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Comparison of the Germinability and Protective Potentials of *Helianthus annuus* Extracts and Copper Sulphate Solution on *Cajanus cajan* Seeds

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ABSTRACT: Proper seed treatment is vital for seed quality improvement and significant increase in crop vield. The aim of this study was to evaluate the germinability improvement and protective potentials of leaf extracts of Helianthus annuus in comparison with CuSO4 on Cajanus cajan seeds. The extracts used were ethyl acetate, hexane and methanol. Prior to use, the seeds of the C. cajan were checked for viability and surface sterilized. The effective concentration that enhanced germinability of the seeds and optimum soaking time in the respective extracts and chemicals were determined. In the different experiments, surface sterilized seeds were soaked in the respective extracts and chemicals at known durations. After planting, the seeds were observed for germination and germination time, germination rate, % germination, plant height and vigor index were calculated, using standard procedures. The fungal species involved in the infectivity studies were Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus and Penicillium sp. From the findings, optimum concentrations of 500 mg/L of the ethyl acetate and hexane extracts and 2500 mg/L of the methanol extract were observed for steeping. Optimum soaking duration of 3-4 h was recorded for steeping in the extracts and CuSO₄ solution. All the extracts showed protective potential on the infected seeds and inhibited the growth of all the test fungal species, except Penicillium. Observations from this study could help in the development of viable alternatives to chemical treatments of seeds, thus enhancing sustainable and eco-friendly agricultural practices and management.

Keywords: Germinability improvement, Protective Potentials, Aspergillus, Helianthus annuus

I. INTRODUCTION

Plants have been used throughout history by human beings because they produce diverse secondary metabolites that defends against environmental stress and additional factors like pest attacks, wounds, and injuries. The intricate secondary metabolites produced by plants have found various therapeutic uses in medicine from time immemorial. The early history of present-day prescription contains depictions of plantderived phytochemicals, a large number of which are still being used [1]. The beneficial effects of seed priming have been documented in cereals, sugar crops, oilseeds and horticultural crops [2]. A few investigations report long-lasting effects on yield-associated advantages in terms of increased growth rates, high dry matter production and produce quality by improving crop protection from biotic and abiotic stresses [1].

The initial remedy for dealing with a large number of diseases affecting plants is prevention. An integration of strategies which involves cultural practices, sanitation and seasonal applications of spray are used for disease prevention. The purpose of sanitation is to remove the cause of future disease by a thorough clean-up program. Proper treatment of seed is a certain element for the improvement of seed quality and it allows a significant increase in crop yield [3].

The adoption of prevention and control of plant diseases with agrochemicals, causes adverse environmental and health hazards. The occurrence of chemicals in the environment could lead to the termination of the natural balance of the ecosystem by eliminating the beneficial microorganisms in the soil. New methods of less expensive seed treatment, which does not require the use of chemicals and is eco-friendly in controlling plant diseases have to be discovered. Medicinal plants contain various compounds which may serve as potential antifungal agents and they serve as alternative, cheap, effective, and safe antifungal for treatment of common fungal infections [4]. The objective of this study was to assess the potential of methanol, hexane and ethyl acetate extracts of *Helianthus annuus*, in comparison with copper sulphate solution in enhancing germinability, vigor and protection on *Cajanus cajan* against selected fungal pathogens.

II. MATERIALS AND METHODS

A. Helianthus annuus extracts

Three leaves extracts (hexane, ethyl acetate and methanol) of *Helianthus annuus* were used for this study. The leaves samples were collected from the environment of the Teaching and Research Farm Landmark University, Omu-Aran, Kwara State, Nigeria. The collected leaves were first washed to remove sand and debris. They were then sun-dried and pulverized using a laboratory blender.

For extraction, known quantities of the dried leaves were placed in 2 L capacity glass beakers and the respective solvents added (1:2 w/v) and allowed to stand for 24 h at room temperature. The extracted solution was filtered, using Whatman No. 1 filter paper, after which the respective supernatant was removed and the filtrate was concentrated in a rotary evaporator (MRC-ROVA 100) and freeze dried with a freeze-drier (LYOTRAP). The dried extracts were kept in clean plastic bottles and kept in the refrigerator until when needed.

B. Seed preparation and experimental setup

The *Cajanus cajan* seeds, was obtained from the laboratory of the Department of Agricultural Sciences (Crops Science Unit) of the Landmark University, Omu-Aran, Kwara, State, Nigeria.

Prior to use, the seeds were surface-sterilized by soaking in sodium hypochlorite (5%) solution for 5 min before soaking in the respectively test extracts and CuSO₄ solution for a fixed time. At the expiration of soaking, five seeds were withdrawn and planted in plastic containers (9.3 cm diameter and 4.2 cm height) containing 40 g of absorbent cotton wool and incubated in the laboratory for 7 d. The seeds were watered daily with 10 mL of distilled water, to keep the blotters from drying up.

Daily germination readings were taken while the plant height readings were taken at the end of the 7-d planting period, after which germination time, germination rate and vigor index values were calculated as follows:

Germination time (d)
=
$$\frac{(G1Xd1) + (G2Xd2) + (G3Xd3) + \dots ... (G7Xd7)}{G1 + G2 + G3 + \dots ... G7}$$

$$\begin{aligned} & \text{Germination rate } (d^{-1}) \\ &= \frac{G1 + G2 + G3 + \cdots \dots G7}{(G1Xd1) + (G2Xd2) + (G3Xd3) + \cdots \dots (G7Xd7)} \end{aligned}$$

Vigor index = plant height X % germination

where G and d represent % germination and day of planting respectively

All experimental setups were carried out in duplicate

C. Determination of optimum concentration and priming duration

For the determination of optimum concentration, five different concentrations (500 mg/L, 1000 mg/L, 1500 mg/L, 2000 mg/L and 2500 mg/L) of the respective extracts and CuSO₄ solution were used. For experimentation, to a 30 mL capacity bottles containing 20 mL of the respective extracts, 15-20 surface-sterilized *Cajanus cajan* seeds were added and allowed to soak for 1 hr. Five seeds were then withdrawn from each of the respectively soaked concentrations and planted for 7 days duration.

For the determination of the effect of priming duration time on germinability of the seeds, five (1 h - 5 h) soaking durations. The surface-sterilized seeds were soaked in a known concentration of the respective extracts and CuSO₄ solution for the respective durations. At the expiration of soaking duration, seeds were planted and readings taken, as described earlier.

D. Determination of protective potential and antifungal testing

Four fungal pathogens (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Penicillium sp.*), obtained from the Microbiology laboratory, were utilized for this study. Prior to use, the fungal pathogens were cultured in sabouraud dextrose agar and incubated for 72 h. At the expiration of incubation, the spores were harvested and suspended in sterile normal saline.

For experimentation, two treatments were setup, which were infected and untreated, infected and treated with the respective extracts and $CuSO_4$ solution. For the infected and untreated setup, the surface sterilized seeds were soaked in the suspended organisms for 1 hr and then planted as described earlier. For the infected and treated treatments, the surface sterilized seeds were first soaked in the suspended organisms for 1 hr

and later transferred to known concentrations of the respective extracts and CuSO₄ solution for treatment for 1 h before planting. Germination rate, time, vigor index and % germination was estimated, as described earlier. The agar diffusion method was used for antifungal testing. To a 100 mL sterile Sabouraud dextrose agar, 2 mL of a broth culture of a respective test fungal pathogen was added and mixed gently before dispensing into Petri dishes 20 mL quantity.

After solidifying, three wells were bored in the agar plates and 0.5 mL of the respective extract or CuSO₄ solution was added to a well and allowed to diffuse before incubation at 25 °C for 72 h. At the expiration of incubation, the zone of inhibition was determined and measured.

III. RESULTS

A. Phytochemical components of the extracts

The presence of phenol, flavonoids, tannins was detected in all the extracts. There was no detection of steroids and anthraquinones and phlobatannins in all the extracts (Table 1).

Phytochemicals	Extracts				
	Methanol	Hexane	Ethyl acetate		
Cardiac glycosides	+	+	-		
Phenols	+	+	+		
Anthraquinones	-	_	-		
Terpenoid	+	-	-		
Saponins	+	_	-		
Steroids	-	_	-		
Alkaloids	_	+	+		
Flavonoids	+	+	+		
Tannins	+	+	+		
Phlobatannins	-	_	-		
(1) and (-) represent detected and undetected respectively					

(+) and (-) represent detected and undetected respectively.

B. Effect of concentration on germinability and vigor In seeds primed in the hexane extract, peak germination was observed after 4 days of planting for seeds primed with 2000 mg/L (70%), 1000 and 2000 mg/L (60%), and control (50%). (Fig. 1). For seeds that were primed in the ethyl acetate extract, highest germination of 60 % was detected at concentration of 500 mg/L while lowest germination of 20% was observed at concentration of 1000 mg/L. When seeds were primed in the methanol extract, a highest and lowest percentage germination of 50% and 20% was observed at concentration of 2500 mg/L and 500 mg/L, respectively. At the respective concentrations of the CuSO₄ solution, germination of 60% was observed to peak after 2 days of planting and was consistent till the end of the planting period (Fig. 1). A highest and lowest vigor index of 4600 and 1500 were observed in seeds that were primed in 500 and 2500 mg/L of the hexane extract, respectively. In seeds that were primed in the ethyl acetate extract, highest and lowest vigor index of 4425 and 292 were observed in seeds that were primed in concentrations of 500 mg/L and 100 mg/L, respectively. In the methanol extract, vigor indices of 2700 and 200 were observed when seeds were at concentration of 2500 and 500 mg/L, respectively. In presence of the CuSO₄ solution, highest vigor index of 6078 was observed in seeds that were primed in 1500 mg/L while seeds that were primed at 1000 mg/L had the lowest vigor index of 400 (Table 2).

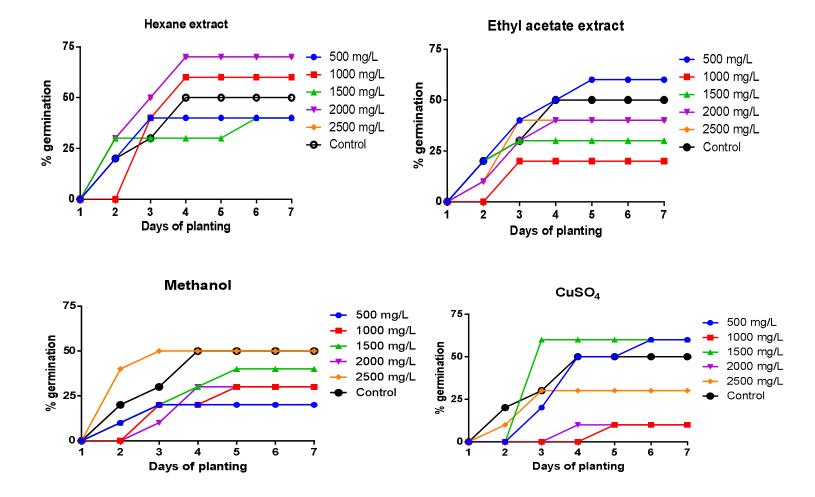


Fig. 1. Germination profile of the Cajanus cajan seeds primed in different concentrations of the respective extracts and CuSO₄ solution.

Table 2: Effect of concentration of the extracts and CuSO ₄ solution on germinability and vigor of the <i>Cajanus</i>
<i>cajan</i> seeds.

Concentration (mg/L)	GT (d)	GR (d⁻¹)	Plant height (mm)	VI
· - ·		Hexane extract		
500	3.7	2.4	115	
1000	4.1	1.4	56	3360
1500	3.3	2.4	57	2268
2000	3.9	2.4	57	4004
2500	3.8	1.4	50	1500
	Et	hyl acetate extract		
500	4.2	2.3	74	4425
1000	4.0	2.4	15	292
1500	3.6	2.4	117	3519
2000	3.9	2.4	38	
2500	3.9	2.4	16	652
		Methanol extract		
500	3.7	2.4	10	
1000	4.2	1.4	71	
1500	4.1	2.4	39	
2000	4.3	1.4	19	
2500	3.6	2.4	54	2700
		CuSO ₄ solution		
500	4.4	2.4	56	3345
1000	5.0	1.4	40	400
1500	4.0	1.4	101	6078
2000	4.5	1.0	140	1400
2500	3.8	2.4	123	3698

GT, GR and VI represent germination time, germination rate and vigor index, respectively

C. Effect priming duration on germ inability and vigor

When seeds were primed in the hexane extract, highest germination of 100 % was observed after 5 d of planting for seeds that were primed for 3 h duration before planting. For ethyl acetate extract, percentage germination of 100% was observed after 6 d of planting for seeds primed for 5 h before planting. A lowest germination of 40% was however observed for seeds that were soaked for 2 h before planting. In seeds that were primed in the methanol extract before planting. highest germination of 80% was observed when seeds were primed for 3 h before planting. At the respective priming durations in the CuSO₄ solution, highest germination of 80 % was observed in seeds that were primed for 5 h before planting while a lowest germination of 40 % was however observed for seeds that were primed for 4 h before planting (Fig. 2).

In seeds that were primed in the hexane extract, highest vigor index of 4000, when seeds were primed for 4 h while lowest vigor index of 600 was observed when the seeds were primed for 2 h before planting. For the ethyl acetate primed seeds, highest and lowest vigor index of 2835 and 696 were observed for seeds primed for 4 h and 3 h, respectively before planting. In the case of the methanol extract primed seeds, highest vigor index of 1680 was observed in seeds that were primed for 3 h before planting while the lowest vigor index of 150 was observed in seeds that were primed for 2 h before planting. In the case of the CuSO₄ primed seeds, highest and lowest vigor index of 6200 and 910 were recorded when the seeds were primed for 4 h and 5 h before planting, respectively (Table 3).

Priming duration	GT (d)	GR (d ⁻¹)	Plant height (mm)	VI
	Hexane e	extract	• • • •	
1 h	4	1.5	30	2700
2 h	4	1.5	12	600
3 h	4	1.5	28	2800
4 h	4	1.5	50	4000
5 h	4	1.5	11	1100
	Ethyl a	acetate extract		
1 h	4.0	1.5	30	2420
2 h	4.0	1.5	13	765
3 h	4.0	1.5	8	696
4 h	4.0	1.5	32	2835
5 h	4.0	1.5	15	1475
	Meth	nanol extract		
1 h	4.3	1.5	22	1100
2 h	4.4	1.5	3	150
3 h	4.0	1.5	21	1680
4 h	4.1	1.5	28	1400
5 h	4.3	1.5	9	540
	CuS	SO ₄ solution		
1 h	4	1.5	41	4100
2 h	4	1.5	12	840
3 h	4	1.5	12	1200
4 h	4	1.5	62	6200
5 h	4	1.5	13	910

Table 3: Effect of priming duration on germinability and vigor of the Cajanus cajan in the hexane extract.

GT, GR and VI represent germination time, germination rate and vigor index, respectively.

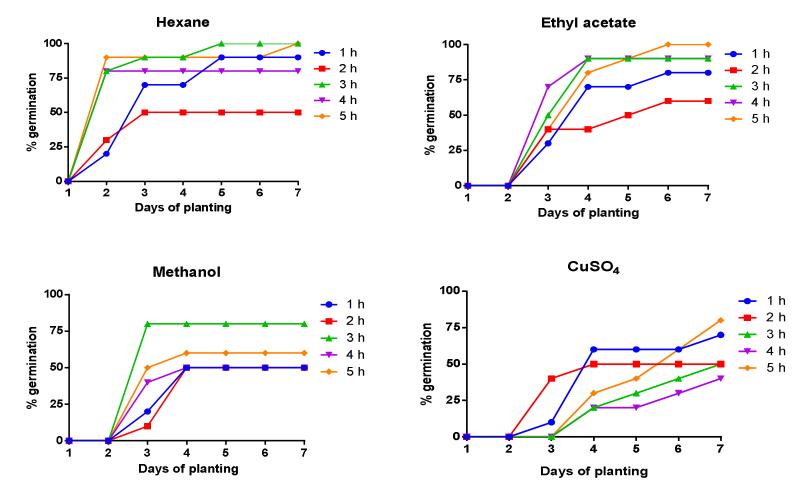


Fig. 2. Germination profile of the Cajanus cajan seeds at the different priming durations in the extracts and CuSO4 solution.

Treatments	Aspergillus niger	Aspergillus flavus	Aspergillus fumigatus	Penicillium
Methanol extract	+(34)	-	+(22)	-
Ethyl acetate extract	+(35)	-	+(22)	-
Hexane extract	+(39)	-	+(28)	-
CuSO₄ solution	+(22)	+(29)	+(18)	+(13)
Water	-	-	-	-

'+' and '-'represent inhibition and no inhibition, respectively.

D. Protective potential of the extracts

All the extracts and CuSO₄ solution were inhibitory to the growth of *Aspergillus niger* and *Aspergillus fumigatus*. Only the CuSO₄ solution showed inhibitory potential against the growth *Aspergillus flavus* and *Penicillium* sp (Table 4).

Values in parenthesis indicate zones of inhibition. Concentration of $CuSO_4$ and respective extracts used were 20,000 mg/L

The seeds infected with *Aspergillus niger* and treated with the methanol extract were observed to have 80% germination, with a germination rate of 2.5 and highest vigor index of 8640. For seeds infected with the *Aspergillus flavus* and treated with methanol extract, there was 70% germination, with a germination rate of 1.5 and a vigor index of 6440 while those infected with the *Aspergillus fumigatus* and treated with the methanol extract had 50% germination, with 2.5 germination rate and vigor index of 4100. A 50 % germination and vigor index of 5600 was observed for seeds treated after infection with the *Penicillium* sp. The infected and untreated setups showed 30, 10, 20 and 20 % germination and vigor indices of 2820, 200, 300 and 60,

for seeds infected with *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Penicillium* sp (Table 5).

When the Aspergillus niger infected seeds were treated with the hexane extract, 80% germination, germination rate of 2.5 and vigor index of 12640 were observed while for those infected with the Aspergillus flavus before treatment, 70 % germination, germination rate of 2.5 and vigor index of 5950 were observed. For the infected seeds with Aspergillus fumigatus before treatment with the hexane extract, 50 %, germination, 2.5 and vigor index of 4850 was reported. In the case of setup infected with the *Penicillium* before treatment 50 % germination, germination rate of 2.5 and a vigor index of 4500 was observed (Table 6).

Seeds infected with Aspergillus niger and treated ethyl acetate extract were observed to have 80% germination, with a germination rate of 1.5 and vigor index of 4400. When they were with the Aspergillus flavus before treatment, there was 70 % germination, germination rate of 2.0 and a vigor index of 7840 was observed.

Table 5: Protective potential of the <i>Cajanus cajan</i> when infected with the test fungal species and treated with
the methanol extract.

Treatments	% germination	GT (d)	GR (d⁻¹)	PH (mm)	VI
	Asj	pergillus niger			•
Infected	30	5	1.0	94	2820
Infected and treated	80	4	2.5	108	8640
	Asp	ergillus flavus			•
Infected	10	11	0.4	20	200
Infected and treated	70	4	1.5	92	6440
	Aspei	rgillus fumigatus	;		
Infected	20	6	0.2	15	300
Infected and treated	50	4	2.5	82	4100
		Penicillium			
Infected	20	5	1.0	3	60
Infected and treated	50	4	2.5	112	5600

GT, GR, PH and VI represent germination time, germination rate, plant height and vigor index, respectively.

 Table 6: Protective potential of the Cajanus cajan when infected with the test fungal species and treated with the hexane extract.

Treatments	% germination	GT (d)	GR (d ⁻¹)	PH (mm)	VI		
		Aspergillus nige	er				
Infected	30	5	1.0	94	2820		
Infected and treated	80	4	2.5	158	12640		
		Aspergillus flavi	IS				
Infected	10	11	0.4	20	200		
Infected and treated	70	4	2.5	85	5950		
	Aspergillus fumigatus						
Infected	20	6	0.2	15	300		
Infected and treated	50	4	2.5	97	4850		
		Penicillium					
Infected	20	5	1.0	3	60		
Infected and treated	50	4	2.5	90	4500		

GT, GR, PH and VI represent germination time, germination rate, plant height and vigor index, respectively.

Table 7: Protective potential of the Cajanus cajan when infected with the test fungal species and treated with the ethyl acetate extract.

Treatments	% germination	GT (d)	GR (d ⁻¹)	PH (mm)	VI
	A	spergillus niger			
Infected	30	5	1.0	94	2820
Infected and treated	80	4	1.5	55	4400
	As	pergillus flavus			
Infected	10	11	0.4	20	200
Infected and treated	70	4	2.0	112	7840
	Aspe	ergillus fumigatus			
Infected	20	6	0.2	15	300
Infected and treated	50	4	2.5	113	5650
		Penicillium			
Infected	20	5	1.0	3	60
Infected and treated	50	4	2.5	65	3250

GT, GR, PH and VI represent germination time, germination rate, plant height and vigor index, respectively.

Table 8: Protective potential of the <i>Cajanus cajan</i> when infected with the test fungal species and treated with
the CuSO₄ solution.

Treatments	% germination	GT (d)	GR (d ⁻¹)	PH (mm)	VI
	As	spergillus niger			
Infected	30	5	1.0	94	2820
Infected and treated	80	4	2.5	85	6800
	As	pergillus flavus			
Infected	10	11	0.4	20	200
Infected and treated	70	4	2.0	102	7140
	Aspe	ergillus fumigatus			
Infected	20	6	0.2	15	300
Infected and treated	50	4	2.5	131	6550
		Penicillium			
Infected	20	5	1.0	3	60
Infected and treated	50	4	2.5	205	10250

GT, GR, PH and VI represent germination time, germination rate, plant height and vigor index, respectively.

In the case of seeds that were infected with *Aspergillus fumigatus* and the *Penicillium* species, before treatment, 50 % germination was observed for both setups while vigor indices 5650 and 3250 were observed (Table 7).

Seeds infected with the *Aspergillus niger* before treatment with the CUSO₄ solution showed 80 % germination and vigor index of 6800 while those infected with the *Aspergillus flavus* before treatment were reported to have 70 % germination and vigor index of 7140. However, when the seeds were infected with the *Aspergillus fumigatus* or the *Penicillium* species had 50 % germination, with vigor indices 6550 and 10250, respectively (Table 8).

IV. DISCUSSION

Seeds used in this study were primed in the respective extracts and extracts and CuSO₄ solution. Adequate priming properly hydrates seeds, thus leading to enhancement of vigor and germination rate [5]. Priming or steeping of seeds before planting is indicated to help in improving germination. During priming, seeds have access to sufficient moisture, which is vital for is essential for rapid germination [6]. Apart from enhancing moisture requirement, steeping is indicated to soften the hard seed coats [7]. In this study, seeds were first steeped in the respective extracts before planting.

In this study, lower concentrations of the extracts were found to be optimum for germinability of the *Cajanus cajans* seeds. A similar observation has been reported by earlier investigators [8]. In a study on the effect of aqueous extracts of seeds of *Psoraleacoryifolia* on seed mycoflora, germination and vigor of maize seeds, it was reported that highest germination (80 %) and vigor index (1398.5) were observed when 20 % of the extract concentration was used, as against when 10, 30, 40 and 50 % of the extract concentration. The study further reports that extract concentration of 50 % inhibited germination [8]. The inhibitory effects of extracts on seed germination and growth is reported to be concentration dependent [9, 10].

Hossain *et al.*, have reported inhibitory effects of extracts of *Albizia lebbeck* on seed germination and development. In their study, the extent of inhibition was observed to increase with increase in extract concentration. The study further reported that lower concentrations of the extract caused stimulatory effects [12].

With respect to steeping duration, this study revealed duration of 3-5 h in the plant extracts and copper sulphate solution. In a study on the use of maize cultivars, optimum steeping time for germinability of the seeds was indicated to be 12 h [12]. Steeping time has also been implicated in the effectiveness of mustard and ginger rhizome extracts, and lemon juice in the inhibition of tomato seed borne pathogen [13]. It is opined that steeping duration greatly influenced the germination of the seeds of litchi. When the seeds were primed for different duration, 26-54 h was observed to be the optimum steeping range for the germination (90-100 %) of litchi seeds [14]. In pitaya seeds, optimum steeping duration of 4-6 h have been reported to increase seed germinability, with further increase in steeping time causing a decrease in germination rate and percentage [15]. Also, steeping of guava seeds in distilled water for 48 h is reported to greatly enhance growth percentage, when compared to 24 h steeping time [16].

Although water sufficient moisture is necessary for rapid seed germination, prolonged soaking is reported to lead to poor growth and germination [17]. High water level could lead to poor aeration and low oxygen availability, which can decrease germination [18]. Ascertaining the ideal soaking time is vital to obtaining maximum

germinability. Premature or late soaking have been reported to decrease the yield of Rhodes grass [19]. Moreover, high water availability could lead to the leaching of vital soluble food reserves in the seeds, exosmosis of enzymes and hormones, reduction in synthesis of proteins and decreased respiration rate [20].

Schemeza *et al.*, reported that long soaking duration is favorable in increasing antifungal efficacy of the aqueous of *Balanites aegyptiaca, Cymbopogon citratus, Cassia occidentalis and Portulaca oleracea.* In a study that assessed the extracts of leaves of *Moringa oleifera* and *Annona muricata* for the control of *Collectotrichum destructivum* on cowpea seeds, although all concentrations of the extracts at the different soaking times were observed to decrease the incidence of *Collectotrichum destructivum*, total control was obtained when seeds were soaked for 18 h time [22].

In this study, all the fungal pathogens initiated their infections on the *Cajanus cajans* seeds. Haikal [24], reported that *Aspergillus niger* and *Penicillium chrysogenum* have the ability of producing metabolites that reduce germination and seedling development. Pathogenic fungi are known to produce phytotoxins that can penetrate seed coats, the endosperm and also the cotyledon [24]. The presence of fungal pathogens has been indicated to significantly reduce seed germination, vigor, root elongation, pre and post emergence and mortality of seedlings [25].

Several plant extracts have been indicated to be efficacious in seed protection [26]. According to Veloz-Garcia *et al.*, extracts of some higher plants are known to exert antifungal activity. Stimulation of germination of seedlings and subduing seed infection has also been observed in rice seedlings treated with *Azadirachta indica* [28]. The extracts of *Vernonia amygdalina*, *Annona muricata* and *Moringa oleifera* have been shown to control the growth of *Collectotrichum destructivum*, with the *Moringa oleifera* extract showing more efficiency [22]. Extracts from *Terminalia arjuna* and *Eucalyptus lanceolotus* have been implicated in improved seed germination and development in tomatoes [29].

V. CONCLUSION

This study examined the germinability and protective potentials of methanol, ethyl acetate and hexane extract of *Helianthus annus* in comparison with $CuSO_4$ on *Cajanus cajan* seeds. From the findings of the study, seed germination Extracts of *Lawsoniainermis*, *Datura stramonium* and *Eucalyptus* sp. are also indicated to inhibit seed-borne pathogens and vigor index were observed to be dependent on the concentrations of the extract used. Concentration of 500 mg/L was observed to be optimum for steeping of the seeds in the ethyl acetate and hexane extracts while optimum concentration of 2500 mg/L was observed for steeping in the methanol extract.

The optimum soaking time of the seeds in presence of the extracts and CuSO₄ solution was observed to be between 3-4 h for soaking in the H_2O_2 solution. In addition, all the extracts showed protective potential on infected seeds of the *Cajanus cajan*. Generally, all the extracts inhibited the growth *Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus*. None of the extracts inhibited the growth of *Penicillium* sp.

Findings of this study could help in the development of viable alternatives to chemical treatments of seeds. This

could be of value in sustainable and eco-friendly agricultural practices and management.

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