EFFECTS OF THREE MEDICINAL PLANT EXTRACTS ON LEAF WILT AND STEM ROT DISEASES OF SESAME (*Sesamum indicum L*).

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ABSTRACT

Leaf curling, wilting and yellowing of sesame plant caused by Fusarium oxysporum and stem rot caused by Rhizoctonia solani are destructive diseases affecting sesame, throughout the world. Despite the success of several synthetic chemicals to curb this menace, their use has been discouraged, due to their negative impact on the ecosystem. This study was therefore designed to annex the use of botanicals, which seems to be a trend in tackling plant diseases. The pathogens were isolated from infected sesame collected from farmers' field; they were morphologically identified using standard mycology keys and hereafter authenticated using molecular tools. Pathogenicity was conducted and the pathogenic effects of both pathogens on sesame were confirmed. Three extracts which are Murraya koenigii, Azadirachta indica and Allium cepa at 50 and 100 g/l were screened using food poisoning technique. Mancozeb and distilled water were used as positive and negative controls respectively. Results revealed that the methanolic extracts of the three plants were highly effective at 50 and 100 g/l against the mycelial radial growth of the tested pathogens. However, M. koenigii extract was more effective at 100 g/l against both F. oxysporum and R. solani with mycelial growth inhibition rate of (100% and 96% respectively) while A. indica had (94.67% and 91%) and A. cepa has (71% and 50%). At 50 g/l the three plant extracts showed some inhibitory effects with A. cepa being the least, having 38.33 %, and 61% inhibitory rate on F. oxysporum and R. solani respectively. M. koenigii being the best in controlling both pathogens (with an inhibitory rate of 100%) can serve as a good candidate for the development of ecofriendly bio-fungicide against these tested plant pathogens.

KEY WORDS: Botanicals, Mycelial, Pathogens

INTRODUCTION

Sesame (*Sesamum indicum* L. Family: Pedaliaceae) is one of the most important oil seed crop cultivated around the world (Al – Yemeni *et al.*, 2000). It is an annual crop which is considered to be one of the most important oil seed (Noorka *et al.*, 2011). It is ranked ninth (9th) among the top thirteen oil seed crops, which make up to 90% of the world edible oil production (Kafiriti and Deckers, 2001). Sesame production is however liable to attack by at least eight economically important fungal diseases (El-Bramawy and Abd Al-Wahid, 2009). *Fusarium oxysporum* is one of the most common, widely distributed and destructive pathogen of sesame. It causes vascular wilt leading to reduction in seed yield (Radha, 2013). It is a soil borne plant pathogen that belongs to the Division Ascomycota, with over 120 known strains or "special forms" each of which is specific to a unique plant host, in which, it causes disease (Köhl and van der Heijden, 2016). *Rhizoctonia* spp. have also been reported in sesame fields, all of which attack seedling, causing damping-off, reduce crop stands in cool, wet soils and may attack the crop later in the growing season. Sesame seedlings may emerge, but seedling diseases may reduce germination

from 70% to 90% (Amber, 2014). Under severe infections, disease has been reported to cause 22 to 53 per cent loss in seed yield (Enikuomehin *et al.*, 2002).

Over time, fungicides have been used by farmers to control the plant diseases in order to improve yield (Gerken *et al.*, 2001). The misuse of fungicides in terms of quantity applied or in deleterious combinations has created a myriad of problems which include pathogen resistance, fungicide residues, destruction of beneficial fauna and environmental pollution (Obeng-Oforiet *al.*, 2002). Public concern over the harmful effects of these chemical compounds on the environment, plants and human health has facilitated the search for safer, environmental friendly control alternatives (AVRDC, 2003).Some plants contain components that are toxic to pathogens when extracted from plant and applied on infected crops (Yasser *et al.*, 2016). These components are called botanical pesticides or botanicals (Yasser *et al.*, 2016). Commonly used botanicals among others include plant extracts such as neem (*Azadirchta indica*, A. juss) and garlic (*Allium sativum*); and essential oils such as nettle (*Urtica spp.*), rue (*Ruta graveolens*, Linn), thyme (*Thymus vulgaris*, Linn), and tea tree (*Melaleuca alternifolia*) (Gurjar *et al.*, 2012).

The biological inhibitions by different natural substances, such as essential oils and plant extracts have been investigated widely against fungal activities (Gurjar*et al.*, 2012 and Yasser *et al.*, 2016). *Murraya koenigii*, *Azadirachta indica* and *Alllium cepa* were selected for this research because several researchers have reported their use in fungi disease management practices (Tunwari *et al.*, 2014; Sanjay *et al.*, 2006; and Gabriel *et al.*, 2014). This study was therefore conducted to ascertain the efficacy of these medicinal plant extracts as cheap alternative bio-fungicide to synthetic fungicide.

MATERIALS AND METHODS

Survey and collection of samples

Surveys on the incidence and severity of Fusarium wilt and Stem rot of sesame plants (*Sesamum indicum*)were carried out in March and April, 2017 in three farms from two L.G.A (Bosso and Bida) areas in Minna, Niger State. A rating scale used by Amadi *et al.* (2009) was employed to assess the incidence and severity of the disease. The Disease Incidence (IC) was calculated using the formula used by Kone *et al.*(2017):

$$IC = \frac{n}{N} \times 100$$

Where n = number of disease plants, N = total number of plant assessed

After the survey, the diseased, non – diseased plant and soil in their rhizosphere were collected in sterile polythene bag, a total of hundred (100) matured sesame plant were sampled. Visual observation of the selected leaf, stem and seed pod were carried out and the disease incidence was recorded following the method of Kone *et al.*(2017). The collected samples were taken to the Laboratory, Department of Biological Sciences, Federal University of Technology, Minna, for the isolation of causative organisms.

Table 1: Rating scale for diseased plant

Scale	Description	Inference
0	No symptoms on leaves	No Infection
1	1 - 25% leaf area covered with lesions	Mild Infection
2	26 - 50% leaf area covered with lesions	Moderate Infection
3	51 - 75% leaf area covered with lesions	Severe Infection
4	76% and above	Very Severe/Devastating

Amadi *et al.* (2009)

Collection, authentication and preparation of plant extracts

The leaves of *Azadirachta indica* (Neem) were collected from Federal University of Technology, Minna, while fresh *Alium cepa* (Onion bulbs) and *Murraya koenigii* (Curry) leaves were bought from vegetable section of Kure Ultra-modern Market Minna, and were authenticated in the Department of Biological Sciences, Federal University of Technology, Minna Niger State. The plants were sterilized by soaking the leaves of *Azadirachta indica* and *Murraya koengii* in 10% sodium hypochlorite (NaCl) for 1 minute, washed thoroughly under tap water, air dried for thirty (30) minutes and later oven dried at 40°C for 4 – 6 hours to attain constant weight. The outer layers of *Allium cepa* was peeled off and discard, the remaining bulb was washed with sterile distilled water, sliced and oven dried at 60°C (Amadi *et al.,* 2009) and there after ground using mortar and pestle to obtain coarse particle (Ayoola *et al.,* 2008).

Methanolic extraction was carried out using soxhlet apparatus. Fifty grams (50 g) each of the grated samples were packed in no. 1 Whatman's filter paper and put into the thimble of the soxhlet apparatus. Three hundred milliliter (300 ml) of methanol was used in a typical run against the plants. The extract was concentrated using the rotary evaporator under reduced pressure (40°C) until dryness (Faridah, 2011).

Phytochemical Analysis

The phytochemical analysis of the plant extracts was done following the review methods of Prashant *et al.* (2011) and Ayoola *et al.* (2008).

Preparation of Potato Dextrose Agar (PDA)

Irish Potato was washed, peeled and 200gram was weighed out. It was boiled for 20 minutes in 200 ml of sterile distilled water. The supernatant was drained into one litre conical flask, 20 g of agar agar and 20 g glucose were added. Distilled water was added to make it up to 1000 ml. The flask was corked with cotton wool wrapped in foil paper. The mixture was sterilized at 121°C for 15 minutes in an autoclave; it was allowed to stand for some minutes (Cheek bearable) before 0.5 g of chloramphenicol was added and carefully stirred (Adebola and Amadi 2010)

Isolation and Identification of Fungal

Infected sesame leaves, seeds and stem with rhizosphere soil were collected in sterile envelopes in sesame farms from Bida and Bosso Local Government Areas of Niger State. They were taken to the Laboratory, Department of Biological Sciences, Federal University of Technology Minna, for pathogens isolation. The collected samples were washed in sterile distilled water and surface sterilized with 70% ethyl alcohol. A portion close to infected areas (3mm x 3mm) were cut using sterile scarpel and aseptically placed in petri dishes containing 20 ml Potato Dextrose Agar (PDA), incubated for seven (7) days at ambient temperature at 12 hours photoperiod (28°C ± 2°C). Serial dilution method was used to isolate the fungi from the soil; one gram of the soil was placed in 9ml of sterile distilled water in test tube and then serially diluted. Dilution factors used were 10^{-2} 10^{-4} 10^{-6} from which 1ml of each diluents was placed on PDA plate (Amadi *et al.*, 2009). Pure cultures of the isolates were obtained after series of sub – culturing in sterile potato dextrose agar (PDA) (Tijani *et al.*, 2014, Adebola and Amadi, 2010). The fungal isolates were examined under light microscope, and morphological characteristics such as mycelial growth, pigmentation and presence of fungal conidia (Microconidia, macroconidia and chlamydospore) were used foridentification, hypothetical names (FO 001, FO 002, FO 001, RH 001 and RH 002) were given.to the isolated fungi (Watananbe and Nakamura, 2004).

Molecular characterization of isolates

The isolated organisms were further identified and characterized molecularly at the International Institutes of Tropical Agriculture (IITA) down to species level. Fungi DNA extraction protocol, PCR analysis (using Internal Transcribe Spacer universal primer set), integrity test, Purification of amplified product andsequencing were done following the standard procedures.Molecular characterization of two strains labeled using hypothetical names where picked at random and molecular toolswere used to further authenticate the strains.

Preparation of Inoculums

Five (5) and Seven (7) days old culture plates of causative organisms were respectively flooded with sterile distilled water and gently agitating, the cultures with an artist's paint brush to dislodge the conidia. *Fusarium oxysporum* inoculum was poured into ten (10) ml of sterile distilled water in 100 ml beakers, stirred with a spatula and the resulting conidia suspensions were first cleared for hyphal debris by filtration, using sterilized muslin cloth and centrifuge for 5 min at 3000 rpm (Ashraf *et al.* 2007). Two hundred (200µl) microlitres of the spore suspension was pipetted in channel of haemocytometer and the numbers of spores in five (5) squares at random were counted using the formula given by Karuna and Kolte, (2005).

$$\text{No. of conidia in the diluted suspension/millilitre} = \frac{\text{Average no of conidia above one large square} \times 1\text{ml}}{0.004\ \text{mm3}}$$

The final concentration was adjusted to 2.70×10^4 conidial/ml (Karuna and Kolte, 2005).

Rhizoctonia solani does not produce spores, thus,30 mL of *R. solani* inoculum (mycelial fragments) were poured into 100 ml of distilled and kept for further use (Abdeljalil,*et al.*, 2016).

Pathogenicity

Pathogenicity test was carried out using five (5) and seven (7) days old cultures of each isolated pathogen (*F. oxysporum* and *R. solani*) to infest three weeks-old sesame plant (cultivar, E_8collected for National Cereal Research Institute, Badeggi). Healthy sesame plants were

transferred in seedling pots from the field into the screen house (Ara *et al.*, 2017). Inoculation was performed by spraying with 50 ml of conidial suspensions (2.70 x 10^4spores/mL) using self-purging noozle sprayer (240 microns) over the soil surface in each pot, close to the sesame seedling roots while the control plants were sprayed with sterile distilled water. The inoculated plants were first kept in humid plastic bags and symptoms of infestation were look out for after ten (10) days of inoculation. The fungi were re - isolated and identified from the diseased plants. (Ara*et al.*, 2017).

***In vitro* assessment of methanolic plant extracts on fungi isolates**

In vitro inhibitory effects of methanolic plant extracts on the growth of the pathogens were tested using food poisoned technique. Observations of colony growth of isolated fungi were done at ambient temperature (28 ± 2°C) at 12 hours photoperiod. Fifteen (15) ml of PDA (double strength) were amended with five (5) ml of methanolic plant extracts (50 g/l and 100 g/l) in 40 sterile plates (Three treatments and two controls), the Controls were set up using mancozeb (positive control) and sterile distilled water (negative control). Mycelial disc five (5) mm in diameter was picked from the peripheral of seven (7) and five (5) days old culture onto the amended PDA plates. The plates were incubated at 27 ± 2°C (ambient temperature), mycelial radial growth of *Rhizoctonia solani* was recorded every 24 hours for 5 days while record was taken for seven days for *Fusarium oxysporum (*Adebola and Amadi, 2010, Miyashira *et al.*, 2010). Percentage growth inhibition rate was calculated using the formula;

$$\text{Percent mycelia inhibition} = \frac{R_1 - R_2}{R_1} \times 100 \quad \text{(Adebola and Amadi, 2010)}$$

Where R_1= radial growth of pathogen in control and R_2= the radial growth of pathogen in the test plate

Data Analysis

Data collected from the *in vitro* experiment using methanolic plant extracts on *Fusarium oxysporum* and *Rhizoctonia solani* in the laboratory were subjected to analyses of variance (ANOVA). Treatment means separation were carried out with the Duncan's Multiple Range test (DMRT) according to Gomez and Gomez (1984) and probability of treatment means being significantly different were set at $P < 0.05$. The analysis was done using Microsoft excel 2010 and statistical packages for social sciences, 20th version.

Results

Disease incidence of the three surveyed farmers' field

Visual observation of the selected leaf, stem and seed pod showed that the three farms surveyed have mild infections. Result of the survey on the disease incidence revealed that sesame in farmers' field of National Cereal Research Institute(NCRI) has 12% infection rate, while that of Pyata (Bosso area) and Government College Staff Quarters Bida, Farmers' field has 19 and 15% infection rate respectively (Table 2).

Table 2: Disease incidence and severity of surveyed site

S/N	Site	Percentage Disease Incidence	Scale / inference
1	NCRI	12%	1 (Mild Infection)
2	Pyata	19%	1 (Mild Infection)
3	G. C. S. Q	15%	1 (Mild Infection)

NCRI: National Cereal Research Institute,, Badeggi, Pyata: Farming area located in Bosso, L. G. A., G.C.S.Q: Government College Staff Quarters, Bida

Phytochemical analysis

The phytochemical constituents of the methanolic extract of *Murraya koengii*, *Azadirachta indica*and *Allium cepa* is presented in Table 3. The analysis revealed the presence of flavonoid, tannin, saponin, alkaloid, terpernoid, phenols, cardiac glycosides in *Murraya koengii* and *Azadirachta indica* while tannin, phenol, phlobatanins were absent in *Allium cepa*.

Table 3: Qualitative phytochemical screening

Active compounds	*Allium cepa*	*Murraya koengii*	*Azadirachta indica*
Flavonoid	+	+	+
Tanins	–	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Terpernoids	+	+	+
Phenols	–	+	+
Phlobatanins	–	+	–
Steroids	+	+	–
Cardiac glycosides	+	+	+
Anthroquinones	+	+	–
Reducing sugar	+	–	+

Keys: (+) Present, (-) Absent

Isolated pathogens from sesame plant

The results from the three sample sites (NCRI, Pyta, and Government College Staff Quarters Bida,"farmer's field") and the number of *F. oxysporum* and *R. solani* isolates are presented in Table 4. A total of six strains (four *F. oxysporum* and two *R. solani*) were isolated from all the samples collected.

Table 4:Number and sources of*Fusarium oxysporum and Rhizoctonia solani*isolated

SN	ISOLATE	LOCALITY/ COLLECTION SITE	SOURCE
1	FO 001	Pyta	Leaf
2	FO 002	Pyta	Soil
3	FO 003	NCRI	Soil
4	FO 004	GCSQ	Soil
5	RS 001	GCSQ	Stem
6	RS 002	GCSQ	Root

Keys: NCRI: National Cereal Research Institute, Badeggi, Pyata: Farming area located in Bosso, L. G. A., G.C.S.Q: Government College Staff Quarters, Bida
FO- *Fusarium oxysporum,* RS- *Rhizoctonia solani*

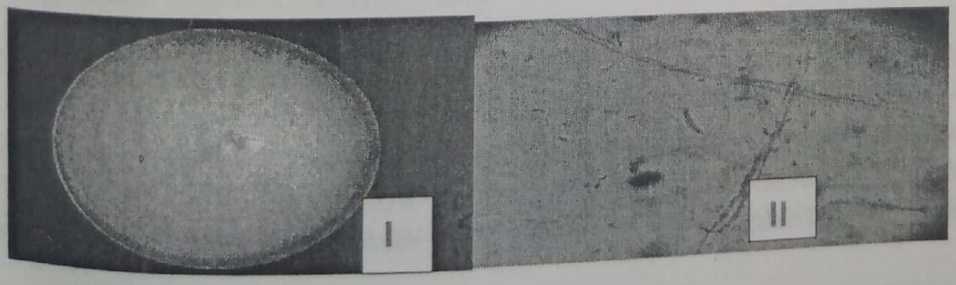

Plate I: Culture plateof *Fusarium oxysporum Oxysporum*

Plate II: Microphotograph of *Fusarium*

Characteristics of *Rhizoctonia solani*isolated

The Characteristics of *Rhizoctonia solani* isolatedfrom soil samples usedare pale creamy color (Plate III), septate hyphae that branch at right angle to each other (Plate IV) and absence of spores (Plate IV).

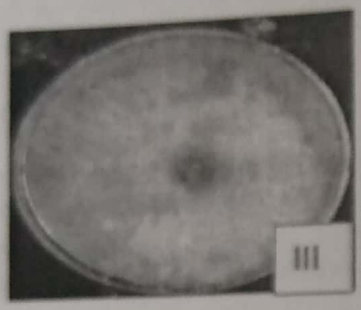

Plate III: Culture plate of *Rhizoctonia solani*

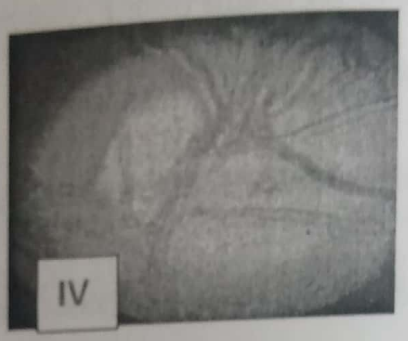

Plate IV: Microphotograph of *Rhizoctonia solani*.

Pathogenicity result

The results of pathogenicity test done revealed that *Fusarium oxysporum* isolated caused the leaves to wilt and curled (Plate III) while *Rhizoctonia solani* caused the stem to rot (plate IV).These symptoms showed that these pathogens were the causative organism of curled, wilted leaf and stem rot in sesame

Plate V: Stem rot caused by *Rhizoctonia solani*Plate VI: Leaf curl and blight caused by *Fusarium oxysporum*

Molecular identification

Molecular characterization using the Internal Transcribed Spacer primer (ITS) set; ITS 1, 5.8 and ITS 2 regions shows that sequences aligned with results from a query of the BLAST database and authenticated the isolate to be *F. oxysporum* accession number accession

KY090783.1 and *Rhizoctonia solani* nucleotide sequence unique banding patterns specific for AG- 3 with accession number KX129968.1 (Table 5 and 6).

Table 5 Nucleotide sequence of isolate RS. 001 from sesame leaf

99% identical *to Rhizoctonia solani* strain AG-3 ACCESSION NUMBER KX129968.1
AAATAAAAGTCGGAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGA
ATTTTAATGAAGAGTTTGGTTGTAGCTGGTCTATTTATTTAGGCATGTGCACACCTCC
CTCTTTCATCCCACACACACCTGTGAACTTGTGAGACAGTTGGGGAATTTATTTGTTA
TTTTTTGTAATAAAATGATAATAAGTCATTGAACCCTTCTGTCTACTCAACTCATATA
AAATCAATTTATTTTAAATGAATGTAATGGATGTAACACATCTCATACTAAGTTTCA
ACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA
ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTT
GGTATTCCTTGGAGCATGCCTGTTTGAGTATCATGAAATCTTCAAAATCAATCTTTTT
GTTAACTCAATTAGTTTGATTTTGGTATTGGAGGTCTTTTGCAGCTTCACACCTGCTC
CTCTTTGTGTATTAGCTGGATCTCAGTGTTATGCTTGGTTCCACTCAGCGTGAAAAAT
TATCTATCGCTGAGGACACTGTAAAAAGTGGCCAAGGTAAATGCAGATGACCGCTT
CTAATAGTCCATTGACTTGGACACTATTATTATGATCTGATCTCAAATCAGGTAGGG
CTACCCCC

Table 5 Nucleotide sequence of isolate FO. 001 from sesame stem

Sample B 98% identical to *Fusarium oxysporum* accession KY090783.1
AAcGaGttTGCATCTCCCTCaCcGCTGTGTaACATACCACTTGtTGCcGCGGCGGATCAGC
CCGCTCCCGGTAAATCCGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATAT
GTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGT
TCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGgcATG
CCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTCGCGTTAA
TTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGTAAA
ACCCTCGTTACTGGTAATCGTCGCGgCCACGCCGTTAAACCCCAACTTCTGAATGTTG
ACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAGGGCGGAGGA
A

***In Vitro* Efficancy of Methanolic Plant Extract against*Fusarium Oxysporum* and *Rhizoctonia solani*of Sesame Plant**

The results showed that methanolic plantextracts of the three botanicals significantly ($p< 0.05$) inhibited the mycelial growth of *Fusarium oxysporum and Rhizoctonia solani*at concentrations of 50 and 100 g/l respectively (Table 7 and 8). *Murraya koengii* extract demonstrated maximum mycelial growth inhibition (100%) of *Fusarium oxysporum*at 100 g/l,whenits concentrations are being compared down the column with 50 g /l of same extract and mancozeb (positive control).

Also *M. koenigii* when compare across the row with other extracts (*Azadirachta indica* and *Allium cepa)* at both concentrations (50 and 100 g/l) hadthe highest inhibitory potential which are 97 % and 100% respectively. *Allium cepa*however, has a lower inhibitory potential on themycelial growth of *Fusarium oxysporum* at 50 g/l (38.33%), although, it moderatelyinhibited the growth of *F. oxysporum* at 100 g/l (50%), never the less, *Azadirachta indica* showed to be a good antagonist against *Fusarium oxysporum* at both concentrationof 50 and 100 g/l (94.67 and 86.67% respectively), (Table 7)

Table 7 *In vitro* of methanolic plant extractsagainst *Fusarium oxysporum* of sesame at day 7 after inoculation

S/n.	Conc. (g/l, x)	Percentage mycelial reduction		
		A. indica	*A. cepa*	*M. koengii*
1	50	$86.67 \pm 3.84^{a}{}_{a}$	$38.33 \pm 4.37^{b}{}_{b}$	$97.67 \pm 1.45^{a}{}_{a}$**
	100	$94.67 \pm 2.40^{a}{}_{a}$	$50.00 \pm 2.08^{b}{}_{a}$	$100 \pm 0.000^{a}{}_{a}$
2	Mancozeb (+)	$45.33 \pm 0.67^{a}{}_{b}$	$45.33 \pm 0.66^{a}{}_{ab}$	$45.33 \pm 0.67^{a}{}_{b}$
3	Water(-)	$0.00 \pm 0.00^{a}{}_{c}$	$0.00 \pm 0.00^{a}{}_{c}$	$0.00 \pm 0.00^{a}{}_{c}$

Results are means of three replicates with their standard error
*Means followed by the same superscript across each row are not statistically different $p< 0.05$.
**Means followed by the same subscript down each column are not statistically different$p< 0.05$.
x = manufacturers recommended application rate
g/l = gram per litre. Controls: + (Postive) , - (Negative)

The results of mycelial radial growth of *Rhizoctonia solani*at 100 g/l when compared with other extracts (*Azadirachta indica* and *Allium cepa*) across the row showed that*Murraya koengii*has the highest inhibitory effect(96%),*Azadirachta indica* has (91%) and *Allium cepa* has (71%) while at 50 g/l almost the same result were recorded against mycelial growth of *Rhizoctonia solani* though not as effective as 100 g/l (Table 8). Down the column at 100 g/l, *Murraya koengii*has 96%,*Azadirachta indica* (91%) and *Allium cepa* (71%) while at 50 g/l, 94%, 77% and 61% inhibitory rate were recorded for the three plant extracts respectively. It can be deduced that 100 g/lis more effective than 50 g/l methanolic extracts of the same plant. However, the positive

control at manufacturer recommended application rate has more effect on *R. solani* than the 50 g/l plant extracts used (Table 8).

Table 8*In vitro* efficacy of methanolic plant extractsagainst *Rhizoctonia solani* of sesame at 5 days after inoculation

S/n.	Conc. (g/l, x)	Percentage mycelial reduction		
		A. indica	*A. cepa*	*M. koengii*
1	50	$77.00 \pm 4.04^{b}_{b}$	$61.00 \pm 1.00^{c}_{b}$	$94.00 \pm 0.58^{a}_{a}$
	100	$91.00 \pm 2.00^{a}_{a}$	$71.00 \pm 0.67^{b}_{a}$	$96.00 \pm 0.33^{a}_{a}$
2	Mancozeb(+)	$86.00 \pm 2.31^{a}_{a}$	$86.00 \pm 2.31^{a}_{a}$	$86.00 \pm 2.31^{a}_{b}$
3	Water(-)	$0.00 \pm 0.00^{a}_{c}$	$0.00 \pm 0.00^{a}_{c}$	$0.00 \pm 0.00^{a}_{c}$

Results are means of three replicates with their standard error
*Means followed by the same superscript across each row are not statistically different $p< 0.05$.
**Means followed by the same subscript down each column are not statistically different$p< 0.05$.
x = manufacturers recommended application rate
g/l = gram per litre. Controls: + (Postive), - (Negative)

Discussion

Leaf curl, wilt and stem rot diseaseincidence observed on sesameplants growing in Pyata, NCRI farmer's field and Government College Staff Quarters farmers' field in Bosso and Bida Local Government Areas of Niger Stateranges between12-19%. This indicated mild infection of the farmers' field by the diseases. Though diseases were not severe, intervention is needed to curb the rapid spread of the diseaseso as not to exceed 50% disease occurrence, this was in conformity with the research ofCunniffe*et al.*(2014), whostated that disease incidenceon citrus having an average of 50%,makes plants become symptomatic approximately within the first 5 years without treatment.Synergistic relationship, between the plant and the disease could lead to outbreak, if not checked(Cunniffe*et al.*, 2014). Application of fungicides in the control and management of plant pathogens has their detrimental effect onenvironmental quality and human health; therefore, botanicals are annexed by plant pathologist to solve this problem.

Phytochemicals analysis of plants used in this study revealed that *Murraya koenigii* and *Azadirachta indica* has alkaloid, tannin and phenols, which are bioactive component already

authenticated for their antimicrobial activities. Chyau, *et al.*,(2002) and Ramakul*et al.*(2012) reported that,the extracts of *Terminaliacatappa*,have high quantity of tannins,cardiac glycosides,saponins,flavonoid,alkaloids,steroids,glycosideand anthraquinone which amount to theirpotency in antimicrobial activity, the bioactive componentactby inhibitionof nucleicacid,protein,cellwallandmembranephospholipidsbiosynthesis. Causal organisms found associated with leaf wilt, curl and stem rot of sesame in minna farmers' field are detected by morphological and molecularly characterization to be *Fusarium oxysporum*' and *Rhizoctonia solani*respectively, this is in agreement with the work of Dong-hua*et al.*(2012) who isolated twenty-five isolates of Fusarium species from wilted sesame (*Sesamum indicum* L.) grown on 25 farms from 22 regions of China whileYang and Li, (2012) reported the isolation of *Rhizoctonia solani* from soil, forest plant and sesame of china provinces.Morphological characteristics of *F. oxysporum* isolated included white mycelium, presence of ovoid microconidia and canoe shaped macroconidia, while that of *R. solani* are pale creamy color and septate hyphae being at right angle to each other, this conforms with the work of Hafizi*et al.*(2013) who revealed that oil palm plant has crown disease infecting their early stage, and its causative organism was *F. oxysporum*, morphological characteristics showedwhite to white-violet, slightly curved and thick macroconidia while Moni *et al.*(2016) and Lal, and Kandhari (2009) stated that *R. solani* have cream or faint brown mycelial; characteristically having hyphal branching at right angle and constriction at the point of branching of the mycelium.

Pathogenicitytest confirmed *F. oxysporum* and *R.solani* to be the causative agent of wilt, curled and stem rot of sesame respecticely, this showed that the disease cycle obeys Koch's postulates,this is in agreement with the work of Ara *et al.*(2017), whose histopathological studies conducted on sesame plants after artificial inoculation with *F. oxysporum* showed symptoms such as chlorotic and wilted leaves which appeared 15–20 days after inoculation.

Authenticationof the isolated organism using molecular tools such as PCR and ITS primers further confirmed the organism to be *F. oxysporum* and *R. solani*. Nucleotide sequences revealed that this organism have similar sequence aligned with results from a query BLAST database. This studies corroborate with the work ofAlbores,*et al.*(2014) who worked on *Fusarium* spp. obtained from gladiolus corms andmolecularly characterized it using polymerase chain reaction and internal transcribed spacer (PCR-ITS) and reported that the sequence of *F. oxysporum* f. sp *gladioli* and isolates T30, T35 and T39 showed a similarity index of 99% with *F. oxysporum*from other regionwhile Moni *et al.*(2016), analysis R. solani isolated from shealth blight diseases of rice using variable numberof tandemrepeat and amplified fragment length polymorphism markers showed that the different isolates within the same local geographic regions of Bangladeshsuggest the presence of different races of *R. solani* including AG- 3 found in this investigation.

In vitro analysis, revealed that *Murraya koenigii* has the best inhibitory potential at both tested concentrations(50 and 100 g/l) followed by *Azadirachta indica* and *Allium cepa*. This may be attributed to the synergetic effect of the bioactive phytochemicals present in the plants used. Tanins, phenols and alkaloidshad been proved to be active compounds against pathogenic microorganisms (Cowan, 1999). This agrees with the work of Gabriel and Vincent (2014); who reported that ethanol aqueous extracts (EE) of *Murraya koengii* had high activity against *Candida albicans*, *Penicillium funiculosum*, *Penicillium camemberti, and Aspergillus niger* at 20 g/ml.Concentrations of the extracts has great impacts on both *Fusarium oxysporum* and *Rhizoctonia* solani, 100 g/l has best inhibitory effects on the isolated pathogens than 50 g/l, which means the higher the concentration the better the inhibitory effect on the organisms, this is

in line with the work of Danish and Robab (2015), whodescribed *M. koenigii* as an effective antifungal against *A. niger* in increasing concentrations of 10% 15% and 20% while Mishra, *et al.*(2010) reported that Shade dried leaves of *Murraya koenigii* extracted using five different solvent is effective against *A.niger, Alternqria solani, Helminthosporium solani,* and *Penicillium notatum.*

However, *Allium cepa* used in this study, have less potential on mycelial radial reduction of the organisms when compared with other extracts this is in line with the work of Shams-Ghahfarokhi *et al.* (2006) that used agar dilution assay to analyze antifungal activity of aqueous extracts from *Allium cepa* and *Allium sativum* against 25 strains of *Malassezia furfur*, and18 strains of *Candida albicans.* Their results showed that the extracts were able to inhibit the growth of all fungi tested in a dose-dependent manner with maximum of 100% inhibition exhibited by *Allium cepa.*

Conclusion

The methanolic extracts of three medicinal plants (*Murray koenigii, Allium cepa* and *Azadirachta indica*) tested in this study showed that the plants have strong potency to curb the menace of leaf curling, leaf wilting, yellowing caused by *Fusarium oxysporum* and stem rot caused by *Rhizoctonia solani*. *Murraya koenigii* and *Azadirachta indica* showed maximum inhibitory potentials against the tested fungi and this may be due to the presence of certain bioactive component present in them. The three plant extracts showed inhibitory effect against the two tested pathogens and thereby preventing the growth of the organisms especially at higher doses. Hence *M. koenigii, A. indica* and *A. cepa* can therefore, serve as alternatives to chemicals. Further studies should be geared toward extraction and purification of the active phyto-constituents of the plants in order to ascertain the most effective.

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