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Evaluation of antifungal plant extracts against cereal and legume seed-borne pathogens for effective management

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Abstract

Sri Lanka as an agricultural country needs to manage the seed-borne fungal pathogens that have caused infections and diseases that result in significant crop losses and a decline in yield and productivity. Therefore, it is imperative to apply pathogen management strategies that are environmentally friendly, and economically feasible such as plant extractions, to reduce seed-borne fungi and increase the quality of the seed. This study was aimed at identifying the antifungal efficacy of *Allium sativum*, *Aloe vera*, *Azadirachta indica*, and *Zingiber officinale* extracts and their effective concentrations to control the seed-borne fungal pathogens; *Aspergillus flavus*, *A. niger*, *Orbilia foliicola*, *Rhizopus oryzae*, and *Talaromyces oumae-annae* isolated from *Arachis hypogea*, *Oryza sativa, Vigna radiata,* and *V. sinensis* respectively. Antifungal efficacy was determined by the agar well diffusion method and poisoned food technique. Plant extracts' effectiveness for seed germination and seed quality was evaluated by pot experiments. *Zingiber officinale* crude extract exhibited the highest antifungal activity against the tested pathogens which was as effective as Captan 50% (WP), a positive control. Further analysis of the results from the pot experiment revealed that *O. sativa*, and *V. radiata* seeds treated with *A. indica*, and *Z. officinale* aqueous extracts showed 100% germination percentage. *Azadirachta indica*, and *Z. officinale* aqueous extracts are the most effective in promoting seed germination and seedling vigor while *A. vera* extract is the least effective extract. Comparing the two different extracts, aqueous extracts significantly promote seed germination and increase seedling vigor.

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INTRODUCTION

Sri Lanka's food supply revolves around cereal and legume crops centered on rice, and pulses followed by fruits and vegetables. Almost 90% of the world's food crops are grown from seeds[1]. During the second part of the 20th century, cereal and legume production in South Asian countries tripled as a result of the Green Revolution[2–4]. It has been found that the seed-borne fungi generate a considerable effect on the reduction of crop productivity as the external and internal distribution of seed-borne pathogens causes seed abortion, seed necrosis, elimination of seed germination capacity, and damage to seedlings[5]. Seed-borne pathogen prevalence has long been known to be influenced by numerous factors including abiotic factors such as temperature, humidity, and moisture content, and also biotic factors like insect damage during seed storage[6]. Different fungal pathogens and fungal like pathogens that attack cereal cultivars belong to a few of the common genera such as *Alternaria, Aspergillus, Cercospora, Fusarium, Phytophthora,* and *Rhizoctonia*[1,7]. The presence of these seed-borne pathogens causes mycotoxin production, and mycotoxin contamination of cereal grain is a worldwide issue for public health and a permanent concern for cereal-food industries[8]. For instance, aflatoxin B1 (AFB1) is generated largely by *A. flavus*, and *A. parasiticus* and it is one of the most carcinogenic, teratogenic, hepatotoxic, and mutagenic mycotoxins[9]. *Fusarium* species occur in several crop species, such as wheat, maize, and sorghum plants, and are also associated with the production of trichothecenes, deoxynivalenol (DON), and its toxic derivatives[10,11]. Mycotoxin production has a detrimental impact on both humans and animals, causing issues with the liver, kidneys, and intestines. Furthermore, chronic disorders are caused by long-term exposure to low levels of aflatoxins, the most common and devastating of which is cancer[12]. Therefore, control of seed-borne fungi becomes an essential aspect to reduce the impacts generated by the seed-borne fungi. In particular, controlling the seed-borne fungi can be accomplished *via* chemical or natural approaches[13]. However, misuse of these chemical techniques, such as synthetic fungicides has resulted in many issues around the world, including environmental, ecological, health, social, and economic sectors. Consequently, switching to natural pathogen management is a more viable and appropriate strategy for the farming community[13,14]. Considerable literature evidence showed that *Aloe vera, Allium sativum, Azadirachta indica*, *Camellia sinensis, Chrysanthemum coccineum, Coffee arabica, Datura stramonium,* and *Z. officinale* plant extracts have a potential to control the seed-borne fungi including *Aspergillus fumigatus* , *A. niger*, *A. flavus*, and *Pyricularia oryzae*[7,14–16]. This study was conducted to address a knowledge gap in the Sri Lankan agricultural environment and to increase public awareness of the fungal species, such as *Arachis hypogea* (peanut), *Oryza sativa* (rice), *Vigna radiata* (mungbean), and *Vigna sinensis* (cowpea). Furthermore, the study would facilitate the ability to find appropriate plant extract treatments and the feasibility of using natural plant products as fungicides to control plant diseases cause by seed-borne fungi.

MATERIALS AND METHODS

Collection of samples

Seeds from *Arachis hypogea* L. (Tissa), *Oryza sativa* L. (Bg251), *Vigna radiata* (L.) R.Wilczek (M16), and *Vigna sinensis* L. (Dhawala) were collected from the Seed Certification and Plant Protection Centre (SCPPC), Gannoruwa, Sri Lanka in July 2021. The *Allium sativum* bulbs, and *Zingiber officinale* rhizomes were collected from the marketplaces in the Maharagama area (Sri Lanka). *Aloe vera*, and *Azadirachta indica* leaves were collected from home gardens in the Maharagama area.

Methanol: Distilled water (4:1) extraction

Respective parts of each plant materials including *A. sativum* bulbs, *A. vera*, and *A. indica* leaves, and *Z. officinale* rhizome 100 g were taken and thoroughly washed with tap water to remove soil and other debris. The leaves, bulbs, and rhizomes were then separated and air-dried under the shade at 25–29 °C until they became dry and crispy. The dried plant materials were powdered using a heavy-duty blender (Panasonic MX-AC 400, India). About 25 g of each air-dried plant material was defatted with 10 mL of n-hexane (C_6H_{14}). Then the solution was extracted with methanol: distilled water (4:1) at room temperature (28 ± 2 °C) by maceration with occasional stirring for 48 h. The macerate was filtered using Whatman filter papers No. 1. Samples were concentrated by a rotary evaporator (IKA HB 10 digital, China) at 37 °C. Each sample was dissolved in Dimethyl Sulfoxide (DMSO), and DMSO-water (50%, v/v) was added to prepare the stock solutions[17,18]. Stock solutions were used to prepared the (10%, 15%, 20%) (w/v) solutions.

Aqueous extraction

Respective parts of each plant materials including *A. sativum* bulbs, *A. vera,* and *A. indica* leaves, and *Z. officinale* rhizome 100 g were taken, and thoroughly washed with tap water to remove soil and other debris. The leaves, bulbs and rhizomes were then separated and air-dried under the shade at 25–29 °C until they became dry and crispy. The dried plant materials were powdered using an electrical heavy-duty blender (Panasonic MX-AC 400, India). About 25 g of air-dried *A. sativum*, *A. vera*, *A. indica*, and *Z. officinale* powder were separately mixed with 75 mL of sterilized distilled water, then solutions were stored in a flask and the extracts were left standing in the dark for 3–4 d. The solutions were then filtered through two layers of muslin cloth[17,19].

Antifungal activity by agar well diffusion method

The antifungal activity of *Allium sativum, A. vera, A. indica*, and *Z. officinale*, plant extracts were evaluated against the identified seed-borne pathogens by the agar well diffusion method with modifications[20]. The 8–10 d old pure cultures of fungal pathogens including; *Aspergillus flavus*, *A. niger*, *Orbilia foliicola*, *Rhizopus oryzae*, and *Talaromyces oumae-annae* were sub-cultured onto PDA medium. Using a sterile cork borer, three 5 mm diameter wells were made on each sub-cultured plate (Fig. 1). Then 10%, 15%, 20% (w/v) of each plant extract were introduced into each well made on the medium.

Then 5 mm diameter well was created on a separate plate and it was filled with fungicide 50% (WP) Captan as the positive control. DMSO-water (50%, v/v), methanol-water (80%, v/v), sterilized distilled water, and n-hexane were introduced into another separate plate containing wells as the negative control.

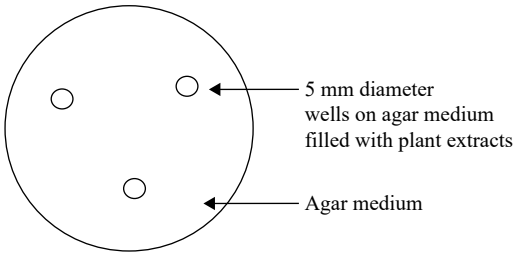

Fig. 1 Diagram of the final step in the agar well diffusion method.

Then the plates were allowed to stand in an upright position for 15 min to allow for the proper diffusion of the extracts into the medium before incubation. The plates were incubated at room temperature (28 ± 2 °C) for about 7 d[21]. The experiment was performed in triplicates to minimize the error ratio. At the end of incubation period, the inhibition zones around each well was measured for 3, 7 d intervals after the inoculation to an accuracy of 0.1 mm and the effect was calculated as a mean of triplicate tests to evaluate the antifungal activity[21,22].

Antifungal activity by the poison food method

Plant extracts of *Allium sativum, A. vera, A. indica*, and *Z. officinale* were evaluated against the identified seed-borne pathogens including; *Aspergillus flavus*, *A. niger*, *O. foliicola*, *R. oryzae*, and *T. oumae-annae*. Plant extracts at 10% (w/v), 15% (w/v), and 20% (w/v) concentrations were used for this study for which 10, 15, and 20 mL of stock solutions were mixed with 90, 85, and 80 mL of sterilized PDA media individually. The amended PDA medium was thoroughly shaken for uniform mixing with leaf extracts. Twenty mililitres of this mixed agar media was poured into sterile petri plates and allowed to solidify. Five millimetre diameter of agar disk of test pathogenic fungi were cut from the 8-10 d old pure cultures using a sterile cork-borer and placed in the center of each petri-plate containing different concentrations of plant extracts (Fig. 2). The experiment was carried out in triplicate[23]. The petri plates with 10 mL of each solution including; fungicide 50% (WP) Captan, DMSO-water (50%, v/v), methanol-water (80%, v/v), sterilized distilled water, and n-hexane were mixed with 90 mL of sterilized PDA media and maintained as controls. Then the plates were incubated at room temperature (28 ± 2 °C) for about 7 d[21]. At the end of the incubation period, the percentage inhibition of mycelial growth was calculated using the equation below[23].

$$\%\text{Inhibition effect} = \frac{\text{Inhibition halo diameter in control} - \text{inhibition halo diameter in treatment}}{\text{Inhibition halo diameter in control}} \times 100$$

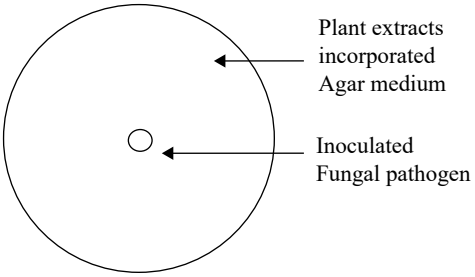

Fig. 2 Diagram of the final step in the poison food method.

Seed germination and seedling vigor

Seed quality was measured in pot experiments with soil sterilized by steam distillation. A total of 20 seeds per sample of *O. sativa*, *V. radiata*, and *V. sinensis* and 10 seeds of *A. hypogea* were soaked in plant extracts which were taken from methanol: distilled water (4:1) for 10 min. The same number of seeds were soaked in DMSO-water (50%, v/v), methanol-water (80%, v/v) and sterilized distilled water, n-hexane for 30 min as the negative control, and in fungicide 50% Captan 50% (WP) for 10 min as the positive control. The liquid was drained off and the seeds were dried under shade before use. A total of 20 seeds of *O. sativa*, *V. radiata,* and *V. sinensis* and 10 seeds of *A. hypogea* were sown per pot. The used potting mixture was Compomix potting soil with equal parts of compost, top soil, coir dust and sand which were sterilized by steam distillation. The seeds were covered with 1–3 cm deep soil layer depending on the seed size. The pots were kept in partial shade and had an average temperature of approximately 30 °C with regular watering. The germination count was recorded every day during the testing period.

Plants were carefully uprooted and gently washed with running water. The percentage of seeds germinating normally, abnormally, healthily and diseased seedlings were recorded according to International Seed Testing Association Rules[24] and infections were counted. Seedling vigor was measured using the shoot and root length[2,25,26]. Shoot lengths from the base of the shoot to the uppermost leaf tip and root length from the collar region to the end of the longest tip were measured[1]. Measurements were taken after three weeks of sowing for *A. hypogea* and after two weeks for the other seed varieties. The germination percentage, germination index, and vigor index were calculated using the below-mentioned equations[2,25,26]. During recording, the normal seedlings and abnormalities of germinating seeds and seedlings, were considered[27].

$$\text{Germination percentage} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100$$

$$\text{Speed of germination index} = \frac{\text{No. of seeds germinated}}{\text{day of first count}} + \frac{\text{No. of seeds germinated}}{\text{day of final count}}$$

$$\text{Vigor index} = \text{Germination percentage (\%)} \times (\text{mean root length} + \text{mean shoot length})$$

Statistical analysis

All experiments were performed in triplicates and all the data were expressed as mean value ± (SE). Analysis of variance (ANOVA) and Tukey's multiple comparison were performed on all transformed data collected in respect of parameters studied on effects of plant extracts at the least significant difference (LSD) which was employed to test for significant differences between treatments at $P \leq 0.05$ using Minitab (version 17).

RESULTS

Antifungal activity by the agar well diffusion method

Extract of *Allium sativum* exhibited moderate antifungal activity against the selected seed-borne pathogens; *Aspergillus flavus, A. niger, O. foliicola, R. oryzae,* and *T. oumae-annae.* Following the results obtained by the agar well diffusion method, a maximum mean inhibition diameter of 2.53 cm was reported for *T. oumae-annae* by the *A. sativum* crude extract after 3 d of incubation. The lowest mean inhibition diameter of 0.40 cm was reported for *A. niger* by 10% (w/v) *A. sativum* extract after 7 d of incubation. The effect of 20% (w/v) *A. sativum* extracts in inhibiting the seed-borne fungal pathogens was considerably higher than the other two *A. sativum* concentrations (10% and 15%). Extract of *A. vera* exhibited low antifungal activity while the *Z. officinale* showed the highest antifungal activity compared with other treatments against the selected seed-borne pathogens; *A. flavus, A. niger, O. foliicola, R. oryzae,* and *T. oumae-annae.* After 7 d of incubation, 10% (w/v) and 15% (w/v) *A. vera* extract could not exhibit inhibition against *A. niger* and was unable to successfully control the *A. niger*. Compared to the other three plant extracts, the best mean inhibition diameters of 1.50 cm and 1.33 cm for *A. niger* was reported by the *A. indica* crude extract and 20% (w/v) *A. indica* extract respectively after the 7 d of incubation. In this experiment Captan 50% (WP) used as the positive control, was capable of significantly arresting the growth of *A. flavus, A. niger, O. foliicola, R. oryzae,* and *T. oumae-annae* compared to the negative controls (Figs 3 & 4).

Antifungal activity by the poisoned food technique

Extract of *Z. officinale* exhibited the highest antifungal activity against the selected seed-borne pathogens and the effect of crude extract of *Z. officinale* in inhibiting the seed-borne fungal pathogens was considerably higher than the other concentrations (Fig. 5). The effect of crude extract and 20% (w/v) *Z. officinale* in controlling *T. oumae-annae* was significantly similar to the effect of commercial fungicide Captan (50% WP). Increasing the concentrations of plant extract caused an increase in the percentage inhibition of all tested fungi (Fig. 6). *Allium sativum* extract percentage inhibition ranged between 50.90% to 98.62% for all five pathogens tested. Maximum percentage inhibition (other than the positive control) of 96.81% was reported for *T. oumae-annae* by the *A. sativum* crude extract while the lowest percentage inhibition (50.90%) was reported for *A. niger* by 10% (w/v) *A. sativum* extract. Furthermore, *Aloe vera* extract percentage inhibition ranged between 37.43% to 98.62% for all five pathogens tested and maximum percentage inhibition (other than the positive control) of 93.61% was reported for *T. oumae-annae* by *A. vera* crude extract and 20% (w/v) *A. vera* extract. The lowest percentage inhibition (37.43%) was reported for *A. niger* by 10% (w/v) *A. vera* extract. The effect of crude extract of *A. vera* in inhibiting the seed-borne fungal pathogens was considerably higher than the other *A. vera* concentrations. The second most effective extraction was *A. indica* and maximum percentage inhibition (other than the positive control) of 96.28% was reported for *O. foliicola* by *A. indica* crude extract. The level of controlling seed-borne fungal pathogens by three different concentrations of *A. sativum* extract, 10% (w/v), 15% (w/v), and 20% (w/v) was statistically significant ($P < 0.05$) compared to the negative controls (0%) in the study.

Seed germination and seedling vigor

Seed varieties including *A. hypogea*, *O. sativa, V. radiata,* and *V. sinensis* were treated separately with aqueous extractions (Table 1). Seed treatment with aqueous extract of *A. sativum* exhibited the highest (80%) germination percentage for *A. hypogea* similar to the germination percentage by captan (50%

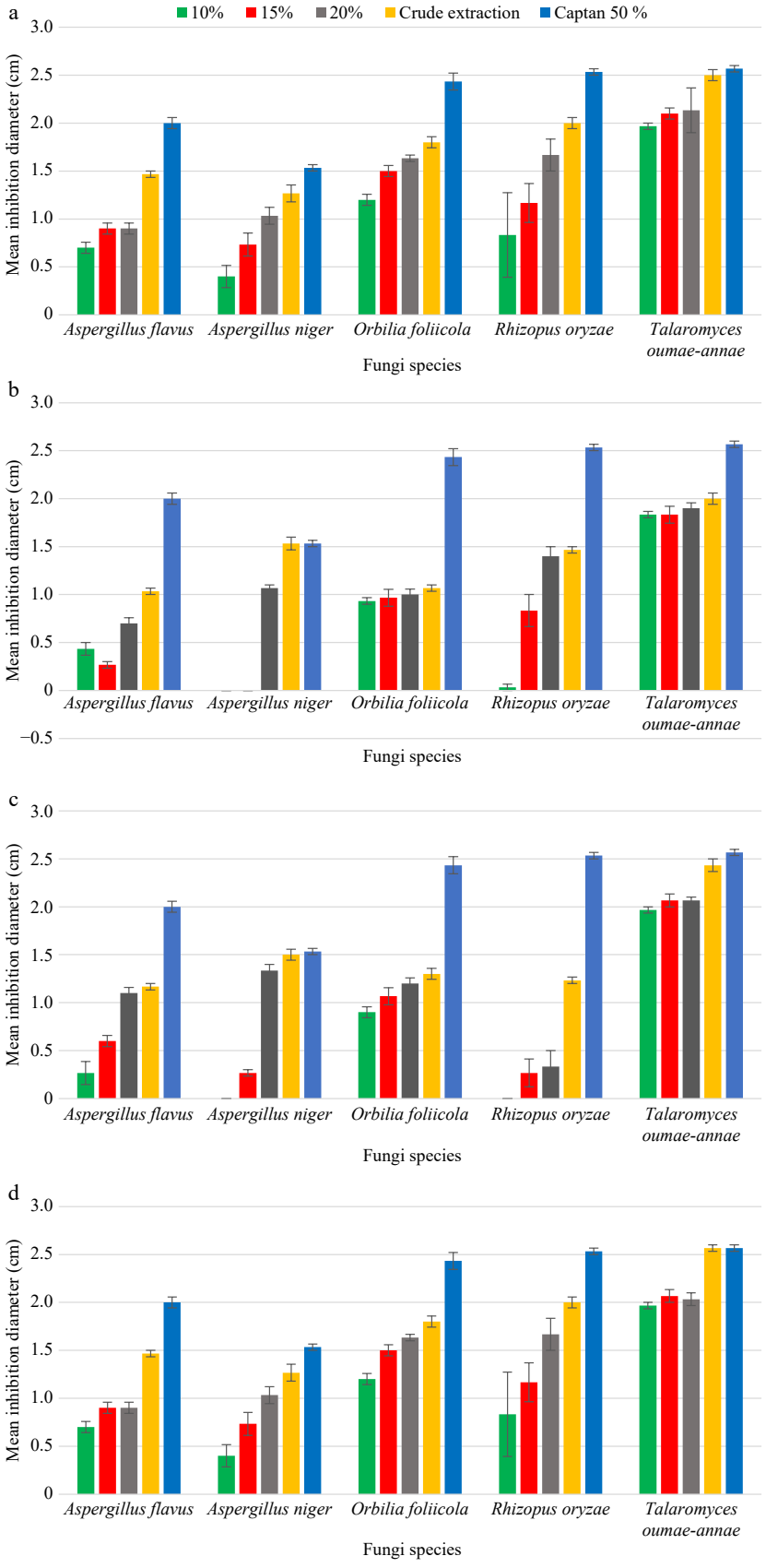

Fig. 3 Effect of different concentrations of (a) *A. sativum*, (b) *A. vera*, (c) *A. indica* and (d) *Z. Officinale* aqueous extractions against all five pathogens after 7 d of incubation.

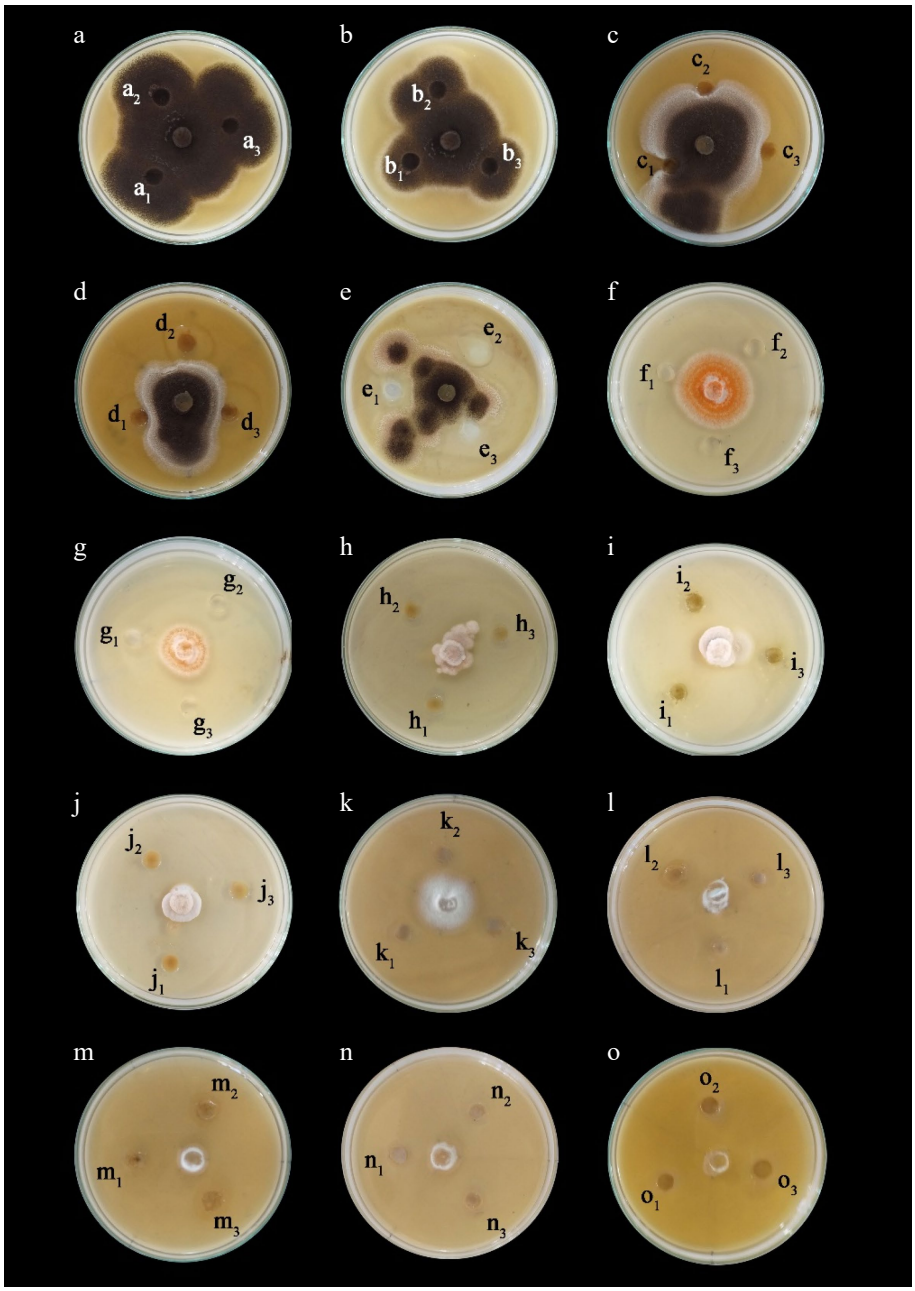

Fig. 4 Effect of different concentrations of plant extracts against the selected seed-borne pathogens; *A. niger, O. foliicola*, and *T. oumae-annae*. (a) *A. niger* in negative controls; a_1-Methanol 80%, a_2-n-hexane, a_3-DMSO 50%; (b) *A. niger* in *A. vera* (b_1-10%, b_2-15%, b_3-20% concentrations); (c) *A. niger* in *A. sativum* (c_1-10%, c_2-15%, c_3-20% concentrations); (d) *A. niger* in *Z. officinale* (d_1-10%, d_2-15%, d_3-20% concentrations); (e) *A. niger* in Captan 50 % (WP) positive control (e_1, e_2, e_3-Captan 50 % (WP) positive control) ; (f) *O. foliicola* in negative controls; f_1-Methanol 80%, f_2-n-hexane, f_3-DMSO 50%; (g) *O. foliicola* in *A. vera* (g_1-10%, g_2-15%, g_3-20% concentrations); (h) *O. foliicola* in *A. sativum* (h_1-10%, h_2-15%, h_3-20% concentrations); (i) *O. foliicola* in *A. indica* (i_1-10%, i_2-15%, i_3-20% concentrations); (j) *O. foliicola* in *Z. officinale* (j_1-10%, j_2-15%, j_3-20% concentrations); (k) *T. oumae-annae* in negative controls; k_1-Methanol 80%, k_2-n-hexane, k_3-DMSO 50%; (l) *T. oumae-annae* in *A. vera* (l_1-10%, l_2-15%, l_3-20% concentrations); (m) *T. oumae-annae* in *A. sativum* (m_1-10%, m_2-15%, m_3-20% concentrations); (n) *T. oumae-annae* in *Z. officinale* (n_1-10%, n_2-15%, n_3-20% concentrations); (o) *T. oumae-annae* Captan 50 % (WP) positive control (o_1, o_2, o_3-Captan 50 % (WP) positive control).

WP). It was higher than the germination given by all other extractions including the negative control. All three extractions including *A. sativum, A. indica,* and *Z. officinale* exhibited the highest (100%) germination percentage for *V. radiata* and lower germination given by *A.vera* extraction. Among the four plant aqueous extracts tested, the maximum vigor index of 2664 was reported from *Z. officinale* while the highest germination index of 5.42 was reported from *A. sativum, A. indica*, and *Z. officinale* extracts for *V. radiata*. *Azadirachta indica* and *Z. officinale* exhibited the highest (100%) germination percentage for *O.sativa* similar to the germination percentage by captan (50% WP).

The lowest germination percentage (70%) was reported for *O.sativa* by aqueous extract of *A. sativum*. The maximum vigor index of 3079 and the highest germination index of 4.72 for

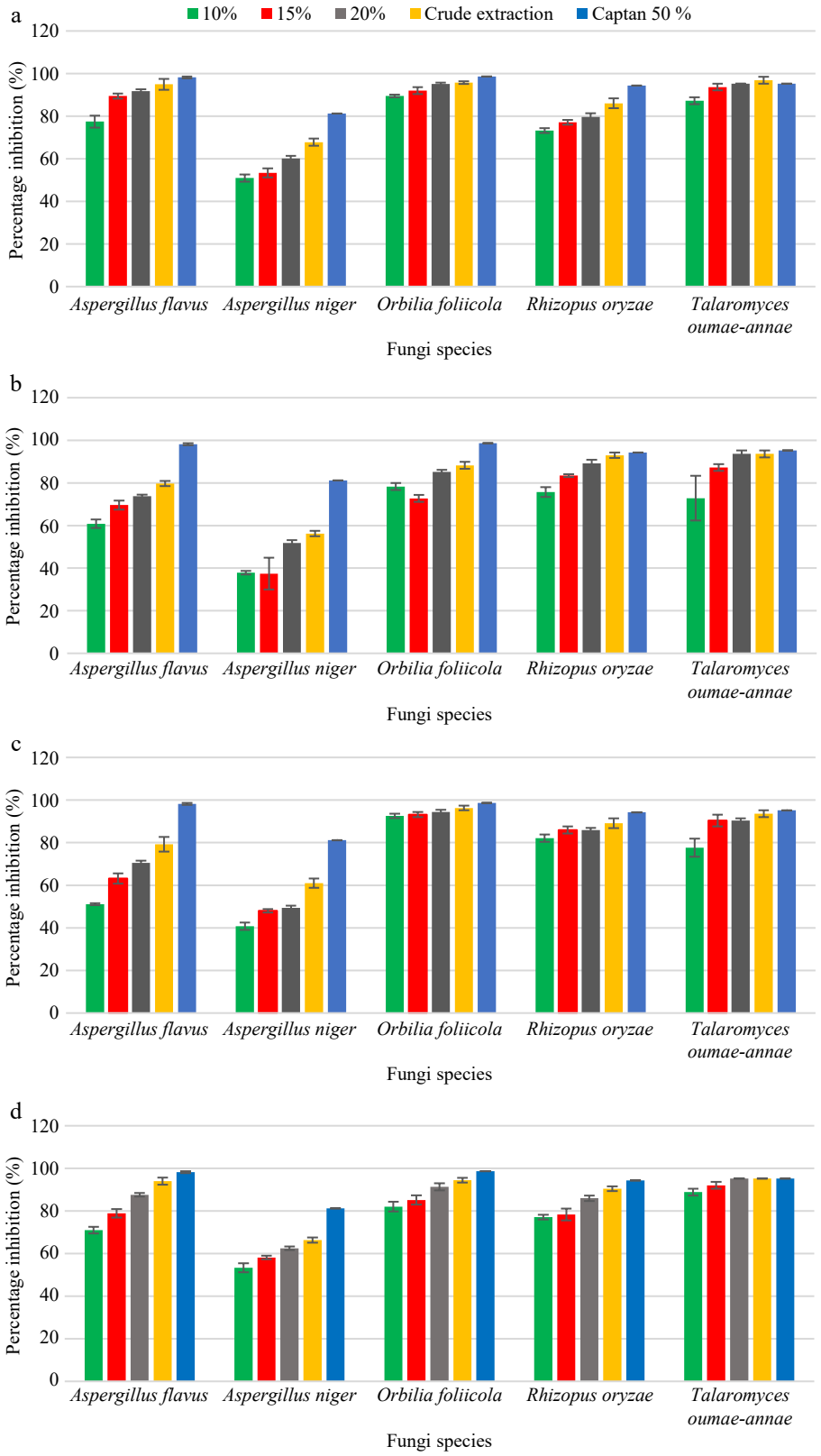

Fig. 5 Effect of different concentrations of (a) *A. sativum*, (b) *A. vera*, (c) *A. indica* and (d) *Z. Officinale* aqueous extractions against all five pathogens after 7 d of incubation.

O.sativa were reported from *Z. officinale*. Seed treatment with aqueous extract of *A. indica* exhibited the highest (30%) germination percentage for *V. sinensis* which is similar to the germination percentage by Captan (50% WP). Among the four plant aqueous extracts the lowest germination percentage (15%) was reported for *V. sinensis* by aqueous extract of *A. vera*. and the maximum vigor index of 686.4 and highest germination index of 1.42 for *V. sinensis* were reported from *A. indica*.

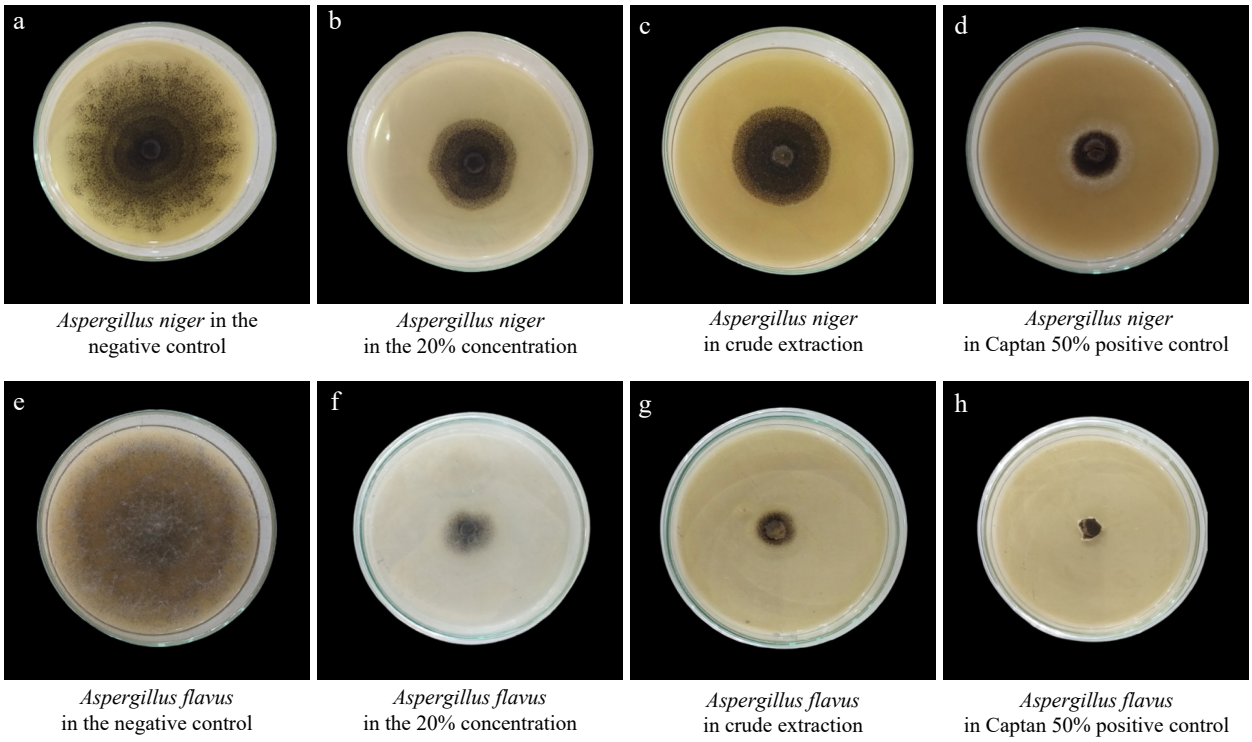

Fig. 6 Effect of different concentrations of *Z. officinale* extract against the selected seed-borne pathogen; *A. niger* and *A. flavus*. (a) *A. niger* in negative control (distilled water); (b) *A. niger* at 20% concentration; (c) *A. niger* in crude extract; (d) *A. niger* in Captan 50 % positive control; (e) *A. flavus* in negative control (distilled water); (f) *A. flavus* in 20% concentration; (g) *A. flavus* in crude extract (h) *A. flavus* in Captan 50 % (WP) positive control.

Table 1. Effect of different plant extracts on seed germination data of *Arachis hypogea*, *Oryza sativa*, *Vigna radiata*, *V. sinensis* seeds.

Plant extract	Seed variety	Storage period (months)	% Germination	% Abnormal seedling	Speed of germination index	Vigor index
Allium sativum	*Arachis hypogea*	>6	80	20	2.17	1912.8
Aloe vera		>6	60	–	0.94	2118
Azadirachta indica		>6	70	–	2.20	1710.8
Zingiber officinale		>6	50	10	1.02	1205
Distilled water		>6	30	30	0.71	713.1
Captan (50%)		>6	80	–	2.31	3329.6
Allium sativum	*Oryza sativa*	>6	70	20	1.33	2170
Aloe vera		>6	80	–	1.66	1565.6
Azadirachta indica		>6	100	–	4.42	2824
Zingiber officinale		>6	100	30	4.72	3079
Distilled water		>6	80	–	1.73	1742
Captan (50%)		>6	100	–	4.73	4385
Allium sativum	*Vigna radiata*	>6	100	–	5.42	2404
Aloe vera		>6	95	–	5.02	2011.2
Azadirachta indica		>6	100	10	5.42	2269
Zingiber officinale		>6	100	–	5.42	2664
Distilled water		>6	100	30	5.40	2306
Captan (50%)		>6	100	20	5.42	2238
Allium sativum	*Vigna sinensis*	>6	20	–	1.08	509.4
Aloe vera		>6	15	–	0.81	396.4
Azadirachta indica		>6	30	–	1.42	686.4
Zingiber officinale		>6	25	–	0.96	627
Distilled water		>6	5	–	0.23	105
Captan (50%)		>6	30	10	1.09	705

Furthermore, the lowest vigor index of 396.4 and the lowest germination index of 0.81 for *V. sinensis* were reported from *A. vera* (Fig. 7).

Effect of plant methanolic extracts on seed quality

Seed treatment with methanolic extract of 15% (w/v), 20% (w/v) *A. indica* exhibited the highest (50%) germination

Fig. 7 Seed germination data of *Arachis hypogea* treated with (a) *Azadirachta indica,* (b) *Aloe vera,* (c) *Allium sativum,* and *V. sinensis* treated with (d) *Allium sativum*, (e) *Aloe vera* and (f) *Zingiber officinale* aqueous extractions.

percentage for *A. hypogea* while the lowest germination percentage (20%) was reported for *A. hypogea* by aqueous extract of 10% (w/v) *A. vera* and 10% (w/v) of *Z. officinale*. Among the four plant methanolic extracts, the lowest germination percentage (30%) and lowest vigor index of 654.9 were reported for *O. sativa* by methanolic extracts of 10% (w/v) *A. vera* extract. Seed treatment with methanolic extract of 20% (w/v) *Z. officinale* exhibited the highest (90%) germination percentage and maximum germination index of 3.53 for *O. sativa* which is lower than the germination percentage by Captan (50% WP). Also, the seed treatment with methanolic extracts of 10% (w/v), 15% (w/v)**,** and 20% (w/v) *A. indica* exhibited the highest (100%) germination percentage for *V. radiata* while the lowest germination percentage (50%) was reported from 10% (w/v), 15% (w/v) *A. vera* and 10% (w/v) of *Z. officinale* extracts. According to the *V. sinensis* germination data methanolic extract of 20% (w/v) *A. sativum* exhibited the highest (55%) germination percentage and maximum vigor index of 1564.8 which is even higher than the germination percentage by Captan (50% WP). The lowest germination percentage (10%) was reported for *V. sinensis* by methanolic extract of 10% (w/v) *A. vera* (Table 2).

DISCUSSION

Seeds are the most vital input for crop production. Fungal pathogens caused by seed-borne infections are one of the most considerable risks to cereals and legume production worldwide, which cause a reduction in both quantity and quality every year[28]. Plants and other natural sources are capable of producing a wide range of complex and structurally diverse compounds, which have low to significant levels of bioactive potential[29,30]. In this present study, fungitoxicity was expressed in terms of percentage inhibition and mean inhibition diameter.

Antifungal activity of four medicinal plant extracts including; *A. sativum*, *A. vera*, *A. indica*, and *Z. officinale* were assayed by the agar well diffusion method and poisoned food technique. Our preliminary results in the current study by these two methods indicate that each of the extracts of four medicinal

Table 2. Effect of different plant extracts on seed germination data of *Vigna sinensis* seeds.

Plant extract	(w/v)	Storage period (months)	% Germination	% Abnormal seedling	Speed of germination index	Vigor index
Allium sativum	10	>6	20	–	1.08	705
	15		35		1.95	1092.4
	20		55		2.20	1564.8
Aloe vera	10	>6	10	–	0.42	273
	15		30		0.94	822.3
	20		20		0.94	470
Azadirachta indica	10	>6	20	–	1.35	670
	15		30		1.62	904.8
	20		20		1.77	250
Zingiber officinale	10	>6	15	–	1.95	397.5
	15		15		2.32	449.8
	20		30		2.32	983.4
Distilled water	–	>6	5	–	0.23	105
n-Hexane	–	>6	4	–	1.11	89.0
Methanol (80%)	–	>6	5	–	0.94	84.6
DMSO (50%)	–	>6	4	–	0.87	77.0
Captan (50%)	–	>6	30	10	1.09	705

plants reduced the natural infection frequency of *Aspergillus flavus*, *A. niger*, *Orbilia foliicola*, *Rhizopus oryzae and Talaromyces oumae-annae*.

It was evident that *A. vera* extract could not exhibit significant antifungal activity against some of the selected seed-borne pathogens such as *A. niger*. It may be due to the low concentration of antifungal compounds in *A. vera* extract or may also be due to the low diffusibility of these antifungal compounds into the agar medium[31]. However, results of the poisoned food technique demonstrated a higher percentage inhibition for *A. niger* by *A. vera* extract. According to the previous literature, *A. vera* plant extract contains several antifungal, anti-inflammatory, antibacterial, and antiarthritic properties[32]. When comparing the two approaches (agar well diffusion and poison food technique) utilized in the current study to assess the *in-vitro* activity of plant extracts in reducing seed-borne fungal infections, it is noteworthy to mention that the poison food technique exhibited a rather significant effect in inhibition, which is also proven from previous studies[33]. The possible reason for this can be due to the higher distribution of antifungal compounds across the agar medium and prevents the growth of microbial strains tested. Results of the present study were aligned with previous literature data and clearly show that *A. vera* extracts exhibited poor antifungal efficacy against *Aspergillus* strains[34].

According to previous literature, considerable inhibition was reported for *A. niger* by *Z. officinale* extract[35]. Several studies also revealed that significant percentage inhibition can be exhibited for *A. niger* by *A. sativum* extract[36]. Observations from our study are in agreement with the above studies exhibiting higher level efficacies in controlling the common fungal pathogens such as *Aspergillus* spp. This inhibition may be due to the basic, low-molecular weight nitrogen-containing alkaloid present in *A. sativum*. These nitrogen-containing alkaloids have an inhibitory effect on fungal enzyme activities and it was able to degrade the chitin-made cell walls of *A. niger*, thus degrading the integrity of the cellular structures and causing the apoptosis of pathogenic cells. Also, the high flavonoid content in *A. sativum* plays a significant role in its antibacterial and antifungal effect against fungal pathogens[36].

In addition, it was found that *Z. officinale* crude extract showed the highest percentage inhibition against *T. oumae-annae* and it was just as effective as the percentage inhibition exhibited by Captan 50% (WP). Therefore, this concentration was found to have a high fungicidal effect on *T. oumae-annae*. However, this requires further study to confirm the exact concentration of *Z. officinale* crude extract which can replace the commercial fungicide Captan 50% (WP). The inhibition of *A. flavus*, *A. niger*, *O. foliicola*, *R. oryzae*, and *T. oumae-annae* was found to increase with the concentration of plant extraction in the agar well diffusion method. Similarly, the poisoned food technique has also reflected a decrease in the fungal mycelial growth with the increase in the concentration of the plant extract.

The ultimate goal of our study was to use these different plant extractions against pathogenic fungi in order to use them as a successful natural fungicidal agent to treat seeds and increase seed quality. The search for antimicrobials from natural sources has received a lot of attention, and researchers are attempting to find compounds that can replace synthetic antimicrobials[37]. For that reason, the pot experiment was conducted to test the quality of the seeds; *A. hypogea, O. sativa, V. radiata,* and *V. sinensis* with different extracts including; *A. sativum*, *A. vera*, *A. indica*, and *Z. officinale*. According to previous literature data, *A. indica* in aqueous extraction enhances the germination of *O. sativa* at 100% strength[38]. Further studies revealed that *A. indica* consistently produced a considerably higher inhibitory effect on radial growth of the seed-borne fungi in *O. sativa*[39].

The aqueous extracts of *Z. officinale* had strong antifungal activity against *A. niger* and enhanced the germination of *O. sativa*[40].

Furthermore, *Z. officinale* was found highly effective in reducing the growth of the seed-borne fungus and enhancing seed germination[21].

According to data from the Seed Certification and Plant Protection Centre (SCPPC, Gannoruwa, Sri Lanka), *V. radiata* showed 75% germination percentage and the present study revealed that the aqueous extracts of *A. sativum*, *A. indica* and *Z. officinale* exhibited a 100% germination percentage for *V. radiata*. However, the germination percentage of *V. radiata* was reduced during methanolic extract treatments. The presence of methanol may be due to the reduction in germination by damaging the seed embryo. Though the mechanism of action of these plant components is unknown, it is apparent that the efficiency of extracts is highly dependent on the type of solvents used[41,42]. On the contrary, this study found that all aqueous and methanolic plant extracts treated *A. hypogea* and *O. sativa* showed significant activity in promoting seed germination and increasing seedling vigor compared to non-treated *A. hypogea* seeds and *O. sativa*[1]. This percentage was higher than the percentage germination reported by the Seed Certification and Plant Protection Centre (SCPPC, Gannoruwa, Sri Lanka), which is 75% for *A. hypogea* and 85% for *O. sativa*. Nevertheless, it was shown that all aqueous and methanolic plant extracts treated *V. sinensis* and only methanolic plant extracts treated *V. radiata* showed lower germination percentage compared to non-treated *V. sinensis* seeds and *V. radiata*[1].

CONCLUSIONS

The study presents the antifungal activity and seed germination potential of plant extracts. Methanolic extract of *Z. officinale* exhibited the highest antifungal activity while the *A. vera* exhibited the lowest antifungal activity against seed-borne pathogens; *A. flavus, A. niger, O. foliicola, R. oryzae,* and *T. oumae-annae* in this study compared with other treatments. Furthermore, *A. indica* and *Z. officinale* aqueous extracts are the most effective extract and *A. vera* extract is the least effective extract in controlling the seed-borne pathogens and promoting seed germination and seedling vigor of *Arachis hypogea*, *Oryza sativa*, *Vigna radiata*, *V. sinensis*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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