American Journal of Plant Biology
2022; 7(1): 41-46
http://www.sciencepublishinggroup.com/j/ajpb
doi: 10.11648/j.ajpb.20220701.16
ISSN: 2578-8329 (Print); ISSN: 2578-8337 (Online)

Effect of Some Seed Borne Fungi on Seed Germination and Seedling Emergence of Soybean and Antifungal Impact of Certain Botanicals

Owolabi Abibat Olayinka, Adegboyega Ayodele Sobowale*, Odebode Adegboyega Christopher

Department of Botany, University of Ibadan, Ibadan, Nigeria

Email address:

delesobowale@yahoo.com (A. A. Sobowale)

*Corresponding author

To cite this article:

Owolabi Abibat Olayinka, Adegboyega Ayodele Sobowale, Odebode Adegboyega Christopher. Effect of Some Seed Borne Fungi on Seed Germination and Seedling Emergence of Soybean and Antifungal Impact of Certain Botanicals. *American Journal of Plant Biology.* Vol. 7, No. 1, 2022, pp. 41-46. doi: 10.11648/j.ajpb.20220701.16

Received: January 11, 2022; **Accepted**: February 7, 2022; **Published**: February 16, 2022

Abstract: An investigation was carried out to examine the effect of some seed borne fungi on seed germination and seedling emergence of soybean. A total of five fungi species comprising four genera namely *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*. *Penicillium chrysogenum* and *Colletotrichum sp* were isolated from the tested seeds. The effects of the mycelial suspension of the isolated fungi on percentage seed germination and seedling emergence was studied. Ten seeds were soaked in mycelial suspension of each fungus for 1hour before placing on Petri-dishes and sowing in bags containing 3 kg sterilized soil in triplicates. It was observed from the study that *Penicillium chrysogenum* had the highest inhibitory effect on seed germination while *Aspergillus niger* had no effect on seed germination. Also, the result showed that all the isolated fungi had a significant effect ($p<0.05$) on length of radicle with *A. flavus* recording the least length of radicle (2.10 cm) as compared with the control (4.75 cm). *Fusarium oxysporum*, *Penicillium chrysogenum* and *A. flavus* significantly reduced shoot length. While all the isolated fungi had significant differences at $p<0.05$ on root length with *P. chrysogenum* recording the least root length (5.15 cm) as compared with 10.13 cm observed on the control. The efficacy of both the aqueous and methanol extracts of *Aframomum melegueta* and *Petiveria alliacea* was assessed against the mycelial growth of the isolated fungi at 10, 20 and 30% concentration. The methanol extract of both plants most especially that of *Aframomum melegueta* performed better in inhibiting the radial growth of *Aspergillus flavus*, *Aspergillus niger*, Fusarium oxysporum and *Colletotrichum sp*.

Keywords: Seed Borne Fungi, Pathogenicity, Seedling Emergence, Biocontrol

1. Introduction

Soybean (*Glycine max* (L.) Merrill) known as "golden bean" is an annual legume categorized under Fabaceae family. Soybean is an excellent source of major nutrients, about 40% of dry matter is protein and 20% fat [1]. Etiosa *et al*. reported that the calcium and iron present in soybean seeds are 300.36mg/100g and 16.4mg/100g respectively [2]. However, several seed borne fungal diseases have been known as one major limiting factor of soybean production [3].

According to Tawale and Pawar, production constraint faced by soybean grower was calculated in frequency, the attack of insect pests and diseases was found to be 74.45% [4]. Seed-borne pathogens are agents that are found internally or externally in seeds and have the potential to cause diseases in plants [5, 6]. Akranuchat *et al.* reported that seed borne pathogens have been involved in seed rots during germination and seedling mortality leading to poor crop stand reduction in plant growth and productivity of crops [7]. Several pathogenic fungi like *Rhizopus, Alternaria, Culvularia, Diaporthe, Mucor, Corynespora, Cercospora, Colletotrichum, Phoma, Pythium, Fusarium, Aspergillus*, and *Cladosporium* have been isolated from soybean seeds [8, 9]. As reported by Welbaum, soya protein contains essential amino acids and its oil is also a rich source of plant fatty acids [10]. These constituents are attractive to a wide range of fungal pathogens to survive and multiply themselves.

Application of synthetic fungicides is the most widely employed method of controlling pathogens and has helped to reduce pathogenic fungi associated with soybean. However, the major problems with the constant use of synthetic fungicide is that resistance can be induced in fungi and increases the risk of high-level toxic residues to health and environment concerns [11]. Seed treatment with living organisms has been confirmed to be an economical and viable method of protecting seeds and seedlings from the activities of pathogens. Plants have been recognized long back as a potential source of bioactive chemicals known as phytochemicals with potential antifungal agent and are biodegradable to non-toxic products [12]. This study, therefore examined the effect of seed-borne fungi on seed germination and seedling emergence of soybean and the antifungal ability of two plant extracts against the isolated fungi.

2. Materials and Method

2.1. Sample Collection

Soybean seeds were obtained from Bodija market. The seed samples were brought to Plant Pathology Laboratory University of Ibadan and stored at room temperature for subsequent uses.

Isolation and identification of fungi

Seed samples were analyzed for detection of seed borne fungi using Blotter technique and Agar plate method. Soybean seeds were randomly picked and surface sterilized using 1% sodium hypochlorite for 1 minute. The seeds were rinsed in three changes of distilled water and placed on dry filter paper to remove excess water. In the Blotter method, 10 sterilized seeds were placed on 3 layered moistened filter papers while in the agar plate method, 10 seeds were put in Petri dishes containing sterilized potato dextrose agar medium in triplicate and incubated for 7 days at 28°C. Identification was done using morphological structures and microscopic characteristics.

2.2. Effect of the Isolated Fungi on Seed Germination and Seedling Emergence

Seven days old culture of each isolated fungus was flooded with 10 ml of distilled water and transferred into sterilized labelled test tubes. Thirty sterilized seeds were soaked in each of the suspension for an hour while seeds soaked in distilled water served as the control. Ten inoculated seeds were plated on a Petri dish containing 3 layers of filter paper moistened with sterile distilled water to test effect of the isolated fungi on seed germination. Their effect on seedling emergence was carried out by sowing ten inoculated seeds in bags containing 3 kg of sterilized soil in triplicate. Observation on seed germination, length of radicle, shoot and root length were recorded after 5 days.

2.3. Collection of Plant Samples

Two plants materials were used for the biocontrol: the seed of *Aframomum melegueta* and the leaves of *Petiveria alliacea*. *A. melegueta* was obtained from Moniya market, Ibadan Oyo state and *Petiveria alliacea* was collected from the nursery unit of the Department of Botany University of Ibadan.

2.4. Preparation of Plant Extract

Fresh leaves of *P. alliacea* and seed of *A. melegueta* were washed with distilled water and air dried in the laboratory. The two plant samples were pulverized separately using household blender. Cold water extraction was done for the aqueous extract in which 140 g of powdered leaf of *P. alliacea* was soaked in distilled water for 72 hours and filtered twice using sterile muslin cloth while 140 g of same leaf was soaked in methanol for 48 hours. The same procedure was used on seeds of *A. melegueta* but 240 g was used for aqueous and methanol extraction. Evaporation of the filtrate of both plants to remove the solvent of extraction was done and crude extract of the plants were stored in reagent bottles in the refrigerator until they were used.

2.5. Determination of Antifungal Activity

The antifungal potential of the samples, in terms of inhibition of radial growth of test fungi, was assessed by Poisoned food technique. Two milliliter from each of the concentration of the extract (10, 20, 30 and 40%) was dispensed separately into 9 cm diameter Petri dishes and 10 ml of already sterilized PDA was poured into each of the plates. The plates were rotated gently to allow even mixing of the extracts and the PDA. A 5 mm disc of 5 days old pure culture of each isolate was inoculated at the point of intersection of two lines drawn at the bottom of each plates and incubated for seven days at 28±2°C. Control experiments were set up without any plant extract. Each concentration was replicated three times. Daily measurements of the mycelial extension of the cultures were determined by measuring culture along two diameters.

2.6. Statistical Analysis

Data collected were characterized and organized into tables using Microsoft excel. Quantitative data were represented as means and standard deviation. Analysis of Variance (ANOVA) was conducted using CoStat 6.451 statistical software. The homogeneity of means was determined using Duncan Multiple Range Test (DMRT).

3. Results and Discussion

A total of five fungi species belonging to four genera were isolated, namely; *Aspergillus niger, A. flavus, Colletothricum sp, Fusarium oxysporum* and *Penicillium chrysogenum*. Agar plate method performed best by isolating all the fungi while *Colletothricum sp* and *Fusarium oxysporum* were not isolated by blotter technique. This agrees with the findings of Alemu and could be due to the carbon compound present in PDA which are known to play two major functions in the

growth of fungi; the supply of carbon for synthesis of compound within the fungi cytoplasm and the production of cellular energy [13]. However, this result contradicts the work of Dawar *et al*. who reported the superiority of blotter technique over agar plate method in chicken pea seeds [14] as well as Shovan *et al.* in soybean seeds [15].

Table 1 shows the effect of the isolated fungi on seed germination and length of radicle. The result showed that *Aspergillus niger* did not reduce seed germination. This is in agreement with the report of Ahammed *et al.* [16] but in contrary with the work of Nahed, [17] who reported that the filtrates of *A. niger* and *Fusarium culmorium* gave the highest reduction in percentage germination. *Penicillium chrysogenum* had the highest inhibitory effect on seed germination as it reduced germination by 26.67% when compared with the control. Similar to this finding, is the report of Garuba *et al*. who observed that *Penicillium chrysogenum* decreased percentage germination of maize by 20.33% [18]. This also supports the work of Rao *et al*. who found out that the filtrates of different *Penicillium* species significantly retarded seed germination, length of radicle and leaf elongation of sorghum [19].

Table 1. *Effect of the isolated fungi on seed germination and length of radicle*

Treatment	Germination (%)	Radicle Length (cm)
Aspergillus niger	100.00	3.59 ± 0.11^{b}
Aspergillus flavus	90.00	2.10 ± 0.07^{c}
Colletotrichum sp	96.67	4.02 ± 0.58^{ab}
Fusarium oxysporum	96.67	3.88 ± 0.46^{b}
Penicillium chrysogenum	73.33	2.76 ± 0.09^{c}
Control	100.00	4.75 ± 0.44^{a}

Means with the same alphabets down the COLUMN are not significantly different at P<0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only

The effect of the isolated fungi on shoot ant root length of soybean seedlings is shown in Table 2. It was observed that *Fusarium oxysporum*, *Penicillium chrysogenum* and *A. flavus* significantly reduced shoot length (14.14 cm, 14.93 cm and 18.33 cm respectively) as compared with the control (24.68 cm). This finding is similar to that of [16] who observed *Fusarium sp* to drastically affect shoot length of seedlings of soybeans. The result obtained with reference to the effect on root length showed that all the isolated fungi had significant effect at p<0.05 on root length with *P. chrysogenum* recording the least root length (5.15 cm) when compared with 10.13 cm recorded in the control. The inhibitory effect of seed borne fungi on radicle and coleoptile growth has been attributed to the production of certain enzymes and toxins produced by these fungi in different crops [20].

Table 2. *Effect of the isolated fungi on shoot and root length of seedlings after 7days of planting*

Treatment	Shoot Length (cm)	Root Length (cm)
Aspergillus niger	22.11 ± 0.53^{b}	6.82 ± 0.25^{b}
Aspergillus flavus	18.33 ± 0.53^{c}	7.71 ± 0.39^{b}
Colletotrichum sp	23.41 ± 0.02^{ab}	6.94 ± 0.94^{b}
Fusarium oxysporum	14.14 ± 0.89^{d}	6.30 ± 0.81^{bc}
Penicillium chrysogenum	14.93 ± 0.59^{d}	5.15 ± 0.09^{c}
Control	24.68 ± 0.04^{a}	10.13 ± 0.22^{a}

Means with the same alphabets down the COLUMN are not significantly different at P<0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only.

Table 3. *Effect of Aframomum melegueta and Petiveria alliacea on the mycelial growth of isolated fungi at 10% concentration.*

F1:	*Aspergillus niger*
F2:	*Aspergillus flavus*
F3:	*Colletotrichum sp*
F4:	*Fusarium oxysporum*
F5:	*Penicillium chrysogenum*

Botanicals	Pathogen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Petiveria alliacea (Aqueous Extract)	F1	1.03 ± 0.11^{b}	2.05 ± 0.14^{a}	3.25 ± 0.14^{a}	4.98 ± 0.11^{a}	6.35 ± 0.14^{a}	7.90 ± 0.14^{a}	8.43 ± 0.04^{a}
	F2	0.98 ± 0.11^{bc}	1.75 ± 0.07^{b}	2.63 ± 0.11^{b}	3.85 ± 0.07^{b}	4.68 ± 0.18^{d}	5.53 ± 0.25^{c}	6.40 ± 0.14^{c}
	F3	0.55 ± 0.07^{fg}	0.83 ± 0.32^{f}	1.60 ± 0.71^{f}	2.83 ± 0.81^{d}	4.03 ± 1.10^{e}	5.30 ± 1.27^{c}	6.53 ± 1.59^{c}
	F4	0.75 ± 0.07^{d}	1.30 ± 0.14^{d}	1.98 ± 0.32^{e}	2.75 ± 0.14^{de}	3.35 ± 0.14^{fg}	3.73 ± 0.11^{f}	4.15 ± 0.14^{ef}
	F5	0.50 ± 0.00^{g}	0.63 ± 0.04^{g}	0.80 ± 0.07^{i}	1.05 ± 0.07^{h}	1.20 ± 0.07^{i}	1.33 ± 0.04^{i}	1.43 ± 0.04^{i}
Petiveria alliacea (Methanol Extract)	F1	0.78 ± 0.04^{d}	1.08 ± 0.04^{e}	1.75 ± 0.14^{ef}	2.55 ± 0.00^{de}	3.23 ± 0.04^{g}	3.83 ± 0.04^{bc}	4.40 ± 0.00^{e}
	F2	0.80 ± 0.07^{d}	1.40 ± 0.28^{d}	2.25 ± 0.64^{cd}	2.85 ± 0.64^{d}	3.45 ± 0.64^{fg}	3.93 ± 0.60^{e}	4.50 ± 0.57^{e}
	F3	0.50 ± 0.00^{g}	0.68 ± 0.04^{g}	0.90 ± 0.07^{hi}	1.05 ± 0.07^{h}	1.33 ± 0.11^{hi}	1.50 ± 0.14^{hi}	1.58 ± 0.11^{hi}
	F4	0.50 ± 0.00^{g}	0.90 ± 0.00^{ef}	1.03 ± 0.04^{h}	1.53 ± 0.11^{g}	2.43 ± 0.04^{ef}	3.10 ± 0.07^{cd}	3.58 ± 0.04^{f}
	F5	0.50 ± 0.00^{g}	1.05 ± 0.07^{e}	1.33 ± 0.04^{g}	1.58 ± 0.04^{g}	1.68 ± 0.04^{h}	1.78 ± 0.04^{h}	1.85 ± 0.07^{h}

Botanicals	Pathogen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Aframomum melegueta (Aqueous Extract)	F1	0.85±0.07^{cd}	1.25±0.49^{b-e}	2.60±0.14^{b}	3.65±0.00^{bc}	4.95±0.07^{c}	5.95±0.07^{c}	7.23±0.25^{b}
	F2	0.90±0.07^{c}	1.50±0.14^{c}	2.28±0.11^{cd}	2.95±0.07^{d}	3.60±0.00^{f}	4.08±0.11^{e}	4.58±0.11^{e}
	F3	0.58±0.04^{f}	1.18±0.04^{e}	2.18±0.18^{d}	3.45±0.14^{c}	4.90±0.21^{c}	6.08±0.18^{bc}	7.28±0.18^{b}
	F4	1.28±0.04^{a}	1.85±0.07^{b}	3.08±0.32^{a}	4.25±0.49^{a}	5.58±0.60^{b}	6.70±0.78^{b}	7.75±0.64^{b}
	F5	0.50±0.00^{g}	0.80±0.07^{f}	0.98±0.04^{h}	1.15±0.07^{h}	1.28±0.04^{i}	1.35±0.00^{i}	1.53±0.04^{hi}
Aframomum melegueta (Methanol Extract)	F1	0.60±0.00^{ef}	0.80±0.14^{f}	1.05±0.00^{h}	1.55±0.35^{g}	1.53±0.11^{hi}	1.75±0.07^{h}	2.05±0.07^{h}
	F2	0.58±0.04^{f}	0.78±0.04^{f}	1.10±0.07^{h}	1.48±0.25^{g}	1.70±0.57^{h}	2.28±0.32^{g}	2.63±0.32^{g}
	F3	0.50±0.00^{g}	0.60±0.07^{g}	0.78±0.04^{g}	1.33±0.25^{gh}	1.83±0.18^{h}	1.98±0.18^{h}	2.23±0.25^{h}
	F4	0.68±0.04^{e}	1.35±0.07^{d}	2.00±0.28^{e}	2.75±0.49^{de}	3.33±0.67^{fg}	4.05±0.85^{e}	4.80±1.06^{d}
	F5	0.50±0.00^{g}	0.63±0.04^{g}	0.80±0.00^{g}	1.18±0.04^{h}	1.60±0.35^{h}	1.68±0.32^{h}	1.75±0.35^{hi}
Control	F1	0.80±0.00^{d}	1.55±0.00^{c}	2.38±0.04^{c}	3.00±0.07^{d}	3.70±0.07^{f}	4.30±0.21^{d}	5.00±0.21^{d}
	F2	1.00±0.00^{b}	1.65±0.21^{bc}	2.15±0.14^{d}	2.58±0.11^{ef}	2.93±0.04^{g}	3.70±0.07^{f}	5.05±0.07^{d}
	F3	0.60±0.07^{ef}	1.18±0.04^{e}	1.85±0.00^{e}	2.35±0.00^{f}	2.63±0.11^{g}	2.80±0.07^{g}	2.85±0.07^{g}
	F4	0.83±0.04^{cd}	1.53±0.39^{c}	2.13±0.18^{d}	2.68±0.25^{e}	3.13±0.53^{g}	3.93±0.74^{e}	4.43±0.88^{e}
	F5	0.55±0.00^{fg}	0.70±0.07^{fg}	0.85±0.07^{i}	0.90±0.14^{i}	1.03±0.11^{i}	1.10±0.14^{i}	1.25±0.14^{i}

Means with the same alphabets down the COLUMN are not significantly different at P<0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only.

Table 3 indicates effect of *Aframomum melegueta* and *Petiveria alliacea* on the mycelial growth of isolated fungi at 10% concentration. It was observed that *A. niger, A. flavus* and *Colletotrichum sp.* were actively inhibited by the methanol extract of *Aframomum melegueta* while *Colletotrichum sp* and *Fusarium oxysporum* were inhibited by methanol extract of *Petiveria alliacea* throughout the days of incubation. At 20%concentration, the extract of both plants failed to inhibite the growth of *Penicillium chrysogenum* as observed in Table 4.

Table 4. *Effect of Aframomum melegueta and Petiveria alliacea on the mycelial growth of isolated fungi at 20% concentration.*

F1:	*Aspergillus niger*
F2:	*Aspergillus flavus*
F3:	*Colletotrichum sp*
F4:	*Fusarium oxysporum*
F5:	*Penicillium chrysogenum*

Botanicals	Pathogen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Petiveria alliacea (Aqueous Extract)	F1	1.00±0.00^{b}	2.10±0.00^{a}	3.45±0.07^{a}	5.28±0.18^{a}	6.55±0.28^{a}	7.98±0.39^{a}	8.48±0.04^{a}
	F2	1.03±0.04^{b}	1.85±0.07^{b}	2.78±0.04^{b}	4.00±0.14^{b}	4.85±0.07^{c}	5.80±0.07^{c}	6.63±0.18^{b}
	F3	0.60±0.00^{d}	1.40±0.07^{cd}	2.73±0.04^{b}	3.48±0.74^{c}	4.88±0.95^{c}	5.90±1.20^{c}	6.75±0.85^{b}
	F4	0.78±0.18^{c}	1.28±0.18^{d}	2.18±0.53^{c}	3.08±0.67^{d}	4.00±0.92^{d}	4.43±0.81^{d}	4.73±0.60^{de}
	F5	0.50±0.00^{e}	0.53±0.04^{i}	0.68±0.11^{gh}	0.80±0.07^{i}	1.10±0.21^{i}	1.20±0.14^{i}	1.30±0.07^{i}
Petiveria alliacea (Methanol Extract)	F1	0.75±0.00^{c}	1.03±0.11^{e}	1.53±0.25^{de}	2.13±0.25^{g}	2.93±0.25^{ef}	3.85±0.28^{e}	4.48±0.39^{e}
	F2	0.78±0.04^{c}	1.08±0.04^{e}	1.78±0.04^{d}	2.25±0.00^{g}	2.95±0.07^{ef}	3.63±0.11^{e}	4.23±0.11^{ef}
	F3	0.50±0.00^{e}	0.50±0.00^{i}	0.53±0.04^{j}	0.58±0.04^{i}	0.78±0.04^{e}	0.88±0.04^{h}	1.00±0.00^{g}
	F4	0.50±0.00^{e}	0.65±0.21^{h}	0.70±0.14^{ij}	0.95±0.07^{hi}	1.23±0.04^{h}	1.68±0.25^{h}	2.28±0.46^{h}
	F5	0.50±0.00^{e}	0.68±0.11^{h}	0.85±0.14^{fg}	0.98±0.11^{hi}	1.05±0.14^{i}	1.13±0.04^{i}	1.25±0.07^{i}
Aframomum melegueta (Aqueous Extract)	F1	0.70±0.07^{cd}	1.25±0.14^{d}	2.28±0.11^{bc}	3.80±0.71^{bc}	5.78±1.52^{b}	6.73±1.31^{b}	7.50±0.64^{ab}
	F2	0.63±0.04^{d}	1.13±0.11^{de}	1.75±0.07^{d}	2.48±0.11^{f}	3.25±0.07^{e}	3.83±0.04^{e}	4.50±0.1^{e}
	F3	0.55±0.07^{e}	0.80±0.14^{fg}	1.43±0.32^{e}	2.35±0.57^{f}	3.63±0.60^{de}	4.85±0.64^{d}	5.83±0.67^{c}
	F4	1.20±0.00^{a}	2.03±0.04^{a}	3.35±0.14^{a}	4.50±0.07^{b}	5.88±0.18^{b}	6.98±0.25^{b}	8.13±0.18^{a}
	F5	0.50±0.00^{e}	0.78±0.25^{g}	0.95±0.14^{f}	1.10±0.14^{h}	1.25±0.14^{h}	1.35±0.14^{hi}	1.50±0.21^{i}
Aframomum melegueta (Methanol Extract)	F1	0.55±0.07^{d}	0.75±0.07^{g}	1.30±0.07^{e}	1.90±0.21^{gh}	2.13±0.39^{cd}	2.38±0.32^{g}	2.53±0.39^{g}
	F2	0.58±0.04^{d}	0.88±0.11^{f}	1.00±0.21^{f}	1.20±0.21^{h}	1.55±0.42^{g}	1.73±0.32^{h}	2.05±0.28^{d-g}
	F3	0.50±0.00^{e}	0.50±0.00^{i}	0.58±0.04^{h}	1.15±0.14^{h}	1.50±0.07^{g}	1.85±0.07^{h}	2.18±0.04^{h}
	F4	0.50±0.00^{e}	0.63±0.18^{h}	1.05±0.71^{f}	1.70±0.57^{gh}	2.65±0.92^{f}	3.15±1.2^{f}	3.93±1.52^{f}
	F5	0.50±0.00^{e}	0.60±0.00^{h}	0.73±0.04^{g}	0.95±0.00^{hi}	1.25±0.14^{h}	1.40±0.21^{hi}	1.50±0.21^{i}
Control	F1	0.80±0.00^{c}	1.55±0.00^{c}	2.38±0.04^{bc}	3.00±0.07^{d}	3.70±0.07^{d}	4.30±0.21^{d}	5.00±0.21^{d}
	F2	1.00±0.00^{b}	1.65±0.21^{bc}	2.15±0.14^{c}	2.58±0.11^{e}	2.93±0.04^{ef}	3.70±0.07^{e}	5.05±0.07^{d}
	F3	0.60±0.07^{d}	1.18±0.04^{de}	1.85±0.00^{d}	2.35±0.00^{f}	2.63±0.11^{f}	2.80±0.07^{g}	2.85±0.07^{g}
	F4	0.83±0.04^{c}	1.53±0.39^{c}	2.13±0.18^{c}	2.68±0.25^{e}	3.13±0.53^{e}	3.93±0.74^{e}	4.43±0.88^{e}
	F5	0.55±0.00^{e}	0.70±0.07^{g}	0.85±0.07^{fg}	0.90±0.14^{hi}	1.03±0.11^{i}	1.10±0.14^{i}	1.25±0.14^{i}

Means with the same alphabets down the COLUMN are not significantly different at P<0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only.

Table 5 shows that *Aframomum melegueta* had higher potency against all the fungi isolated except on *Penicillium chrysogenum*. This is in line with the work of Okoi *et al*. who observed that *A. melegueta* to have the highest percentage inhibition on isolated fungi from carrot [21]. Similar to this is the findings of Okigbo R. N. and Ogbonnaya who reported the efficacy of *A. melegueta* extract in the control *A. niger, F. oxysporum* and *Botrydiplodia theobromae* causing rot in yam [22]. The effectiveness of methanol extracts of the two plants over the aqueous extracts supported the report of Adejumo, that methanolic extracts of botanicals possess high antimicrobial properties [23]. This is likely to be due to the solubility of the active compounds in methanol.

Table 5. *Effect of Aframomum melegueta and Petiveria alliacea on the mycelial growth of isolated fungi at 30% concentration.*

F1:	*Aspergillus niger*
F2:	*Aspergillus flavus*
F3:	*Colletotrichum sp*
F4:	*Fusarium oxysporum*
F5:	*Penicillium chrysogenum*

Botanicals	Pathogen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Petiveria alliacea (Aqueous Extract)	F1	0.55±0.07^{e}	1.03±0.04^{e}	2.20±0.00^{bc}	4.05±0.14^{a}	5.45±0.07^{a}	7.08±0.04^{a}	8.13±0.25^{a}
	F2	0.85±0.14^{b}	1.48±0.11^{b}	2.80±0.28^{a}	4.20±0.35^{a}	5.13±0.39^{a}	6.33±0.60^{b}	7.00±0.42^{ab}
	F3	0.50±0.00^{e}	0.68±0.04^{e}	0.80±0.00^{gh}	0.95±0.07^{h}	1.05±0.07^{i}	1.33±0.04^{h}	1.55±0.07^{h}
	F4	0.50±0.00^{e}	0.63±0.11^{f}	1.13±0.25^{f}	1.98±0.04^{f}	2.90±0.00^{e}	3.50±0.07^{f}	4.25±0.00^{e}
	F5	0.50±0.00^{e}	0.63±0.11^{f}	0.78±0.11^{gh}	1.05±0.14^{h}	1.13±0.18^{i}	1.20±0.14^{h}	1.25±0.14^{i}
Petiveria alliacea (Methanol Extract)	F1	0.65±0.00^{d}	1.08±0.11^{c}	1.48±0.18^{e}	1.80±0.35^{f}	2.43±0.39^{f}	3.50±0.42^{f}	4.08±0.39^{e}
	F2	0.73±0.04^{c}	0.98±0.04^{cd}	1.58±0.04^{e}	2.23±0.04^{ef}	2.95±0.07^{e}	3.68±0.04^{ef}	4.43±0.11^{d}
	F3	0.50±0.00^{e}	0.50±0.00^{g}	0.50±0.00^{i}	0.55±0.00^{i}	0.78±0.04^{i}	0.95±0.14^{i}	0.98±0.11^{i}
	F4	0.50±0.00^{e}	0.50±0.00^{g}	0.63±0.18^{h}	0.85±0.21^{hi}	1.23±0.46^{hi}	1.78±0.53^{gh}	2.28±0.60^{g}
	F5	0.50±0.00^{e}	0.65±0.14^{ef}	0.88±0.18^{g}	1.08±0.18^{h}	1.15±0.14^{i}	1.23±0.11^{h}	1.35±0.07^{h}
Aframomum melegueta (Aqueous Extract)	F1	0.70±0.07^{c}	1.18±0.18^{bc}	2.45±0.71^{b}	3.75±1.2^{b}	4.48±0.46^{b}	5.35±0.21^{c}	6.35±0.21^{b}
	F2	0.60±0.00^{d}	0.98±0.11^{cd}	1.70±0.14^{d}	2.43±0.18^{e}	3.30±0.14^{cd}	3.93±0.04^{e}	4.60±0.07^{cd}
	F3	0.50±0.00^{e}	0.50±0.00^{g}	0.50±0.00^{i}	0.53±0.04^{i}	0.73±0.25^{i}	1.18±0.39^{fg}	1.80±0.85^{h}
	F4	0.50±0.00^{e}	0.50±0.00^{g}	0.78±0.04^{gh}	1.50±0.07^{g}	2.50±0.14^{f}	3.45±0.21^{f}	4.73±0.18^{cd}
	F5	0.50±0.00^{e}	0.85±0.14^{d}	1.10±0.07^{f}	1.40±0.14^{g}	1.65±0.21^{h}	1.75±0.21^{gh}	1.93±0.18^{gh}
Aframomum melegueta (Methanol Extract)	F1	0.55±0.00^{e}	0.68±0.11^{e}	1.25±0.07^{f}	1.75±0.07^{fg}	2.15±0.07^{g}	2.25±0.00^{g}	2.60±0.14^{g}
	F2	0.50±0.00^{e}	0.60±0.07^{f}	0.75±0.21^{g-i}	0.95±0.21^{h}	1.20±0.28^{hi}	1.38±0.32^{h}	1.60±0.49^{h}
	F3	0.50±0.00^{e}	0.50±0.00^{g}	0.50±0.00^{i}	0.55±0.00^{i}	0.83±0.11^{i}	0.90±0.07^{i}	1.15±0.14^{i}
	F4	0.50±0.00^{e}	0.75±0.35^{de}	1.03±0.53^{fg}	1.65±0.57^{g}	2.15±0.42^{g}	2.68±0.18^{g}	3.35±0.07^{f}
	F5	0.50±0.00^{e}	0.58±0.04^{f}	0.63±0.04^{h}	0.78±0.04^{hi}	0.85±0.00^{i}	0.98±0.04^{i}	1.03±0.04^{i}
Control	F1	0.80±0.00^{b}	1.55±0.00^{a}	2.38±0.04^{b}	3.00±0.07^{c}	3.70±0.07^{c}	4.30±0.21^{d}	5.00±0.21^{c}
	F2	1.00±0.00^{a}	1.65±0.21^{a}	2.15±0.14^{c}	2.58±0.11^{d}	2.93±0.04^{e}	3.70±0.07^{ef}	5.05±0.07^{c}
	F3	0.60±0.07^{d}	1.18±0.04^{bc}	1.85±0.00^{d}	2.35±0.00^{e}	2.63±0.11^{f}	2.80±0.07^{g}	2.85±0.07^{g}
	F4	0.83±0.04^{b}	1.53±0.39^{a}	2.13±0.18^{c}	2.68±0.25^{d}	3.13±0.53^{d}	3.93±0.74^{e}	4.43±0.88^{d}
	F5	0.55±0.00^{e}	0.70±0.07^{e}	0.85±0.07^{g}	0.90±0.14^{h}	1.03±0.11^{i}	1.10±0.14^{hi}	1.25±0.14^{i}

Means with the same alphabets down the COLUMN are not significantly different at P<0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only.

4. Conclusion

The mycelial suspension of all the isolated fungi significantly reduced not only the length of radicle in-vitro but also the root and shoot length of soybean seedlings sown in bags. This suggests that these fungi are not only a storage fungi affecting the quality of stored soybean seeds, but also are pathogenic to the seedlings of soybean. The two extracts most especially *A. melegueta* showed significant result in controlling *A. niger, A. flavus, Fusarium oxysporum* and *Colleotrichum sp*. Hence, can be used to check the growth of these fungi.

References

[1] Lakshmeesha, T. R., Sateesh, M. K. Vedashree, S. and Mohammad, S. S. (2013). Antifungal activity of some medicinal plants on Soybean seed-borne *Macrophomina phaseolina. Journal of Applied Pharmaceutical Science* 3 (02): 084-087.

[2] Etiosa, O. R., Chika, N. B. and Benedicta A. (2018). Mineral and proximate composition of soya bean. *Asian Journal of Physical and Chemical Sciences* 4 (3): 1-6.

[3] Hartman G. L. (2015). Compendium of Soybean Diseases and Pests. 5th ed. The American Phytopathological Society; St Paul, MN. USA. Worldwide importance of soybean pathogens and pests; pp. 4-5.

[4] Tawale J. B. and Pawar B. R. (2011). Constraint and suggestion of soybean production in mahashtra. *Agriculture Update* 6 (2)*:* 11-13.

[5] Gupta S, Dubey A, Singh T. 2017. *Fusarium semitectum* as a dominant seed-borne pathogen in Albizzia lebbeck (Linn.) Benth., its effect on location and transmission studies. Res J For 11: 13-18.

[6] Pedraza LA, Bautista J, Uribe-Vélez D. 2018. Seed-born Burkholderia glumae infects rice seedling and maintains bacterial population during vegetative and reproductive growth stage. Plant Pathol J 34 (5): 393-402.

[7] Akranuchat P, Noimanee, P. Krittigamas, N. Horsten D. V. and Vearasilp. S. (2007). Control of seed borne fungi by radio frequency heat treatment as alternative seed treatment in barely (*Hordeum vulgare*). *Conference on International Agricultural Research for Development*. October 9-11. University of Gottingen, Germany.

[8] Kinnikar A, Desai P, Jahagirdar S. 2015. Identification and detection of seed-borne diseases of soybean using image processing-a survey. Intl J Emerg Technol Comput Sci Electr 14 (2): 363-368.

[9] Escamilla D, Rosso ML, Zhang B. 2019. Identification of fungi associated with soybeans and effective seed disinfection treatments. Food Sci Nutr 7 (10): 3194-3205.

[10] Welbaum, G. E., 2006. Natural defense mechanisms in seeds. *In*: Basra, A. S. (ed.), *Handbook of Seed Science and Technology*, pp: 451–73. Food Product Press: An Imprint of the Haworth Press, New York.

[11] Carsten, S. J. and Hjort, H. M. (2014). Agricultural soils, pesticides and microbial diversity. *Current Opinion in Biotechnol*ogy, 27: 15–20.

[12] Dubey, N. K., Shukla, R., Kumar, A., Singh, P. and Prakash, B. (2010): Prospects of botanical pesticides in sustainable agriculture. *Current Science* 98: 479–480.

[13] Alemu, K. (2014). Seed Borne Fungal Pathogen Associated with Soybean (*Glycine max* L.) and their Management in Jimma, Southwestern Ethiopia. *Journal of Biology, Agriculture and Healthcare* 4 (25): 14-19.

[14] Dawar, S., Farzana, S and Ghaffar, A. (2007). Seed borne fungi associated with chick pea in Pakistan. *Pakistan Journal of Botany*. 39 (2): 637–643.

[15] Shovan, L. R, Bhuiyan, M. K. A., Sultana, N., Begum, J. A., Pervez, Z. (2008). Prevalence of fungi associated with soybean seeds and pathogenicity tests of major seed-borne pathogens. *International Journal of Sustainable Crop Production* 3 (4): 24-33.

[16] Ahammed S. K., Anandam R. J., Prasad Babu G., Munikrishnaiah M. and Gopal K. (2006). Studies on seed mycoflora of soybean and its effect on seed and seedling quality characters. *Legume Research*, 29 (3): 186–190.

[17] Nahed Z. H. (2008). Effect of Filtrates of Pathogenic Fungi of Soybean on Seed Germination and Seedling Parameters. *Journal of Applied Sciences Research*, 4 (1): 48-52.

[18] Garuba, T., Abdulrahman, A. A., Olahan, G. S., Abdulkareem, K. A. and Amadi, J. E. (2014). Effects of fungal filtrates on seed germination and leaf anatomy of maize seedlings (*Zea mays* L., Poaceae). *Journal of Applied Science and Environmental Management* 18 (4): 662-667.

[19] Rao, V. K., Girisham, S. and Reddy, S. M. (2014). Influence of different species of Penicillium and their culture filterates on sed germination and seedling growth of sorghum. *Journal of Biochemical Technology,* 5 (4): 832-837.

[20] Rajput, N. A., Pathan, M. A., Rajput, A. Q., Jiskani, M. M., Lodhi, A. M., Rajput, S. A. and Khaskheli M. I. (2010). Isolation of fungi associated with shisham trees and their effect on seed germination and seedling mortality. *Pakistan Journal of Botany,* 42: 369-374.

[21] Okoi, A. I., Udo, S. E., Eka M. E., Kingsley H. E., Alobi, N. O. and Magdalene O. (2013). Antifungal Activity of Extracts of Scent Leaf (*Ocimum gratissimum)* and Alligator Pepper *(Aframomum melegueta)* on the Postharvest Decay of Carrot in Calabar, Nigeria. *Journal of Biology, Agriculture and Healthcare* Vol. 3, No. 14 ISSN 2224-3208.

[22] Okigbo R. N. and Ogbonnaya, U. O. (2006). Antifungal effects of two tropical plant leaf extracts *(Ocimum gratissimum* and *Aframomum melegueta*) on postharvest yam (*Dioscorea* spp.) rot. *African Journal of Biotechnology,* 5 (9): 727-731.

[23] Adejumo T. O. (2011). Evaluation of botanicals as biopesticides on the growth of *Fusarium verticillioides* causing rot diseases and fumonis in production of maize. *Journal of Microbiology and Antimicrobials*. 4 (1): 23-31.