Journal of Agriculture and Ecology Research International
10(1): 1-11, 2017; Article no.JAERI.29753
ISSN: 2394-1073

SCIENCEDOMAIN *international*
www.sciencedomain.org

Seed Vigour Enhancement and Protective Potential of *Annona senegalensis* Leaf Extracts against Fungal Pathogens of Sorghum Seeds

O. B. Akpor[1*] and A. O. Salami[1]

[1]Department of Biological Sciences, Landmark University, PMB 1001, Omu-Aran, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author OBA designed the study, interpreted the results, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AOS performed the experiment, interpreted the results and contributed to the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAERI/2017/29753
Editor(s):
(1) Petropoulos Spyridon, Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Greece.
Reviewers:
(1) Azhar Mehmood, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan.
(2) Cletus Anes Ukwubile, University of Ibadan and Department of Science Laboratory Technology, Federal Polytechnic, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/17289

Original Research Article

Received 27th September 2016
Accepted 26th October 2016
Published 20th December 2016

ABSTRACT

Medicinal plants are in nature and are cultivated worldwide and also commercially. This study was aimed at assessing the protective potential of leaf extracts of *Annona senegalensis* against selected fungal pathogens of sorghum seeds. Before the use of the seeds, they were checked for viability and surface sterilized. In this study, the effective concentration, optimum soaking time, protective potential of the extract and antibacterial effect of the extracts were carried out. During each setup, at the end of the planting period, percentage germination, germination index, germination capacity, germination rate, germination time and seed vigour were calculated. The most effective concentrations of the aqueous, n-hexane, ethanol extracts of *Annona senegalensis* and copper sulphate solution were observed to be 2000 mg/L, 7000 mg/L, 2000 mg/L and 5000 mg/L respectively while the best soaking times of the seeds in the copper sulphate solution, aqueous and n-hexane extracts of *Annona senegalensis* were 30 min while for ethanol extract was 60 min. The infected seeds that were treated with the extracts showed high level of germination and seed vigour. This study was able to reveal the potential of *Annona senegalensis* in the

**Corresponding author: E-mail: akpor.oghenerobor@lmu.edu.ng;*

enhancement of germination and seedling vigour as well as the protective effect against fungal pathogens. This study can be exploited to control the growth of storage or spoilage fungi thus reducing the dependence on the synthetic fungicides.

Keywords: Annona senegalensis; seed vigour; germination; leaf extract; fungal pathogens.

1. INTRODUCTION

Plants play a vital role in the life of humans. Many of these indigenous medicinal plants are used as spices and food plants; they are also sometimes added to foods meant for pregnant mothers for medicinal purposes [1]. According to the World Health Organization, three-quarter of the world's population rely greatly on the use of traditional medicine as their primary health care foundation [2]. Resistance to antibiotics has increased greatly in the modern years and is posing an ever increasing therapeutic problem. One of the means to reduce the resistance to antibiotics is by the use of antibiotic resistance inhibitors from plants. New sources of antimicrobial agents could be obtained from natural or higher plants with unique mechanisms of action [3,4,5].

A wide variety of bioactive metabolites are produced by plants and they serve as defense mechanisms against pests. Bioactive compounds also called secondary metabolites consist of low molecular weight compounds that are crucial for the survival of the producing organism and those from higher plants could serve as defense agents against invading microorganisms. These groups of compounds are vital for the structure of plants and confers resistance against microorganisms and aid the preservation of the integrity of the plant during constant exposure to environmental stress, such as ultraviolet radiation, dehydration and high temperatures [6,7].

The initial remedy for dealing with a large number of diseases affecting plants is prevention. An integration of strategies which involves cultural practices, sanitation and seasonal applications of spray are used for disease prevention. The purpose of sanitation is to remove the cause of future disease by a thorough clean-up program [8]. Proper treatment of seed is a certain element for the improvement of seed quality and it allows a significant increase in crop yield [9,10].

The prevention and control of plant diseases is generally carried out with chemicals, which could pose adverse environmental and health hazards. The presence of chemicals could also lead to the termination of the natural balance of the ecosystem by destroying the beneficial microbes in the soil [11]. The new methods of seeds treatment which have to be less expensive, non-chemical and eco-friendly in controlling plant diseases have to be discovered [12,13]. Medicinal plants contain various compounds which may serve as potential antibacterial agents and they serve as an alternative, cheap, effective, and safe antibacterial for treatment of common bacterial infections. The ability of the plant extracts as antimicrobial agent is due to of their antioxidant properties which are correlated with their phenolic contents [14].

Annona senegalensis is indicated to have the ability to produce certain metabolites that can act against the fungal pathogens of the sorghum seeds. Flavonoids produced by the *Annona senegalensis* have the ability to inhibit the germination of spores of pathogens of plants and therefore has been proposed to be used against fungal pathogens [15]. This study was aimed at evaluating the vigour enhancement and protective potentials of the extracts of *Annona senegalensis* against selected fungal pathogens of sorghum.

2. MATERIALS AND METHODS

2.1 Test Extracts

The plant used for the study was *Annona senegaliensis* leaves. The leaves of the plant were obtained from the environment of Landmark University, Omu Aran, Kwara State. The leaves were first washed with clean tap water to eliminate sand and other debris and air-dried, before pulverizing using a laboratory grinder.

For extraction, known quantities of the ground leaves were soaked for 24 h in respective beakers containing known quantities of the extraction solvent to be used. The extraction solvents used for the study were water, ethanol and n-hexane, which were referred to as aqueous, ethanol and n-hexane extracts, respectively. At the end of the 24 h extraction period in the respective solvents, the extracts

were filtered using Whatman no.1 filter paper. While the supernatants were discarded, the respective filtrates were concentrated in a rotary evaporator (MRC-ROVA 100) and freeze dried in a free-drier (LYOTRAP).

2.2 Test Seeds

Sorghum, also known as guinea corn seeds were used for the study. The seeds were purchased from the local market in Omu-Aran, Kwara State, Nigeria. The seeds were stored in cellophane bags and kept in a freezer, until when needed.

Prior to use, seeds soaked in water to remove floating ones, which were perceived to be non-viable. This was followed by surface-sterilization of the seeds with 5% sodium hypochlorite (v/v) in distilled water for 5 min in 5% sodium hypochlorite. The surface sterilized seeds afterwards rinsed several times with distilled water to remove the residual sodium hypochlorite. The viable seeds were kept in a plastic bag in the refrigerator till use. Prior to use, all seeds were confirmed to be viable.

2.3 Determination of Optimum Concentration

A total of ten different concentrations (1000 mg/L to 10000 mg/L) of the respective crude extracts or copper sulphate solution were used for the study. To each universal bottle containing 20 mL of the known concentrations of the respective extracts or copper sulphate, approximately 20 surface sterilized seeds were added and allowed to stand for 30 min. At the end of the 30 min soaking time of the seeds in the extracts or copper sulphate solution, seven seeds were withdrawn at each concentration and planted on blotters that were placed in transparent plates with dimensions of 9 cm diameters and 4 cm heights. The respective blotters were pre-soaked in 50 mL of distilled water. In this study, the blotters used were absorbent cotton wools.

The plates containing the planted seeds were arranged on trays in the presence of fluorescent light and observed for daily germination of seeds for eight days. On a daily basis, 10 mL of distilled water were added to each plate containing the blotters and planted seeds to keep the environment moistened. At the termination of the planting period, the seedling height, % germination, germination rate, germination time, germination capacity, germination index and seeds vigour of each of the slots were estimated.

2.4 Determination of Optimum Soaking Time

Using the optimum concentration for each of the respective extracts or copper sulphate solutions in respective beakers, the surface sterilized seeds were added. Every 30 min, for a duration of 180 min, seven seeds were removed from each extract and planted in transparent plates containing the blotters. The plates were then incubated under fluorescent light for germination. On a daily basis germination profile of the seeds were recorded. In order to keep the environment moist, approximately 10 mL of distilled water was added to each plate containing the planted seeds on a daily basis. Apart from germination values that were recorded daily, at the end of the planting period, seedling height, % germination, germination rate, germination time, germination capacity, germination index and seeds vigour of each of the slots were estimated.

2.5 Assessment of Protective Potential of the Extracts

Using the optimum concentration and soaking time in each extracts, the protective potential of the extracts against selected fungal pathogens was investigated. The fungal pathogens used for the experiment were *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*. The isolates were already sub cultured on sabouraud dextrose broth after confirming them as pure cultures on sabouraud dextrose agar plates after incubation at 25℃ for 72 h. The experimental groups were as follows: seeds soaked in the respective extracts only; seeds soaked in broth cultures of the respective fungal pathogens; seeds that were soaked in the broth cultures of the fungal pathogens before soaking in the extracts and seeds soaked in distilled water only.

In all experimental groups, apart from the groups that were first soaked in broth cultures of the pathogens before treatment with the extracts, the soaking time before planting was 60 min. In the group that were first infected before treatment, the soaking time was 60 min each in the broth cultures of the pathogens and in the extracts.

As was carried out in the optimum concentration and soaking times, after planting, daily germination values were recorded while at the expiration of incubation, seedling height, % germination, germination rate, germination time, germination capacity, germination index and seeds vigour of each of the slots were estimated.

2.6 Statistical Analyses

All statistical analyses were carried out using the SPSS statistical software package. Comparison of means were determined using the One-Way Analysis of Variance Test at 95% confidence interval. All experimental set were in duplicate.

3. RESULTS AND DISCUSSION

3.1 Effect of Extracts Concentration

As shown in Table 1, at the end of the planting period, at different concentrations of the aqueous extracts of *Annona senegalensis*, germination capacity, germination index, and germination rate was observed to range from 0.09-4.25, 1.15- 7.87 and 0.19-0.20 d^{-1} respectively. For germination capacity and germination rate, the highest and lowest values of extracts concentration were recorded at 2000 mg/L and 4000 mg/L. At the different concentrations of extract, in the case of germination time, % germination and seed vigour values showed values from 5.00–5.75 days, 7.15–78.57 % and 0–422.36, respectively. The highest values for germination time, % germination and seed vigour were recorded at concentrations of 1000 mg/L, 2000 mg/L and 6000 mg/L, respectively (Table 1). Generally, % germination at aqueous extract concentration of 2000 mg/L was observed to be significantly higher than most of the other concentrations ($p \leq 0.05$).

Table 1. Germination and vigour parameters of the sorghum seeds at the different concentrations of the extracts

Concentration (mg/L)	GC	GI	GR (d^{-1})	GT (d)	%G	SVI
Aqueous extract						
1000	0.29	1.94	0.19	5.35	21.43	108.65
2000	4.25	7.81	0.19	5.26	78.57	200.36
3000	0.40	3.19	0.20	5.11	28.57	90.57
4000	0.09	1.15	0.20	5.00	7.15	0.00
5000	1.04	5.26	0.19	5.20	50.00	162.51
6000	1.92	6.59	0.19	5.25	64.29	422.36
7000	0.58	3.88	0.19	5.15	35.72	247.51
8000	0.87	4.65	0.19	5.15	42.86	224.13
9000	0.58	3.88	0.19	5.15	35.72	57.15
10000	0.87	3.90	0.19	5.33	42.86	111.42
n-hexane extract						
1000	0.46	2.46	0.18	5.52	28.58	21.44
2000	0.38	1.36	0.18	5.46	21.43	32.15
3000	0.46	1.96	0.17	5.80	28.58	37.15
4000	0.29	2.58	0.20	5.00	21.43	0.00
5000	0.38	1.45	0.18	5.64	21.43	48.22
6000	1.34	2.72	0.17	5.93	42.86	180.01
7000	0.87	3.26	0.18	5.66	42.86	81.43
8000	0.75	2.35	0.16	6.15	42.86	166.30
9000	0.58	2.61	0.18	5.69	35.72	116.08
10000	0.29	0.60	0.15	6.88	21.43	23.58
Ethanol extract						
1000	0.75	3.90	0.19	5.42	42.86	50.15
2000	1.63	5.91	0.19	5.22	57.15	211.44
3000	0.46	1.99	0.18	5.56	28.58	85.73
4000	0.00	3.91	0.17	5.74	57.14	128.57
5000	0.00	0.00	0.00	0.00	0.00	0.00
6000	000	0.00	0.00	0.00	0.00	0.00
7000	0.00	0.00	0.00	0.00	0.00	0.00
8000	0.00	0.00	0.00	0.00	0.00	0.00
9000	0.00	0.00	0.00	0.00	0.00	0.00
10000	0.09	0.22	0.14	7.00	7.15	19.65

The soaking time of the seeds in the extract was 30 min. Values are averages of duplicate samples. GC, GI, GR, GT, % G and SV represent germination capacity, germination index, germination rate, germination time, % germination and seed vigour index, respectively

At the different concentrations of the n-hexane extract, germination capacity, germination index and germination rate at the end of the planting period were observed to range from 0.29 – 1.34, from 0.60–3.26 and from 0.15–0.20 d^{-1}, respectively. The highest and lowest values for the germination capacity were recorded at 6000 mg/L and 10000mg/L, respectively, while that for germination rate was observed at 4000 mg/L and 10000 mg/L, respectively. At the expiration of the period of planting, germination time, % germination and seed vigour values at the different concentrations of the n-hexane extract were observed to range from 5.00–6.88 d, from 21.43–42.86% and from 0–180.01, respectively (Table 1). Although the highest germination was observed at concentration of 7000 mg/L, this was not observed to be significantly different from the other concentrations used for investigation ($p \leq 0.05$).

In the case of ethanol extract, at the end of the planting period, the germination index, germination rate, and germination capacity values were observed to range from 0 – 5.91, 0 – 0.19 d^{-1} and 0 – 1.63, respectively. The highest values for the germination rate and capacity, which were at extract concentration of 2000 mg/L were observed to be 0.19 and 1.63, respectively. The % germination, seed vigour and germination time values was observed to range from 0 – 57.15 %, 0- 211.44, and 0 - 7.00 d, respectively. The highest values of germination time, seed vigour and % germination were recorded at extract concentration of 10000 mg/L for germination time and 2000 mg/L for seed vigour and % germination (Table 1). The % germination at the end of the planting period was observed to be significantly higher than most of the other concentrations used for investigation ($p \leq 0.05$).

In the present study, optimum concentrations of the extracts that enhanced germination and vigour of the sorghum seeds were observed to be 2000 mg/L for the aqueous and ethanol extracts, 7000 mg/L for the n-hexane extract and 5000 mg/L for the copper sulphate solution. In a study by Kiran and coworkers [16], when evaluating the aqueous extract of seeds of *Psoralea corylifolia* on seed mycoflora, germination and vigour of maize seeds, a 20 % concentration of the extract was observed to be more effective that a 50% concentration. In the study, a 20 % extract concentration was reported to show high increase in seed germination (80 %) and seed vigour index (1398.5) than at extract concentrations of 30%, 40% and 50%. At 50% extract concentration, seed germination was observed to be inhibited. High extract concentrations above the optimum is reported to be phytotoxic to seeds [16].

In a laboratory study to assess the growth inhibitory effect of different concentrations leaf extracts of *Albizia lebbeck* and their possible phytotoxicity on some common agricultural crops, it was reported that the aqueous extracts of *Albizia lebbeck* leaf caused both stimulatory and inhibitory effect on germination, root and shoot elongation and development of lateral roots of receptor plants. The results of the bioassays showed that the inhibitory effects were more pronounced at higher concentrations of the extracts while at lower concentrations, stimulatory effects were observed [17]. The inhibitory effects of extracts on seed germination and growth have also been reported to concentration dependent, as the degree of inhibition increases with increase in concentration of the extracts [18].

In a study on the effect of aqueous leaf extract of *Ocimum basilicum* and *Artemisia absinthium* on tomato seed quality, plant leaf extracts 10 % (w/v) was used. In presence of the 10 % extracts concentrations, 40% and 60% germination were observed for the tomato seeds treated with the *Ocimum basilicum* and the *Artemisia absinthium*, respectively. Also, maximum shoot lengths of 6.5 cm and 6 cm were observed for the tomato seeds in presence of the *Ocimum basilicum* and *Artemisia absinthium*, respectively [19].

3.2 Effect of Soaking Time

At the different soaking period of the sorghum seeds in the aqueous extract before planting, the values for germination capacity, germination index and germination rate at the end of the planting period were observed to range from 0.28 – 6.50, from 1.70 – 7.89, and from 0.15 – 0.17 d^{-1}, respectively. The highest and lowest values of germination capacity and index were recorded at 60 min and 150 min, respectively while % germination, seed vigour and germination time of the seeds showed a range from 21.43% - 92.86%, from 14.29 – 311.72 and from 5.76 – 6.68 d. The highest values of % germination and seed vigour were seen at soaking time of 60 min (Table 2). The % germination at 60 min soaking time was observed to be significantly higher than germination at other soaking times ($p \leq 0.05$).

When soaked at different times in the n-hexane extract, germination capacity, germination index and germination rate at the expiration of the planting duration ranged from 0.08–1.73, from 0.27–3.70, and from 0.13–0.16 d^{-1}, respectively. The highest and lowest values of germination capacity were documented at 60 min and 90 min soaking time while those for germination index were 30 min and 90 min, respectively. The % germination, seed vigour and germination time ranged from 7.15%- 42.86%, from 8.93–209.99 and from 6.13–7.50 d, respectively. The highest % germination was recorded at 30 min soaking time while that for seed vigor was recorded at 60 min soaking time (Table 2). Although % germination at 30 min and 60 min were not observed to differ significantly, they were significant higher than those at other soaking times ($p \leq 0.05$).

For seed soaked in the ethanol extract, the values for germination capacity, index and rate were observed to range from 0.58–3.38, from 2.57–4.65 and from 0.15–0.16 d^{-1}, respectively. The minimum and maximum values of germination capacity and germination index were observed at soaking times of 180 min and 30 min. The range of values for % germination, seed vigour and germination time were from 35.72% to 64.29%, from 28.57 to 136.61 and from 6.30 to 6.91 d. The highest values for % germination and seed vigour were observed at soaking times 30 min (Table 2). The % germination at 180 min soaking time was observed to be significantly lower than germination at other soaking times ($p \leq 0.05$).

For seeds soaked in normal saline solution containing suspended cells of *Pseudomonas aeruginosa*, at the end of the planting period, the values of germination capacity, germination index and germination rate showed a range from 0.87 to 3.86, from 1.40 to 5.40 and from 0.13 to 0.16 d^{-1}, respectively. The minimum and maximum values for the germination capacity were observed at 180 min and 150 min while that for germination index was observed at 90 min and 150 min, respectively. The ranges of % germination, seed vigour and germination time were recorded as 42.86%-85.72%, 21.43–235.12, and 6.13–7.50 days respectively. The highest values of % germination and seed vigour was noticed at soaking time 180 min. (Table 3).

Table 2. Germination and vigour parameters of the sorghum seeds at the different soaking times in the extracts

Soaking time(min)	GC	GI	GR (d^{-1})	GT (d)	%G	SVI
Aqueous extract						
30	1.92	6.98	0.17	5.76	64.29	265.18
60	6.50	7.89	0.16	6.15	92.86	311.72
90	0.46	1.72	0.16	6.45	28.58	14.29
120	0.40	1.78	0.15	6.68	28.57	119.05
150	0.28	1.70	0.16	6.26	21.43	171.47
180	3.38	4.16	0.15	6.60	64.29	53.55
n-hexane extract						
30	1.33	3.70	0.16	6.13	42.86	58.94
60	1.73	3.30	0.16	7.00	42.86	209.99
90	0.08	0.27	0.13	7.50	7.15	8.93
120	0.67	1.32	0.14	7.17	28.57	132.37
150	0.58	2.60	0.16	6.40	35.72	125.01
180	0.75	1.22	0.14	7.25	35.72	17.86
Ethanol extract						
30	3.38	4.65	0.16	6.38	64.29	136.61
60	3.00	3.30	0.16	6.30	42.86	123.21
90	1.33	3.39	0.15	6.51	57.14	28.57
120	1.45	2.69	0.15	6.91	50.00	118.75
150	1.92	3.82	0.15	6.70	64.29	112.5
180	0.58	2.57	0.16	6.32	35.72	89.29

Values are averages of duplicate samples. The concentration of extract used was 2000 mg/L for the aqueous and ethanol extracts and 7000 mg/L for the n-hexane extracts.

GC, GI, GR, GT, %G and SV represent germination capacity, germination index, germination rate, germination time, % germination and seed vigour index, respectively

At the end of the planting period, when the seeds were soaked in suspended cells of Bacillus *subtilis* in normal saline solution, germination capacity, germination index and germination rate were observed to range from 0.58 to 6.5, from 2.41 to 7.48 and from 0.15 to 0.17 d^{-1}, respectively. The minimum and maximum values for germination capacity and germination index were observed at 180 min and 60 min and at 30 min and 120 mi, respectively. The range of values for the % germination, seed vigour and germination time were from 35.72% to 92.86, from 0 to 694.01 and from 5.97 to 6.53 d, respectively (Table 3).

When seeds were soaked in distilled water, at the end of the planting period, the values of germination capacity, germination index and germination rate were observed after the planting period to range from 0.17 to 4.17, from 0.94 to 6.76 and from 0.15 to 0.17 d^{-1}, respectively. The highest and lowest values of germination capacity and germination index were recorded at soaking times 30 min and 150 min, respectively. The % germination, seed vigour index and germination time of the seeds at the end of planting showed a range from 14.29 % to 78.57 %, from 3.57 to 319.78 and from 5.93 to 6.72 d while the highest values of % germination and seed vigour index occurred at soaking time 30 min (Table 3).

This study revealed optimum soaking time of the seeds in the extract to be 60 min in the aqueous extract of the *Annona senegalensis* leaves and 30 min in both the ethanol extract, n-hexane extract and the coper sulphate solution. The present study investigated soaking times of 30, 60 90, 120, 150 and 180 min of the seeds in the extracts. Earlier workers have used similar soaking time in similar studies. When investigating the potency of mustard and ginger rhizome extracts, lemon juice, Atella (residue of traditional Ethiopian beer) and cow urine in controlling tomato seed borne pathogen, *Xanthomonas campestris pv. vesicatoria* (Xcv), soaking times 30 min, 3 h, 12 h or 24 h [20].

Germination can be affected, from chemicals that get into the seed, after seed soaking to soften the hard seed coat [21,22]. In a study that evaluated the aqueous extracts of leaves of *Moringa oleifera and Annona muricata* for the control of *Collectotrichum destructivum* on cowpea seeds, soaking times of the seeds in the extract before planting were indicated to be of 6,

Table 3. Germination and vigour parameters of the sorghum seeds at the different soaking times in *Pseudomonas aeruginosa* suspension in normal saline

Soaking time(min)	GC	GI	GR (d^{-1})	GT (d)	%G	SVI
***Pseudomonas aeruginosa* suspension**						
30	1.92	3.87	0.15	6.63	64.29	53.55
60	1.04	4.26	0.16	6.13	50.00	166.65
90	1.92	5.40	0.16	6.17	64.29	96.43
120	1.04	2.92	0.15	6.75	50.00	83.50
150	0.87	1.40	0.13	7.50	42.86	21.43
180	3.86	4.57	0.15	6.50	85.72	235.12
***Bacillus subtilis* suspension**						
30	4.17	7.48	0.17	5.97	78.57	694.01
60	0.58	3.32	0.17	6.00	35.72	35.72
90	0.87	2.88	0.16	6.42	35.72	0.00
120	0.58	2.41	0.15	6.55	35.72	38.11
150	1.45	2.45	0.15	6.52	35.72	142.86
180	6.5	7.18	0.16	6.28	92.86	286.74
Distilled water						
30	4.17	6.76	0.16	6.08	78.57	319.78
60	1.63	5.57	0.17	5.93	57.15	85.72
90	1.33	3.65	0.16	6.15	42.86	75.01
120	3.70	3.83	0.15	6.72	64.29	109.29
150	0.17	0.94	0.15	6.55	14.29	3.57
180	1.04	3.49	0.15	6.48	50.00	237.51

Values are averages of duplicate samples. GC, GI, GR, GT, %G and SV represent germination capacity, germination index, germination rate, germination time, % germination and seed vigour index respectively

12 and 18 h. In the investigation, all concentrations of the plant extracts used at the different time soaking hours reduced the incidence of *C. destructivum*. Although complete control was obtained with at 18 h soaking period. There were significant differences in the incidence of *C. destructivum* on seed treated with the different concentrations of the extracts of *A. muricata* and at different time of exposure, soaking periods. The incidence of *Collectotrichum destructivum* was indicated to be reduced significantly to 18.6% on the cowpea seeds treated with 10% extracts for 18 h while complete control was attained in seeds that were soaked for 18 h and 12 h at 20 and 30% of the extract, respectively [23]. In evaluating the effectiveness of the aqueous extracts of *Balanites aegyptiaca*, *Cymbopogon citratus*, *Cassia occidentalis* and *Portulaca oleracea* at different soaking times of 6 12, 24 and 48 h, Schémaeza et al. [24] reported that long duration of soaking and maceration were favourable in increasing the antifungal efficiency of the extracts.

3.3 Protective Potential of the Extracts against Pathogens

When the seeds were soaked in cell suspensions of *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*, no germination was obtained. All the pathogens were ascertained to initiate infections of the seeds and initiate infection. For seeds soaked in the extracts only, germination capacity, germination index, % germination and seed vigour were observed to range among the extracts from 0.58 to 6.00, from 1.82 to 5.81, from 35.72% to 85.71% and from 142.88 to 495.40, respectively (Table 4).

In seeds that were first infected with the fungal pathogens before treating with the aqueous extract before planting, % germination, seed vigour, germination index, germination capacity, germination rate and germination time at the end of the planting period were observed to range from 50% to 64.29%, from 385.71 to 425.00, from 4.26 to 4.31, from 1.04 to 3.38, from 0.16 d^{-1} to 0.17 d^{-1} and from 5.80 d to 6.29 d, respectively. When seeds were first infected and soaked in the n-hexane extract before planting, % germination, seed vigour, germination index, germination capacity, germination rate and germination time at the end of the planting period were observed to range from 28.58% to 50.00%, from 185.79 to 435.00, from 1.09 to 2.93, from 0.46 to 1.04, from 0.15 d^{-1} to 0.16 d^{-1} and from 6.06 d to 6.64 d, respectively. For the seeds that were infected, then treated with the ethanol extract, % germination, seed vigour, germination index, germination capacity, germination rate and germination time at the end of the planting period were observed to range from 28.57% to 78.57%, from 237.91 to 403.84, from 1.81 to 5.60, from 0.40 to 4.17, from 0.17 d^{-1} to 0.16 d^{-1} and from 5.51 d to 5.95 d, respectively (Table 4).

In the present study, when the seeds were soaked in broth suspended cells of the *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* no germination was observed throughout the period of planting. The presence of fungal pathogens (*Aspergillus* species) significantly reduces the germination of seeds as well as shoot and root elongation causing pre and post emergence mortality of seedlings [25]. Similar observation has been reported in similar studies by Hayden and Maude, [26].

Pathogenic fungi are known to produce mycotoxins which can penetrate seed coats, endosperm and cotyledon [27]. In a study on the examination of the effects of 7-day- old fungal filtrates of *Penicillium chrysogenum* and *Aspergillus niger* isolated from maize seeds on the morphology and percentage germination of maize seeds soaked in culture filtrates before planting on blotters, the results showed that the percentage germination of the seeds treated with culture filtrates of the fungi were significantly lower than that of the control [28].

Inhibition of seed germination by *Aspergillus* species has also been reported by Ibraheem et al. [29]. In their investigation, they observed that seed germination by *Aspergillus niger*, *Aspergillus flavus* and *Alternaria alternate* may be due to the production of toxic metabolites in the broth during growth. Haikal, [30] has also reported that *Aspergillus niger* and *Penicillium chrysogenum* have the ability of producing metabolites that reduce germination and seedling development.

In this study, it was observed that seeds treated with any of the extract or copper after initially infected with the fungal pathogens showed high percentage germination and seed vigour. Previous studies on cowpea seeds with aqueous extract of the leaves of *Vernonia amygdalina*, *Annona muricata* and *Moringa oleifera*, revealed that the extracts controlled the growth of *Collectotrichum destructivum*, with the *Moringa oleifera* extract showing more effectiveness [23].

Table 4. Germination and vigour parameters of the pre-infected and treated seeds before planting

Treatments	GC	GI	GR (d^{-1})	GT (d)	%G	SVI
Seeds pre-infected with broth cultures of the fungal pathogens only						
Aspergillus niger	0.00	0.00	0.00	0.00	0.00	0.00
Aspergillus flavus	0.00	0.00	0.00	0.00	0.00	0.00
Aspergillus fumigatus	0.00	0.00	0.00	0.00	0.00	0.00
Seeds pre-treated with the extracts only						
Aqueous	0.87	2.74	0.17	5.93	42.86	368.55
n-hexane	3.58	3.45	0.17	6.00	57.15	350.87
Ethanol	0.58	1.82	0.17	6.05	35.72	142.88
Seeds that were first infected before treating with the aqueous extract						
Aspergillus niger	3.38	4.31	0.17	5.80	64.29	385.71
Aspergillus flavus	1.04	3.12	0.17	5.94	50.00	425.00
Aspergillus fumigatus	1.04	2.46	0.16	6.29	50.00	412.50
Seeds that were first infected before treating with the n-hexane extract						
Aspergillus niger	1.04	2.93	0.16	6.06	50.00	435.00
Aspergillus flavus	1.04	2.05	0.15	6.58	50.00	270.00
Aspergillus fumigatus	0.46	1.09	0.15	6.64	28.58	185.79
Seeds that were first infected before treating with the ethanol extract						
Aspergillus niger	3.08	4.22	0.18	5.51	50.00	358.50
Aspergillus flavus	0.40	1.81	0.17	5.95	28.57	237.99
Aspergillus fumigatus	4.17	5.60	0.17	5.76	78.57	403.84

Values are averages of duplicate samples. GC, GI, GR, GT, %G and SV represent germination capacity, germination index, germination rate, germination time, % germination and seed vigour index respectively

Several studies proved the efficacy of diverse plant extracts as vaibale sources of eco-friendly and natural fungicides [31,32,33]. It was observed by Kahkashan and Biswas [34] that the extracts of plant parts like bark of *Terminalia arjuna*, *Eucalyptus lanceolotus*, tubers of *Cyperus rotundus*, leaves of *Withamia sominifera*, *Datura stromonium* and *Parthenium hysterophorus*, cloves of *Allium sativum*, bulb of *Allium cepa*, fruits of *Emblica officinalis* and rhizome of *Zingiber officinale* when used for seed treatment, significantly increased seed germination and development of tomato plants in both glass house and blotters.

4. CONCLUSION

This study, which examined the protective potential of *Annona senegalensis* leaf extracts and copper sulphate against selected fungal pathogens of sorghum seeds as well as their effects on vigour and germination of the seeds was able to reveal optimum concentrations of the aqueous, n-hexane and ethanol extracts of *Annona senegalensis* that will enhance germination and seedling vigour of sorghum seeds to be 2000 mg/L, 7000 mg/L, 2000 mg/L and 5000 mg/L respectively. Further increases in the concentrations did not improve germination.

The optimum soaking time of the seeds in the extracts was 30 min. All the pathogens were able to introduce infections and inhibit germination in the respective seeds when soaked before planting. The extracts were observed to effectively treat seeds that were initially infected with the pathogens. Seeds treated with the extracts had a higher germination percentage, seed vigour, and other germination parameters than seeds that were soaked in water only.

The study revealed that the aqueous, ethanol and n-hexane extracts obtained from the leaf of *Annona senegalensis* could increase germination and other growth parameters of the seeds when treated with them before planting on blotters. It also showed that seeds germinated better when treated or soaked in aqueous extract of *Annona senegalensis* suggesting it as an effective extract of *Annona senegalensis*. Furthermore, results indicate that ethanol and water are better solvents than n-hexane for the extraction of the antibacterial properties of the *Annona senegalensis*.

Although further studies still need to be carried out in green house and field, the study was able to provide insights to the germination and vigour enhancement, enhancement properties, as well

as the protective potential of the extract under the experimental conditions investigated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Remedies in South-western Nigeria. African Journal of Biotechnology. 2006; 7(5):1078-1081.
2. Atawodi SE, Bulus T, Mamman M. Bioassay guided fractionation and anti-trypanosomal effect of fractions and crude aqueous and methanolic extracts of *Terminalia avicennioides* (Guill& Perr). Prts. International Journal of Biology. 2011; 3(3):19-30.
3. Nostro Germano MP, Angelo V, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology. 2000;30(50):379-384
4. Alagesaboopathi C. Antimicrobial screening of selected medicinal plants in Tamilnadu, Indian. African Journal of Microbiology Research. 2011;5:617-621.
5. Radhika B, Murthy JV, Nirmala Grace D. Preliminary phytochemical analysis and antibacterial activity against clinical pathogens of medicinally important orchid *Cymbidium aloifolium* (l.) Sw. International Journal of Pharmaceutical Sciences and Research. 2013;4(10):3925-393.
6. Fabry W, Okemo PO, Ansorg R. Antibacterial activity of East African medicinal plants. Journal of Ethnopharmacology. 1998;60(1):79-81.
7. Hadacek F. Secondary metabolites as plant traits: Current assessment and future perspectives. Critical Reviews in Plant Sciences. 2002;21:273-322.
8. Bernard M. Prevention of plant disease; 2010.
   Available:ucanr.ed/sites/mgslo/newsletters/prevention_of_plant_disease28073.html
9. Meah MB, Islam MR, Islam MM. Development of an integrated approach for the management of phomopsis blight and fruit rot of eggplant in Bangladesh. Annual Research Report. 2004;57-58.
10. Celar F, Valic N. Effects of *Trichoderma* spp and *Glicladium roseum* culture filtrates on seed germination of vegetables and maize. Journal of Plant Disease Protection. 2005;112(4):343-350.
11. Mukhopadhyay AN. Bio control of soil borne fungal plant pathogens-current status, future prospect and potential limitations. Indian Phytopathology. 1994; 47:119-126.
12. Suriyavathana M, Usha V, Shanthanayaki M. Studies on phytochemical analysis and antioxidant activity of selected medicinal plants from Kolli hills. Journal of Pharmacy Research. 2010;2:260-262.
13. Pal GK, Kumar P, Kumar P. Enhancing seed germination of maize and soyabean by using botanical extracts and *Trichoderma harzianum*. International Journal of Current Discoveries and Innovations. 2013;2(3):72-74.
14. Cesar G. Phenolic compounds of plant origin and human health: Wiley, Hoboken N.J; 2009.
15. Harborne B, William A. Advances in flavonoid research since 1992. Phytochemistry. 2000;55:481-504.
16. Kiran B, Lalitha V, Raveesha KA. Evaluation of aqueous extract of seeds of *Psoralea corylifolia* L. on seed mycoflora, seed germination and seedling vigor of maize seeds. International Journal of Life Science and Pharmacy Research. 2012; 2(2):40-45.
17. Hossain MK, Ahmed R, Uddin MB. Growth inhibitory effects of different concentrations of water extracts of *Albizia lebbeck* on some agricultural crops. Paper Submitted to the XII Worlds Forestry Congress 2013, Quebec City, Canada; 2013.
18. Marraiki N. Investigating the effect of aqueous medicinal leaf extract on tomato seed quality. Biotechnology Research Asia. 2013;10(2):843-847.
19. Ayeni MJ, Kayode J. Laboartory studies on the effects of aqueous extract from *Sorghum bicolour* stem and *Zea mays* (roots and tassel) on the germination and seedling growth of okra (*Abelmoscus esculentus*). Advances in Agriculture. 2014;2014:1-6.
20. Kebede S, Ayalew A, Yusuf M. Efficacy of plant extracts, Traditional materials and antibacterial chemicals against *Xanthomonas campestrus* pv. Vesicatoria on tomato seeds. African Journal of Microbiological Research. 2013;720:2395-2400.
21. Finkelstein RR. Hormones in seed development and germination. *In*: Plant

hormones: Biosynthesis, signal transduction and action, Davies, PJ. (ed.). Kluwer Academic Publishers; 2004.
22. Kucera B, Cohn MA, Leubner-Metzger G. Plant hormone interactions during seed dormancy release and germination. Seed Science Research. 2005;15:281–307.
23. Akinbode OA, Ikotun T. Efficacy of certain plant extracts against seed-borne infection of *Collectotrichum destructivum* on cowpea (*Vigna uniguculata*). African Journal of Biotechnology. 2008;7(20):3683-3685.
24. Schemaeza B, Irenee S, Elisabeth Z, Toudou A. Efficacy of plant extracts and seed soaking duration on treatment of sorghum seeds naturally infected by *Collectotrichum graminicola* and *Phoma sorghina*. Archives of Phytopathology and Plant protection. 2012;45(12):1405-1410.
25. Gupta R, Khokhar MK, Lal R. Estimation of deteriorative effect of *Aspergillus niger* on seed germination and seedling vigour. International Journal of Plant Science. 2014;9(2):333-336.
26. Hayden NJ, Maude RB. The role of seedborne *Aspergillus niger* in transmission of black mold of onion. Plant Pathology. 1992;41: 573-581.
27. Patil DP, Muley SM, Pawar PV. Impact of fungal culture filtrate (mycotoxins) on seed germination of some pulses. World Journal of Science and Technology. 2012;2(8):01-02.
28. Garuba T, Abdulrahman AA, Olhang GS, Abdulkareem KA, Amadi JE. Effects of fungal filtrates on seed germination and leaf anatomy of maize seedlings (*Zea mays* L. Poaceae). Journal of Applied Sciences and Environmental Management. 2014;18(4):662-667.
29. Ibraheem SA, Okesha AM, Mlhathem KT. Inter-relationship between protein and oil content of soybean seed with some associated fungi. Journal of Agriculture and Water Resources Research Plant Production. 1987;6:53-66.
30. Haikal NZ. Effect of Filtrates of Pathogenic Fungi of Soybean on Seed Germination and Seedling Parameters. Journal of Applied Sciences Research. 2008;4:48-52.
31. Arokiyaraj S, Martin S, Perinbam K, Marie AP, Beatrice V. Free radical scavenging activity and HPTLC finger print of *Pterocarpus santalinus* L. – an *in vitro* study. Indian Journal of Science and Technology. 2008;1(7):1-7.
32. Brindha V, Saravanan A, Manimekalai R. Drug designing for ring finger protein 110 involved in adenocarcinoma (human breast cancer) using casuarinin extracted from *Terminalia arjuna*. Indian Journal of Science and Technology. 2009;2(2):22-26.
33. Gurjaret MS, Ali S, Akhtar M, Singh KS. Efficacy of plant extract in disease management. Agricultural Science. 2012; 3(3):425-433.
34. Kahkashan AS, Biswas SK. Effect of plant extracts as seed treatments on growth parameters, seedlings mortality and biochemical changes in tomato. International Journal of Bio-Resource & Stress Management. 2013;4(1):47-48.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17289