Assessment of the Protective Potential of Annona senegalensis Leaf Extract against Selected Fungal Pathogens of Sorghum, Tomato and Okra Seeds

Akpor OB[1*], Nwogwugwu JC[1], Adejobi OI[1], Oyebanji AO[1, 2] and Babalola OO[3]

Abstract— The aim of this study was to access the protective potential of Annona senegalensis leaf extract against selected fungal pathogens of selected seedlings (sorghum, tomato and okra). Prior to use, the seeds were surfaced sterilized and viability test carried out. In the study, the effective concentration of the extract, optimum soaking time in the extract and infective concentration of the fungal pathogens were determined. The outcome of the effective concentration of the extract revealed the germination percentage of the sorghum seeds to range from 71% to 93.36%. For the okra seeds the result ranged from 38% to 86%, while for that of the tomato seeds the germination profile was observed to range from 28.5% to 71%. With respect to the optimum soaking time, the end result for the germination of the sorghum seeds was seen to be high at100 % which were recorded in most of the soaking time seen at 30 min, 90 min, 120min, 180 min, 210 min. Lowest value of 78.5 % was seen at 240 min soaking time in the extract. In the case of tomato seeds, the germination result revealed a variation from 28.5 % to 71 %, with the lowest and highest seen at soaking time of 240 min and 30 min respectively. The study was able to reveal the protective potential of the extract of the seeds against the fungal pathogens.

Index Terms— Annona senegalenisi, effective concentration, germination index, infective dose, fungal pathogens, seed protection, vigor index

1 INTRODUCTION

pLANTS play essential roles in human lives. The traditional knowledge of the use of plants accumulated over the years and is passed from ancestors to new generation; this is important to scientist and has brought about discovery of new drugs (Kolawole *et al*., 2013). The systematic screening of different plants species with the aim of discovering new bioactive compound is a daily activity in many industries. (Adebayo, 2001). In recent years, derived substances from plant are indicated to have versatile application (Ncube *et al*., 2008).

Annona senegalensis, commonly known as African custard-apple, wild custard apple and wild soursop, is a species of flowering plant in the custard apple family, Annonaceae. It is a member of the family of plants that is used mostly as ethnomedicinal prescriptions for various ailments, such as diarrhoea, stomach upset, anthelmintic, veneral diseases, veterinary and other commercial uses. The leaves of the plant are used to protect maize, millet and sorghum locally against the likes of weevils. Also in ethno-medicine, the leaves are used as antitrypanosomic and anti drepanocitory (Ngamo *et al*., 2007; Ogbadoyi, 2007).

The majority of investigation on *Annona senegalensis* have been focused on medicinal and phytochemical properties (Ogbadoyi, 2007; Magadula *et al*., 2009; Farid *et al*., 2002; Nebie *et al*., 2005). In part of Northern Cameroon, it is reported that *Annona senegalensis* leaves are used locally to protect maize, millet and sorghum against weevil attacks (Ngamo *et al*., 2007).

Although chemicals appear to be effective, their use is being discouraged due to associated human health and environmental problems such as pest resistance to insecticide, environmental pollution, high cost of purchase, non-availability as well as hazards to farmers (Talukder and Howse, 1995). These drawbacks have necessitated the need for sustainable alternatives that are easily biodegradable, environmentally friendly and safe to both producers and consumers (Ewete *et al*., 1996; Akob and Ewete, 2007). The challenge of finding a good alternative to replace these conventional fungicides has led to bioprospecting for plants with natural fungicidal potency.

The purpose of this study was to assess the protective effect of the ethanolic leaf extract of *Annona senegalensis* against selected fungal pathogens of sorghum, tomato and okra.
2 Procedure for Paper Submission

2 MATERIALS AND METHODS

The plant extract used for the study was *Annona senegalensis* leaf extract extracted in 95 % ethanol. The leaves of the plant were obtained from the environment of Landmark University, Omu-Aran, Kwara State.

The *Annona senegalensis* leaves were collected in clean plastic bags, transported to the laboratory and washed with clean tap water to remove sand and other debris. The washed leaves were sun-dried for one week before pulverising, using a laboratory grinder. After pulverisation, they were then extracted by soaking in the ethanol (1:2 w/v) for 24 h in a beaker before filtering the extract in a beaker, using Whatman No 1 filter paper.

The ethanol was separated from the extract in a rotary evaporator before concentrating using a freeze drier. The dried extract was kept as stock in glass bottles at room temperature till needed.

2.1 Test seeds and viability testing

Three different seeds (okra, sorghum and tomato) were used for the study. The seeds were purchased from a local agro-

stores located within Omu-Aran community in Kwara State, Nigeria. Before use, the purchased seeds were first surfaced sterilised using 5 % sodium hypochlorite (v/v). Surface sterilisation was carried out by soaking the seeds to be used in the sodium hypochlorite for 5 min after which they were washed several times to remove the sodium hypochlorite.

For viability testing, the surface-sterilised seeds were soaked in sterile distilled water for 30 min after which the 7 seeds each were planted in transparent plastic plates (6 cm diameter) containing 2.7 g of absorbent cotton wool (to serve as blotter) that 30 ml of distilled water have been added. The planted seeds were then observed daily for germination for 7 days. Seeds were confirmed to be viable if over 80 % germination was obtained. All seeds used for the study were ascertained to be viable before usage.

2.2 Determination of effective concentration

To determine the effective concentration of the extract that will enhance germination and other growth parameters of the seeds, 10 different concentrations of the extract (1000 mg/L to 10,000 mg/L) were prepared.

After surface-sterilising the seeds, a total of 20 respective seeds were soaked in 10 ml of the respective concentrations in universal bottles for 30 min. At the expiration of the soaking period, 5 seeds each were planted in duplicate in the plastic plates containing the cotton wool that 30 ml of water has been added.

On a daily basis, for seven day duration, the number of germinated seeds and number of germinated seeds that showed leave emergence were recorded. At the end of the seven days, the shoot and root lengths of the seedlings were measured. Also measured or calculated were the wet weights of the seeds, germination index and seed vigor index.

2.3 Determination of optimum soaking time

This was determined by first surface-sterilising the seeds and soaking at a known concentration of the extract. Every 30 min, for 240 min, approximately 10 respective seeds were withdrawn and planted as described previously.

On a daily basis, for seven day duration, the number of germinated seeds and number of germinated seeds that showed leave emergence were recorded. At the end of the seven days, the shoot and root lengths of the seedlings were measured. Also measured or calculated were the wet weights of the seeds, germination index and seed vigor index.

2.4 Determination of infective dose of the test pathogens

Three fungal pathogens were used for the study. The pathogens were *Aspergillus niger, Aspergillus fumigatus and Aspergillus flavus.* Before use, the test fungal were first cultured on saboraud dextrose agar plates to ascertain their purity, after which they were sub-cultured in sabourand dextrose broth and allowed grow at 25^{o}C ± 2^{o}C for 72 h.

In determining the infective dose of each isolate, different dilutions of the broth cultures of the organisms with known inoculums population were prepared. The surface sterilised seeds were then soaked in the respective dilutions of the isolates for 30 min before planting. Daily records of the germination and leaf emergence were taken till the end of the seven days planting period.

Minimum dilution of the organism that inhibited germination and leaf emergence of each seed was indicated as the infective concentration of that isolate for the particular seedling.

2.5 Determination of the infective effect of the extract

To determine this, 5000mg/L concentration of the extract was used for treatment. After surface sterilisation, the respective seeds were soaked for 30 min in different treatments: water only, extract only, different broth cultures of the pathogens, equivalent mixture of both the broth culture and extract, and broth cultures of the pathogens and then the extract. At the expiration of soaking, the seeds were planted as described previously. The planted seeds were checked daily for seven days for germination and leaf emergence. At the end of the seven days planting period, wet weight, germination index and seed vigor index were estimated.

2.6 Estimation of germination and seed vigor index

Germination index was calculated by counting the nnumber of seedlings emerging daily from day of planting the seeds till the time germination is complete. Thereafter germination index (G.I.) was computed by using the formula G.I = n/d

Where, n = number of seedlings emerging on day'd'

d = day after planting

The seed lot having greater germination index is considered to be more vigorous.

- *Akpor OB is currently a Senior Lecturer in the Department of Biological Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria. E-mail: akpor.oghenerobor@lmu.edu.ng*
- *Nwogwugwu JC is a final student in the Department of Biological Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria.*
- *Adejobi OI is a Lecturer in the Department of Biological Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria*
- *Oyebanji AO is a Researcher in the Operations Research Department, Nigerian Stored Products Research Institute, Ilorin, Kwara State, Nigeria*
- *Babalola OO is a Principal Lecturer in the Department of Science Technology, Federal Polytechnic, Ado-Ekiti, Nigeria*

Vigor index was calculated by determining the germination percentage and seedling length of the same seed lot. Seed vigor index was calculated by multiplying the % germination at the end of planting with the summation of the root length (RL) and the shoot length (SL).

Seed vigor index = (RL + SL) x % germination

3 RESULTS

3.1 Effect of extract concentration

As shown in Table 1, germination profile of the sorghum seeds at the expiration of the planting period was observed to range from 71 % to 93.36 %. The highest germination was observed at extract concentration of 3000 mg/L. With respect to leaf emergence in the germinated seeds, the result revealed a range from 43 % to 93 %, with the lowest and highest observed at concentrations of 1000 mg/L and 7000 mg/L, respectively. Generally, germination and seed vigor indices were observed to range from 86.28 to 144.29 and from 1396.13 to 3036.07, respectively. The lowest and highest germination index were observed at extract concentrations of 3000 mg/L and 4000 mg/L respectively while the lowest and highest vigor index were observed at extract concentrations of 1000 mg/L and 5000 mg/L, respectively (Table 1).

With respect to the tomato seeds, at the conclusion of the period of planting, germination was observed to range from 38 % to 86 % with the highest and lowest seen at extract concentrations of 3000 mg/L and 8000 mg/L, respectively. In case of leaf emergence, highest value of 71 % was recorded at 3000 mg/L while a lowest value of 24 % was observed at 8000 mg/L extract concentration. For the seedling height and wet weight, the result ranged from 8.5 mm to 14.58 mm and from 6.21 mg to 8.71 mg, respectively. The lowest and highest seedling heights were revealed at extract concentration of 5000 mg/L and 7000 mg/L, respectively while the lowest and highest wet weights were observed at concentrations of 1000 mg/L and 9000 mg/L, respectively (Table 1).

Germination of the okra seeds at the termination of the planting showed variation from 28.5 % to 71 %. The lowest germination was revealed at extract concentration of 7000 mg/L while the highest was concentration of 2000 mg/L. In the case of leaf emergence by the germinated seeds, the outcome showed a range from 7 % to 43 %, with highest and lowest observed at concentrations of 1000 mg/L and 7000 mg/L, respectively. The result of the height of the seeds showed a range from 17 mm to 39.5 mm. The highest and lowest wet weights were observed at extract concentrations of 1000 mg/L and 4000 mg/L, respectively. In general, the lowest and highest germination index were seen at extract concentrations of 2000 mg/L and 7000 mg/L, respectively while the lowest and highest vigor index were observed at extract concentrations of 7000 mg/L and 10,000 mg/L, respectively (Table 1).

3.2 Effect of soaking time

As shown in Table 2, at the different soaking time in the extract, 100 % germination (at 30 min, 90 min, 120 min, 180 min and 210 min) was recorded at the end of the planting period except at 150 min and 240 min when 86 % and 78.5 % germination were recorded, respectively. In the case of the plant height and wet weight, the results observed ranged from 18.59 mm to 41.15 mm and from 28.22 mg to 48.35 mg, respectively. Generally, germination and seed vigor indices showed variation from 133.23 to 153.45 and from 1459.70 to 4115, respectively (Table 2).

In the case of the tomato seeds, at the different soaking times in the extract, germination at the expiration of the planting period revealed a variation from 28.5 % to 71 %, with lowest and highest observed at soaking times of 240 min and 30 min, respectively. Also the % leaf emergence of the germinated seeds ranged from 14 % to 71.5 %, with the highest and lowest values recorded after 120 min and 240 min, respectively. In the case of plant height, a lowest value of 9.33 mm and a highest value 29.58 mm were observed at 240 min and 90 min, respectively. With respect to the wet weight, the highest and lowest values of 3.65 mg and 2.11 mg were observed at 210 min and 240 min, respectively. For the germination and seed vigor indices, values were observed to range from 24 to 49.26 and from 285.19 to 2099.83, respectively (Table 2).

For the okra seeds, at the end of the seven days planting period, germination and leaf emergence profile at the different soaking times in the extract ranged from 57 % to 68.5 % and from 7 % to 57 %, respectively. In the case of plant height and wet weight, the outcome ranged from 38.15 mm to 114.36 mm and from 550.90 mg to 2896 mg, respectively. Germination index of the okra seed showed the highest of 114.36 after 30 min while the highest vigor index of 2896.65 was also observed at 30 min (Table 2).

3.3 Effect of inoculum size

When infected with the *Aspergillus flavus*, germination was observed to range from 57 % to 100 %, from 29 % to 100 % and from 29 % to 71 %, for the sorghum, tomato and okra, respectively. A lowest vigor index of 1333, 377 and 203 was observed when cells were infected with 1.33 X 104 propagules/ml, 1.16 X 104 propagules/ml and 1.16 X 104 propagules/ml, for the sorghum, tomato and okra seeds, respectively (Table 3).

For the sorghum seeds in the presence of the *Aspergillus fumigatus*, germination index was observed to range from 113.09 to 159.28 with highest value observed when infected with 3.33 $x10^3$propagules/mL and lowest value observed when infected with 2.67 $x10^3$propagules/ml. Also vigor index of the sorghum seeds ranged from 1161 to 8333 with lowest and highest observed when infected with 1.33 $x10^3$ propagules/ml 6.67 $x10^2$ propagules/ml (Table 4). In the case of tomato, the seed vigor index result ranged from 140.65 to 1221.20, with highest and lowest observed in seeds soaked in water and those infected with 4.00 x 10^3propagules/mL, respectively. Generally, in the presence of okra, germination index was observed to range from 315.19 to 1204 in when infected with 6.67 x 10^3 propagules/ml and 4.00 $x10^3$propagules/ml, respectively (Table 4).

As indicated in (Table 5), in the presence of the *Aspergillus niger*, % germination of the sorghum seed was observed to vary from 14 % to 100 %, with the lowest observed when infected with 1.44 X 10^3 and 1.10 X 10^3 propagules/ml. A lowest seed vigor index of 238 was observed when cells were infected with 1.10 X 10^3 propagules/ml (Table 5).

In the case of the tomato seeds, germination profile when infected with the *Aspergillus niger* was observed to range from 14 % to 100 %, with highest and lowest values recorded when infected with 4.7 X

10^3 propagules/ml and 7.18 X 10^3 propagules/ml, respectively. For the okra seeds, germination profile showed a variation from 14 % to 71 %, with the highest and lowest germination observed at 4.78 x 10^3 propagules/ml and 1.44 x 10^4 propagules/ml, respectively (Table 5).

Table 1: Effect of extract concentration on germination and other growth parameter of the seeds

Conc. (mg/L)	Germination (%)	Emergence (%)	Plant height (mm)	Wet weight (mg)	Germination index	Seed vigor index
Sorghum						
0	71 (±0)	57 (±0)	24.81 (±2.82)	35.14 (±0.19)	101.73 (±2.83)	1761.15 (±45.68)
1000	86 (±0)	43 (±19.79)	28.40 (±2.38)	41.35 (±2.73)	129.19 (±32.66)	2441.97 (±204.93)
2000	78.5 (±10.61)	57.5 (±40.31)	26.72 (±10.20)	37.78 (±5.76)	102.26 (±12.49)	2115.01 (±548.12)
3000	93.86 (±9.89)	71 (±0)	27.25 (±5.30)	42.28 (±5.46)	86.28 (±46.96)	2560.50 (±762.96)
4000	71 (±0)	50 (±9.89)	35.55 (±10.54)	42.71 (±0.41)	144.29 (±93.53)	2524.05 (±748.04)
5000	93 (±9.89)	57.5 (±40.31)	35.5 (±50.20)	40.32 (±1.61)	138.55 (±12.82)	3036.07 (±934.89)
6000	93 (±9.89)	78.5 (±30.41)	28.5 (±20.50)	37.71 (±1.41)	93.79 (±13.97)	1645.57 (±347.28)
7000	93 (±9.89)	78.5 (±10.61	20.21 (±4.35)	41.35 (±6.97)	122.35 (±35.72)	1900.59 (±604.44)
8000	93 (±9.89)	93 (±9.89)	26.67 (±8.96)	43.5 (±4.95)	102.08 (±9.21)	2435.50 (±546.22)
9000	85.5 (±20.51)	78.5 (±30.41)	19.83 (±3.08)	35.57 (±6.26)	95.12 (±38.69)	1664.95 (±155.63)
10000	78.5 (±0)	64 (±9.89)	17.91 (±1.77)	35.21 (±1.12)	104.86 (±6.54)	1396.13 (±50.58)
Tomato						
0	43 (±19.79)	21.5 (±10.61)	27.88 (±4.07)	113.93 (±3.74)	47.57 (±12.55)	1238.87 (±726.72)
1000	57 (±0)	43 (±0)	31.75 (±3.54)	122.07 (±16.87)	51.39 (±5.04)	1809.75 (±120.91)
2000	71 (±0)	43 (±19.79)	36.75 (±14.76)	104.78 (±3.34)	75.75 (±2.59)	2880.11 (±1047.77)
3000	35.5 (±30.41)	28.5 (±20.51)	39.5 (±2.12)	116.99 (±29.09)	43.46 (±29.92)	1413 (±1226.12)
4000	43 (±19.79)	35.5 (±30.41)	29.33 (±17.44)	99.14 (±6.67)	44.27 (±30.16)	1433.81 (±1330.50)
5000	64 (±9.89)	29 (±0)	32.52 (±8.04)	115.49 (±27.98)	73.33 (±12.32)	2053.06 (±38.79)
6000	64.5 (±50.20)	43 (±60.81)	25.34 (±18.72)	120.73 (±25.63)	61.93 (±56.60)	2097.50 (±2474.16)
7000	28.5 (±20.51)	7 (±9.89)	17 (±17.67)	100.07 (±5.15)	27.66 (±29.05)	303.25 (±155.21)
8000	35.5 (±50.20)	14.5 (±20.50)	12.05 (±10.04)	109.43 (±4.04)	37.88 (±53.56)	855.55 (±1209.93)
9000	50 (±50.91)	21.5 (±10.61)	33.25 (±7.42)	97.07 (±22.93)	47.69 (±45.21)	1599.5 (±1569.07)
10000	64 (±9.89)	21.5 (±10.61)	34.17 (±2.59)	101.35 (±6.46)	64.69 (±6.69)	2185.40 (±323.28)
Okra						
0	71 (±0)	43 (±19.79)	13.7 (±8.91)	7.21 (±1.12)	34.43 (±10.41)	982.7 (±618.44)
1000	57 (±19.79)	43 (±19.79)	9.33 (±2.36)	6.21 (±0.29)	31.04 (±23.00)	555.19 (±319.34)
2000	57 (±19.79)	57.5 (±40.31)	11.75 (±0.59)	6.79 (±0.89)	44.95 (±3.09)	675.28 (±265.99)
3000	86 (±0)	71 (±0)	14.08 (±2.47)	7.93 (±1.11)	41.01 (±2.47)	1210.88 (±212.83)
4000	64	50	14.25	7.14	28.15	882.25

	(±9.89)	(±9.89)	(±6.01)	(±0.81)	(±1.90)	(±243.59)
5000	64 (±9.89)	35.5 (±50.20)	8.5 (±3.89)	7.28 (±1.61)	24.66 (±17.23)	732.13 (±370.35)
6000	64 (±9.89)	28.5 (±20.51)	11.12 (±4.07)	7.57 (±2.02)	27.88 (±10.76)	838.29 (±10.03)
7000	64.5 (±30.41)	43 (±0)	14.58 (±6.72)	7.29 (±0.40)	25.46 (±4.04)	468.13 (±251.91)
8000	38 (±12.73)	29 (±0)	11.88 (±2.65)	6.64 (±0.30)	17.37 (±1.27)	583.4 (±142.27)
9000	64 (±9.89)	35.5 (±30.41)	9.4 (±3.67)	8.71 (±0)	30.43 (±1.85)	863.38 (±676.88)
10000	78.5 (±30.41)	57.5 (±40.31)	10.09 (±4.72)	7.29 (±0.40)	41.4 (±19.79)	982.7 (±618.44)

All values are averages of duplicate samples. Values in parenthesis represent ± standard deviation. Emergence column indicate % leaf emergence of the growing seedling after the seven days planting period. Conc. represent extract concentration (mg/L)

3.4 Protective potential of the extract

In assessing the protective potential of the extract on the sorghum seeds against the fungal pathogens, the results revealed vigor index values of 2332.50, 2616.50, 1803.83, 1625.65 and 1561.37 in seeds soaked in water, extract, infected with *Aspergillus flavus*, infected with *Aspergillus fumigatus* and infected with *Aspergillus niger*, respectively. Vigor index values for seeds that were first infected with *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger*, before treatment in the extract were observed to be 3510.50, 1776 and 3945, respectively. Generally, germination profile of the sorghum seeds showed a variation from 78.50 % to 100 % (Table 6).

In the case of the okra seeds, germination at the expiration of the planting period was observed to range from 35.5 % to 71 %, among the different treatments. The vigor index values for the respective treatments were 612.40 (for water only), 727.35 (for extract only), 834.35 (for seeds infected with *Aspergillus flavus* only), 312.25 (for seeds infected with *Aspergillus fumigatus* only), 453.44 (for seeds infected with *Aspergillus niger* only), 1340.12 (for seeds infected with *Aspergillus flavus* before treatment with extract), 671.19 (for seeds infected with *Aspergillus fumigatus* before treatment with extract) and 658.75 (for seeds infected with *Aspergillus niger* before treatment with extract). All the untreated infected seeds showed negligible or no leaf emergence throughout the planting period (Table 6).

As shown in Table 6, germination profile of the tomato seeds at the end of germination was observed to range from 35.50 % to 71 %, with the lowest value observed in untreated seeds infected with *Aspergillus niger*. The seed vigor values for the water and extract only treatments were observed to be 111.36 and 480.62, respectively. For the untreated infected seeds, vigor index values were observed to be 500. 22, 739.96 and 359.24, for seeds infected with *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger*, respectively. In the case of the infected and then treated seeds, vigor values were found to be 788.10 (for *Aspergillus flavus*), 830.25 (for *Aspergillus fumigatus*) and 697.12 (for *Aspergillus niger*).

Table 2: Effect of soaking time on germination and other growth parameters of the seeds

Time (min)	Germination (%)	Emergence (%)	Plant height (mm)	Wet weight (mg)	Germination index	Seed vigor index
Sorghum						
30	100 (±0)	86 (±0)	20.03 (±0.89)	36.64 (±14.24)	149.69 (±13.56)	2003 (±66.46)
60	93 (±9.89)	78.5 (±10.61)	23.79 (±10.18)	48.35 (±7.17)	138.55 (±12.82)	2212.93 (±100.87)
90	100 (±0)	93 (±9.89)	33.21 (±2.72)	41.71 (±3.83)	153.45 (±8.25)	3320.50 (±0)
120	100 (±0)	100 (±0)	27.64 (±0.47)	40.93 (±1.11)	147.19 (±17.09)	2764 (±0)
150	86 (±0)	86 (±0)	25.01 (±1.73)	37.79 (±5.96)	117.65 (±6.84)	2150.43 (±0)
180	100 (±0)	78.5 (±10.61)	41.15 (±2.72)	36.71 (±0.19)	133.23 (±5.30)	4115 (±0)
210	100 (±0)	71 (±0)	22.75 (±1.06)	40.36 (±3.13)	145.63 (±9.40)	2275 (±0)
240	78.5 (±10.61)	43 (±0)	18.59 (±1.79)	28.28 (±1.41)	118.79 (±8.05)	1459.70 (±18.97)
Tomato						
30	71 (±0)	64 (±9.89)	13.2 (±0.50)	3.22 (±0.50)	49.26 (±3.30)	937.2 (±682.78)
60	57 (±19.78)	57 (±19.79)	19.26 (±3.58)	3.29 (±0.40)	33.01 (±10.76)	1054.99 (±174.81)
90	71 (±0)	64 (±9.89)	29.58 (±7.32)	3.22 (±1.11)	29.19 (±12.86)	2099.83 (±519.62)
120	85.5 (±20.51)	71.5 (±20.51)	14.9 (±4.95)	2.79 (±0.30)	43.77 (±0.49)	1223.2 (±117.66)
150	85.5 (±20.51)	71.5 (±20.51)	19.25 (±8.84)	3.43 (±1.22)	41.07 (±12.06)	1555.25 (±360.98)
180	43 (±0)	43 (±0)	14.17 (±4.01)	3.22 (±0.50)	24 (±1.97)	609.09 (±172.39)
210	50 (±29.69)	50 (±29.69)	16.05 (±3.61)	3.65 (±1.72)	32.59 (±20.25)	856.05 (±656.97)
240	28.5 (±20.51)	14 (±0)	9.33 (±1.88)	2.11 (±0.56)	37.69 (±2.97)	285.19 (±244.93)
Okra						
30	78.5 (±30.41)	50 (±29.69)	36.9 (±2.89)	104.07 (±13.23)	114.36 (±43.03)	2896.65 (±88.15)
60	64 (±9.89)	21.5 (±10.61)	23.12 (±9.25)	100.07 (±26.77)	86.94 (±4.45)	1479.68 (±91.56)
90	43 (±0)	21.5 (±10.61)	39.17 (±6.84)	99.14 (±8.68)	54.76 (±5.98)	1684.09 (±0)
120	57 (±19.79)	14 (±0)	20.16 (±3.54)	68.99 (±2.23)	42.41 (±16.09)	1149.12 (±70)
150	78.5 (±30.41)	36 (±9.89)	27.3 (±8.87)	78.21 (±13.02)	99.23 (±57.83)	2143.05 (±269.61)
180	64 (±9.89)	7 (±9.89)	22.45 (±4.31)	65.64 (±4.14)	38.15 (±22.92)	1436.80 (±42.7)
210	28.5 (±20.51)	14.5 (±20.51)	19.33 (±9.43)	69.57 (±2.83)	38.15 (±22.41)	550.90 (±193.43)
240	78.5 (±30.41)	14.5 (±20.51)	19.31 (±0.38)	79.79 (±7.38)	65.67 (±22.92)	1515.83 (±11.61)

All values are averages of duplicate samples. Values in parenthesis represent ± standard deviation. Emergence column indicate % leaf emergence of the growing seedling after the seven day planting period

Table 3: Effect of different inoculum populations of *Aspergillus flavus* on germination and other growth parameters of the seeds

Inoculum size (Propagules/ml)	Germination (%)	Emergence (%)	Wet weight (mg)	Germination index	Seed vigor index
Sorghum					
$1.33x10^4$	86	71	42.42	459.48	1333
$1.16x10^4$	57	57	26.14	144.11	1790.37
$8.83x10^3$	100	43	39.42	136.98	4500
$6.63x10^3$	86	71	22.85	115.48	5130.76
$4.42x10^3$	86	71	36.42	122.48	1404.38
$2.21x10^3$	86	57	41	152.28	3719.50
Soaked in water	86	14	36.42	129.48	1419
Tomato					
$1.33x10^4$	29	43	6.28	21.59	362.50
$1.16x10^4$	29	14	7.57	26.69	377
$8.83x10^3$	86	14	9.57	65.31	761.10
$6.63x10^3$	100	43	8.28	67.56	1500
$4.42x10^3$	86	43	8.28	61.93	737.02
$2.21x10^3$	71	57	8.85	53.92	1249.60
Soaked in water	57	57	5.42	26.35	664.62
Okra					
$1.33x10^4$	57	0	42.42	90.79	783.75
$1.16x10^4$	29	0	26.14	22.30	203
$8.83x10^3$	29	0	39.42	46.19	391.50
$6.63x10^3$	43	14	22.85	61.49	616.19
$4.42x10^3$	43	14	36.42	94.42	573.19
$2.21x10^3$	71	0	41	46.19	667.40
Soaked in water	71	0	36.42	129.48	823.60

All values are averages of duplicate samples.. Emergence column indicate % leaf emergence of the growing seedling after the seven day planting period

Table 4: Effect of different inoculum populations of *Aspergillus fumigatus* on germination and other growth parameters of the seeds

Inoculum size (Propa-gules/ml)	Germination (%)	Emergence (%)	Wet weight (mg)	Germination index	Seed vigor index
Sorghum					
$4.00x10^3$	86	43	31.85	129.48	1511.88
$3.33x10^3$	100	57	11.28	159.28	2066
$2.67x10^3$	71	86	29.57	113.09	981.93
$2.00x10^3$	86	71	32.21	129.48	1161
$1.35x10^3$	100	43	42.42	152.28	8333
$6.67x10^2$	86	43	33.21	129.48	2163.76
Soaked in water	100	29	34.28	147.61	1987
Tomato					
$4.00x10^3$	29	14	6.28	12.77	140.65
$3.33x10^3$	86	29	7.57	26.69	688
$2.67x10^3$	86	57	9.57	50.63	1032
$2.00x10^3$	29	29	9.14	22.02	169.65
$1.35x10^3$	43	29	4.85	37.32	548.25
$6.67x10^2$	57	43	4.85	41.65	769.50
Soaked in water	71	57	8.28	58.59	1221.20
Okra					
$4.00x10^3$	86	43	118.85	125.23	1204
$3.33x10^3$	100	0	92.28	83.79	612.75
$2.67x10^3$	71	57	105.85	117.48	802.38
$2.00x10^3$	86	04	86.57	106.09	667.40
$1.35x10^3$	100	0	102.71	68.49	318.20
$6.67x10^2$	86	0	87.42	61.49	315.19
Soaked in water	100	0	90	46.19	420.50

All values are averages of duplicate samples. Emergence column indicate % leaf emergence of the growing seedling after the seven day

planting period

Table 5: Effect of different inoculum populations of *Aspergillus niger* on germination and other growth parameters of the seeds

Inoculum size (Propagules/ml)	Germination (%)	Emergence (%)	Wet weight (mg)	Germination index	Seed vigor index
Sorghum					
$1.44x10^4$	14	71	25.28	33.69	378
$1.10x10^4$	14	71	26	38.60	238
$9.57x10^3$	86	43	52.14	136.98	1234.10
$7.18x10^3$	86	14	33	136.98	1238.40
$4.78x10^3$	100	86	38.57	159.28	1250
$2.39x10^3$	86	43	41	136.98	1075
Soaked in water	71	57	25.28	33.69	378
Tomato					
$1.44x10^4$	71	0	8.71	41.32	557.35
$1.10x10^4$	57	43	7.57	53.92	1011.75
$9.57x10^3$	86	14	5.14	19.60	1139.50
$7.18x10^3$	14	14	2.85	10.63	154
$4.78x10^3$	100	12.75	57	65.31	1275
$2.39x10^3$	86	29	6.28	19.60	602
Soaked in water	71	29	7.28	36.99	875.43
Okra					
$1.44x10^4$	14	14	108.57	155.78	852
$1.10x10^4$	14	14	102	159.28	710
$9.57x10^3$	86	43	100.85	155.78	716.38
$7.18x10^3$	86	57	97.42	125.03	350
$4.78x10^3$	100	57	107.28	159.28	1260.76
$2.39x10^3$	86	57	86.14	139.14	61.33
Soaked in water	71	29	42.28	129.18	71.50

All values are averages of duplicate samples. Emergence column indicate % leaf emergence of the growing seedling after the seven day planting period

Table 6: Protective potential of the extract on the sorghum seed against the fungal pathogens

Treat-	Germination	Emergence	Plant height	Wet weight	Germination	Seed vigor

ments	(%)	(%)	(mm)	(mg)	index	index
Sorghum						
Water only	100 (±0)	78.5 (±10.60)	23.32 (±3.21)	35.85 (±3.83)	155.78 (±4.94)	2332.50 (±147.03)
Extract only	100 (±0)	86 (±0)	26.16 (±1616)	37.14 (±5.65)	159.28 (±0)	2616.50 (±209.33)
A	85.5 (±20.50)	50 (±29.69)	22.45 (±12.31)	41.5 (±0.70)	129.18 (±32.66)	1803.87 (±1518.59)
B	93 (±9.89)	64 (±9.89)	17.48 (±5.68)	32.57 (±4.85)	139.14 (±3.06)	1625.64 (±355.67)
C	78.50 (±10.60)	64 (±9.89)	19.89 (±11.04)	35.92 (±3.33)	131.71 (±6.92)	1561.37 (±1104.50)
D	100 (±0)	64 (±9.89)	35.10 (±13.35)	40.42 (±5.25)	159.28 (±0)	3510.50 (±1335.72)
E	100 (±0)	64 (±9.89)	17.76 (±4.82)	35.92 (±3.33)	155.78 (±4.94)	1776 (±48.22)
F	100 (±0)	57 (±0)	39.45 (±8.42)	44.42 (±2.82)	125.03 (±16.89)	3945 (±1079.58)
Okra						
Water only	57 (±19.79)	0 (±0)	10.4 (±1.97)	99.56 (±10.10)	76.60 (±10.16)	612.40 (±318.76)
Extract only	50 (±29.60)	14.5 (±20.50)	13.35 (±4.03)	89.42 (±13.73)	62.72 (±50.95)	727.35 (±598.00)
A	71 (±0)	0 (±0)	11.75 (±4.59)	95.71 (±0)	69.77 (±11.05)	834.25 (±326.32)
B	35.5 (±30.40)	0 (±0)	9.25 (±1.06)	0 (±0)	59.28 (±52.31)	312.25 (±243.59)
C	36 (±9.89)	7 (±9.87)	12.58 (±0.11)	83.99 (±11.91)	40.35 (±9.23)	453.44 (±128.60)
D	71 (±0)	43 (±0)	18.87 (±4.41)	41.49 (±48.49)	52.13 (±22.79)	1340.12 (±313.77)
E	36 (±9.89)	21.5 (±10.60)	20.33 (±12.26)	96.28 (±25.25)	39.75 (±1.50)	671.19 (±240.14)
F	57 (±19.79)	0 (±0)	11.25 (±1.76)	85.99 (±7.47)	75.25 (±3.30)	658.75 (±323.50)
Tomato						
Water only	71 (±0)	29 (±0)	16 (±5.37)	8.35 (±1.11)	44.35 (±5.28)	111.36 (±381.55)
Extract only	43 (±19.79)	43 (±0)	11.62 (±1.94)	6.49 (±0.10)	42.37 (±26.77)	480.62 (±146.54)
A	50 (±9.89)	43 (±0)	10.29 (±2.88)	6.35 (±0.09)	25.97 (±30.61)	500.22 (±42.38)
B	50 (±29.69)	43 (±19.79)	14.41 (±1.29)	6.71 (±0.80)	40.19 (±10.50)	739.96 (±492.80)
C	35.50 (±30.40)	14 (±7)	11.45 (±3.11)	7.07 (±3.53)	28.16 (±6.59)	359.24 (±237.59)
D	71 (±0)	57 (±19.79)	11.10 (±2.68)	6.99 (±0.60)	31.26 (±7.10)	788.10 (±190.77)
E	64 (±9.89)	50 (±9.89)	13 (±0.35)	5.21 (±0.29)	33.94 (±0.17)	830.25 (±106.06)
F	64 (±9.89)	57 (±0)	11.12 (±3.00)	6.21 (±1.11)	33.72 (±4.45)	697.12 (±82.20)

All values are averages of duplicate samples. Values in parenthesis represent ± standard deviation. Emergence column indicate % leaf emergence of the growing seedling after the seven day planting period. A, B and C represent treatments with only *A. flavus*, *A. fumigatus and A. niger*, respectively. D, E and F represent seeds that were first infected with *A. flavus*, *A. fumigatus and A. niger* before treatment in the extract

4 DISCUSSION

The present study made use of three seedlings, which are sorghum, tomato and okra. They are one of the most important grain legumes in the farming systems of Nigeria. Among the three seeds used in this study, sorghum seeds when soaked in the extract were observed to have high germination percentage viability along with the leaf emergence percentage of the seeds. All the extract concentrations of the seeds had germination capacities that were greater than 71 %. The minimum certified germination standard for sorghum is75 % (Coulibaly, 1987). Vigor index was consistently higher than that of the other seedlings. We can say that sorghum seeds had grew better in the presence of the extract.

In the present study, seeds treated with the extract showed higher germination percentage and leaf emergence than those of the control. It will be good to deduce that the extract has a positive effect on the seedlings and they were not prone to infection. Harmen and Taylor, 1988 reported that primed seeds grow more rapidly and emerge evenly over a great range of temperature conditions, and when this happens there will be greater seedling vigor.

The best soaking time of the tomato seeds in the extract were 120 min and 150 min compared to that of the control. However the best soaking time when the seed were soaked in water was observed to be 90 min. Also for that of okra, the study showed the germination percentage was moderate when seeds were soaked in extract concentration and also in the control (soaked in distilled water). The seedling height of the treated and untreated seeds gave same trend of result as the findings of Delouche, (1980).

It was observed that at all the soaking times of the three seeds; we can say that only the sorghum seeds had maximum growth in both extract and control, the extract didn't have any effect on sorghum seeds. This study has shown that the leaf of *Annona senegalensis* is effective as oviposition deterrence and is in agreement with the work of Anita *et al*., (2012)

In comparing the profile of the three seedlings when infected with a test fungal pathogen, *Aspergillus flavus*, sorghum seeds still seems to have overcome the infectivity. This shows that they were not susceptible to infection when it was simulated. As for tomato, the seed should moderate growth from $8.83x10^3$ to $2.21x10^3$ propagules, while the rest propagules were not able to inhibit the infectivity. It will be reasonable to deduce that okra and tomato were susceptible to the fungal infection than that of the sorghum seeds. When the seedlings were infected with *Aspergillus fumigatus,* it was observed that sorghum and okra seedlings were able to inhibit the fungal pathogen by growing efficiently and having consistent emergence on all the seeds.

It was observed in this study, that when the seeds were soaked in the extract, the germination percentage and leaf emergence was higher than the others when the seeds were soaked in the test fungal pathogens. It will be right to deduce that the chemical has a positive effect on the sorghum seeds. Even though Mereddy *et al*., 2000 reported that longer priming times result in lower germination and vigor indices as a result of fungal contamination, this was not observed in this study.

This study revealed that even though the okra seeds were infected with test fungal pathogen they still showed consistent growth, but the seeds showed no emergence. It can be deduced that the fungal pathogen has a negative effect on the okra seeds, even when they were being treated with the extracts. The result showed that the seedlings height of the seeds when they were infected is higher than when the seeds were treated and then infected.

Although planting was done by the same means, germination was seen to still fall within the same range. The seeds that were treated gave high germination growth that is even higher than the minimum official germination standard for the seeds (Coulibaly, 1987). Furthermore, even though the untreated seeds were able to grow normally; they were still prone to fungal infection. It was reported that treatment on seed varieties have shown a substantial yield increase from 30% to 100% compared to the treated seeds (Pedune-Benin, 1999). In this study, germination was observed within a week of planting which corroborated the finding of earlier investigators (Craufurd *et al*., 1998).

5 CONCLUSION

The present study on *Annona senegalensis* leaf extract revealed that the extract had positive effect on the growth of the seeds In the course of determining the effect of extract concentration on the seeds, the results showed that sorghum seeds have the maximum germination seed profile in a range of 71% to 93.36% when compared to the other seedlings.

The outcome of this study showed that the protective potential of the extract was positive in the sense that it proved that even though the seedlings were being infected it did not stop the growth of the seeds, the leaf extract of *Annona senegalensis* proved to protect the seedlings against the fungal pathogen as all the seedlings have consistent growth.

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