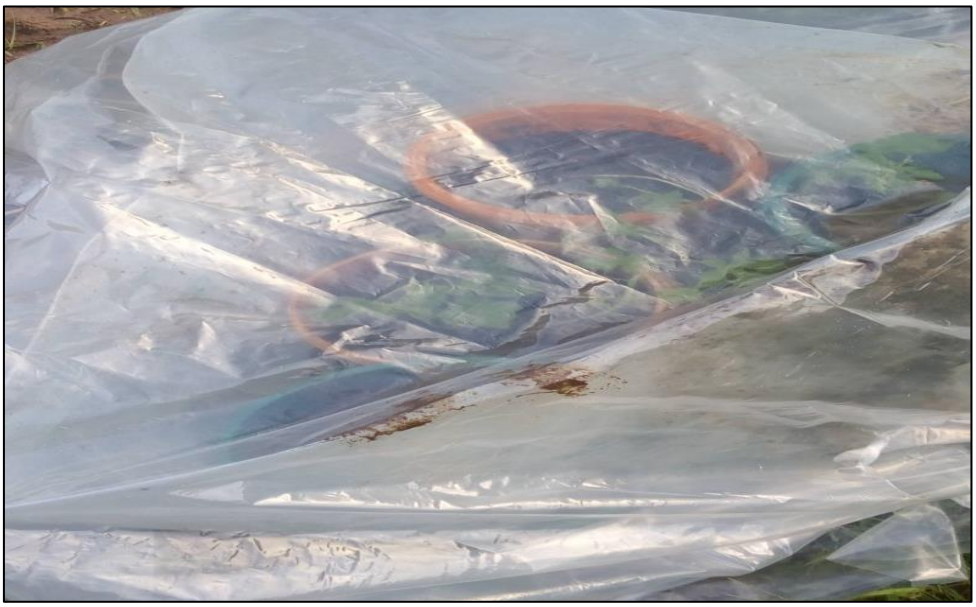

Plate-7. Sweet pepper seedlings after inoculation in a humid chamber

Plate-8. Sweet pepper seedlings showing symptoms of infection

8. Effects of Plant Extract by Ethanol Extraction, their Concentration and Synthetic Fungicide on Percentage Inhibition of the Radial Growth of *Fusarium Solani* 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 solani*, where *A. indica* (35.97%) performed significantly (0.05) better than *A. melegueta* (31.20%) but same with Benlate the inhibition value of (34.79%). All the two plant extracts as well as the synthetic fungicide did better than control.

Concentration level of 75% did significantly (0.05) better than 50%. Table 1 also showed that there was a significant interaction effect where *A. melegueta* × 50% (41.60%) and *A. melegueta* × 75% (52.00%). There was no significant interaction effect in *A. indica* and Benlate.

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

FUNGICIDES				
	Plant extracts		Synthetic fung.	
Concentration	***A. melegueta***	***A. indica***	**Benlate**	**Mean Conc.**
50%	41.600	52.020	51.290	48.300
75%	52.000	55.890	53.070	53.660
0%	00.000	00.000	00.000	00.000
Mean fungicide	31.200	35.970	34.790	
$LSD_{0.05}$ (fungicide) = 2.682 $LSD_{0.05}$ (Conc.) = 2.682 $LSD_{0.05}$ (Fungicide*Conc.) = 4.646				

9. Effects of Plant Extract by Ethanol Extraction, their Concentration and Synthetic Fungicide on Percentage Inhibition on the Radial Growth of *Fusarium Solani* at Day 2 in Culture

Table 2 shows that there was no significant difference among the effects of the two plant extracts and synthetic fungicide in inhibiting radial growth of *Fusarium solani* at day two. But the table showed that highest inhibition value was obtained in *A. indica* (40.84%) followed by Benlate (40.68%) while the least value was with *A.melegueta* (38.43%). All the treatments did better than control.

Concentration level of 75% did significantly better than of 50%. Also the Table shows that there was no significant interaction effect except at 50% *A.melegueta* (53.58%).

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

FUNGICIDES				
	Plant extracts		Synthetic fung.	
Concentration	***A. melegueta***	***A. indica***	**Benlate**	**Mean Conc.**
50%	53.580	60.410	59.04	57.680
75%	61.710	62.120	62.98	62.270
0%	00.000	00.000	00.000	00.000
Mean fungicide	38.430	40.840	40.680	
$LSD_{0.05}$ (fungicide) = 3.545 $LSD_{0.05}$ (Conc.) = 3.545 $LSD_{0.05}$ (fungicide*Conc.) = 6.141				

10. Effects of Plant Extracts by Ethanol Extraction, their Concentrations and Synthetic Fungicide on Percentage Inhibition on the Radial Growth of *Fusarium Solani* at Day 3 in Culture

Table 3 shows that there a significant difference among the inhibition effects of the three treatments where *A. indica* (50.13%) performed significantly (0.05) better than *A. melegueta* (46.79%) and Benlate with (47.65%) respectively. The two plants extract and Benlate performed better than control.

For concentration, 75% concentration level performed significantly (0.05) higher than 50% level but both did better than control. For *A. melegueta*, there was no significant difference in interaction effect between the two interaction levels, but 75% × *A. melegueta* had higher inhibition value than 50% × *A.melegueta*. For *A. indica*, the same trend was observed where 75% × *A. indica* had higher inhibition. For Benlate there was has high significant difference between the interaction effect of the levels where 75%× Benlate performed significantly (0.05) higher than 50%.

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

FUNGICIDES				
	Plant extracts		Synthetic fung.	
Concentration	***A. melegueta***	***A. indica***	**Benlate**	**Mean Conc.**
50%	69.290	74.500	69.320	71.040
75%	71.070	75.890	73.640	73.530
0%	00.000	00.000	00.000	00.000
Mean fungicide	46.790	50.130	47.650	
$LSD_{0.05}$ (fungicide) = 1.702 $LSD_{0.05}$ (Conc.) = 1.702 $LSD_{0.05}$ (fungicide*Conc.) = 2.949				

11. Effects of Plant Extracts by Acetone Extraction, their Concentrations and Synthetic Fungicide on Percentage Inhibition on the Radial Growth of *Fusarium Solani* at Day 1 in Culture

Table 4 shows that there was significant difference in the effect of plant extracts and synthetic fungicides, where *A. indica* (35.53%) performed significantly (0.05) better than *A. melegueta* (30.98%) but statistically same as Benlate with inhibition value of (34.79%).

Table 4 also showed that concentration level of 75% did significantly (0.05) better than concentration level of 50%.

The result also showed that there a significant difference in the interaction of the two levels of concentrations in *A. melegueta* (50% × $A.melegueta$) and (75%× *A. melegueta*) with values of (40.93%) and (52.00%) respectively but there was no significant difference in the interaction effect between the two concentration levels in both *A. indica* and Benlate but concentration level of 75% had better interaction effect that of 50%.

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

FUNGICIDES				
	Plant extracts		Synthetic fung.	
Concentration	***A. melegueta***	***A. indica***	**Benlate**	**Mean Conc.**
50%	40.930	51.910	51.290	48.040
75%	52.030	54.680	53.070	53.250
0%	00.000	00.000	00.000	00.000
Mean fungicide	30.980	35.530	34.790	
$LSD_{0.05}$ (fungicide) = 3.162 $LSD_{0.05}$ (Conc.) = 3.162 $LSD_{0.05}$ (fungicide*Conc.) = 5.476				

12. Effects of Plant Extracts by Acetone Extraction, their Concentrations and Synthetic Fungicide on Percentage Inhibition on the Radial Growth of *Fusarium Solani* at Day 2

Table 5 shows that there was no significant difference in the effect of plant extracts and Benlate in radial growth inhibition of *Fusarium solani* at day two. *A. indica* had the highest inhibition value of (40.89%) followed by (40.84%) in *A.melegueta* while the least was obtained in Benlate with inhibition value of (40.68%). All the treatment did better than control.

Just as earlier observed concentration level of 75% (62.06%) did significantly (0.05) better than concentration of 50% with inhibition value of (60.34%).

There was no significant difference in the interaction effect at all levels . Just as in other result interaction level of 75% performed better than that of 50%.

Table-5. Effect of plant extracts by acetone extraction, their concentration and synthetic fungicide on percentage inhibition on the radial growth of *Fusarium solani* at Day 2 in culture

FUNGICIDES				
	Plant extracts		Synthetic fung.	
Concentration	***A. melegueta***	***A. indica***	**Benlate**	**Mean Conc.**
50%	60.690	61.280	59.040	60.340
75%	61.830	61.380	62.980	62.060
0%	00.000	00.000	00.000	00.000
Mean fungicide	40.840	40.890	40.680	
$LSD_{0.05}$ (fungicide) = 3.142 $LSD_{0.05}$ (Conc.) = 3.142 $LSD_{0.05}$ (fungicide*Conc.) = 5.441				

13. Effects of Plant Extracts by Acetone Extraction, their Concentrations and Synthetic Fungicide on Percentage Inhibition on the Radial Growth of *Fusarium Solani* at Day 3 in Culture

Table 6 shows that there was a significant difference in the effects of plant extract and Benlate in inhibiting the radial growth of *Fusarium solani* at day three. *A. indica* (49.95%) and *A. melegueta* (48.03%) had the highest growth inhibition value being significantly higher than Benlate with inhibition value of (47.65%).

Table 6 also shows that there was a significant difference among the three concentration levels where concentration of 75% performed significantly$_{0.05}$ better than concentration of 50% with value of (73.84%) and (71.79%) respectively. The two concentrations did better than control.

The result also showed that there a significant difference in the interaction of the two levels of concentrations in Benlate (50% × Benlate) and (75%× Benlate) with values of (69.32%) and (73.64%) respectively but there was no significant difference in the interaction effect between the two concentration levels in both *A. indica* and *A. melegueta* but concentration level of 75% had better interaction effect that of 50%.

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

FUNGICIDES				
	Plant extracts		Synthetic fung.	
Concentration	***A. melegueta***	***A. indica***	**Benlate**	**Mean Conc.**
50%	71.430	74.610	73.640	71.790
75%	72.670	75.230	69.320	73.840
0%	00.000	00.000	00.000	00.000
Mean fungicide	48.030	49.950	47.650	
$LSD_{0.05}$ (fungicide) = 1.161 $LSD_{0.05}$ (Conc.) = 1.161 $LSD_{0.05}$ (fungicide*Conc.) = 2.010				

14. Discussion

14.1. Seed Health / Germination Test

The result of the germination / seed health test showed that the two varieties of *Capsicum annum* had different germination rates. The reasons for disparity in germination rates of sweet pepper varieties from localities may be multifaceted. These could be as a result of differences in crop management practices varying prevalence of pest and diseases in the areas, socio-economic differences with its attendant effect on ability to afford the purchase of varying inputs that aid in high quality output. Management practices also affect the quality of seeds; poor management practices leads to unhealthy and less vigorous seeds while good management practices will lead to healthy and more vigorous seeds

This result is similar to the report of who observed that there were significant differences in the quality of rice seeds in Central Luzon, Phillipines. They observed that there were varied reasons for the disparity in the quality of rice seeds in the area and the resultant effect on yield. According to them, the varieties, the size of the farm and the extent of participation in rice farming affect the establishment and yield of rice. They pointed out that among the biophysical factors, source of irrigation water and weed, pest and diseases pressure are significant determinants of rice yield and quality. The result also is similar to Diaz, *et al.* [34], who reported that studies on framers' seed management practices reveal that in most cases, they do not purchase certified seeds as farm input.

14.2. Isolation of Seed Borne Fungal Organism

The fungal organism isolated from the seeds of Nsukka and Otuocha pepper were: *Aspergillus flavus* (plate 1), *Aspergillus nidulans* (plate 3), *Aspergillus niger* (plate 7), and *Fusarium solani* (plate 5) which is in line with the works of Ahmed and Ravinder [6], McLaren, *et al.* [35]. Saad, *et al.* [36], observed that *Aspergillus flavus* (plate 1) and *Fusarium solani* (plate 5) were associated with damage to plumule, radial and hypocotyl of germinating seedlings.

14.3. Pathogenicity Test

The result of the comparison of symptoms observed in the field and those that manifested after artificial inoculation in the improvised greenhouse showed a great similarity in terms of lesions seen on the vegetative parts of the *Capsicum annum* plants. 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 Markson, *et al.* [37] and Akhtar, *et al.* [38]. All these workers used artificial inoculation of fungi spores isolated from infected tissue of various plant species into healthy ones and later re-isolated the same fungi inoculated with the confirmatory characteristics as were seen in this investigation.

14.4. Effect of Plant Extract, their Concentration and Synthetic Fungicide on Radial Growth of *Fusarium Solani*

The results of effects of plant extracts, their concentration and synthetic fungicide (Benlate) on percentage inhibition on the radial growth of *Fusarium solani* isolated from the two varieties of *Capsicum annum* showed that *A. indica* had the highest inhibition effect which was significantly(0.05) higher than *A. melegueta* and statistically same with Benlate.

This is in agreement with the finding of Opara and Obani [39], who reported that extract of *A. indica* and *Z. officinale* were the most effective respective respectively in reducing disease severity of bacteria spot diseases of Solanum. They observed that the plant extract from *A.indica* was as good as the synthetic, Benomyl and Streptomycin.

The result this research showed that the antifungal activity of the phytochemicals increase in concentration. There was increase in effectiveness as the concentration increased from 50% active ingredient to 75%. This is in agreement with Derbaiah, *et al.* [40], who reported in their experiment, efficacy and safety of some plant extracts against tomato early blight disease caused by *Alternaria solani*, that increasing concentration of the botanic extract led to increasing the toxicity of the active bio-compounds. This is also in consonance with the works of Amadioha and Obi [41] and Udo, *et al.* [42].

The result also revealed that Benlate was very effective in reducing mycelial growth of *Fusarium solani* isolated from *Capsicum annum.* Their result is similar to the work of Tunwari, *et al.* [43], who observed that some synthetic fungicides: Metalxyl, Captan, Thiobendazole and Benomyl were very effective in controlling the seventy of gray leaf spot disease Sorghum (*Sorghum bicolor*) than untreated check. This result also agrees with the report of Mtisi [44], and Iwuagwu, *et al.* [15] who have demonstrated the effectiveness of synthetic fungicide and positive return per hectare from the use of the fungicides.

According to Ibiam, *et al.* [45], Benomyl, Bavistin, fernasen D and Apron plus 50DS being synthetic fungicides would have inactivated or killed the pathogens in the seeds or seedlings as they germinated. They also stated that these fungicides could have increased the resistance of seeds or seedlings or must have interfered with pathogenic process, thus blocking the development of symptoms in the seeds or seedlings.

15. Conclusion

Seed borne fungi were isolated from pepper seeds using blotter method. From this research, it was discovered that the major organism isolated are fungal and therefore, it is consequently been implicated to be the major cause of seed deterioration, leading to poor viability and loss in seedling vigour. Also the variety, source of plant and age of plant could affect the rate of germination.

The plant extract use were very effective in use were very effect in reducing the radial growth of the fungus (Fusarim solani). Also Azadirachta indica performed better than Benlate and therefore can be use as an alternative to Benlate.

Recommendations

Farmers should be able to purchase their seed from reputable or certified seed outlets. Farmers should conduct viability or germination test to ensure adequate plant estimate in the field.

Azadirachta indica was as good as synthetic fungicide (Benlate). More studies should be conducted on plant extract to study the bio-compounds to be able to commercialize their production and make them available for farmers to reduce the rate of application of synthetic fungicides which is detrimental to human health, the environment and is not easily biodegradable.

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