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Research Article

PROTECTIVE POTENTIAL OF *ANNONA SENEGALENSIS* BARK EXTRACT AGAINST FUNGAL PATHOGEN INDUCED VIABILITY LOSS OF SEEDS OF OKRA, SORGHUM AND TOMATO

***Akpor O.B.[1], Omole A.O.[1] and Babalola O.O.[2]**
[1]Department of Biological Sciences, Landmark University, PMB 1001, Omu Aran, Kwara State, Nigeria
[2]Department of Science Technology, Federal Polytechnic, P.M.B 5351, Ado Ekiti, Ekiti State, Nigeria
**Author for Correspondence*

ABSTRACT
This study was aimed at exploring the potential of ethanolic stem bark extract of *Annona senegalensis* in enhancing germination in okra, sorghum and tomato seeds and protecting against selected fungal pathogens. During each experimental setup, at the end of the seven days planting period, the following parameters were investigated: % germination, % leaf emergence, plant height, wet weight, germination index and seed vigor index. The results revealed that treated seeds have comparable better growth than untreated or infected seeds. The most effective concentration of the extract was observed to be 8000 mg/L, 6000 mg/L and 5000 mg/L, for the sorghum, okra and tomato seeds, respectively. Optimum soaking time was observed to be 240 min for the tomato seed and 150 min for the sorghum and okra seeds. Although infected seeds were observed to still show growth and leaf emergence, germination and seed vigor index was remarkably lower in those seeds. The study was able to reveal the potential of the extract for possible germination and vigor enhancer in the test seeds. This could serve as departure point for integrated pest management program.

Keywords: *Annona Senegalensis, Fungal Pathogen, Germination, Seed Vigor Index*

INTRODUCTION
Okra (*Abelmoschus esculenta*), Guinea corn or Sorghum (*Sorghum bicolour Solanium granarium*) and Tomato (*Solanum lycopersicum*) or (*Lycopersicum esculentum*) are important food crops in Nigeria and are planted for the nutritious, economic and industrial material values. However, they are prone to pest and diseases including fungal diseases with adverse consequence for yield of crops, farmers' livelihood and economy.

The continued challenge justifies various strategic efforts including sanitation and cultural crop rotation, chemical pesticides (synthetic and organic). With the toxicity problems and health risk concern for many chemicals, focus is shifting to organic, especially of plant origin as encouraged by successes in herbal medicine that focuses on plant parts and product for treatment of diseases.

However, these important crops are prone to diseases pre and post-harvest, including the seed borne diseases caused by fungal pathogens, with adverse consequences for yield of crops and livelihood of farmers and economy. Some insect pests of Okra are flea beetle (*Podagrica sp.*), white fly (*Bemisia tobaci*) and cotton stainer (*Dysdercus superstitus*) (Obeng-Ofori and Sackey, 2003); viruses and fungal diseases of Okra are leaf curl virus (OLCV), okra mosaic virus (OMV), (Krishnareddy *et al.*, 2003) and *Fusarium* wilt and Damping off. Damping off caused by *Pythium sp. and Rhizoctonia sp.* kills seedlings before or soon after emergence. *Fusarium* wilt is caused by the fungus *Fusarium oxysporum* f. sp. *vasinfectum*, which can persist in the soil for a very long time and contaminate seeds.

Prevention is the finest remedy for dealing with most plant diseases and many diseases cannot be controlled effectively as soon as it becomes severe. A combination of strategies involving sanitation, cultural practices, and seasonal spray applications are being used for disease prevention. Sanitation aims to remove the source of future disease infections by means of a thorough clean-up program (Bernard, 2010).

Proper seed treatment is an indisputable fact to improve the quality of seeds and have significant increase in crop yield (Meah *et al.*, 2004; Celar and Valic, 2005). Crop diseases are often seed borne and caused by fungi, and are therefore responsible for poor quality of seeds and consequently decrease yields in many

crops (Perelló *et al.,* 2013; Makovitzki *et al.,* 2007). These workers noted that plant diseases are the main cause of yield problems faced by the farmers. Hitherto, prevention and control of plant diseases are mainly by chemical means, with adverse effects including environmental pollution, health hazards and breaking of the natural ecological balance by destroying beneficial soil microbes (Mukhopadhyay, 1994).

It is vital to explore new methods of treating seeds of plant disease that are less expensive, non-chemical and eco-friendly in controlling pre and post-harvest diseases (Pal *et al.,* 2013; Suriyavathana *et al.,* 2010). The use of medicinal plants has become prominent, as the world is rich with medicinal plants as safe and effective method of treating seed-borne infections. Seed treatment is one of the cheapest and safest ways to control seed borne diseases by eradicating the seed-borne inoculum (Sen, 2000).

However, extracts and materials for treatment need quantification for economy of use of material for the required treatment. Thus, activity against selected fungal pathogens and consequence for seed viability indices need to be assessed for justifying any need for the treatment as well as to guide or determine recommendation of protocols, such as factors in treatment methodology like effective soaking time. This study aimed to evaluate the protective effect of the stem bark extract of *A. senegalensis* against fungal pathogens of selected seeds; okra, sorghum and tomato and investigate associated protocols.

Thus, the present study was designed for treatment of some fungal induced viability loss of Okra, Sorghum and Tomato seed, to improve germinability and vigour using ethanolic extract of stem bark of *Annona senegalensis* and the fungi: *Aspergillusflavus, A. fumigatus* and *A. niger* as test organisms.

MATERIALS AND METHODS

Plant Extract and Test Seeds

The plant extract used for the study was *Annona senegalensis* stem bark ethanolic extract. The stem bark from the plant was obtained within the environment of Landmark University, Omu-Aran, Kwara State.

The *Annona senegalensis* stem bark were collected in clean plastic bags, taken to the laboratory and washed with clean tap water to remove sand and other debris. They were then sun-dried for 7 days and then pulverized with a laboratory grinder.

After pulverization, the extract was obtained by soaking in ethanol (1:2 w/v) for 24 h in a beaker before filtering into a beaker, using Whatman No. 1 filter paper. The ethanol was separated from the extract using a rotary evaporator, before concentrating using a freeze drier. The dried extract was kept as stock in glass bottles at room temperature till needed.

Three different seeds (okra, sorghum and tomato) were used for the study. The seeds were purchased from a local agro-store located within Omu-Aran community in Kwara State. Before use, the purchased seeds were first surfaced sterilized using 5 % sodium hypochlorite (v/v). Surface sterilization 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-sterilized seeds were soaked in sterile distilled water for 30 min after which 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 has 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.

Determination of the Effective Concentration

Different concentrations of the extract was prepared ranging from 0 mg/L to 10,000 mg/L, having 0 mg/L as the control. An approximate 2.77g of non-absorbent cotton wool was weighed and placed in plastic cups (60 mm in diameter), and soaked with 40 mL of water. The seeds were first of all surfaced sterilized, then transferred into the already prepared concentrations, and allowed to stay in for 30 min before planting on the cotton wool, which served as blotters. The experiment was left to run for seven days and the daily germination of the seeds and leaf emergence were recorded, water was added daily only if necessary. At

the expiration of the experiment, the plant height, wet weight, final germination and leaf emergence was recorded.

Determination of Optimum Soaking Time of the Extract

The most effective concentration for each seed was used for this investigation. The seeds were soaked in the concentration for different times ranging from 30 min to 240 min. The seeds were planted on the already prepared cotton wool after every 30 min. Daily germination and leaf emergence were recorded. To avoid drying of the blotters, water was added uniformly every two days. The experiment was terminated after seven days and the different growth parameters were measured and recorded and were used to calculate the germination index and seed vigor index.

Determination of Infective Dose of the Pathogens

The fungal pathogens used for this experiment were, *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus niger*. Prior to being used for the study, the isolates were first cultured in sabouraud dextrose agar plates to ensure they were pure cultures before sub-culturing them into saboraud dextrose broth. The broth culture was diluted into different concentrations using sterilized distilled water, with the inoculum size in each dilution ascertained by culturing into plates using the standard pour plating technique. They were surface-sterilized and soaked for 30 min, before planting as described earlier. The experiment was ran for seven days, at the end of which the final germination and other growth parameters were measured, as described in previous sections.

Assessment of the Protective Effect of the Extract

To assess this, the seeds were soaked in different treatments, which were: water only, extract only, respective broth cultures of the fungal pathogens, in an equal mixture of both the broth culture and the extract, and a treatment where the seeds were infected by soaking in the broth cultures before treatment in the extract. At the end of soaking, the respective seeds were planted as described in previous sections. The experiment was allowed to run for seven days, with daily germination and leaf emergence recording. The final germination and other growth parameters were measured and recorded at the end of the experiment. All experimental setups and measurements were carried out in duplicates.

Calculation of Germination and Seed Vigor Index

Germination index was calculated by counting the number of seedlings emerging daily from day of planting the seeds till the time germination is complete. Thereafter a 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.

Seed 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) * % germination

RESULTS AND DISCUSSION

Results

Effect of Extract Concentration

At the end of the seven days planting period, the germination profile of the okra seeds at the different concentrations of the extract was observed to range from 21% - 78.5 %, with the lowest and highest observed at extract concentrations of 4000 mg/L and 1000 mg/L respectively. The % leaf emergence of the seedlings ranged from 14 % - 50 % with the lowest emergence observed at extract concentration of 2000 mg/L. In the case of the seedling height, a lowest value of 21.13 mm was observed at extract concentration of 10,000 mg/L while a highest value of 37.63 mm was observed for the control (0 mg/L). With respect to the wet weight, the values ranged from 110.50 mg to 173.73 mg. The germination and seed vigor indices were found to range from 47.58 to 83.73 and from 1009.16 to 2768.13, respectively (Table 1).

For the sorghum seeds, the germination profile showed a range from 64 % - 93 %, with lowest observed at extract concentrations of 2000 mg/L. The highest leaf emergence of 93 % was observed at concentration of 8000 mg/L. At the expiration of the planting period, seedling height was observed to range from 17.99 mm to 35.55 mm, with lowest and highest values at extract concentrations of 6000 mg/L and 4000 mg/L, respectively. For the wet weight of the seedlings, the observed values ranged from 35.14 mg to 46.32 mg. The highest germination index and seed vigor index of 97.27 (at concentrations of 7000 mg/L and 8000 mg/L) and 2560.50 (at concentration of 3000mg/L) were observed, respectively (Table 1).

Table 1: Germination and other growth parameters of the seeds at different concentrations of the extract

Conc. (mg/L)	Germination (%)	Leaf emergence (%)	Seedling height (mm)	Wet weight (mg)	Germination index	Seed vigor index
			Okra			
0	71.50	50.00	37.63	173.73	83.73	2768.13
1000	78.50	43.00	27.64	128.14	77.52	2100.24
2000	57.00	14.00	21.79	110.50	57.98	1276.47
3000	57.00	35.50	31.45	132.43	60.46	1798.95
4000	21.50	21.50	51.50	118.00	27.62	1156.00
5000	50.00	21.50	22.03	114.86	59.31	1120.33
6000	36.00	14.50	29.09	133.57	32.01	1009.16
7000	43.00	36.00	26.00	147.58	47.58	1118.00
8000	57.00	36.00	24.73	121.86	70.31	1308.61
9000	78.50	35.50	30.00	124.29	64.63	2421.00
10000	57.00	35.50	21.13	123.86	54.71	1204.13
			Sorghum			
0	71.00	46.00	25.11	35.14	63.93	1782.46
1000	86.00	64.5	18.40	41.35	91.49	2403.34
2000	64.00	50.00	27.97	37.78	91.49	1815.21
3000	93.00	78.50	27.25	42.28	91.16	2560.50
4000	71.00	64.00	35.55	42.71	68.17	2524.05
5000	93.00	57.50	33.37	46.32	85.61	1848.69
6000	93.00	78.50	17.99	37.64	89.36	1645.57
7000	93.00	78.50	20.21	44.49	97.27	1900.59
8000	93.00	93.00	24.37	43.50	97.27	2237.70
9000	85.50	73.00	20.75	35.57	83.88	1730.63
10000	78.50	58.50	20.24	35.21	93.66	1561.92
			Tomato			
0	57.00	29.00	8.38	6.65	34.51	477.38
1000	71.00	7.00	7.50	5.22	65.23	532.50
2000	29.00	7.00	7.25	4.93	43.24	210.25
3000	36.00	14.50	10.00	7.07	40.34	311.00
4000	50.00	35.50	8.90	6.42	58.56	463.20
5000	57.00	36.00	9.75	7.36	46.76	555.75
6000	64.00	43.00	10.33	7.43	71.87	668.33
7000	86.00	50.00	10.33	7.65	52.43	888.38
8000	64.00	21.50	9.45	5.72	67.74	604.45
9000	42.50	21.50	10.30	6.29	45.76	446.3
10000	57.00	21.50	11.38	6.86	50.78	648.38

Plant height and wet weight were calculated as averages of seven seedlings. All values are averages of duplicate samples. Soaking time of the seeds in the extract was 30 min.

The trend in germination, for the tomato seeds showed to range from 29 % - 86 % with the lowest and highest observed at concentrations of 2000 mg/L and 7000 mg/L. The lowest leaf emergence was observed to be 7 % (at extract concentrations of 1000 mg/ L and 2000 mg/L), and the highest was observed to be 50

% (at extract concentrations of 7000 mg/L). The seedling height and wet weight ranged from 7.25 mm to 11.38 mm, and 4.9 mg to 7.65 mg, respectively. The germination and seed vigor indices were observed to range from 34.51 to 71.87 and 210.25 to 888.38 respectively (Table 1).

Effect of the Soaking Time

With respect to soaking time of the okra seeds, lowest germination of 21.5 % was observed at 90 min while highest germination of 85.5 % was observed at 210 min. The % leaf emergence of 64 % was observed to be highest in seeds soaked for 150 min. At the end of the 7 days planting period, the plant height was observed to range between 5.09 mm and 40.72 mm while the weight was highest at 104.29 mg for seeds soaked for 60 min. The germination and seed vigor indices were observed to range from 63.81 to 104.96 and 659.50 to 2743.77 respectively. The lowest and highest germination index were observed in seeds soaked for 30 min and 180 min, respectively while the lowest and highest seed vigor index were observed in seeds soaked for 240 min and 150 min, respectively (Table 2).

Table 2: Germination and other growth parameters of the seeds at different soaking time in the extract

Time (min)	Germination (%)	Leaf emergence (%)	Plant height (mm)	Wet weight (mg)	Germination index	Seed vigor index
			Okra			
30	50.00	50.00	16.63	93.50	63.81	828.63
60	43.00	29.00	22.32	104.29	57.74	959.55
90	21.50	14.50	5.09	86.22	26.99	218.66
120	64.50	7.00	14.28	75.79	27.64	948.15
150	64.00	64.50	40.72	94.86	114.54	2743.77
180	78.50	7.00	20.37	73.5	104.96	1617.89
210	85.50	28.50	15.78	70.29	94.95	1437.35
240	32.00	7.00	21.00	74.22	65.79	659.5
			Sorghum			
30	93.00	78.50	27.74	41.76	147.61	2586.19
60	85.50	85.50	27.25	34.93	125.99	2391.00
90	93.00	85.50	28.37	34.64	132.69	2618.38
120	93.00	71.00	26.80	33.21	136.19	2472.44
150	78.50	50.00	24.52	32.50	133.24	1897.88
180	78.50	64.00	24.61	36.65	128.16	1956.71
210	93.00	78.50	17.84	34.15	128.94	1659.81
240	100.00	93	27.38	35.79	126.64	2737.5
			Tomato			
30	50.00	36.00	9.67	3.07	25.05	490.50
60	85.50	64.00	12.38	4.36	43.24	1023.63
90	57.00	50.00	14.60	3.27	36.17	807.63
120	64.00	50.00	20.53	3.08	33.10	1371.53
150	50.00	43.00	14.71	2.40	27.58	728.78
180	43.00	36.00	19.71	3.36	27.05	847.53
210	50.00	50	10.75	4.86	34.99	553.25
240	36.50	36.00	14.35	3.00	29.81	415.10

Plant height and wet weights were calculated as averages of seven seedlings. All values are averages of duplicate samples. Concentration of the extract was 5000 mg/L, 6000 and 8000 mg/L for the tomato, okra and sorghum seeds, respectively.

For the sorghum seeds, highest and lowest germination of 100 % and 78.5 %were observed at soaking time of 240 min and 150 min, respectively. A lowest leaf emergence of 50 % was observed at 150 min while a highest leaf emergence 93 % was observed at 240 min. The plant heights ranged between 17.84 mm and

27.74 mm for soaking times of 210 min and 30 min respectively while the wet weight ranged between 32.5 mg and 36.65 mg for soaking times of 150 min and 180 min, respectively. With respect to the seed vigor index, a soaking time of 240 min was revealed to give the highest value of 2737.50 while a lowest value of 1659.81 was revealed at 210 min (Table 2).

In the case of the tomato seeds, at the different soaking times investigated, germination was shown to range from 36.5 % to 85 %, with the lowest and highest at 240 min and 60 min, respectively The plant height showed a range from 9.67 mm to 20.53 mm for soaking times of 30 min and 120 min, respectively while lowest and highest wet weights of 3.00 mg and 4.86 mg were observed at 420 min and 210 min, respectively. A germination index that ranged from 25.05 to 43.24 was observed at 30 min and 60 min, respectively while a seed vigor index of between 415.10 and 1371.53 was observed at 240 min and 120 min, respectively (Table 2).

Effect of Inoculum Population

As shown in Table 3, when infected with the different population of *Aspergillus flavus*, % germination, plant height, germination index and seed vigor index of the okra seeds were observed to range from 29 % to 86 %, from 12.00 mm to 24.30 mm, from 46.19 to 90.79 and from 348.00 to 2089.80, respectively.

Table 3: Germination and other growth parameters of okra seeds when infected with population of the test fungal pathogens

Population	Germination (%)	Leaf emergence (%)	Plant height (mm)	Wet weight (mg)	Germination index	Seed vigor index
			Aspergillus flavus			
1.33×10^4	29.00	29.00	12.67	85.43	50.19	367.43
1.16×10^4	29.00	43.00	12.00	92.00	60.74	348.00
8.85×10^3	57.00	14.00	15.30	89.00	49.33	872.10
6.63×10^3	57.00	43.00	18.30	85.43	77.63	1043.10
4.42×10^3	71.00	14.00	17.00	81.14	46.19	1207.00
2.21×10^3	86.00	12.00	21.67	100.14	46.19	1863.62
0	86.00	14.00	24.30	66.86	90.79	2089.80
			Aspergillus niger			
1.44×10^4	71.00	14.00	18.67	81.71	40.96	1325.57
1.19×10^4	71.00	29.00	19.33	87.14	26.44	1372.43
9.57×10^3	71.00	0.00	16.66	104.86	94.42	1182.86
7.18×10^3	57.00	29.00	20.60	77.57	72.93	1174.20
4.78×10^3	71.00	29.00	16.00	91.43	94.42	1136.00
2.39×10^3	86.00	43.00	19.33	103.43	95.69	1662.38
0	86.00	14.00	24.00	102.86	106.92	2064.00
			Aspergillus fumigatus			
4.00×10^3	29.00	29.00	12.30	45.00	10.49	356.70
3.33×10^3	29.00	0.00	17.00	50.00	38.69	493.00
2.67×10^3	43.00	14.00	17.97	71.00	50.53	772.71
2.00×10^3	43.00	14.00	14.00	82.71	61.49	602.00
1.33×10^3	57.00	14.00	13.67	81.29	76.79	779.19
6.67×10^2	86.00	29.00	17.27	73.71	110.49	1485.22
0	86.00	29.00	17.75	101.50	115.49	1526.50

Plant height and wet weight were calculated as averages of seven seeds. Population is expressed as propagules/mL. All values are averages of duplicate samples. Concentration of extract used was 6000mg/L. '0' represent the uninfected control treatments

In presence of the different population of *Aspergillus niger*, growth profile of the okra seeds was observed to range from 29 % to 86 % (% germination), from 12.30 mm to 17.75 mm (plant height), from 26.44 to 106.92 (germination index) and from 1136.00 to 2064.00 (seed vigor index). In all the parameters investigated, highest values were recorded in the control seeds that were not infected with the organism (Table 3).

When infected with the different population of the *Aspergillus fumigatus*, germination profile of the okra seeds ranged from 29 % to 86 % while the plant height, germination index and seed vigor index were observed to range from 12.30 mm to 17.75 mm, 10.49 to 115.49 and 356.70 to 1526.50, respectively (Table 3).

Table 4: Germination and other growth parameters of sorghum seeds when infected with population of the test fungal pathogens

Population	Germination (%)	Leaf emergence (%)	Plant height (mm)	Wet weight (mg)	Germination index	Seed vigor index
			Aspergillus flavus			
1.33×10^4	86.00	29.00	12.5	26.57	136.98	1372.56
1.16×10^4	86.00	43.00	13.83	31.57	124.48	1533.38
8.85×10^3	57.00	29.00	19.33	35.29	61.13	1085. 85
6.63×10^3	71.00	43.00	15.96	26.14	113.02	908.80
4.42×10^3	86.00	43.00	22.50	34.14	136.99	1677.00
2.21×10^3	71.00	57.00	9.75	38.71	113.09	2112.25
0	71.00	43.00	16.60	35.43	88.13	1178.6
			Aspergillus niger			
1.44×10^4	71.00	29.00	12.50	38.71	79.93	887.5
1.19×10^4	71.00	43.00	13.83	24.86	97.93	981.93
9.57×10^3	86.00	71.00	19.33	31.57	117.49	1372.43
7.18×10^3	86.00	57.00	15.96	38.86	120.74	1372.56
4.78×10^3	100.00	57.00	22.50	37.86	127.87	2250
2.39×10^3	71.00	57.00	9.75	28.29	99.09	692.25
0	86.00	43.00	20.16	36.14	129.49	1733.76
			Aspergillus fumigatus			
4.00×10^3	43.00	43.00	20.07	40.00	104.54	863.01
3.33×10^3	86.00	43.00	38.14	38.14	124.49	2519.82
2.67×10^3	71.00	43.00	35.14	35.14	87.43	1643.65
2.00×10^3	57.00	29.00	36.43	36.43	90.79	2052.00
1.33×10^3	86.00	43.00	28.14	28.14	122.49	1204.00
6.67×10^2	100.00	14.00	27.29	27.29	68.49	2729.00
0	86.00	43.00	35.00	35.00	129.49	1668.4

Plant height and wet weight were calculated as averages of seven seedlings. Population is expressed as propagules/mL. All values are averages of duplicate samples. Concentration of the extract was 8000 mg/L. '0' represent the uninfected control treatments.

The % germination of the sorghum seeds when infected with *Aspergillus flavus* was observed to range from 57 % - 86 % while the leaf emergence was between 29 % - 43 %. The plant height and weight showed a range from 9.75 mm to 22.50 mm and from 26.14 mg - 38.71 mg, respectively while the highest germination and seed vigor indices were observed to be 136.99 and 2112.25, respectively (Table 4).

When infected with the *Aspergillus niger*, % germination showed a range from 71 % to 100 % while plant height was observed to range from 12.50 mm to 22.50 mm. With respect to the germination index and seed vigor index at the different inoculum population of the *Aspergillus niger*, a range of 79.93 to 129.47 and 887.5 to 2250 was observed, respectively (Table 4).

In the presence of the *Aspergillus fumigatus*, the germination and leaf emergence of the sorghum seeds ranged from 43 % to 100 % and from 14 % to 43 %, respectively. The seed vigor index ranged from 623.5 - 2519.82 at inoculum population of 6.67×10^2 propagules/mL and 3.33×10^3 propagules/mL respectively (Table 4).

As shown in Table 5, in the presence of the different respective population of the *Aspergillus flavus* on the tomato seeds, the % germination was observed to range from 29 % to 71 %, with the lowest and highest observed at 6.63×10^3 propagules/mL and the uninfected control treatments, respectively. The plant height, germination index and seed vigor index were observed to range from 11.25 mm to 17.25 mm, from 18.28 to 44.46 and from 326.25 to 1136.00, respectively (Table 5).

The tomato seeds infected with the different population of *Aspergillus niger* showed germination that ranged from 29 % to 86 %. The plant height, germination index and seed vigor index values were observed to range from 11.50 mm to 16.00 mm, from 15.28 to 63.59 and from 333.50 to 1261.62, respectively (Table 5).

In the case of the tomato seeds infected with the *Aspergillus fumigatus*, germination was observed to range from 29 % to 71 %, with highest observed in the uninfected control treatment seeds. The plant height and germination index were observed to range from 10.25 mm to 16.80 mm and from 15.28 to 48.09, respectively while the seed vigor index showed a range from 326.25 to 1192.80 (Table 5).

Table 5: Germination and other growth parameters of tomato seeds when infected with population of the test fungal pathogens

Population	Germination (%)	Leaf emergence (%)	Plant height (mm)	Wet weight (mg)	Germination index	Seed vigor index
			Aspergillus flavus			
1.33×10^4	43.00	29.00	13.50	8.29	20.28	580.50
1.16×10^4	43.00	29.00	11.00	10.57	32.66	473.00
8.85×10^3	43.00	14.00	15.75	8.86	32.66	677.25
6.63×10^3	29.00	0.00	11.25	8.43	18.28	326.25
4.42×10^3	57.00	29.00	17.25	8.57	32.54	983.25
2.21×10^3	57.00	43.00	16.75	6.57	42.49	954.75
0	71.00	43.00	16.00	6.86	44.46	1136.00
			Aspergillus niger			
1.44×10^4	57.00	0.00	16.00	7.86	27.41	912.00
1.19×10^4	71.00	29.00	19.50	6.86	63.59	1384.50
9.57×10^3	29.00	14.00	11.50	8.14	15.28	333.50
7.18×10^3	57.00	14.00	13.33	7.14	21.11	759.81
4.78×10^3	57.00	29.00	12.75	8.00	36.29	726.75
2.39×10^3	71.00	29.00	15.67	7.00	48.09	1112.57
0	86.00	14.00	14.67	8.43	42.47	1261.62
			Aspergillus fumigatus			
4.00×10^3	43.00	14.00	12.60	4.86	23.03	541.80
3.33×10^3	29.00	14.00	13.67	8.00	15.28	396.43
2.67×10^3	29.00	14.00	11.25	7.43	18.28	326.25
2.00×10^3	43.00	29.00	10.25	5.86	25.41	440.75
1.33×10^3	43.00	29.00	11.20	6.29	25.41	481.60
6.67×10^2	57.00	29.00	11.50	8.14	26.28	655.50
0	71.00	43.00	16.80	9.29	48.09	1192.80

Plant height and wet weight were calculated as averages of seven seedlings. Population is expressed as propagules/mL. All values are averages of duplicate samples. Concentration of the extract was 5000 mg/L. '0' represent the uninfected control treatments.

Protective Potential of the Extract

When investigating the protective effect of the extract on the okra seeds against the test fungal pathogens, the results revealed that the germination of the seeds infected but not treated was observed to range from 43 % - 93 %. The leaf emergence ranged from 14 % to 50 % while the plant height ranged from 9.15 mmto 15.65 mm.

Table 6: Germination and other parameters of the treatment and infection of okra seeds

Treatments	Germination (%)	Leaf emergence (%)	Plant height (mm)	Wet weight (mg)	Germination index	Seed vigor index
			Okra			
Water only	50.00	14.00	7.75	89.43	53.83	373.5
Extract only	71.50	21.50	18.88	123.07	70.05	1309.33
A1	93.00	50.00	15.65	84.43	111.31	1468.40
A2	78.50	50.00	24.58	103.50	101.42	1931.58
A3	43.00	21.50	9.15	76.79	51.74	423.55
B1	21.50	14.50	10.25	76.72	20.10	259.75
B2	50.00	35.50	16.32	84.72	49.40	801.65
B3	50.00	35.50	13.54	75.43	47.50	663.28
C1	21.50	14.50	10.65	73.15	22.62	211.35
C2	57.00	21.50	17.36	82.22	58.30	999.18
C3	50.00	14.50	16.75	87.57	58.14	821.75
			Sorghum			
Water only	93.00	50.00	16.52	30.00	133.64	1492.12
Extract only	71.50	43.00	15.00	39.00	110.39	1257.75
A1	93.00	64.50	28.91	38.43	148.14	2735.04
A2	93.00	64.00	17.17	28.5	135.01	1596.81
A3	93.00	57.00	17.57	32.17	148.14	1631.90
B1	78.50	43.00	24.02	46.45	110.66	1807.82
B2	85.50	57.00	21.94	38.71	118.79	1796.2
B3	93.00	71.00	22.58	36.29	140.89	2071.65
C1	85.50	57.00	22.20	31.72	111.19	1944.50
C2	86.00	57.00	21.91	35.65	129.74	1884.26
C3	85.50	57.00	21.33	35.57	126.86	1834.30
			Tomato			
Water only	71.50	43.00	8.98	6.64	ND	642.07
Extract only	71.50	14.50	10.59	4.79	ND	757.19
A1	43.00	21.50	6.85	6.50	ND	294.55
A2	41.50	50.00	6.15	6.93	ND	255.23
A3	43.00	35.50	7.80	6.05	ND	335.40
B1	57.00	21.50	7.38	7.86	ND	420.38
B2	71.50	43.00	10.25	7.36	ND	714.75
B3	50.00	28.50	8.60	9.07	ND	430.00
C1	50.00	36.00	12.13	7.50	ND	671.88
C2	78.50	50.00	9.14	8.58	ND	713.61
C3	50.00	36.00	10.13	7.07	ND	528.13

A1, A2 and A3 represent seeds infected with only Aspergillus flavus, Aspergillus niger and Aspergillus fumigatus, respectively. B1, B2 and B3 represent seeds that were first infected with Aspergillus flavus, Aspergillus niger and Aspergillus fumigatus, respectively before treatment with the extract. C1, C2 and C3 represent seeds that were first treated with the extract and then infected with Aspergillus flavus, Aspergillus niger and Aspergillus fumigatus, respectively. Plant height and wet weight were calculated as averages of seven seedlings. All values are averages of duplicate samples. Concentration of the extract was 5000 mg/L, 6000 and 8000 mg/L for the tomato, okra and sorghum seeds, respectively. 'ND' indicate not determined

The seed vigor indices of the infected seeds were remarkably low for those infected with *Aspergillus fumigatus*. The seeds treated with the extract were observed to have higher germination, leaf emergence and germination vigor index (Table 6).

For the sorghum seeds, the infected and treated were observed to have the highest germination, ranging from 93 % to 100 %. The leaf emergence was highest in seeds infected before treatment, and least in treated before infection. The germination index and seed vigor index was higher in the treated seeds. The seeds treated before infections were observed to have the highest wet weight and seed vigor index (Table 6).

For the tomato seed, germination among the different treatments was observed to range from 41.50 % to 78.50 %. The lowest germination was observed in the untreated tomato seeds that were infected with *Aspergillus niger*. The lowest seed vigor indices of 294.55, 255.23 and 335.40 were observed in the untreated infected tomato seeds with *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus*, respectively (Table 6).

Discussion

In this study, ethanolic extracts derived from *Annona senegalensis* stem bark was used. The choice of the extract was deliberate. Farmers have been faced with difficulties of fungal infections, and using fungicides for treatment proves to be very expensive, but treatment of this should start from the seeds before planting. The use of chemicals for treatment is not appropriate as these have toxic residue which does not degrade in the soil, causes severe health difficulties, and also reduce the microbial population in the soil. Hence, the use of natural means to treat seeds helps to prevent fungal diseases, reduce soil pollution, protect soil microbes (non-target organisms) and also provide cheaper ways for farmers to carry out these treatment processes before planting. High crop yield is determined by the germination and quality of seeds but may also be influenced negatively through infestation by pathogens during poor storage. Pre-treatment is vital before planting, as the use of infected seeds without any treatment against these infecting pathogens can cause a decline in the germination. The infection will continue to act on the plant growth thereby affecting crop yield (Odofin, 2010; Hofs *et al.,* 2004; Zida *et al.,* 2008).

The effective concentration of the extract was observed to range from 4000 mg/L to 8000 mg/L giving the highest % germination and seed vigor index for all the three seedlings. Aqueous leaf extract of *Ocimum basilicum* and *Artemisia absinthium* influenced the seed quality parameters of tomato seeds, which includes % germination (40 % - 67 %) and shoot length of 6.5 cm and 6 cm long (Marraiki, 2013). The treatment of sorghum seeds with extracts from *Acacia gourmaensis* and *Eclipta alba* prompted an increase in seedling weight and in the field trials, the later brought about a clear increase in the grain yield (Zida *et al.,* 2008). Seeds treated with *Acalypha wilkesiana* and *Moringa oleifera* had significant germination and radicle length than those treated with sodium hypochlorite (Nwangburuka *et al.,* 2013).

The processes that control seed germination include external and internal factors. The external factors such as temperature, moisture content, light and presence or absence of chemical compounds, the internal factors such as the genotype all influence the seed germination. Germination can be affected, from chemicals that get into the seed, after seed soaking to soften the hard seed coat (Finkelstein, 2004; Kucera *et al.,* 2005).

In this investigation, the seeds soaked in the plant extract had higher % germination and seedling heights compared to the seeds soaked in water, at the different soaking times. The treatment also resulted in significant increase of the seed vigor. Seeds treated with different concentrations of aqueous extract of *Psoralea corylifolia,* promotes the germination and vegetative growth of maize seeds, and also a significant decrease in mycoflora (Kiran *et al.,* 2012). *Lawsonia inermis* and *Datura stramonium* are plants whose extracts have inhibited pathogens, also, aqueous extract of *Eucalyptus* spp has inhibited seed-borne pathogens (*Drechslera hawaiiensis* and *D. tetramera*) and the in vitro growths of *A. alternate* (Bajwa and Iftikhar, 2005).

In this study, the extract promoted plant growth, leaf emergence and enhanced the seedling weight. This observation was irrespective of the seeds investigated. Earlier workers have reported increase in seedling weight after treatment of rice seeds, with plant extracts (Mishra and Sinha, 2000). The effect the plant extract has on managing the seed-borne fungi is possibly what favors the plant growth. Nonetheless, other

factors may influence the process, because plant growth was promoted even though extracts were not effective in managing the seed pathogens.

The seeds infected with the *Aspergillus* species and untreated to determine the most effective inoculum dose, still showed significant % germination but the seedling height was low and also had poor leaf emergence. The lower inoculum doses and controls for tomato seeds gave significantly high germinations and leaf emergence as compared to the higher doses. A new source for drugs for the future, are traditional medicinal plants and there has been an increase in the research of the effect of plant extracts (Prakash-Rout *et al.,* 2009). An antimicrobial evaluation of the stem bark of *Nauclea latifolia,* showed to be effective against *Staphylococcus aereus, Bacillus subtilis,* E*cherichia coli, Pseudomonas aeruginosa Shigella dysentariae, Candida albicans,* and *Aspergillus niger* (Anowi *et al.,* 2012). Over the years, research has shown that there is a high rate of fungal contamination in okra (Nwangburuka *et al.,* 2012).

The ability of the plant extract to inhibit fungal growth makes it more suitable for treatment before planting and for post-harvest preservation of okra seeds. Also extracts of *Acalypha* showed high antifungal potential in culture in the report by Ezekiel *et al.,* (2009). Increase in the concentration of the *Moringa oleifera* extract, increases the inhibitory activity against the seed-borne mycoflora of okra (Nwangburuka *et al.,* 2012). In this study, the stem bark extract inhibited the fungal pathogens at high levels. The treatment of wheat seeds with garlic juice, increases the germination rate, the shoot length, vigor index and inhibition of mycoflora of the seedlings. The study concludes that the use of certain medicinal plant extracts may enhance seed quality germination and vigor or may improve plant quality.

Conclusion

The present study, which was aimed at assessing the potential of *Annona senegalensis* stem bark ethanolic extract in enhancing viability and protection of okra, sorghum and tomato seeds against fungal pathogens was able to reveal the following:

- Optimum concentration of the extract that will enhance germination was observed to be 8000 mg/L, 6000 mg/L and 5000 mg/L for the sorghum, okra and tomato seeds, respectively. Further increases in the concentrations did not enhance germination.
- The ideal soaking time of the seeds in the extract before planting was revealed to be 240 min for the tomato seed and 150 min for the sorghum and okra seeds
- All the pathogens were able to initiate infections in the respective seeds when soaked at high inoculum population before planting. At low fungal, germination and other growth parameters of the seeds were not remarkably affected.
- The extract was observed to effectively treat seeds that were initially infected with the pathogens before treating in the extract.
- Seeds treated with the extract had a relatively higher germination percentage, seedling height, and seed vigor than seeds that were only soaked in water.

In conclusion, the present study was also able to reveal that *Annona senegalensis*ethanolic extract could increase germination and other growth parameters of the okra, sorghum and tomato seeds when pre-treated with it before planting.

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