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Fungicidal Effect of *Azadiracta Indica* and *Zingiber Officinale* Extracts in the Control of *Fusarium Oxysporum* and *Rhizoctonia Solani* on Tomato (*Solanum Lycopersicum*) Fruits

Sani S and Gwa V I*

Department of Crop Production and Protection, Faculty of Agriculture and Agricultural Technology, Federal University, Dutsin-Ma, PMB 5001, Katsina State, Nigeria

***Corresponding Author:** Gwa V I, Department of Crop Production and Protection, Faculty of Agriculture and Agricultural Technology, Federal University, Dutsin-Ma, PMB 5001, Katsina State, Nigeria.

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Abstract

Studies were carried out on the in-vitro evaluation of plant extracts in the control of some fungal pathogens from tomatoes (*Solanum lycopersicum* L) fruits collected from Darawa, Dutsin-Ma and Makera settlements in Dutsin-Ma Local Government Area of Katsina State, Nigeria using neem (*Azadirachta indica*) and ginger (*Zingiber officinale*) extracts. Rotted tomato fruits were collected from same locations and taken to Biological science laboratory for isolation, identification and subsequently pathogenicity test. Fusarum oxysporum, *A. niger, Rhizoctonia solani, F. moniliforme* and *A. flavus* were isolated from the rotten tomato fruits. Pathogenicity tests carried out confirmed that all the isolates were pathogenic on the tomato fruits. The most virulent pathogens (*R. solani* and F. oxysporum) were controlled, using the two plant extracts at 40g/l, 80g/l and 120g/l levels of concentrations respectively. The result obtained showed that *A. indica* was more effective in inhibiting the mycelial growth of R. solani at 40g/l (44.90%) and at 120g/l (97.22%) compare with *Z. officnale* which reduce the mycelial growth of pathogens to 43.83% and 63.31% at 40g/l and 120g/l respectively. The A. indica also proved to be more potent in controlling *F. oxysporum* at 40g/l, 80g/l and 120g/l with percentage growth inhibition of 37.51%, 40.56% and 45.48% respectively compared with 22.91%, 35.00% and 42.73% of *Z. officinale*. It is therefore, concluded that extracts of A. indica and *Z. officinale* can be used to manage fungal growth of tomato fruits by farmers since they have inhibitory effect on fungal pathogens and is easily available and cheap.

Keywords: *Fungicidal effect; Extract; Azadiracta indica; Zingiber officinale; Fusarium oxysporum; Inhibition*

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Introduction

Tomato (*Solanum lycopersicum*) is grown all over the world (Agrios, 2005). According to Wachira., *et al.* (2014), tomatoes are widely grown and consumed. Tomatoes are the world second most important vegetable crop after potato (Dimphna, 2016). It is estimated that the crop is grown on more than 5 million hectares of land with a production of nearly 129 million tonnes worldwide (Bright, 2012) out of

which Nigeria produces 1.5 million tonnes annually. Although the production of tomatoes is mostly concentrated in the northern part, it is consumed throughout the country (Kuntama., *et al*. 2007). The crop is grown for both fresh domestic uses as well as for export market but there is increasing demand for processed tomato products in Nigeria and beyond. The crop supplies a range of nutrients which makes it a widely accepted vegetable by consumers. It is characteristically rich in calcium, phosphorus, magnesium, copper, niacin, iron, foliate, vitamin A, B6, vitamin E, vitamin B2, vitamin C, iron and carbohydrates (Wamache, 2005).

The quality, quantity and to some reasonable extent, profitability of tomatoes are affected by insect pests and pathogens (Tijjani., *et al*. 2014). Tomatoes are also affected by disease-causing pathogens including bacteria, fungi, viruses and nematodes (Tijjani., *et al*. 2014). These pests and disease pathogens reduce the quality, quantity of the tomato fruits produced the shelf life of the tomato fruits as well as reduce its marketability income of famers (Goufo., *et al*. 2008). Synthetic chemicals have been used to manage pathogens in different crops. The major problems of these synthetic chemicals are that they cause environmental pollution and destabilize the ecosystem. Pesticides of plant origin are the best and most important because unlike the synthetic pesticides they are easily degradable, they are non-toxic to humans and the environment, they are target specific, are easily available and do not have residual effects on produce (Okigbo and Nmeka, 2005). In addition, pesticides of plant origin offer solutions to pest resistance, environmental and water body pollution, public concerns about food safety and improve agricultural productivity (Mishra., *et al*. 2015). It is against this backdrop that the study was carried out to isolate, identify and test the pathogenicity of fungal organisms associated with rot of tomato fruits as well as to inhibit the growth of these fungal pathogens with leaves of *A. indica* and rhizomes of *Z. officinale* extracts in vitro.

Materials and Methods

Description of Study Area

The experiment was conducted in the Biological Science Laboratory, Federal University, Dutsin-Ma, Local Government Area (LGA) in Katsina State, Nigeria (latitude 12° 27' 18'' N and longitude 07° 29' 29'' E) in 2016.

Collections of Tomato Fruits

Diseased samples of tomato fruits showing various rot symptoms were collected from three locations of Dutsin-ma Local Governments Area in Katsina State namely: Darawa, Dutsin-Ma and Makera at forth nightly interval and the samples were packaged in sterile polythene to prevent to prevent further attack by insects and pathogens.

Isolation and identification of pathogens

Diseased samples were washed under running tap water after which the samples were chopped into small pieces of about 2-3 mm in diameter and kept in a sterile Petri dish. The pieces were dipped into 5% hypochlorite solution for about 20 seconds. The pieces of the tomatoes were transferred into Petri dishes containing sterile distilled water and were washed thoroughly in three successive changes of sterile distilled water. About 15 ml of the molten PDA was poured in Petri dishes of 9 cm in diameter and allowed to solidify. After solidification, four pieces of the diseased tomato tissues were aseptically placed at different distance in the Petri dishes. The Petri dishes were tightly covered with a masking tape to prevent contamination by air-borne pathogens. The dishes were incubated at ambient room temperature (33°C-37°C) for 7 days to allow for the growth of fungi.

Preparation of sub-culture

The pathogens that grew on the Petri dishes were sub-cultured after incubation period of 7 days to have a pure culture of the isolates. This was done by transferring the fungi mycelial on agar plates containing the medium by using an inoculation needle to place the mycelial at the centre of the Petri dishes. The Petri dishes were tightly sealed with a masking tape and thereafter incubated for 7 days. When growth was established, growth patterns were determined based on microscopic and morphological characteristics and were

compared with existing authorities (Ahmed and Ravinder, 1993; Burgess., *et al*. 2008). Test fungi in this study were *Rhizoctonia solani* and *Fusarium oxysporum* which were mostly isolated in the locations.

Determination of frequency of occurrence of isolates

Records of the organisms isolated were kept on periodic basis to determine the frequency of occurrence of the isolates. Since isolation and characterization were carried out at fortnightly interval, the number of times each fungal pathogen was isolated at fortnightly interval was expressed as a percentage of the total of all the different fungal organisms isolated over the period (Okigbo and Ikediugwu, 2000), which was calculated as follows:

$$\%\ frequency\ of\ occurence\ = \frac{x}{n} \times \frac{100}{1}$$

Where,

x = number of times an individual isolate has occurred over the period

n = total number of fungal organisms isolated in the study area over the period

Pathogenicity test for isolated fungi

Fresh and healthy-looking tomato fruits were collected from the markets and were surface sterilized by dipping them in 5% sodium hypochlorite for 20 seconds and then rinsed with four successive changes of sterile distilled water. The healthy-looking fruits were then wounded with a sterile needle. Mycelial disc from *R. solani* and *F. oxysporum* were carefully lifted from the pure culture of the respective plates and introduced directly into the wounded tissues of healthy tomato fruits. In another experiment, three tomato fruits were each surface sterilized and wounded with a sterile needle and inoculated separately with sterile distilled water instead of the mycelial of *R. solani* and *F. oxysporum*. This served as the control experiment for both *R. solani* and *F. oxysporum*. The inoculated fruits and the control were placed separately lined with a moist filter paper and cover with aluminium foil and incubated at ambient room temperature (33°C-37°C). The fruits were observed for symptoms of rot development after five days of incubation.

Preparation of plant extracts using leaves of *A. indica* and rhizomes of *Z. officinale*

The methods of Gwa and Akombo, (2016) and Gwa and Nwankiti, (2017) were adopted for this experiment. Accurately, 40g, 80g and 120g of powdered leaves of neem (A. indica) and rhizomes of ginger (Z. officinale) were measured respectively using an electric weighing machine. Sterile water was heated using hot plate to a temperature of 100°C. 1-litre of the sterile distilled water was each measured using a measuring cylinder and was poured into conical flasks containing the neem leaves powder and ginger rhizome powder at different level of concentrations respectively. The mixtures were vigorously stirred and left to settle for 24 hours, after which they were filtered through three layers of muslin cloth. Concentrations of 40 g/L, 80 g/L and 120 g/L of the neem leaves and ginger rhizomes were prepared accordingly. 5 ml each of the prepared plant extracts at the different level of concentrations were used to amend in 15 ml of potato dextrose agar.

Effect of *A. indica* and *Z. officinale* in inhibiting the mycelial growth of *R. solani* and *F. oxysporum in vitro*

The method of Amadioha and Obi (1999) was used to measure the fungitoxicity of the extracts. This method involved direct treatment of potato dextrose agar (PDA) medium with plant extracts before inoculation of fungus. This involved creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The point of intersection indicates the centre of the plates. These were done before dispensing PDA into each of the plates. The prepared medium was poured into sterilized Petri dishes and 5 ml of each plant extracts at different levels of concentration were poured into Petri dishes containing the media separately, mixed well and allowed to solidify, the solidified medium was inoculated centrally at the point of intersection of the two perpendicular lines drawn at the bottom of the plate with discs (5 mm diameter) which was obtained from one-week old cultures of *R. solani* and *F. oxysporum*. Three

dishes were plated with extract of each plant at different concentrations. The control experiment had 5 ml of sterile distilled water added to the PDA plates in place of plant extracts respectively, the treatment and control plates were replicated three times and were incubated for 4 days at ambient room temperature (33°C-37°C) and measurement of mycelial radial growth as radius of growing fungal colony were undertaken at intervals of 1 day for 4 days using a transparent ruler. The absence of growth in any of the plates was indication of the potency of the extract against the test fungal. Fungitoxicity was determined as percentage growth inhibition (PGI) according to the method described by Gwa and Akombo, (2016).

$$PGI = \frac{(R-R1)}{R} \times 100$$

Where PGI = Percentage growth inhibition

R = Distance of fungal growth from the point of inoculation to the colony margin in the control plate

R_1 = Distance of fungal growth from the point of inoculation to the colony margin in treated plate.

Experimental design and data analysis

Data collected were subjected to Analysis of variance (ANOVA) using GenStat Discovery Edition 12 for ANOVA and means separation, Minitab Release 17 for descriptive statistics and Graph Pad Prism 6 for trend graphs. Statistical F-tests were evaluated at $P \leq 0.05$. Differences among treatment means for each measured parameter were separated using Fisher's least significant difference (FLSD) (Cochran and Cox, 1992).

Results

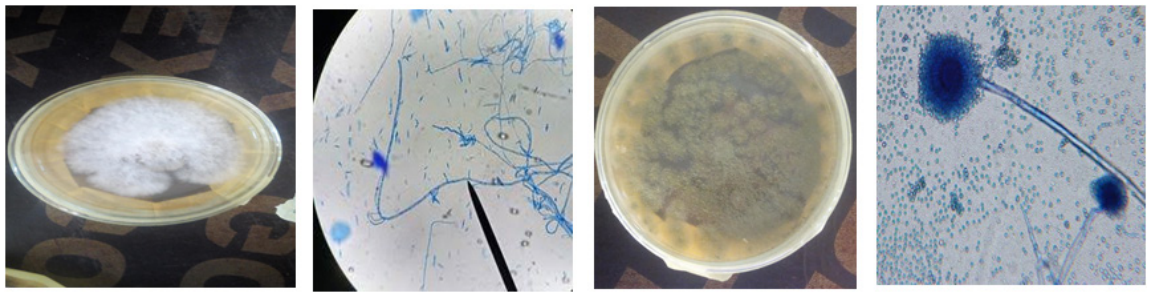
Isolation, identification and pathogenicity tests

Results presented in Plates I shows some cultures and photomicrographs of the fungi pathogens isolated from tomato fruits. Results presented in Figure 1 show the frequency of the fungi organisms that were isolated and identified in the different locations. *F. oxysporium, F. monilliforme, A. niger, A. flavus* and *R. solani* were identified. Among the pathogenic fungi, *F. oxysporum* was the most frequently occurring fungus constituting 67% in Darawa, 51.36% in Dutsin-ma and 33.30% in Makera. *A. niger* was the second most encountered pathogen constituting 4.17% in Darawa, 21.01% in Dutsin-ma, and 31.85% in Makera. The percentage frequency of occurrence of *A. flavus* was 13.69%, 4.17%, and 28.10% in Darawa, Dutsin-ma and Makera regions, respectively. *R. solani* recorded percentage frequency of 9.52% in Darawa, 12.94% in Dutsin-ma, and 2.22% in Makera. The least occurred isolate was *F. monilliforme* with percentage frequency of occurrence of 4.17% in Darawa, 10.50% in Dutsin-ma and 4.44 % in Makera. Fungi pathogenic organisms were identified based on their morphological growth patterns as well as microscopic characteristics and were compared with existing authorities

Table 1 show that there was no significant difference in the occurrence of the isolated pathogens in all the locations. However, there was a significant difference in the total number of fungi pathogens isolated in all the study locations with the highest number of fungi pathogens in Dutsin-ma (12.33) followed by Makera (9.67) and least number of fungi organisms occurred in Darawa (5.67).

Pathogenicity Test

The study revealed that the fungi organisms isolated from the infected tomato fruits were pathogenic on the healthy tomato fruits. A. niger was more virulent where the inoculated fruits were completely rotten at the end of the fifth day of incubation. The fruits were completely disintegrated with extensive mycelial growth forming a dark colour covering the fruit skin. Fruits inoculated with *F. oxysporum* had water-soaked lesions with some white to pink mycelial while fruits inoculated with *A. flavus* had whitish cheesy like lesions. Samples inoculated with *F. monilliforme* had small water soaked lesion with slightly brownish appearance on the inoculated areas while tomato fruits inoculated with *R. solani* had small hard dark lesion around the inoculated area. The fruits that were not inoculated with any fungi pathogen however, showed no signs and symptoms of rot.



A B C D

Plate 1: *Culture of F. oxysporum (A), Photomicrogragh of F. oxysporum (B), culture of A. flavus (C) and photomicrograph of F. oxysporum (D) showing conidia on conidiophores (x10).*

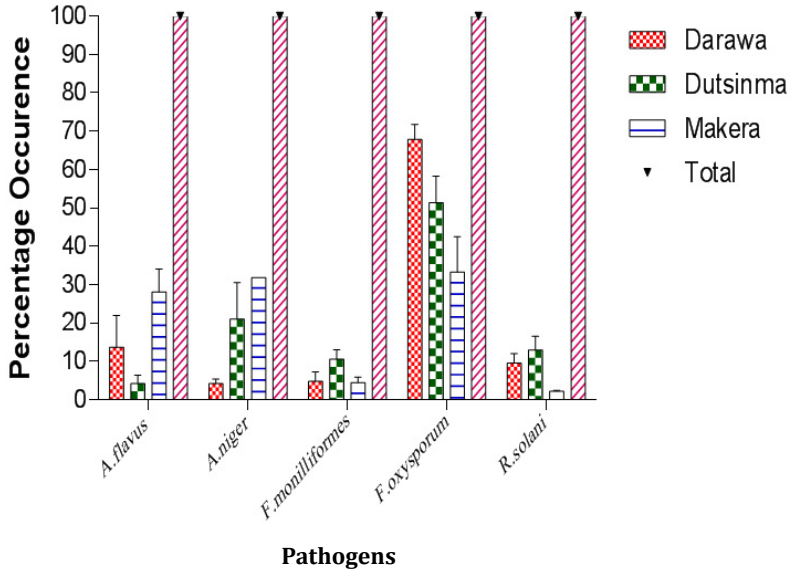


Figure 1: *Percentage Frequency of Occurrence of Fungal Pathogens at Different Locations.*

Pathogens	Locations			P-Value
	Darawa	Dutsinma	Makera	
A.flavus	1.00 ± 0.57	0.33 ± 0.03	2.00 ± 0.57	0.14^{ns}
A.niger	0.33 ± 0.03	2.33 ± 0.88	3.00 ± 1.00	0.12^{ns}
F.monilliformes	0.33 ± 0.03	2.33 ± 0.20	0.66 ± 0.06	0.62^{ns}
F.oxysporum	3.33 ± 1.33	5.66 ± 0.33	3.67 ± 1.86	0.45^{ns}
R.solani	0.66 ± 0.06	2.00 ± 1.53	0.33 ± 0.03	0.48^{ns}
Total	5.67 ± 1.86^{a}	12.33 ± 3.38^{b}	9.67 ± 2.91^{ab}	0.04

Means on the same row with different superscript are statistically significant (P ≤ 0.05); ns = not significant

Table 1: *Number of fungi pathogens isolated from different locations.*

A B C D E

Plate 2: *Tomato fruit inoculated with A. niger (A), A. flavus (B), R. solani (C), F. oxysporum (D) and Control without fungus mycelial (E).*

Efficacy of *A. indica* and *Z. officinale* on Radial Growth inhibition of *R. solani*

The result presented in Table 2 revealed that the plant extracts inhibited the growth of test fungi, although the rate of inhibition varied with different extracts and concentrations used. However, growth inhibition in all the test pathogens took a similar trend in all extracts as the concentration of the tested plant extracts were found to increase with increase in the concentration as incubation period increased. The highest concentration of 120 g/L was the most potent and had the highest inhibition on the pathogens. At concentration of 120 g/L, *Z. officinale* produced lower effect on *R. solani* compared with *A. indica*. The effectiveness of the extracts differed significantly ($P \leq 0.05$) comparing them at 120 g/L on mycelial growth inhibition of R. solani. The effectiveness of neem and ginger extracts did not differ significantly ($P \leq 0.05$) at 40 g/L and 80 g/L. *A. indica* inhibited the mycelial growth of *R. solani* recording percentage growth inhibition of 44.90% compared with *Z. officinale* which inhibited the growth of *R. solani* by 43.38%. Neem crude plant extract was the most effective on R. solan but less effective on *F. oxysporum*. There was however, no significant difference ($P < 0.05$) between the extracts at each level of comparison when they were tested on *F. oxysporum* (Table 3).

Concentration (g/L)	Plant Extract		df	T-Value	P-Value
	A. indica	*Z. officianale*			
40	44.90 ± 11.80	43.38 ± 6.88	17	0.11	0.91
80	41.07 ± 9.27	61.84 ± 6.49	19	1.84	0.08
120	97.22 ± 2.78	63.31 ± 6.79	14	4.62	< 0.01*

*indicates statistical significance at 95% CL

Table 2: *Percentage Growth Inhibition of R. solani at different levels of Concentrations of A. indica and Z. officinale.*

Concentration (g/L)	Plant Extracts and Growth Inhibition (%)		df	T-Value	P-Value
	A. indica	*Z. officianale*			
40	37.51 ± 6.97	22.91 ± 4.92	19	1.71	0.10
80	40.56 ± 6.30	35.00 ± 7.24	21	0.58	0.56
120	45.48 ± 6.79	42.73 ± 6.67	21	0.29	0.77

Table 3: *Percentage Growth Inhibition of F. oxysporum at different levels of Concentrations of A. indica and Z. officinale.*

Measurement of radial mycelial growth of *F. oxysporum* in potato dextrose agar amended with *Z. officinale*

Growth of *F. oxysporum* on potato dextrose agar amended with *Z. officinale* is presented in figure 2. The results indicated that mycelial extension was higher in the control (0 g/L of *Z. officinale*). This study reveals that the radial growth decreases with increase in the concentration of ginger. At 120 g/L the radial mycelial growth was lowest compared to 80 g/L and 40 g/L respectively. Results presented in figure 3 revealed the growth inhibition of *F. oxysporum* throughout the period of incubation. It showed that radial mycelial growth increased with increase in incubation period but decreased with increase in concentration. At 120 g/L the radial mycelial growth was lowest compared to 80 g/L and 40 g/L.

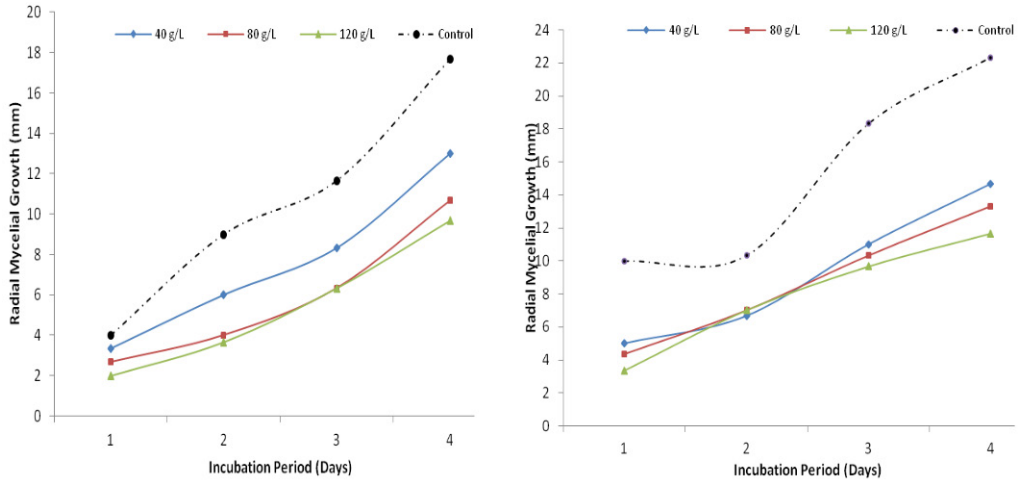


Figure 2: *Radial Mycelia Growth of F. oxysporum on Potato Dextrose Agar amended with Z. officinale (A) and A. indica (B) after 4 days of incubation.*

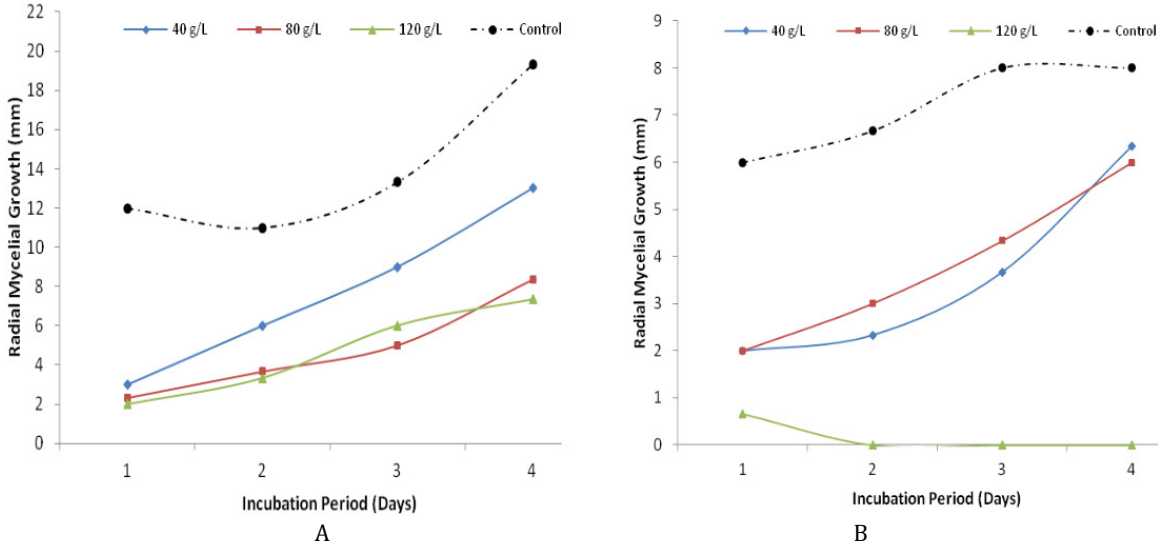


Figure 3: *Radial Mycelial Growth of R. solani on Potato Dextrose Agar amended with Z. officinale (A) and A. indica (B) after four days of incubation.*

The results presented in Figure 3 show the growth inhibition of R. solani on potato dextrose agar amended with Z. officinale. The results revealed that mycelial growth inhibition was lowest in the control plates beginning from first day of incubation at 12 mm and rose to 19 mm on the fourth day. *R. solani* was most inhibited at concentration of 80 g/l and 120 g/l with mycelial growth reduction of 4 mm and 3 mm at first day of incubation and 12 mm and 10 mm at fourth day of incubation respectively. *Figure 5* shows a similar trend of growth inhibition of *R. solani* on potato dextrose agar amended with A. indica extracts at different concentrations. The result indicated that the pathogen grew more in the control plates that were not inoculated with A. indica. Growth was only observed in plates that were inoculated with the pathogen at first day of incubation in 120 g/L of *A. indica*.

Discussion

The isolated fungi pathogens were *F. oxysporum, F. moniliforme, R. solani, A. niger, A. flavus.* The most frequently occurring fungi were *F. oxysporum* followed by A. niger while the least was *F. moniliforme* in all the locations. The result of the pathogenicity test from this study revealed that all tomato fruits showed symptoms of rot on the inoculated healthy tomato fruits while the uninoculated (control) fruits showed no symptoms of rot. However, the rate of rot varied from one tomato fruit to another depending on the pathogen inoculated. These fungi organisms have been previously found to be associated with rotted tomato fruits. Similar results were obtained by Tijjani., *et al.* (2013) who used some plant extracts to inhibit the growth of *Rhizopus stolonifer* on mechanically injured tomato fruits. Results obtained showed that both *A. indica* and *Z. officinale* extracts inhibited the mycelial growth of *F. oxysporum* and *R. solani*. Neem leaf extract was found to be the most effective on *R. solani*. This is like the findings of Mugao, (2015) who reported that neem leaf extracts were more effective in the inhibition of Fusarium spp growth in tomato fruits. This also agreed with the report of Hycenth, (2008) who evaluated the effect of different plant extracts on *R. stolonifer* and found out that all the extracts effectively suppressed mycelial growth of R. stolonifer. According to Meena and Mariappan, (1993), neem leaf extracts inhibited the growth and spore germination of seed microflora including *A. tenuis, A. flavus, C. lunata, F. moniliforme* and *R. stolonifer*. Sharma and Jandaik (1994) reported that different extracts from neem leaves have inhibitory effect on R. solani. Cassava anthracnose caused by *C. gloeosponiodes* was controlled using neem extracts (Fokunang., *et al*. 2000). Hoque., *et al*. (2007) reported that neem contains a compound known as mahmoodin which is active against gram-positive and gram-negative bacteria. According to Suleiman (2010), neem extracts controlled the growth of fungi Alternaria solani causal organism of yam rot.

Stangarlin., *et al*. (2011) revealed that aqueous extract of ginger at different concentrations had effect on the mycelial growth and sclerotia production of Sclerotina sclerotium in vitro. The anti-microbial property of ginger in reducing the mycelial growth of fungal pathogens agreed with the results of this study. The inhibitive effect was proportional to the concentration of the crude extract used: the higher the concentration the higher the inhibitory effect. According to Ijato, (2011) extracts of *Z. officinale* and Ocimum gratissimum were mycotoxic to *F. oxysporum, A. flavus* and *A. niger* that caused post-harvest rot of yam tubers and that the effectiveness of the extracts increased with increase in concentration as was observed in this study. Gwa and Akombo, (2016) used extracts of *P. nigrum, Z. officinale, A. indica, C. papaya and N. tabacum* and inhibited the mecylial growth of *A. flavus in vitro*. The authors found out that the growth of the pathogen decreased as the concentrations of the extracts were increased. Similar results were obtained by Gwa and Nwankiti, (2017) who reduced the growth of Colletotrichum sp isolated from yam tubers with extracts of P. *nigrum, Z. offcinale, A. indica, C. papaya* and *N. tabacum in vitro.*

Conclusion

F. oxysporum, F. moniliforme, R. solani, A. niger, and *A. flavus* are responsible for rot of tomato fruits in Darawa, Dutsin-ma, and Makera. Extracts of *A. indica* and *Z. officinale* origin showed inhibitory activities on both *R. solani* and *F. oxysporum.* However, the concentration of 120 g/L was found to be effective compared to 80 g/L and 40 g/L for both *R. solani* and *F. oxysporum*. It is therefore concluded that extracts of plant origin especially *A. indica* and *Z. officinale* should be considered in managing fungi pathogens of tomato fruits by farmers.

Conflict of Interest Disclosure

The authors declare that there is no conflict of interest regarding the publication of this paper.

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