Antifungal Effects of Botanical Extracts Against Black Mold of Shallot Bulbs caused by *Aspergilus niger*

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ABSTRACT— ***Black mold, caused by* Aspergillus niger*, is a major postharvest disease of shallots in Ghana. This study investigated the efficacy of spices (plant materials/botanicals) such as cloves (Syzygium aromaticum), habanero pepper (Capsicum chinense), West African pepper (Piper guineense), grains of paradise (Aframomum melegueta) and black pepper (Piper nigrum) in managing the disease. Two cultivars of shallot bulbs in Ghana were used; namely, pink and pale brown. The antifungal activity of 25, 50, 75, and 100 % concentrations of each plant extract were assessed in vitro against A. niger mycelia growth. The efficacy of the plant extracts (cloves, habanero pepper, West African pepper, grains of paradise and black pepper) in managing the black mold disease of shallot bulbs was assessed in vivo by dipping bulbs in 100 % concentrations of each plant extract 21 days. Factorial experimental design was used in both in vitro and in vivo experiments; each plant extract treatment had three replications at four concentration levels, with three replications each for both positive and negative controls arranged in a completely randomised design. The study revealed that all extracts significantly ($P \leq 0.05$) inhibited mycelial growth at various concentrations (25,50,75, and 100 %) compared to the negative control treatments, but clove extracts completely suppressed growth at all concentrations. In vivo, aqueous extracts of cloves, black pepper, and grains of paradise reduced disease intensity and bulb weight loss, whereas habanero and West African pepper were less effective. Disease severity and weight loss were consistently higher under enclosed storage compared to open storage.***

Keywords— Synthetic fungicide, Plant extracts, Disease Intensity, Shallot bulb weight loss, Black mold, Open storage condition, Enclosed storage condition, mycelia growth inhibition, phytochemical.

1. INTRODUCTION

Shallot (*Allium cepa* Var. *aggregatum* G. Don.) is an important vegetable crop that is cultivated worldwide. It is a valuable source of nutrients and has been used in the traditional medicine of many cultures for its various therapeutic properties. The value of exports of shallots and other alliaceous vegetables from Ghana summed up to $118,000.00 in 2019, and their sales in Ghana went up by 329% compared to 2018 [5]. The crop is mainly grown by smallholder farmers, who employ traditional farming practices. However, the lack of access to improved varieties, post-harvest losses, and limited market opportunities are some of the challenges facing shallot production in Ghana.

Black mold disease in shallots is a major post-harvest challenge that affects the quality and shelf-life of the crop [14]. Post-harvest losses of shallots due to black mold disease can be significant. In Ghana, the magnitude of post-harvest loss of shallot due to black mold is not known; however, according to a study by [15], black mold disease caused by the fungus *A. niger* can result in up to 30 % post-harvest losses of shallots. The pathogen can infect the shallots during the harvesting process or through wounds during storage, leading to rotting and discoloration of the bulbs.
The disease can also lead to the production of mycotoxins, which can be harmful to human health if consumed in large quantities. In addition to the direct effects on shallot quality and shelf life, black mold can also have negative economic impacts on farmers and the agricultural industry as a whole. According to a study [23], black mold can reduce the market value of shallots and other crops, leading to financial losses to farmers and other stakeholders. Synthetic fungicides are

commonly used to control fungal infections in stored shallot bulbs. However, their use can have several negative effects on the bulbs. One of the main effects of using synthetic fungicides on stored shallot bulbs is the potential for residues to remain on the bulbs after treatment [18] [21]. These residues can have harmful effects on human health if the bulbs are consumed [10].

Additionally, the continual application of synthetic fungicides may result in the emergence of fungal strains that are resistant to them, making it more challenging to manage infections in the future [1] [4]. Furthermore, the usage of synthetic fungicides could damage the ecosystem by polluting the soil and water supplies [24]. It is therefore recommended to use an environmentally friendly method in managing the disease.

Recent research has shown that plant extracts such as garlic, ginger, and turmeric possess antifungal properties that can inhibit the growth of *A. niger* in shallots [25] [22]. These natural extracts may be a safer and more environmentally friendly alternative to synthetic fungicides since they contain biologically active chemicals that are known to possess antifungal activity against diverse fungal pathogens [12].

Furthermore, farmers will have easy access to these resources if local products are effectively investigated in agricultural areas, such as bio-fungicides for the prevention of shallot black mold under storage conditions. This will guarantee that shallots are stored in a way that is both colorable and affordable, as well as increase shallot lifespan of shallot bulbs, farmer revenue, and food security. Implementing crop and store management techniques to reduce the effects and further spread of diseases requires an assessment of the plant's health condition and early disease identification. Also, since shallot is a spice, exploring the efficacy of other spices in managing the black mold disease will make the shallot healthier and more palatable, aside from increasing the shelf life. The objectives of the study were to: i. evaluate *in vitro* the fungitoxicity of the aqueous and ethanol plant extracts against the mycelia growth of *A. niger*, and ii. determine *in vivo*, the efficacy of aqueous plant extracts in the management of shallot black mold disease.

2. MATERIALS AND METHODS

2.1 Source of shallot bulbs and plant materials

Pale brown and pink shallot cultivars cultivated in Ghana were used in this study. They were sourced from farmers' gates and markets in Anloga and Agbogbloshie market in the Volta and Greater Accra regions of Ghana, respectively. The dried fruits of black pepper (*Piper nigrum*) and West African pepper (*Piper guineense*), fresh fruits of habanero pepper (*Capsicum chinense*), dried flowers of cloves (*Syzygium aromaticum*), and grains of paradise (*Aframomum melegueta*) seeds used for this study were bought from the Aboabo market in Tamale. Each plant material was transported to the laboratory in sterile and labelled paper bags.

2.2 Preparation of plant materials for extraction

The dried fruits of West African pepper, black pepper, and clove flowers were separated and sieved. The shells of grains of paradise's seeds were broken off. To make drying easier, fresh habanero peppers were sliced into 1 cm pieces. These plant materials were then air-dried separately for 10 days at room temperature on lab plates in the shade. After that, a Sufre German blender was used for blending each material for 5 minutes. The acquired powder was then sieved with a sieve of pore size 0.4 mm to produce a fine powder, which was then kept until use.

2.3 Media preparation

The analytical grade Potato Dextrose Agar (PDA) medium was prepared in accordance with the directions provided by the supplier (Sigma-Aldrich Company, Spain). In a 1 L Erlenmeyer flask containing 39 g of powdered PDA, 1 L of distilled water was added. About 250 mg of chloramphenicol was added to prevent bacterial growth. The suspension was thoroughly dissolved by boiling the Erlenmeyer flask in a water bath while it was covered with non-absorbent cotton wool. After that, it was autoclaved for 15 minutes at 121°C and 1.03 kg/cm2 of pressure.

2.4 Isolation and identification of *Aspergillus niger*

Healthy shallot bulbs and shallot bulbs with symptoms of black mold disease were brought to the laboratory in sterile paper bags. These shallot bulbs were washed separately with tap water to remove debris, cut into pieces (1 cm fragments), surface sterilized with 0.1% sodium hypochlorite for three minutes, and rinsed three times alternately with sterile distilled water. The pieces were then inoculated at five equidistant spots on PDA in a 90 mm Petri dish and incubated for 7 days at ambient temperature (28 ± 2 °C). Growing mycelia were sub-cultured onto fresh PDA, and further sub-culturing was done until pure cultures of *A. niger* were obtained.

Slides of 7-day-old mycelia from pure cultures of *A. niger* were examined under a compound microscope (Leica DME, Leica Microsystems, Shanghai, China). They were confirmed as *A. niger* by comparing their morphological and cultural characteristics with images and descriptions.

2.5 Preparation of plant extracts for *in vitro* studies

Plant extracts were pulverized into powdered forms as described in section 3. For each powdered dried plant material, 10 g was weighed into a dry conical flask and 300 mL of ethanol was added. This was then placed on a shaker (LAB-LINE

Instrument, Inc., Melrose Park, ILL Orbit Shaker) for 24 hours. The extract was then filtered through Whiteman No. 1 filter paper, and the filtrate was evaporated to dryness using the rotary evaporator. For each sample, 1 mg/ml of stock solution was prepared. Each sample was then analyzed in triplicate.

2.6 Preparation of plant extracts for *in vivo* studies

Plant extracts were prepared according to the method of [9] with some modifications. Two hundred (200) g of powdered plant material (prepared as described in Section 3.3) was steeped in 1 L of sterile distilled water (w/v), stirred vigorously with a glass rod, and allowed to stand at room temperature for 24 h. After this time, the supernatant was then filtered through a double-layer cotton cloth. The extract obtained served as the stock solution (100%).

2.7 Preparation of synthetic fungicides

The synthetic fungicide Bon Victory (Mancozeb 640 g/kg + Metalaxyl 80 g/kg WP) was used for the study. It was prepared at the rate of 2.0 g of the fungicide to 1 litre of distilled water.

2.7.1 Antifungal activity of plant extracts against *A. niger* mycelia growth

About 2.0 g of the aqueous and ethanol crude extracts of the plant materials were each added to 10 ml of sterile distilled water in a McCartney tube that was capped and then shaken for 5 minutes to mix the content homogeneously. This formed the stock solutions (100 % extract concentration). The stock solution was then diluted with various volumes of sterile distilled water to obtain 25, 50 and 75 % concentrations for each extract.

The antifungal activity of the various concentrations (25, 50, 75 and 100 %) of the plant extracts was evaluated against the mycelia growth of *A. niger* by the poisoned food technique of Grover and Moore (1962). Five (5) ml of each plant extract concentration was poured into a 9 cm diameter Petri dish, and then 20 ml of melted PDA medium was added. The mixture was then swirled for uniform mixing before the PDA solidified. A mycelial disk (5 mm) of *A. niger* pure culture was placed in the centre of the Petri dish and incubated at room temperature (28 ± 2 ^{0}C) for 5 days. Mycelia growth was determined by measuring the colony radius on the fifth day of inoculation with a transparent ruler along two mutually perpendicular lines on the bottom of the Petri dish and recording the average per Petri dish [20]. Potato Dextrose Agar, to which sterilized distilled water was added, served as the negative control. The positive control consisted of a PDA medium modified with the synthetic fungicides Bon Victory (Mancozeb 640 g/kg + Metalaxyl 80 g/kg WP) prepared at the rate of 2.0 g/L water. Each treatment had three replications. The treatments were arranged in a Completely Randomized Design. Per cent inhibition of mycelial growth was determined using the formula of [6]. Mycelia growth inhibition = $\frac{C-T}{C} \times 100$

where C = Radial growth of fungus in control

T = Radial growth of fungus in treatment

2.7.2 Antifungal activity of plant extracts against black mold of shallot

This was determined according to the method of [7] with some modifications. The following experimental set ups (set ups A and B) were used for this study.

2.7.3 Antifungal activity of plant extracts against black mold disease development in *A. niger* inoculated shallot bulbs (Set up A)

Healthy, clean shallot bulbs were taped with pieces of masking tape, labelled according to treatments and then weighed. The shallot bulbs were surface sterilized with 70 % ethanol, followed by immersion in *A. niger* spore suspension (1 × 10 6 /ml) for one minute. The *A. niger* inoculated shallot bulbs were then air-dried for 5 minutes, after which they were dipped separately in the stock solution (100 % concentration) of the various plant extracts for 2 min. The shallot bulbs were then incubated at room temperature (28 ± 2 ^{0}C) under the following separate conditions;

i. 12 hours light/12 hours darkness cycle in an open cardboard box for 21 days.
ii. 24 hours darkness cycle in an enclosed cardboard box for 21 days.

The positive and negative controls were shallot bulbs dipped in the synthetic fungicide (Mancozeb 640 g/kg + Metalaxyl 80 g/kg WP) and sterile distilled water respectively. Each treatment was replicated thrice.

2.7.4 Antifungal activity of plant extracts against black mold disease development in shallot bulbs (Set up B).

Healthy shallot bulbs were surface sterilized with 70 % ethanol. The shallot bulbs were then dipped in the stock solution (100 % concentration) of the various plant extract for 2 minutes, removed and dried in a laminar air flow chamber. The shallot bulbs were then incubated at room temperature (28 ± 2 ^{0}C) under the following separate conditions;

i. 12 hours light/12 hours darkness cycle in an open cardboard box for 21 days.
ii. 24 hours darkness cycle in an enclosed cardboard box for 21 days.

The positive control consisted of shallot bulbs dipped in synthetic fungicide (Mancozeb 640 g/kg + Metalaxyl 80 g/kg WP) and negative control those dipped in sterile distilled water. Each treatment had three replications.

The stored shallot bulbs in set-ups A and B were weighed every 7, 14, and 21 days after incubation and a change in weight was recorded. The black mould disease intensity was calculated using the formulae of [12].

$$\text{Percent disease intensity (PDI)} = \frac{\textit{Sum of all numerical rating (s)}}{\textit{Total No. of bulb assessed by maximum no.of rating (s)}} \times 100$$

2.8 Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) using GenStat (12th edition). The LSD (Least Significant Difference) test was used to separate treatment means at a 5% level of significance. The analyzed results were presented in tables.

3. RESULTS

3.1 Effect of plant extracts on mycelia growth

3.1.1 Effect of aqueous plant extracts on mycelia growth

There was total inhibition of mycelia growth of *A. niger* by the positive control treatment. Also, the aqueous extracts of cloves at all four levels of concentrations (25,50,75 and 100 %) totally inhibited the mycelia growth of *A. niger*. Generally, there were significant differences ($P \leq 0.05$) in *A. niger* mycelia growth among the various aqueous plant extract treatments. The *A. niger* mycelia growth inhibition ranged from 35.71 to 100.00 %. Aqueous extracts of cloves recorded the highest *A. niger* mycelia inhibition of 100.00 % for its 25, 50, 75 and 100 % extract concentrations, while the lowest of 35.71 % was recorded for black pepper at 25 %. There was no significant difference ($P \leq 0.05$) in the *A. niger* mycelia growth recorded for aqueous extracts of black pepper at 75 % (0.85), grains of paradise at 50 % (0.81mm) and West African pepper at 25 % (0.84mm). There was no significant difference between the *A. niger* mycelia growth for aqueous black pepper extract at 100 % (0.74mm) and grains of paradise at 100 % (0.71mm). There was also no significant difference ($P \leq 0.05$) between the *A. niger* mycelia growth recorded for aqueous extracts of grains of paradise at 75 % (0.76mm) and West African pepper at 75 % (0.76mm) (Table 1).

3.1.2 Effects of ethanol plant extracts on mycelia growth

Treatment of the positive control and ethanol extract of cloves totally inhibited the mycelia growth of *A. niger*. There were significant differences ($P \leq 0.05$) in the *A. niger* mycelia growth among the various ethanol plant extract treatments. The *A. niger* mycelia growth inhibition ranged from 57.14 % to 100.00 %. Ethanol extracts concentrations of habanero pepper at 100%, grains of paradise at 100%, and cloves at all four levels of concentrations (25 %, 50 %, 75 % and 100 %) totally inhibited the mycelia growth of *A. niger*. There were no significant differences ($P \leq 0.05$) in *A. niger* mycelia growth recorded for ethanol extracts of grains of paradise at 25%, West African pepper at 50 %, 75 % (0.59mm, 0.54mm), respectively, and habanero pepper at 25 % (0.30mm). There was also no significant difference ($P \leq 0.05$) in *A. niger* mycelia growth for black pepper at 25 %, grains of paradise at 75 % (0.37mm). Also, there were no significant differences ($P \leq 0.05$) in the *A. niger* mycelia growth recorded for black pepper at 75 % (0.30mm), black pepper 100 % (0.26mm) and habanero pepper at 50 % (0.25mm) (Table 1).

Table 1: Effect of plant extracts on mycelia growth of *A. niger*

Treatment	Mycelia growth (mm)		% growth inhibition	
	Aqueous	Ethanol	Aqueous	Ethanol
Positive control	0.00 a	0.00 a	100.00	100.00
BP@25 %	0.90 j	0.36 cd	35.71	74.29
BP@50 %	0.81 ij	0.32 c	39.29	77.14
BP@75 %	0.85 hi	0.30 bc	42.14	78.57
BP@100 %	0.74 fg	0.26 bc	47.14	81.43
CL@25 %	0.00 a	0.00 a	100.00	100.00
CL@50 %	0.00 a	0.00 a	100.00	100.00
CL@75 %	0.00 a	0.00 a	100.00	100.00
CL@100 %	0.00 a	0.00 a	100.00	100.00
GP@25 %	0.84 ij	0.53 e	40.00	62.14
GP@50 %	0.81 hi	0.36 cd	42.14	74.29
GP@75 %	0.76 gh	0.37 cd	45.71	73.57
GP@100 %	0.71 fg	0.00 a	49.29	100.00
WAP@25 %	0.84 ij	0.60 e	40.00	57.14
WAP@50 %	0.81 hi	0.59 e	42.86	57.86
WAP@75 %	0.76 gh	0.54 e	45.71	61.43
WAP@100 %	0.70 f	0.49 de	50.00	65.00
HP@25 %	0.63 e	0.30 e	55.00	78.57
HP@50 %	0.52 d	0.23 bc	62.86	83.57
HP@75 %	0.76 c	0.17 b	45.71	87.86
HP@ 100%	0.26 b	0.00 a	81.43	100.00
Negative Control	1.40 k	1.40 f	0.00	0.00
F (pr)	<0.001	<0.001		

Data was log transformed. Means in the same column followed by different letter(s) are significantly different as determined by Tukey's test.

Key: BP= Black Pepper (*Piper nigrum)*, GP = Grains of Paradise (*Aframomum melegueta)*, CL= Cloves (*Syzygium aromaticum)*, WAP = West African Pepper (*Piper guineense)*, HP = Habanero Pepper (*Capsicum chinense).*

3.2 Effect of aqueous plant extracts on black mould percent disease intensity and weight loss in shallot bulbs

3.2.1 Effect of aqueous plant extracts on black mould disease intensity of *A. niger* inoculated shallot bulbs stored under open and enclosed conditions

Generally, the shallot bulbs stored under enclosed conditions recorded higher black mold disease intensity than those stored under open conditions at 7, 14 and 21 days after inoculation for each of the treatments of the pink and pale brown shallot bulbs cultivars (Tables 2 and 3). For each of the pink and pale brown shallot bulb cultivar treatments, both the open and enclosed storage methods recorded a progressive increase in black mold disease intensity as the storage period moved from 7 to 21 days after inoculation (Tables 2 and 3). At 7 days after inoculation, black mold disease intensity was not recorded for both the open and enclosed storage methods of the positive control and the cloves treatments of the pink shallot bulb cultivar (Table 2). However, for the pale brown shallot bulb cultivar, at 7 days after inoculation, all the treatments recorded black mold disease intensity, with the positive control recording 6.67 % for both the open and enclosed storage methods, and the cloves recording13.33 % and 20.00 % for the open and enclosed storage methods, respectively (Table 3).

For each of the pink and pale brown shallot bulb cultivars, the negative control treatments of the open and enclosed storage methods each recorded a higher disease intensity than their corresponding treatments at 7, 14 and 21 days after inoculation (Tables 2 and 3). For the pink shallot bulbs cultivar, the negative control treatment of the enclosed storage method recorded the highest disease intensity (66.67 %) at 21 days after inoculation, which was followed by the enclosed storage method treatments of grain of paradise (46.67 %), and then West African pepper (40.00 %) (Table 2). At 21 days after inoculation among the pink shallot bulb cultivars, the least disease intensity was recorded for the positive control treatments (13.33 %), followed by clove open and positive control enclosed storage, each of which recorded 20.00 % and then cloves enclosed storage (26.67 %) (Table 2).

Table 2: Percent disease intensity of plant extract treated *A. niger* inoculated pink shallot bulb cultivar stored under open and enclosed conditions

Treatment	Percent disease intensity							
	7 DAI		14 DAI		21 DAI		Pool means	
	Open	Enclosed	Open	Enclosed	Open	Enclosed	Open	Enclosed
Positive control	0.00	0.00	6.67	13.33	13.33	20.00	6.67	11.33
Black Pepper	13.33	20.00	26.67	40.00	40.00	46.67	26.67	35.56
Cloves	0.00	0.00	6.67	20.00	20.00	26.67	8.89	15.56
Habanero	6.67	6.67	13.33	20.00	26.67	33.33	15.56	20.00
West Africa Pepper	6.67	13.33	26.67	26.67	33.33	40.00	22.22	26.67
Grains of Paradise	13.33	20.00	26.67	33.33	33.33	46.67	24.44	33.33
Negative Control	20.00	26.67	40.00	46.67	53.33	66.67	37.78	46.67

DAI = Days after inoculation/storage.

Table 3: Percent disease intensity of plant extract treated *A. niger* inoculated pale brown shallot bulb cultivar stored under open and enclosed conditions

Treatment	Percent disease intensity							
	7 DAI		14 DAI		21 DAI		Pool means	
	Open	Enclosed	Open	Enclosed	Open	Enclosed	Open	Enclosed
Positive control	6.67	6.67	13.33	13.33	33.33	40.00	17.78	20.00
Black Pepper	26.67	40.00	40.00	53.33	46.67	73.33	37.78	55.55
Cloves	13.33	20.00	20.00	26.67	33.33	40.00	22.22	28.89
Habanero	20.00	20.00	33.33	26.67	40.00	53.33	31.11	33.33
West Africa Pepper	20.00	26.67	33.33	46.67	40.00	66.67	31.11	46.67
Grains of Paradise	20.00	26.67	33.33	53.33	46.67	66.67	33.33	48.89
Negative Control	26.67	53.33	46.67	67.67	53.33	80.00	42.22	67.00

DAI = Days after inoculation/storage.

3.2.2 Effect of aqueous plant extracts on weight loss of *A. niger* inoculated pale brown shallot bulb cultivars stored under open and enclosed conditions

The highest pale brown shallot bulb cultivar weight loss was recorded for the enclosed stored negative control treatment (0.97 g) at 21 days after inoculation, and the lowest by the open stored positive control treatment (0.09 g) at 7 days after inoculation (Table 4). For all the treatments, the *A. niger* inoculated pale brown shallot bulbs stored under enclosed conditions recorded higher weight losses than those stored under open conditions (Table 4). Also, each of the treatments of the pale brown shallot bulbs stored under both the enclosed and open conditions recorded a progressive increase in weight loss from 7 to 21 days after inoculation (Table 4). The highest pale brown shallot bulb weight loss was recorded for the enclosed stored negative control treatment (0.97 g) and the lowest by the open stored positive control treatment (0.09 g) (Table 4). There were significant differences ($P \leq 0.05$) in the weight loss in the various treatments of the pale brown shallot bulbs stored under enclosed and open conditions at 7, 14 and 21 days after inoculation (Table 4). The negative control treatments of the pale brown shallot bulbs stored under both enclosed and open conditions at 7, 14 and 21 days after inoculation recorded significantly higher ($P \leq 0.05$) weight loss than their corresponding treatments (Table 4). Among the plant extract treatments, the lowest shallot bulb weight loss at 7, 14 and 21 days after inoculation for both enclosed and open storage conditions was recorded for the cloves treatments (Table 4). Among the plant extract treatments, at 7 days after inoculation, pale brown shallot bulb weight loss recorded for the open stored cloves treatment was significantly lower ($P \leq 0.05$) than those of grains of paradise (0.19 g) and black pepper (0.20 g) and for the enclosed storage, cloves also recorded a significant lower ($P \leq 0.05$) bulb weight loss (13 g) than those of black pepper (0.35 g) and grains of paradise (0.34 g) (Table 4). There were no significant differences ($P \leq 0.05$) between the pale brown shallot bulb weight loss recorded for the cloves and positive control treatments for each of the enclosed and open storages at 7, 14 and 21 days after inoculation (Table 4).

Table 4: Effect of aqueous plant extracts on weight loss of A. niger inoculated pale brown shallot bulb cultivar stored under open and enclosed conditions

Treatment	Weight Loss (g)						Pooled Mean	
	Day 7		Day 14		Day 21			
	open	enclosed	Open	enclosed	Open	enclosed	Open	Enclosed
Black Pepper	0.20 b	0.35 b	0.32 c	0.47 bc	0.40 b	0.76 c	0.31	0.53
Cloves	0.11 a	0.13 a	0.15 a	0.27 a	0.24 a	0.38 a	0.17	0.26
Habanero Pepper	0.16 ab	0.19 ab	0.27 b	0.36 ab	0.37 b	0.49 ab	0.27	0.35
West African Pepper	0.15 ab	0.28 ab	0.28 bc	0.47 bc	0.40 b	0.57 b	0.28	0.44
Grains of Paradise	0.19 b	0.34 b	0.28 bc	0.46 bc	0.38 b	0.58 b	0.28	0.46
Negative Control	0.30 c	0.61 c	0.47 d	0.81 d	0.61 c	0.97 d	0.46	0.80
Positive control	0.09 a	0.12 a	0.12 a	0.26 a	0.20 a	0.36 a	0.14	0.25
F(pr)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		

Means in the same column followed by different letter (s) are significantly different as determined by Tukey's tests.

3.2.3 Effect of aqueous plant extracts on weight loss of healthy pale brown shallot bulb varieties stored under open and enclosed conditions

For each of the treatments, there was an increase in shallot bulb weight loss as time proceeded from 7, 14, to 21 days after inoculation (Table 5). For each of the treatments at 7, 14 and 21 days after inoculation, the enclosed stored bulbs recorded higher weight loss than those stored under open conditions (Table 5). The weight loss recorded for the negative control (0.17 g) of the open storage at 7 days after inoculation was significantly higher ($P \leq 0.05$) than that of the other treatments, except that of black pepper (0.14 g) (Table 5). The weight loss recorded for the enclosed storage treatments of cloves (0.16 g) and the positive controls was significantly lower ($P \leq 0.05$) than that of the negative control (0.24 g), but not the other treatments (Table 5). At 14 days after inoculation, the negative control of the open storage recorded a significantly higher ($P \leq 0.05$) pale brown shallot bulb weight loss (0.35 g) than the other treatments (Table 5). Also, at 14 days after inoculation, treatment of the black pepper recorded a significantly higher ($P \leq 0.05$) pale brown shallot bulb weight loss (0.28 g) than those of habanero pepper, West African pepper, grains of paradise which each recorded a weight loss of 0.22 g, cloves (0.21 g) and the positive control (0.20 g) (table 5). For the enclosed storage at 14 days after inoculation, the weight loss recorded for the treatment of the negative control (0.44 g) was significantly higher ($P \leq 0.05$) than that recorded for the other treatments, except those of black pepper (0.36 g) and grains of paradise (0.36 g) (Table 5). At 21 days after inoculation of the open storage, treatment of the negative control recorded a significantly higher ($P \leq 0.05$) pale brown shallot bulb weight loss (0.44 g) than the other treatments, except that of the black pepper (0.40 g) (Table 5). The enclosed storage at 21 days after inoculation had the treatment of the positive control recording a significantly lower ($P \leq 0.05$) pale brown shallot bulb weight loss (0.33 g) than those of the other treatments, except that of cloves (0.35 g) (Table 5). The weight loss recorded for the treatment of cloves (0.35 g) at 21 days after inoculation was significantly lower ($P \leq 0.05$) than that of black pepper (0.53 g), grains of paradise (0.49 g) and the negative control (0.59 g) (Table 5).

Table 5: Effect of aqueous plant extracts on the weight loss of a healthy pale brown shallot bulb cultivar stored under open and enclosed conditions

Treatment	Weight Loss (g)						Pooled Mean	
	Day 7		Day 14		Day 21			
	Open	Enclosed	open	enclosed	Open	Enclosed	Open	Enclosed
Black Pepper	0.14 ab	0.21 ab	0.28 b	0.36 ab	0.40 bc	0.53 cd	0.27	0.37
Cloves	0.09 a	0.16 a	0.21 a	0.34 a	0.32 a	0.35 ab	0.21	0.28
Habanero Pepper	0.11 a	0.17 ab	0.22 a	0.29 a	0.33 ab	0.41 b	0.22	0.29
West African Pepper	0.11 a	0.21 ab	0.22 a	0.30 a	0.34 ab	0.40 b	0.22	0.30
Grains of Paradise	0.11 a	0.21 ab	0.22 a	0.36 ab	0.35 ab	0.49 c	0.23	0.35
Negative Control	0.17 b	0.24 ab	0.35 c	0.42 b	0.44 c	0.59 d	0.32	0.42
Positive control	0.08 a	0.15 a	0.20 a	0.32 a	0.30 a	0.33 a	0.19	0.27
F(pr)	0.002	0.020	<0.001	<0.001	<0.001	<0.001		

Means in the same column followed by different letter (s) are significantly different as determined by Tukey's tests.

3.2.4 Effect of aqueous plant extracts on weight loss of *A. niger* inoculated pink shallot bulb cultivars stored under open and enclosed conditions

The highest pink shallot weight loss was recorded by the enclosed storage treatment of the negative control (0.72 g) at 21 days after inoculation, followed by that of the enclosed storage of black pepper (0.55 g) also at 21 days after inoculation (Table 6). The lowest pink shallot weight loss was recorded for treatment of the positive control (0.04 g), followed by that of cloves (0.05 g), both at 7 days after inoculation under open conditions (Table 6). At 7 days after inoculation under open storage, the pink shallot bulb weight loss recorded for the treatments of the positive control (0.04 g) and cloves (0.05 g) were significantly lower ($P \leq 0.05$) than those of the other treatments (Table 6). Also, at 21 days after inoculation under open condition, the pink shallot bulb weight loss recorded for habanero pepper (0.22 g), West African pepper (0.19 g) and grains of paradise (0.19 g) were significantly lower ($P \leq 0.05$) than those of black pepper (0.26 g), and the negative control (0.24 g) (Table 6). For the enclosed storage at 7 days after inoculation, the pink shallot bulb weight loss recorded for the positive control (0.20 g) were significantly higher ($P \leq 0.05$) than those of black pepper (0.32 g), grains of paradise (0.38 g) and the negative control (0.50 g) but not those of cloves (0.22 g) and West African pepper (0.28 g) (Table 6). The pink shallot bulb weight loss recorded under open storage at 14 days after inoculation for treatments of the positive control (0.28 g) and cloves (0.30 g) each were significantly lower ($P \leq 0.05$) than those recorded for the other treatments (Table 6). The pink shallot bulb weight loss recorded for habanero pepper (0.29 g) at 14 days after inoculation under open storage was significantly lower ($P \leq 0.05$) than that of black pepper (0.43 g) and the negative control (0.42 g). At 14 days after inoculation under enclosed storage condition, there were no significant differences ($P \leq 0.05$) among the bulb weight loss recorded for treatments of cloves (0.30 g), habanero pepper (0.33 g), West African pepper (0.32 g) and the positive control (0.28 g) which each were significantly lower ($P \leq 0.05$) than those recorded for black pepper (0.53 g), grains of paradise (0.49 g) and the negative control (0.71 g) (Table 6). The pink shallot bulb weight loss recorded for the negative control (0.71 g) at 14 days after inoculation under enclosed storage was significantly higher ($P \leq 0.05$) than that recorded for the other treatments (Table 6).

The pink shallot bulb weight loss recorded for treatments of cloves (0.19 g) and positive control (0.18 g) each was significantly lower ($P \leq 0.05$) than those recorded for black pepper (0.51 g), habanero pepper (0.37 g) West African pepper (0.39 g), grains of paradise (0.48 g) and the negative control (0.63 g) (Table 6). Also, at 14 days after inoculation under open storage, the pink shallot bulb weight loss recorded for treatments of habanero pepper (0.37 g) and West

African pepper (0.39 g) each was significantly lower ($P \leq 0.05$) than those recorded for black pepper (0.51 g), grains of paradise (0.48 g) and the negative control (0.63 g) (Table 6). The pink shallot bulb weight loss recorded for the negative control treatment (0.63 g) was significantly higher ($P \leq 0.05$) than that of the other treatments (Table 6).

The pink shallot bulb weight loss recorded for treatments of cloves (0.32 g), habanero pepper (0.35 g), West African pepper (0.34 g) and the positive control (0.29 g) each was significantly lower ($P \leq 0.05$) than those of black pepper (0.55 g), grains of paradise (0.51 g) and the negative control (0.72 g) (Table 6). Also, at 14 days after inoculation under the enclosed storage, the pink shallot bulb weight loss recorded for treatments of black pepper (0.55 g) and grains of paradise (0.51 g) were significantly lower ($P \leq 0.05$) than that of the negative control (0.72 g) (Table 6).

Table 6: Effect of aqueous plant extracts on weight loss of *A. niger* inoculated pink shallot bulb varieties stored under open and enclosed conditions

Treatment	Weight Loss (g)						Pooled Mean	
	Day 7		Day 14		Day 21			
	Open	Enclosed	Open	Enclosed	Open	Enclosed	Open	Enclosed
Black Pepper	0.27 cd	0.31 d	0.37d	0.38 cd	0.40 cd	0.43 bc	0.35	0.37
Cloves	0.12 a	0.17 ab	0.16 a	0.24 ab	0.21 a	0.31 a	0.16	0.24
Habanero Pepper	0.13 a	0.21 abc	0.18 ab	0.30 abc	0.23 a	0.35 ab	0.18	0.29
West African Pepper	0.18 ab	0.26 cd	0.24 bc	0.29 ab	0.33 bc	0.34 ab	0.23	0.30
Grains of Paradise	0.21 bc	0.24 bc	0.30 c	0.32 bc	0.32 b	0.38 ab	0.28	0.31
Negative Control	0.29 d	0.32 d	0.38 d	0.44 d	0.45 d	0.52 c	0.37	0.43
Positive control	0.11 a	0.17 a	0.15 a	0.23 a	0.19 a	0.30 a	0.15	0.23
F(pr)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		

Means in the same column followed by different letter (s) are significantly different as determined by Tukey's tests.

3.2.5 Effect of aqueous plant extracts on weight loss of healthy pink shallot bulb varieties stored under open and enclosed conditions

The weight loss in the healthy pink shallot bulbs for each of the treatments stored under open and enclosed conditions recorded progressively increase weight loss from 7 to 21 days of storage (Table 7). For each of the treatments, the enclosed storage recorded higher pink shallot bulb weight loss than their corresponding treatment stored under open conditions (Table 7). At 7 days of the enclosed storage, the pink shallot bulb weight loss recorded by the positive control (0.17 g) was significantly lower ($P \leq 0.05$) than those of black pepper (0.31 g), West African pepper (0.26 g), grains of paradise (0.24 g) and the negative control (0.32 g) but not treatments of cloves (0.17 g) and habanero pepper (0.21 g) (Table 7). Under enclosed storage at 7 days, the pink shallot bulb weight loss recorded for the treatment of negative control (0.32 g), with the exception of that of black pepper (0.31 g) and West African pepper (0.26 g) was significantly higher ($P \leq 0.05$) than those of the other treatments (Table 7).

At 7 days of open storage, there were no significance differences ($P \leq 0.05$) in weight loss recorded for treatments of cloves (0.12 g), habanero pepper (0.13 g), West African pepper (0.18 g) and the positive control (0.11 g) (Table 7). The pink shallot bulb weight loss recorded for the negative control (0.29 g) under open storage at day 7 was significantly higher ($P \leq 0.05$) than that of the other treatments except black pepper (0.27 g) (Table 7).

At day 14 of the enclosed storage, the pink shallot bulb weight loss recorded for treatment of the negative control (0.44 g) with the exception of the black pepper (0.38 g) was significantly higher ($P \leq 0.05$) than that of the other treatments (Table 7). At 14 days of enclosed storage, there were no significant differences ($P \leq 0.05$) among the pink shallot bulb weight loss recorded among treatments of cloves (0.24 g), habanero pepper (0.30 g), West African pepper (0.29 g) and the positive control (0.44 g) (Table 7).

Under the open storage at 14 days, the pink shallot weight loss recorded for treatment of the positive control (0.15 g), with the exception of those of cloves (0.16 g) and habanero pepper (0.18 g) was significantly lower ($P \leq 0.05$) than those of the other treatments (Table 7). The pink shallot bulb weight loss recorded by the negative control (0.38 g) at 14 days under open storage was significantly higher ($P \leq 0.05$) than those of the other treatments except that of black pepper (0.37 g) (Table 7).

At day 21 of the enclosed storage, there were no significance differences ($P \leq 0.05$) among the pink shallot bulb weight loss recorded for treatments of cloves (0.31 g), habanero pepper (0.35 g), West African pepper (0.34 g), grains of paradise (0.38 g) and the positive control (0.30 g) (Table 7). The pink shallot bulb weight loss recorded for the negative control at day 21 of the enclosed storage was significantly higher ($P \leq 0.05$) than that of the other treatments except black pepper (0.43 g) (Table 7).

At day 21 of the open storage, the pink shallot bulb weight loss recorded for the treatment of the negative control (0.45 g) with the exception of black pepper (0.40 g) was significantly higher ($P \leq 0.05$) than those of the other treatments (Table 7). There was no significant difference ($P \leq 0.05$) among the pink shallot bulb weight loss at day 21 of the open storage for treatments of the positive control (0.19 g), cloves (0.21 g) and habanero pepper (0.23 g), each of which was significantly higher than those of the other treatment (Table 7).

Table 7: Effect of aqueous plant extracts on weight loss of healthy pink shallot bulb varieties stored under open and enclosed conditions

Treatment	Weight Loss (g)						Pooled Mean	
	Day 7		Day 14		Day 21			
	open	enclosed	open	enclosed	open	enclosed	Open	Enclosed
Black Pepper	0.26 d	0.32 bc	0.43 c	0.53 b	0.51 c	0.55 b	0.40	0.47
Cloves	0.05 a	0.22 ab	0.10 a	0.30 a	0.19 a	0.32 a	0.11	0.28
Habanero Pepper	0.22 bc	0.19 a	0.29 b	0.33 a	0.37 b	0.35 a	0.29	0.29
West African Pepper	0.19 b	0.28 abc	0.34 bc	0.32 a	0.39 b	0.34 a	0.31	0.31
Grains of Paradise	0.19 b	0.38 c	0.34 bc	0.49 b	0.48 c	0.51 b	0.34	0.46
Negative Control	0.24 cd	0.50 d	0.42 c	0.71 c	0.63 d	0.72 c	0.43	0.64
Positive control	0.04 a	0.20 a	0.08 a	0.28 a	0.18 a	0.29 a	0.10	0.26
F(pr)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		

Means in the same column followed by different letter (s) are significantly different as determined by Tukey's tests.

4. DISCUSSION

4.1 Effect of plant extracts on *A. niger* mycelia growth

All of the aqueous and ethanol extracts of black pepper, grains of paradise, cloves, West African pepper, and habanero pepper inhibited the development of *A. niger*'s mycelia, which may be attributable to the phytochemicals in these plant extracts. The development of fungal mycelia has been demonstrated to be influenced by a number of plant extracts [8].

Clove extract's ability to totally inhibit the growth of *A. niger* mycelia can be due to the variety of phytochemicals (Flavonoids, Saponins, Phenolics, and Phytosterols (Triterpenes)) contained in it. Phenolic chemicals have the ability to prevent microbial development on mycelia. Phytochemical substances, according to [2], serve as a physical barrier, slow the growth of infections, and also trigger the plant's defensive mechanism against invading microbes.

It was also noted that *Botrytis cinereal* and *Venturia inaequalis*, two important fungi that cause the infamous blue-gray mold illness, had been proven to be resistant to saponins isolated from the fruit pericarp of *Sapindus mukorossi*. According to [19], the flavonoids found in the majority of the plant extracts employed in this study (cloves, black pepper, grains of paradise, habanero pepper, and West African Pepper) displayed antifungal effects on *Aspergillus flavus*, Aspergillus niger, and *Trichophyton mentagrophytes*.

4.2 Effect of plant extracts on stored shallot bulbs

Generally, treatment with the plant extracts (cloves, black pepper and grains of paradise) reduced weight loss in Shallot bulbs (both cultivars pale brown and pink) compared to their respective negative controls. This suggests that these extracts may be utilized as a botanical fungicide to treat black moulds on shallot bulbs kept in storage since they lessened the intensity of the black moulds. This is corroborated by research by [7], who found that shallot bulbs with high severity black mould lose significantly more weight than those with low severity or no black mould.

This finding can be attributed to the presence of the photochemical components in these plant extracts, which may have had a fungitoxic impact on the development of *A. niger's* mycelia and spores or increased the plant's resistance to infection. According to a study by [11], certain phytochemicals have a role in the development of systemic acquired resistance, which results in a defensive mechanism that enables plants to defend themselves against infections.

The increase in percentage weight loss in shallot bulbs of the negative control compared to those of the different plant extract treatments supported the plant extracts' antifungal efficacy against the black mould that affects shallot bulbs. According to studies by [7], who reported similar findings in stored onion bulbs, the weight loss of pink and pale brown shallot cultivars in storage increased gradually over time (7 to 21 days).

The higher black mold disease intensity and weight loss recorded for the enclosed stored shallot bulbs than those stored under open conditions could be attributed to an increase in humidity in the enclosed environment resulting from poor ventilation. High humidity is a major factor that facilitate the growth and development of *A. niger* to establish infection in it host. Also, the rise in humidity and temperature in the enclosed storage environment increased the intensity of the black mold disease of the pale brown and pink shallot bulb cultivars; resulting in increased weight loss of the bulbs. This is supported by [15] who reported a positive correlation between black mold disease of onion and bulb weight loss. Treatments of shallot bulbs inoculated with *A. niger* spores had the Pale Brown cultivar recording higher black mold disease intensity than those of the Pink cultivar, which showed that the former cultivar is more susceptible to the black mold disease than the latter.

5. CONCLUSION

This study demonstrated that plant extracts possess promising antifungal potential against *Aspergillus niger*, the causal agent of black mould disease in shallots. Both aqueous and ethanol extracts of cloves, habanero pepper, black pepper, grains of paradise, and West African pepper significantly inhibited mycelial growth compared to the negative control. Among them, clove extracts consistently showed the strongest antifungal activity, achieving complete inhibition at all concentrations.

In vivo assays further revealed that aqueous extracts of black pepper, cloves, and grains of paradise effectively reduced disease intensity and weight loss in both pink and pale brown shallot cultivars, performing comparably to the synthetic fungicide. In contrast, aqueous extracts of West African pepper and habanero pepper were less effective in managing black mould under storage conditions.

The findings of this study reveal the potential of clove, black pepper, and grains of paradise extracts as eco-friendly alternatives to synthetic fungicides for the management of black mould in shallots. Therefore, it is recommended that shallots be stored in a well-ventilated open space. Further research should be conducted to develop plant extracts such as cloves, black pepper, and grains of paradise into to commercial botanical fungicide. Studies should also be conducted to determine the effects of various combinations of cloves, black pepper, and grains of paradise on the mycelia growth of *A. niger* and in managing the black mould disease of shallot bulbs (pink and pale brown cultivars). This is due to the fact that combining various plant extracts would probably increase their antifungal effectiveness compared to using only one.

6. REFERENCES

1. Bastos, R. W., Rossato, L., Goldman, G. H., & Santos, D. A. (2021). *Fungicide effects on human fungal pathogens: Cross-resistance to medical drugs and beyond.* PLoS Pathogens, *17*(12), 1–26. https://doi.org/10.1371/journal.ppat.1010073.
2. Chaube, H. S., and Pundhir, V. S. (2009). *Crop diseases and their management*. PHI Learning Private Limited.
3. Collin K. Campbell, Elizabeth M. Johnson and David W. Warnock (2013). *Identification of Pathogenic Fungi* (2nd ed.). John Wiley and Sons ltd, Publication.
4. Damicone, J., & Smith, D. (2009). *Fungicide Resistance Management*. *EPP-7633* http://osufacts.okstate.edu
5. Economy, T. (2021). Ghana | *Imports and Exports* | *World* | *Onions, shallots, garlic, leeks* | *Value (US$) and Value Growth, YoY (%)* | 2008 - 2019. Annual International Trade Statistics by Country (HS). https://trendeconomy.com/data/h2/Ghana/0703.
6. Hokkanen HMT, Kotiluoto R. 1992. Bioassay of the side effects of pesticides on Beauveria bassiana and Metarhizium anisopliae: standardized sequential testing pro-cedure, IOBC/WPRS Bull. 11:148-151.
7. Kapadiya, H. J., Pathak, D. M., & Patel, D. R. (2013). *Effect of artificial injuries and fresh neck cutting against*

black mould (Aspergillus niger) on onion bulb. Frontier *6*(2), 422–424.

8. Krutmuang, P., Rajula, J., Pittarate, S., Chatima, C., Thungrabeab, M., Mekchay, S., & Senthil-Nathan, S. (2022). *The inhibitory action of plant extracts on the mycelial growth of Ascosphaera apis, the causative agent of chalkbrood disease in Honey bee.* Toxicology Reports, *9*(March 2020), 713–719. https://doi.org/10.1016/j.toxrep.2022.03.036.
9. Kuberan T, Balamurugan A, Vidhyapallavi R, Nepolean P, Jayanthi R, Beulah T,Premkumar R. 2012. *In vitro evaluation certain plant extracts against Glomerella cin-gulata causing brown blight disease of tea.* World J Agric Sci. 8:464–467.
10. Kumar, V., Neeraj, Sharma, S., & Sagar, N. A. (2015). *Post Harvest Management of Fungal Diseases in Onion - A Review.* International Journal of Current Microbiology and Applied Sciences, *4*(6), 737–752.
11. Loon, L. C. Van, Rep, M., & Pieterse, C. M. J. (2006). *Significance of Inducible Defense-related Proteins in Infected Plants*. https://doi.org/10.1146/annurev.phyto.44.070505.143425
12. Mckinney, H. H. (1923). *Influence of soil temperature and moisture on infection of wheat seedlings by Helminthosporium sativum*. Journal of Agricultural Research, v. 26, n. 5, p. 195-217.
13. Mikaili, P., Maadirad, S., Moloudizargari, M., & Aghajanshakeri, S. (2013). *Ijbms-16-1031- Garlic and Shallot.Pdf*. Iranian Journal of Basic Medical Sciences, *16*, 1031–1048.
14. Milind Ladaniya (2023). *Postharvest disease management with fungicides*. Citrus Fruit (Second Edition). Academic Press,16, 563-594, ISBN 9780323993067, https://doi.org/10.1016/B978-0-323-99306-7.00005-0. (https://www.sciencedirect.com/science/article/pii/B9780323993067000050)
15. Ocamb C. M. and Gent D. H. (2023). *Pest Management Handbooks Onion (Allium cepa)-Black Mold*. Pacific Northwest. https://pnwhandbooks.org/plantdisease/host-disease/onion-allium-cepa-black-mold.
16. Prajapati, B., & Patil, R. K. (2016). *Bio-efficacy of fungicides in management of black mould rot (aspergillus niger) of onion*. Elsevier.
17. Prajapati, B., & Patil, R. K. (2016). *Impact of Inoculation Methods on Development of Black Mould Rot of Onion Incited by Aspergillus niger Van Tieghem Impact of Inoculation Methods on Development of Black Mould Rot of Onion Incited by Aspergillus niger Van Tieghem*.
18. Rianosa, R., Hartal, H., & Setyowati, S. (2020). *Effectiveness of the Ageratum (Ageratum conyzoides) Leaf Extract as Botanical Fungicide Against Twisted Disease of Shallot.* Jurnal Lahan Suboptimal : Journal of Suboptimal Lands, *9*(1), 1–10. https://doi.org/10.33230/jlso.9.1.2020.436
19. Sibi G., Wadhavan R., Singh S., Dhananjaya K., Ravikumar K. R., less H. M.. (2013). *Biological control of onion black mold by Indian culinary spices under in vitro conditions.* Asian Journal of Pharmaceutical and Clinical Research.
20. Singh J, Tripathi NN. 1999. *Inhibition of storage fungi of blackgram (Vigna mungo L.) by some essential oils.* Flavour Fragr J. 14(1):1-4.
21. Siswanto, N., Bintoro, N., Karyadi, J. N. W., & Rahayoe, S. (2022). *Effect of postharvest handling of shallots in supporting the sustainability of the availability of shallot seeds.* IOP Conference Series: Earth and Environmental Science, *1059*(1). https://doi.org/10.1088/1755-1315/1059/1/012072
22. Suharti, T., Nugraheni, Y. M. M. A., Suita, E., & Sumarni, B. (2020). *Effect of plant extracts and chemical fungicide on viability and percentage of seed-borne fungal infection on calliandra (Calliandra callothyrsus) seed*. *IOP* Conference Series: Earth and Environmental Science, *533*(1). https://doi.org/10.1088/1755-1315/533/1/012040
23. Swett C.L., Aegerter B.J., Turini T.A., & A. I. P. (2019). *Onion and Garlic Pest Management Guidelines Black Mold Aspergillus niger*. In *UC IPM* Agriculture. UC ANR Publication.
24. Wightwick, A., Walters, R., Allinson, G., Reichman, S., & Menzies, N. (2010). E*nvironmental Risks of Fungicides Used in Horticultural Production Systems*. Fungicides. https://doi.org/10.5772/13032
25. Zahari, R., Halimoon, N., Sajap, A. S., Ahmad, M. F., & Mohamed, M. R. (2014). *Bioantifungal activity of selected medicinal plant extracts against root rot of fungal disease.* Journal of Plant Sciences, *2*(1), 31–36. https://doi.org/10.11648/j.jps.20140201.16