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Effect of *Jatropha curcas* and *Taxodium distichium* extracts on *Sclerotium cepivorum* the cause of onion white rot

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ABSTRACT
The present study was undertaken to investigate the effects of using different extracts of two types of trees (*Jatropha curcas* seeds and *Taxodium distichium* cones). The solvents used were distilled water and ethanol. The determination of bioactive components of each extract was carried out using gas chromatography/mass spectrometry (GC/MS) analysis. In general, ethanolic extract was more effective on both linear growth and sclerotial numbers and mycelium characteristics of *Sclerotium cepivorum* than either of the cold or hot water extracts compared to control. Under in vitro conditions, treated with 20% ethanol solvent showed a fungicidal effect. Meanwhile, fungistatic effects appeared at 5% for reduction of mycelium growth. In the greenhouse experiments, the results indicated that onion transplants treated with ethanolic extract of taxodium and jatropha at 20% resulted in a significant reduction in the disease with a significant increase in bulb yield compared with control treatments. This report is one of the few papers that uses taxodium cones and jatropha seeds as antifungal substances against plant pathogens, especially *S. cepivorum* under worldwide and Egypt conditions.
Keywords: *Jatropha curcas*, onion white rot (OWR), plant extracts, *Sclerotium cepivorum* and *Taxodium distichum*

INTRODUCTION
Sclerotium cepivorum is the main pathogen that causes onion white rot (OWR). It belongs to the mycelia sterilia fungi. The first description of onions was by Berkeley (1841) in the United Kingdom. *S. cepivorum* is classified as a root inhabiting fungus (Valdés-Santiago *et al*., 2021). *S. cepivorum* only attack onion, garlic, leek and other plants belonging to Alliacea causing a considerable loss in quantity and quality of yield. This pathogen is able to attack the onion roots from the seedling stage to the harvest stage, which results in the plants dying during the season even before the stage of onion storage (Elshahawy and Saied, 2021). Disease recycling incidence depends on sclerotia as the only means of survival structures (Ahmed and Ahmed, 2015) that can remain in the soil in absence of a host for 15- 20 years. Germination of *S. cepivorum* sclerotia stimulate by Allium plants -specific root exudates called alkyl cysteine sulphoxides which soil microorganisms playing the main role in this (Amin *et al.* 2014) then, it can begin to grow and germinate through onion roots, causing white rot symptoms (Maude 2006 and Akter *et al.,*2021). Therefore, the longevity of sclerotia in the soil makes it difficult to resist white rot disease.

During the 1980s, dicarboximide fungicides, iabrodone and vinclozolin, were the most common fungicides to control white rot on onions and garlic. Nevertheless, Dicarboximide fungicides may lose their effectiveness in some areas of use, which increases the incidence of white rot disease. (Tyson *et al*., 1999) due to the occurrence of degradation in soil. (Lassalle *et al*., 2014). Later, control of onion white rot infection was achieved by folicur (Tohamy et al., 1992; Dilbo *et al*., 2015). Due to concerns of health and environmental hazards and many pathogens that could develop resistance against fungicides; there is a great demand for safer, alternative and effective control agents. Many studies have been made by researchers to discover new compounds from various kinds of sources such as animals, plants and micro-organisms, and one of these resources is folk medicinal plants (Okereke and Wokocha, 2007). *J. curcas* L. is one of the plants that belong to the FamIly Euphorbiaceae. The plant was first discovered in Mexico, then expanded throughout Asia and Africa. Traditionally, in several subtropical and semi-arid areas, *J. curcas* seed extracts showed the properties effects of anti mollusks, insects, and fungi (Gupta *et al.* 2011, Meshram *et al.* 1996, Liu *et al.* 1997, Ahuchaogu *et al*. 2014 and Angaye (2015).). Also, under in vitro conditions, *J. curcas* extract showed growth-inhibitory activity to *Pyricularia oryzae* causative of rice blast disease (Durgeshlal *et al.*, 2019).

T. distichum Rich. FamilyTaxodiceae commonly recognized as marsh cypress one of the best wood which is usually manufactory for housing construction for the reason that it was confrontation to insects assault especially termites (Scheffrahn *et al*., 1988). Cones of tress are important to conifer self-propagation, moreover, it is recommended that cones include some phytochemical mechanism that defends from microbial infection (El Tantawy *et al.*,1999). On the other hand, our present information, are only available little attention has been paid to the antifungal activities of taxodium against plant pathogenic fungi, and only Kusumoto *et al.* (2010) stated that twelve diterpenoids extracted from *T. distichum* showed

antifungal properties against both *Trametes versicolor* and *Fomitopsis palustris* which causes white-rot and brown-rot, respectively. Also, Abietane-type diterpenes are one of the main components of taxodium cones (Su *et al.,* 2013). Consequently, natural antifungal substances from *T. distichum* cones are beneficial to the ecosystem, phytochemistry, and agrochemistry.

Thus, the main aim of the present work is to investigate the potency control of *S. cepivorum* growth and sclerotial production under in vitro conditions as well as OWR disease under greenhouse conditions using different extracts from *J. curcas* seeds and *T. distichum* cones.

MATERIALS AND METHODS

Treatment Method:

1-Plant Material:

Fresh *T. distichum* cones and *J. curcas* seeds were collected from AL-Gemeza research station -and Agricultural Research Center fields in November and March (2016) respectively. Cones and seeds were washed with tap water several times till all the foreign material and soil particles detached from the seed surface and afterwards shade-dried under room conditions. The dried in the open-air samples were perfectly ground utilizing a grinder machine plus separately subjected used for ethanolic, cold and hot aqueous extractions.

2. Laboratory experiments:

Aqueous extraction:

1-Cold aqueous extraction:

The ground cones and seed powder (500 g) were separated then shaken in 1 litre of distilled water on a shaker at room conditions until homogeneity for 24 h. The two extracts were filtered by Buckner funnel and filter paper **(**Wagner *et al.*, 1989).

2-Hot aqueous extraction:

The ground powder of cones and seed were shaken in 1 litre respectively after that distilled by boiling water in the shaker at room temperature. The extracts were filtrated using a Buckner funnel and filter paper (Wagner *et al.,* .1989**).**

Ethanol extract:

The powdered cones and seeds were individually extracted to exhaustion in a soxhlet apparatus using ethanol (70%). The extracts were singly filtered through Whitman filter paper No.1. (Rangari, 2002). The extract was evaporated with a rotary evaporator at low temperature (40°-50°C) and reduced pressure to provide crude ethanol extracts to determinate phytochemical. Ethanolic extract was evaporated using a rotary evaporator apparatus at low temperature (40°-50°C) and low pressure to provide determinate phytochemical.

Effect of different extracts on mycelial growth and sclerotial numbers:

Cold, hot aqueous and ethanolic extraction of the two materials (*J. curcas* and*T. distichum*) at the concentrations of 5,10 and 20% were examined for their effect on linear growth and sclerotial numbers production of *S. cepivorum* using poison food technique method (PFT) (Qadoos *et al.,* 2016). The prepared concentrations were added to 100 ml PDA before solidification, poured in Petri plates and inoculated at the center with 0.5 cm. in diameter discs of 7days-old cultures of the tested fungus and incubated 20 ± 2 oC for 5days. Medium free from extracts was used as a control. Four replicates were used for each treatment. Mycelial growth was measured when fungal growth covered the surface of any plate meanwhile sclerotial number was calculated 21 days after incubation. The reduction percentage for the *S. cepivorum* mycelial growth was calculated as follows:

Reduction in mycelial growth % = (C-t / C) 100

Where:

C = Linear growth in control treatment

t= Linear growth in each treatment

Effect of jatropha and taxodium alcohol extracts on the sclerotial germination:

Ethanolic extraction of the two materials (*J. curcas* and *T. distichum*) at 20% was examined for its effect on sclerotial germination of *S. cepivorum*. Ten sclerotia were collected from a three-week-old culture of *S. cepivorum* plates. Sclerotia were immersed in both of the aforementioned extractions for 5, 15, 30, 45, and 60 minutes, then planted on PDA surface medium plates. Sclerotia were dipped in sterile distilled water as a control treatment. Each treatment included four

replicates. Germination data were collected after ten days of incubation at 20 C° (Elshahawy *et al*., 2019).The percentage of reduction was calculated as mentioned earlier.

Microscopic examination:

To examine the effects of the two material extracts on the morphogenesis of mycelia of *S. cepivorum* 0.5 cm. from the center of the dish was cute with a cork borer, which was then for transmission electron microscopy (TEM). The samples were examined with a field emission electron microscope (TESLA BS-300) in the Faculty of Agriculture, Cairo University, Egypt.

Determination components of different extracts from the two tested plant samples:

To determine the components of the two tested plant samples, jatropha and taxodium content, were conducted at the Regional Center of Food and Feed Stuff, Agriculture Research Center, Giza, Egypt. Gas chromatography/mass spectrometry (GC/MS) analysis was carried out using (MSD, Agilent 7000) equipped with a polar HP -5 ms (5% -phenyl methyl polysiloxane) capillary column (30m×0.25mm i.d. and 0.25 µm film thickness).

3-Greenhouse experiment:

A pot experiment was conducted in the onion, garlic, and oil crop greenhouse, Plant Pathology Res. Inst., ARC, during the 2018/2019 season. Plastic pots (25-cm-diam) filled with sterilized sand-clay soil (1:1 v/v) and infested with 2% w/w *S.cepivorum* inoculum fungus, 7 days before transplanting. Four pots were used for every treatment and control. Five transplants of cv. Behery (55-60-days old) were transplanted in each pot in1st week of November and irrigated when needed. Transplants were dipped for 15 min. in 20% solution of *J. curcas* and *T. distichum* separately just before transplanting. Growing plants were sprayed at 6 and 12 weeks by the same concentrations. As a chemical check treatment, transplants were dipped for five min. in Folicure 25% (25 ml/L water) solution just before transplanting and then sprayed at 6 and 12 weeks with 1.87.5 ml/L water. Meanwhile, as a quality control measure, transplants were dipped in sterilised water before being implanted.

Determination of % disease incidence:

Disease incidence was observed at the 2 and 4 months after transplanting then, infection percentages by onion white rot were calculated according to Hovius *et al.* (2004) as follows:

Infection (%) = No. of plants infected with white rot / Total No. of transplants X100.

White rot reduction efficacy (%) = White rot % in control – White rot % in each treatment / White rot % in control X 100.

Also, at the end of season onion plants were taken at harvest, plants from each pot were collected to record crop properties (weight, perimeter) and bulb yield.

4- Analytical statistics:

The recorded data for each treatment were subjected to the ANOVA test by using SPSS computer software. Means separation was made by using least significant differences (L.S.D.) at a 5% level of significance (Leech *et al*., 2008).

RESULTS

1- Jatropha seeds extract content:

The data tabulated in Table1 shows that ethanol separated thirty-three compounds. pyridoxal, indanol-5 and cumaldehyde were the main components of these compounds of *J. curcas* which represent about 30 % of the total extract. Fatty acids were represented in erucic acid, junperic acid, oleic acid and pyruvic acid at 2.79, 2.53, 2.47 and 1.78 %.On the other hand, thymol was the major content (6.68%) of cold water extract followed by retinyl propionate (6.44%), cumaldehyde (5.48%) and homovanillic acid (5.02%). On the contrary of ethanol extract, we can observe that Indanol-5 was absent in both cold and hot extracts. Concerning hot water extract, thirty-seven compounds were separated. glycerylmonooleate was the high concentration (8.63%) followed by retinyl propionate (7.64%), arachidonic acid methyl ester (4.41%) cannabidiol 4.05%. Meanwhile, thymol represented at (3.06%).

Table 1: Compounds separated from ethanol, cold and hot water extracts of *J. curcas* seeds.

Components	Ethanol			Cold water			Hot water		
	R.T. (min)	Area	%	R.T. (min)	Area	%	R.T. (min)	Area	%
Cumaldehyde	4.332	14869501.87	2.98	4.332	20392131.76	5.48	4.332	11455499.84	1.70
Indanol-5	4.399	66339615.04	13.29	-	-	0.00	-	-	0.00
Phenol 4 (2-propenyl)	-	-	0.00	-	-	0.00	4.502	4482424.48	0.67
3.5-di-t-Butylcateghol	-	-	0.00	-	-	0.00	-	-	0.00
Thymol	4.993	12499829.25	2.50	4.993	24847093.58	6.68	4.993	20578567.05	3.06
Dimethyl caffeic acid	5.375	11298812.26	2.26	5.375	4698194.49	1.26	5.375	8933616.82	1.33
Ischomogenol	5.482	11361877.18	2.28	-	-	0.00	-	-	0.00
Hydrocinnamic acid	5.545	10790835.23	2.16	5.545	5875271.98	1.58	5.545	11011071.97	1.64
2.3.5.8a- pentamethyl-6.7.8.8a-tetrahydro- 5h- chromen-8-ol	6.263	12975200.39	2.60	6.263	6435345.01	1.73	6.263	15012636.53	2.23
Homovanillic acid	6.790	14428307.24	2.89	6.790	18679907.49	5.02	6.790	5993493.14	0.89
y-Terpinene	-	-	0.00	-	-	0.00	-	-	0.00
B-Carene	-	-	0.00	-	-	0.00	-	-	0.00
m- Hydroxybenzoic acid	7.954	8490705.26	1.70	7.954	2376271.37	0.64	7.954	11958576.5	1.78
Gentisic acid	8.471	12617765.3	2.53	8.471	4971611.41	1.34	8.471	15436589.4	2.30
Carvacrol	-	-	0.00	-	-	0.00	-	-	0.00
Levomenthol	9.525	15426437.68	3.09	9.525	5691217.26	1.53	9.525	16479432.57	2.45
m-Cresol 5-ethyl	9.861	5908428.37	1.18	9.861	7903396.03	2.13	9.861	11036391.36	1.64
Tetra-O-menthylfisetin	10.116	10038123.36	2.01	10.116	6310094.42	1.70	10.116	8797103.25	1.31
Methyl farnesate	10.335	12672714.75	2.54	10.335	6050264.43	1.63	10.335	8413226.8	1.25
a-Guaiene	-	-	0.00	-	-	0.00	-	-	0.00
p-Cresol.2.2ethylenebis (6-tert-butyl)	11.191	14112755.66	2.83	11.191	13721115.88	3.69	11.191	22119446.98	0.00
Benzoic acid. 2.6-dihydroxy	11.283	12255799.47	2.46	11.283	4993620.08	1.34	11.283	10906897.56	1.62
3.4-Dihydroxymandelic acid	-	-	0.00	-	-	0.00	-	-	0.00
Anisole p-tert-butyl	-	-	0.00	11.410	7720209.51	2.08	-	-	0.00
a-Gurionene	11.676	7879768.45	1.58	11.676	5662130.46	1.52	11.676	9104740.29	1.35
4-tert-Octyl-o-cresol	-	-	0.00	11.991	11044196.51	2.97	-	-	0.00
Apigenin8-C-glucoside	12.571	7879768.45	1.58	12.571	5397376.87	1.45	12.571	8789713.73	1.31
Heptadecanoic acid. Methyl ester	12.779	10317861.21	2.07	12.779	8871216.44	2.39	12.779	12935545.08	1.92
Nonadecanoic acid. Methyl ester	13.674	8220660.4	1.65	13.674	3991064.03	1.07	13.674	10152236.51	1.51
Erucic acid	13.940	13924627.41	2.79	13.940	7505465.31	2.02	13.940	12885009.45	1.92
Ethyl linoleate	14.445	16174166.5	3.24	14.445	8390979.11	2.26	14.445	18848902.34	2.80
Oleic acid	14.654	12312534.3	2.47	14.654	6730024.12	1.81	14.654	19098406.72	2.84
11.14-Eicosadienoic acid. Methyl ester	14.846	11670241.27	2.34	14.846	15026765.57	4.04	14.846	17636169.45	2.62
Stigmasterol	-	-	0.00	15.213	9280688.44	2.50	15.213	27839879.89	4.14
Junperic acid	15.330	12640155.18	2.53	15.330	6863206.34	1.85	-	-	0.00
5B.7BH.10a-Eudensn-11-en-1a-ol	-	-	0.00	15.932	5634881.75	1.52	15.932	12732931.98	1.89
Docosanoicacid	16.108	11020255.02	2.21	16.108	7875616.32	2.12	16.108	16366696.74	2.44
Cannabidiol	-	-	0.00	16.667	10295501.63	2.77	16.667	27209672.54	4.05
Arachidonic acid methyl ester	-	-	0.00	-	-	0.00	17.152	29629466.85	4.41
Pelagonidincation	17.743	8020923.1	1.61	-	-	0.00	-	-	0.00
GlycerylMonooleate	-	-	0.00	17.838	6724641.28	1.81	17.838	58014694.6	8.63
Stearic acid	-	-	0.00	-	-	0.00	18.164	14555638.25	2.17
Phytol	18.489	8871541.65	1.78	18.489	6773063.9	1.82	18.489	13405460.34	1.99
Pyruvic acid	18.932	8871541.65	1.78	18.932	8541490.31	2.30	18.932	17332052.25	2.58
Isolongifolol	-	-	0.00	-	-	0.00	19.313	19468161.42	2.90
2-Hexadecanal	20.134	7050965.48	1.41	20.134	6580059.67	1.77	20.134	25220627.57	3.75
Pyridoxal	20.303	70617104.49	14.15	20.303	5303807.02	1.43	20.303	12041718.97	1.79
Vitamin E	-	-	0.00	-	-	0.00	21.040	2163337.01	0.32
Dodecame 2.6.10-trimethyl	21.397	7868321.41	1.58	21.397	10247476.92	2.76	21.397	23669237.01	3.52
Retinyl propionate	22.515	14186450.16	2.84	22.515	23934470.78	6.44	22.515	51348985.74	7.64
Total fractions	33			36			37		

2-Taxodium cones extract content:

According to Table (2), data showed components of *T. distichum* cones extracts of hot and cold water additions to ethanol. Forty-three compounds were separated from the ethanol extract. The major components of the ethanol solvent were heptadecanoic acid, methyl ester phenol -4, indanol-5 and p-cresol.2.2-methylenebis. These contents represent 31.65 % of the total ethanol extract. On the other hand, cold water extracts showed 39 separate compounds. The total extract contains 31.32% dodecame 2.6.10-trimethyl (14.77%), vitamin E (8.92%), cumaldehyde 3.81%, and indanol-5 (3.82%).Meanwhile, forty-two compounds were separated in the hot water extract. Only five compounds have been represented by about 25% of the total extract: { glyceryl monooleate (8.19%), docosanoic acid (7.13%), stearic acid (3.48%) cannabidiol (3.16%) and arachidonic acid methyl ester (3.06%).

Table 2: Compounds separated from ethanol, cold and hot water extracts of *T. distichum* cones.

Components	Ethanol			Cold water			Hot water		
	R.T. (min)	Area	%	R.T. (min)	Area	%	R.T. (min)	Area	%
Cumaldehyde	4.332	6540788.79	0.53	4.332	50293656.77	3.81	4.3332	41627262	1.89
Indanol-5	4.399	60282188.9	5.69	4.399	50435586.89	3.82	4.399	49076733	2.23
Phenol 4 (2-propenyl)	4.502	75735482.9	7.15	-	-	0.00	-	-	0.00
3.5-di-t-Butylcateghol	4.855	11461414.4	1.08	-	-	0.00	-	-	0.00
Thymol	4.993	15086739.7	1.42	-	-	0.00	-	-	0.00
Dimethyl caffeic acid	5.375	17963910.3	1.70	5.375	17550170	1.33	5.375	16580806	0.75
Isohomogenol			0.0	---	---	0.00	---	---	0.00
Hydrocinnamic acid	5.545	15006889.4	1.42	5.545	12338430.84	0.93	5.545	30189260	1.37
2.3.5.8a- pentamethyl-6.7.8.8a- tetrahydro- 5h-chromen-8-ol	6.263	25423019.8	2.40	6.263	18044864.37	1.37	6.263	33412175	1.52
Homovanillic acid	6.790	24466425.9	2.31	6.790	23438775.06	1.78	6.790	31917815	1.45
y-Terpinene	7.006	27251946	2.57	-	-	0.00	-	-	0.00
B-Carene	7.059	30815690.4	2.91	---	---	0.00	---	---	0.00
m- Hydroxybenzoic acid	7.954	12376158.9	1.17	7.954	13266132.59	1.00	7.954	28088672	1.28
Gentisic acid	8.471	21857953.3	2.06	8.471	16012786.24	1.21	8.471	21763098	0.99
Carvacrol	8.955	11999354.7	1.13	---	---	0.00			0.00
Levomenthol	9.525	26911153.3	2.54	9.525	33208938.08	2.51	9.525	57955362	2.64
m-Cresol 5-ethyl	9.861	14854278.4	1.40			0.00			0.00
Tetra-O-menthylfisetin	10.116	13883292.4	1.31	10.116	15540638.99	1.18	10.116	27526674	1.25
Methyl farnesate	10.335	13143940.1	1.24	10.335	8753740.36	0.66	10.335	51353000	2.34
a-Guaiene	10.632	34089952.7	3.22	---	---	0.00	---	---	0.00
p-Cresol.2.2-methylenebis(6-tert-butyl)	11.191	45278138.3	4.27	11.191	8749806.85	0.66	11.191	37244432	1.69
Benzoic acid. 2.6-dihydroxy	11.283	15120332.1	1.43	11.283	7868062.96	0.60	11.283	27186192	1.24
3.4-Dihydroxymandelic acid	11.304	32327780.3	3.05	11.304	8130379.17	0.62	11.304	36741446	1.67
Anisole p-tert-butyl	11.410AA	43102271.1	4.07	11.410	8133832.8	0.62	11.410	24409665	1.11
a-Gurionene	11.676	21462676.9	2.03	11.676	9072915.59	0.69	11.676	36846967	1.68
4-tert-Octyl-o-cresol	11.991	28011083.5	2.64	11.991	16143660.23	1.22	11.991	38548570	1.75
Apigenin8-C-glucoside	12.571	27073748.9	2.56	12.571	3810784.32	0.29	12.571	44952915	2.04
Heptadecanoic acid. Methyl ester	12.779	110872643	10.47	---	---	0.00	12.779	49644011	2.26
Nonadecanoic acid. Methyl ester	13.674	19719022.7	1.86	13.674	5923451.07	0.45	13.674	24442543	1.11
Erucic acid	13.940	20162871.7	1.90	13.940	12496333.27	0.95	13.940	68753220	3.13
Ethyl linoleate	14.445	20162871.7	1.90	-	-	0.00	14.445	61629964	2.80
Oleic acid	14.654	31400114.6	2.96	14.654	27306210.9	2.07	14.654	50535232	2.30
11.14-Eicosadienoic acid. Methyl ester	14.846	19943692.1	1.88	14.846	16985193.32	1.29	14.846	44805845	2.04
Stigmasterol	-	-	0.00	15.213	30678299.68	2.32	15.213	59404209	2.70
Junperic acid	15.330	16292139.2	1.54	15.330	15725505.59	1.19	15.330	57294549.83	2.61
5B.7BH.10a-Eudensn-11-en-1a-ol	15.932	14551071.7	1.37	15.932	26207791	1.98	15.932	50281419.09	2.29
Docosanoicacid	16.108	13884699.8	1.31	16.108	26377990.61	2.00	16.108	156717255.8	7.13
Cannabidiol	-	-	0.00	----	----	0.00	16.667	69503225.16	3.16
Arachidonic acid methyl ester	-	-	0.00	17.152	36235660.95	2.74	17.152	67303021.62	3.06
Pelagonidincation	17.743	14825718.5	1.40	17.743	39937066.47	3.02	17.743	36480280.25	1.66
GlycerylMonooleate	-	-	0.00	----	----	0.00	17.838	180086307.7	8.19

Table 2 continued									
Stearic acid	-	-	0.00	18.164	3691048.48	0.28	18.164	76549501.67	3.48
Phytol	18.489	10559826.5	1.00	18.489	25044539.78	1.90	18.489	51260421.03	2.33
Pyruvic acid	18.932	18870111.2	1.78	18.932	33783079.97	2.56	18.932	47351257.99	2.15
Isolongifolol	19.313	10373401.8	0.98	19.313	15603772.42	1.18	19.313	50417649.86	2.29
2-Hexadecanal	20.134	5850783.86	0.55	20.134	29278532.64	2.22	20.134	24310723.59	1.11
Pyridoxal	20.303	15769737.2	1.49	20.303	21744766.3	1.65	20.303	22303461.68	1.01
Vitamin E	-	-	0.00	21.040	118002004.3	8.94	21.040	62340284.02	2.84
Dodecame 2.6.10-trimethyl	21.397	17722075.6	1.67	21.397	195017644.6	14.77	21.397	55962796.21	2.55
Retinyl propionate	21.882	10395066.1	-----	21.882	240936136.1	-------	21.882	54497212.78	------
Quercetin 3.4.4-trimethyl ether	22.515	17444464	------	22.515	78691080.64	------	22.515	141559119	------ --
Totalfractions	43			38			42		

3- Effect of different jatropha extracts on mycelial growth of *S. cepivorum* and sclerotial production:

The data in Table 3 show the effects of jatropha cold, hot, and solvent extracts on *S. cepivorum* mycelia growth and sclerotial production. Ethanolic extract of jatropha seeds showed complete inhibition of *S. cepivorum* growth at 10 and 20% concentrations, whereas, both of the previous concentrations of cold extract obtained (16.94) and (28.61 %) inhibition, respectively. The concentration of 5% in both cold and hot extracts did not have any effect on mycelia growth inhibition, whereas the higher concentrations (10 and 20%) had a weak effect. Concerning the effect of jatropha extracts on sclerotia formation, data in table 3 also clearly shows that jatropha alcohol extract had the superior effect in preventing sclerotia formation, obtaining 100 % inhibition of sclerotial number at 10 and 20 % up to 14 days after incubation, followed by 20 and 10 of cold extract (69.81 reduction percent). The percentage of sclerotial yield production changed according to fraction extract and concentration. The highest percentage was found at 10% and 20% concentrations, which were the most effective across all three extracts. In general, ethanol extract was the best treatment against *S. cepivorum* mycelial growth, followed by cold and then hot water extract (Table 3). The effect of solvent jatropha extracts on mycelial growth of *S. cepivorum* was also examined by TEM of ultrathin sections of mycelium, which revealed many dead areas in the mycelium cell treated with jatropha extracts compared to the untreated control (Fig. 1).

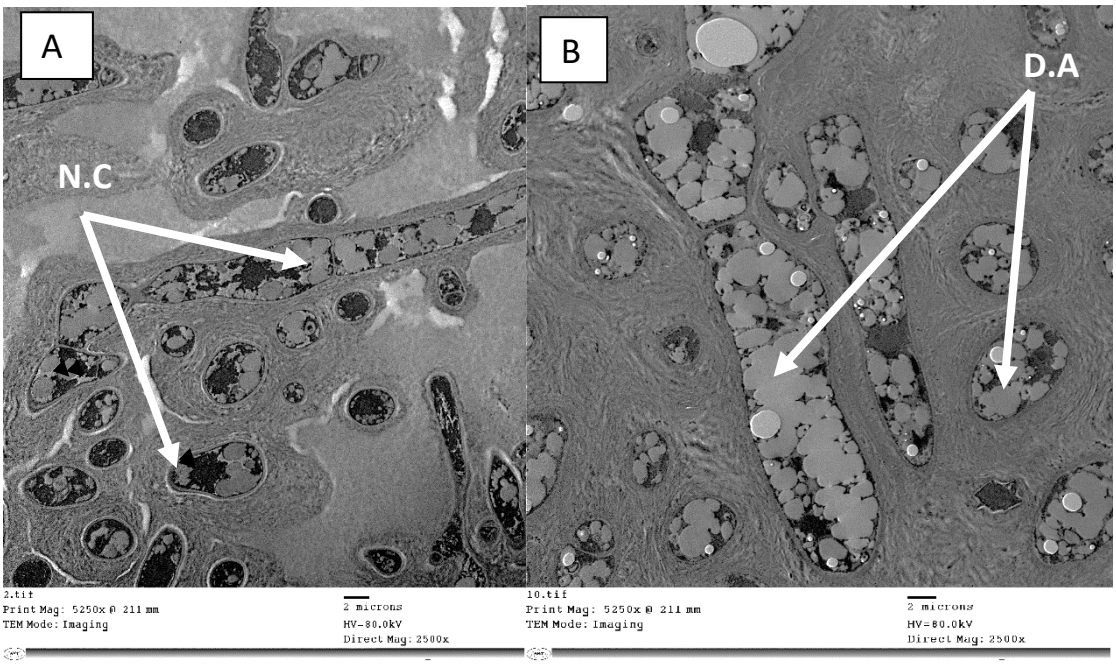

Fig. 1. Effects of jatropha alcohol extract (20 %) on the ultrastructure of *S. cepivorum w*as confirmed by TEM of a particular thin segment of mycelium. (A) Control (B) jatropha alcohol extract .N.C= (Normal cell) D.A =(Dead area).

Table 3: Effect of different jatropha extracts and concentrations on the linear growth and sclerotial numbers of *S. cepivorum* under in vitro conditions.

Treatment (T)	Concentration %(C)	Linear growth (mm) [a]	Reduction (%)[b]	Sclerotial numbers [a]	Reduction (%)[b]
Jatropha cold (T1)	2.5	90h	0.00	4520.00 h	23.85
	5	86.50g	3.89	3760.00 d	36.66
	10	74.75c	16.94	2736.00 c	69.81
	20	64.25b	28.61	2736.00 c	69.81
Means (T1)		81.20 b		3748.80 b	
jatropha Hot (T2)	2.5	90.00h	0.00	5328.00 k	10.24
	5	90.00h	0.00	4664.00 l	21.43
	10	84.5f	6.11	4376.00 g	26.28
	20	79.25e	11.94	3880.00 e	34.64
Means (T2)		86.75 c		4836.80 c	
jatropha Alcohol (T3)	2.5	90.00h	0.00	4904.00 j	17.39
	5	76.5d	17.22	4192.00 f	29.38
	10	0.00a	100.0	0.00 a	100.00
	20	0.00a	100.0	0.00 a	100.00
Means (T3)		51.30 a		3006.40 a	
Control	2.5	90.00h	0.00	5936.00 d	0.00
	5	90.00h	0.00	5936.00 d	0.00
	10	90.00h	0.00	5936.00 d	0.00
	20	90.00h	0.00	5936.00 d	0.00
MEAN	T1	90.00h	-------	5172.00 d	------
	T2	85.75 c	-------	4638.00 c	------
	T3	62.31 b	-------	3262.00 b	------
	T4	58.50 a	------	2902.00 a	------
L.S.D	Treat	2.02	------	217.00	------
	Conc.	1.04	-----	112.06	------
Treat x Conc.		0.90	------	97.05	**------**

Values with the same letter(s) in the same column are not significantly different

[a] Mean of 4 replicates (plates) .[b] Reduction % = (control-treatment /control) x100

4- Effect of different taxedium extracts on mycelial growth of *S. cepivorum* and sclerotial production:

The data tabulated in Table 4 and Fig.(2) showed that the mycelial growth of *S. cepivorum* was significantly reduced in the presence both of hot, cold water in addition to ethanol solvent *T. distichum* cones extracts. The superior effect on *S. cepivorum* growth was obtained by ethanolic alcohol. A high concentration (20 %) could completely inhibit mycelial growth with 100% followed by 10, 5 and 2.5% which obtained 88.89, 65 and 63.61.0 % reduction, respectively. On the other hand, the weak effect was cleared by both cold and hot water extracts an except for hot water extract at 20% which gave a 16.39 % reduction. In addition, Figure 2 shows that visible changes are seen in the degraded cell wall in the mycelium treated with taxodium extracts compared to the cell wall in the control treatment.

Regarding the effect of three taxodium extracts on sclerotial production numbers of *S. cepivorum*, we can observe that all of taxodium extracts obtained encourage results as a fungistatic effect on sclerotial production. Regarding the effect of three taxodium extracts on the sclerotial production numbers of *S. cepivorum*, we can observe that all of the taxodium extracts obtained have a fungistatic effect on sclerotial production. whereas ethanol extract reduces sclerotial production numbers significantly until a low concentration (2.5%). The hot extract was the second one, followed by the cold extract. Whereas, ethanol extract shows a high significantly reduction of the sclerotial production numbers until the low concentration (2.5%). The hot extract was the second one followed by cold extract (Table 4).

Table 4: Effect of different taxodium extracts and concentrations on the linear growth and sclerotial numbers of *S. cepivorum* under *in vitro* conditions.

Treatment (T)	Concentration %(C)	Linear growth (mm) [a]	Reduction (%)[b]	Sclerotial numbers [a]	Reduction (%)[b]
Taxodium cold (T1)	2.5	90.0 i	0.00	5216 k	12.13
	5	90.0 i	0.00	4728 j	20.35
	10	86.25 h	4.17	4576 hi	22.91
	20	85.0 f	5.55	4456 g	24.93
Mean (T1)		88.25 c	-------	4982.40 c	------
Taxodium Hot (T2)	2.5	90.0 i	0.00	4504 gh	24.12
	5	90.0 i	0.00	3688 f	37.87
	10	85.5 fg	5.01	3016 e	49.19
	20	75.25 e	16.39	2152 d	63.75
Mean (T2)		86.15 b	------	3859.20 b	-------
Taxodium Alcohol (T3)	2.5	32.75 d	63.61	1560 c	73.72
	5	31.5 c	65.0	560 b	90.57
	10	10.0 b	88.89	0.00 a	100.00
	20	0.0 a	100.00	0.00 a	100.00
Mean (T3)		32.85 a	-------	1611.20 a	--------
Control	2.5	90.0 i	0.00	5936.00 i	0.00
	5	90.0 i	0.00	5936.00 i	0.00
	10	90.0 i	0.00	5936.00 i	0.00
	20	90.0 i	0.00	5936.00 i	0.00
Mean	T1	75.69 cd	-------	4304.00 d	------
	T2	75.38 c	-------	3728.00 c	------
	T3	67.94 b	-------	3382.00 b	------
	T4	62.56 a	------	3136.00 a	------
L.S.D	Treat.	1.34	------	187.23	------
	Conc.	0.69	-----	96.68	------
Treat x Conc.		0.60	------	83.73	

Values with the same letter(s) in the same column are not significantly different

[a] Mean of 4 replicates (plates) .[b] Reduction % = (control-treatment /control) x100

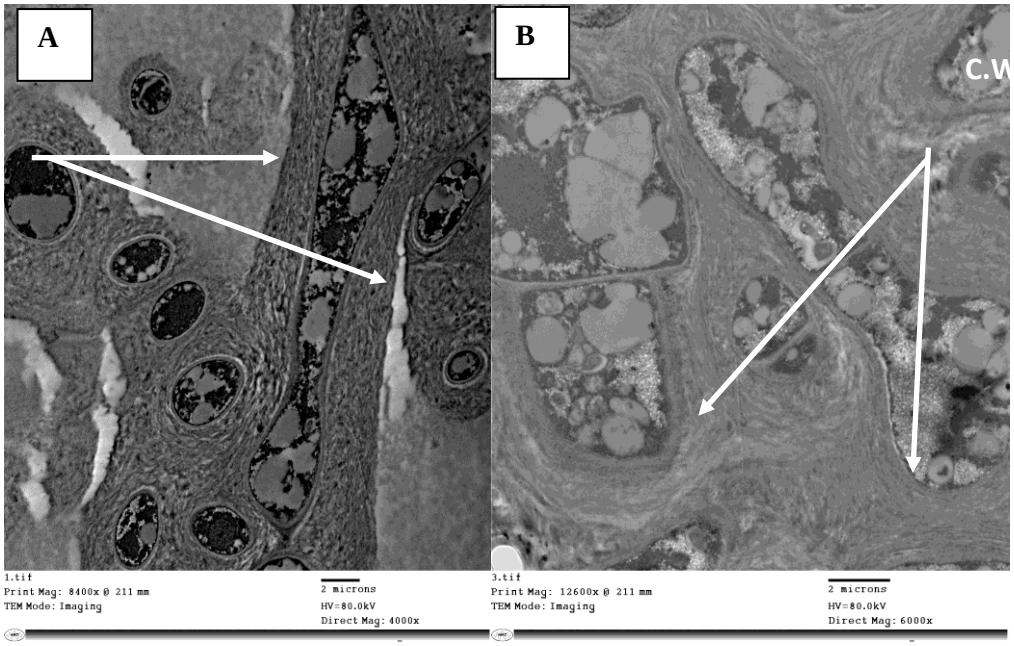

Fig. 2. Effects of taxodium alcohol extract (20%) on the ultrastructure of *S. cepivorum w*as confirmed by TEM of a particular thin segment of mycelium. (A) Control (B) Taxodium alcohol extracts C.W=Cell wall.

5-Effect of jatropha and taxodium alcohol extracts on the sclerotial germination of *S. cepivorum* under lab. conditions.

Data presented in Table 5. cleared that sclerotial germination of *S. cepivorum* was not affected by increasing the soaking period in both jatropha and taxodium alcohol extracts (20%) up to 30 minutes. On the other hand, jatropha extract at 45 and 60 min. soaking period inhibited 12.5 and 15 % fromsclerotia meanwhile, only at 60 min. sclerotial germination of *S. cepivorum* was affected by taxodium extract (12.5 %). Generally, both of the two extracts were influential reduction at different periods on sclerotial production.

Table 5: Effect of jatropha and taxodium alcohol extracts at 20% concentration on the sclerotia germination of *S. cepivorum* under lab. conditions.

Treatment (T)	Soaking period after(Minute) (P)	Sclerotial germination (%)	Reduction (%)[b]
Jatropha	5	100	----------
	15	100	----------
	30	100	----------
	45	87.5	12.5
	60	85.0	15
	MEAN(T1)	94.5	
Taxodium	5	100	----------
	15	100	----------
	30	100	----------
	45	100	----------
	60	87.5	12.5
	MEAN(T2)	97.5	
MEAN(P)	5	100	----------
	15	100	----------
	30	100	----------
	45	93.75	----------
	60	86.25	----------
Control		100	----------
L.S.D	Extracts(T)	4.244	
	Soaking period(P)	1.726	
	TxP	1.119	

6-Effect of jatropha and taxodium alcohol extracts on onion white rot percentage infection and bulb yield under greenhouse conditions:

The effect of jatropha and taxodium alcohol extracts and chemical treatment Folicure (Tebuconazole 25 % EC) on onion white rot percentage infection under greenhouse conditions are tabulated in Table (6). Concerning data, both jatropha and taxodium alcohol extracts caused a significant reduction of onion white rot disease incidence compared with control. Jatropha extract obtained a 30% disease percentage with a 57.14% reduction percentage moreover, taxodium extract gave 35.71 % disease reduction. Pots treated with folicure 25 % as chemical control recorded a high reduction effect of OWR disease percent (78.57%).

Regarding the productivity of onion bulbs, the same trend was found as the treatment with jatropha and taxodium extracts and the chemical treatment in the weight and perimeter of the onion bulbs with a non-significant increase compared to the check treatment. However, the improvement of OWR disease reduction led to a significant final onion bulb yield increase.

Table 6: Effect of jatropha and taxodium alcohol extracts at (20%) and Folicure (25%) on the percentage of white-rot infection, perimeter, the weight of onion bulbs and yield under greenhouse conditions.

Treatment	Disease Incidence (%)[a]	Reduction (%)[b]	Mean perimeter of bulb(cm)[a]	Mean weight of bulbs (gm)[a]	Yield (g/pot)[a]	Yield increasing %
Jatropha Alcohol extract	30.0	57.14	7.39	7.35	29.05	53.87
Taxodium Alcohol extract	45.0	35.71	7.75	7.78	20.05	33.17
Folicure (25%)	15.0	78.57	8.38	8.55	33.83	60.39
Control	70.0	-----	6.88	7.78	13.4	------
L.S.D	15.07	----	N.S	N.S	6.0	------

[a] Mean of 4 replicates (plates), b Reduction% = (Control-treatment /Control) x100

DISCUSSION

Plant extracts had been used successfully to control some plant diseases such as *Alternaria solani* causing early blight disease on potato and tomato (Wszelaki and Miller 2005; Sallam and abo Elyousr, 2012), Stemphylium blight and purple blotch disease of onion (Abdel-Hafez, 2014), Cercospora leaf spot disease of sesame (Tunwari and Nahunnaro, 2014), and Root Rots on Chickpea (Mansour *et al.,* 2017).

The crude extracts of both *T. distichium* and *J. curcas* were analyzed using GC-MS to detect the main chemical components that could play a key role in the suppression of the tested fungi. The data of chemical analysis proved the presence of many aliphatic and aromatic compounds in the different extracts. It was observed that the numeral moreover categories of these forms depended on the variety of the frictions (watery or solvent). This could be reflected by the difference in the inhibitory rate of the different extracting solvents of the same plant (Dinh *et al.,* 2019).

In this study, ethanolic extract and each of the hot and cold watery extracts of *T. distichium* cones and *J. curcas* seeds could suppress the growth of *S. cepivorum*, which is the causal agent for allium plant infection with white rot disease. The results were in agreement with those obtained by Kusumoto *et al.* (2010), Seweta *et al*. (2013), and Cordova-Albores *et al.* (2014). Meanwhile, Durgeshlal *et al*. (2019) reported that the *J. curcas* seed extracts were found to have the highest antifungal property against rice blast caused by *Pyricularia grisea.*

The presented results and figures showed that taxodium and jatropha extract restricted the linear growth of *S. cepivorum*, causing morphological anomalies such as coiling and atrophy, and lysis of the fungal cells. These results were confirmed by Hashem *et al*. (2016) and Lee (2020). Also, a high amount of indole 5 was found in the taxodium and jatropha solvent extracts by 5.69 % and 13.29%, respectively. These amounts may play a role in a positive effect on *S. cepiviorum* growth being reduced. The results were in harmony with Xu *et al.* (2010) who determined that within 9 derivatives of the indole, the indole 5 derivative showed a high reduction against each of *Alternaria alternate, A. brassicae, Helminthosporium sorokinianum, Fusarium graminearum, F. oxysporum* f. sp. *cucumarinum, F. oxysporum* f. sp*. vasinfectum*, and *P. oryzae*, followed by the two and then eight derivatives, respectively. Consequently, they stated that the activity of the compound depends on the substituent position on the indolyl ring.

Some scientists reported that hydroxyethyl derivatives of carvacrol, thymol, and eugenol had significantly lower antifungal activities relative to those of the original compounds (Mastelic *et al.,*2008.) while those derivatives had advanced free fundamental hunt behavior furthermore defensive belongings as antioxidants. This proposes that the antifungal movement depends on the compound of the terpenoid phenols and particularly, the attendance of a free hydroxyl group moreover an aromatic ring.

Jatropha seeds contain numerous components such as cuminaldehyde, thymol, y-terpinene, phytic acid, toxic compounds known as phorbol esters, and a high protein content, all of which have a significant activity such as potent cytotoxic, molluscicidal, insecticidal, and fungicidal properties (Meshram *et al*., 1996; Muniz 2020; Liu *et al.* 2008);

Concerning taxodium extract, many studies should an anti-fungal effect against human diseases (Kusumoto *et al.,*2009 and 2010) however, a few studies that address its effect on plant pathogens, Ogunwande *et al.* (2007), so this study is considered one of the few studies in this field. On the other side, terpene is an important separated from taxodium cones that have a toxic effect on the fungal disease by making complex sterols in fungal membranes, thus guiding to the absence of cell membrane solidity (Morrissey and Osbourn, 1999). Subsequently, from the analysis data of taxodium and jatropha extracts, heptadecanoic acid was isolated. GC-MS separated and identified various fatty acids (FAs) at different percentages. Fatty acids, everywhere in nature, play a crucial role in the life process. (FAs) belong to a physiologically important class of molecules involved in cell energy storage, membrane structure, and various signalling pathways (Fig. 1 and 2).

Some researchers examined the antifungal activity mechanisms of some fatty acids. One of these mechanisms focused on fungal membrane disturbance (Lee, 2020). The antifungal activities of several fatty acids and their probable mechanisms as antifungal sources have been previously examined. For example, Lee (2020) found that capric acid caused a high reduction of *Candida albicans* due to a disrupted or destroyed plasma membrane caused by hydrostatic pressure within the cell, Furthermore, a similar mechanism was presented in the antifungal activity of (cis)-9-heptadecenoic acid against Phytophthora infestans (Avis and Belanger, 2001). Despite that, Siyun *et al.* (2008) stated that neither the germination spores nor linear growth of *Alternaria solani , Colletotrichum lagenarium , Fusarium oxysporum* f. sp. *cucumerinum*, as well as *F. oxysporum* f. sp. *lycopersici* were affected by oleic acid. This is contrary to Walters *et al.* (2004) who reported that oleic acid sourced a significant decrement in a mycelial expansion of *Pythium ultimum* as well as *Crinipellis perniciosa (Moniliophthora perniciosa*) at 1,000 mol/L. Moreover, Xu *et al.* (2012) suggested that interfering fungal sphingolipidbio synthes of acetylenic fatty acid leads to antifungal activity. Up to date, most studies of the antifungal mechanisms of fatty acids have focused on the inhibitory effects against human pathogenic fungi, whereas the antifungal activities against phytopathogens are still unknown.

Regarding the effect of *J. curcas* and *T. distichum* extracts on the yield of onion plants, pots which were treated with 20 %of the two extracts after inoculation with *S. cepivorium* showed increasing bulb weight of each pot. These were in harmony with Siva *et al.* (2008), Ijato *et al.* (2010) and Akanmu *et al*. (2014) found that *J. curcas* extract at 0.15, 0.30 and 0.45 mg/ml significantly reduced the incidence and severity of wilt millet disease caused by *F. anthophilum* and *F. oxysporum* under field conditions in comparison to the control treatments. Meanwhile, at 0.30 mg/ml, data cleared a significant increase in the vegetative characters of millet plants (plant height, number of leaves and leaf area) than the untreated plants. Later, Etaware *et al.* (2019) reported that a high dosage of *J. curcas* extract showed a significantly increase in the vegetative parameters (plant height and leaf area) of tomato plants infested with both *Aspergillus sclerotirium, A. glaucus, A. terreus,* and *A. fumigatus.*

In addition to the previous jatropha biocide activity, da Silva *et al.*(2012) studied the allelopathic effects of different *J. curcas* organs against various plant species like soybean seeds which were grown in the presence of the root exudates of *J. curcas*. The main allelopathic signals produced by *J. curcas* are promotion root growth and seed germination time and speed.

CONCLUSION

In general, the authors believe that the presence of cuminaldehyde, carvacrol, indole-5 fatty acids, and others present in taxidum and jatropha extracts may play an important role against *S. cepivorum*. Consequently, they suggested that *J. curcas* and *T. distichum* stand out as promising means for biofertilizer function and manage pests and diseases that affect agriculture output. Based on that, further research and studies should be conducted on the chemical derivatives of some plant extracts as an alternative or complementary source to the effect of fungicides, insecticides and others. Based on present findings, further research and studies should be conducted on the chemical derivatives of some plant extracts as an alternative or complementary source to the effects of fungicides, insecticides, and others.

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تأثير مستخلصات أشجار التاكسوديم و الجاتروفا على الفطر سكليروشيوم سيبفورم المسبب لمرض العفن الأبيض في الجنس للبصل

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الملخص

أجريت الدراسة الحالية لمعرفة تأثير استخدام المستخلصات المختلفة لنوعين من أشجار جاتروفا وتاكسوديم. تم الاستخلاص بواسطة الماء المقطر ومذيب إلايثانول. كما تم تحديد المكونات النشطة بيولوجيًا لكل مستخلص باستخدام تحليل كروماتوجرافيا الغاز / تحليل الطيف الكتلي (GC / MS). أثبتت التجارب المعملية أن المستخلص الإيثانولي أكثر فاعلية فى التأثير التثبيطي للنمو الميسليومى وكذلك على التعداد الكلى للأجسام الحجرية للفطر سكليروشيوم *Sclerotium cepivorum* من مستخلصي الماء البارد أو الساخن مقارنةً بالكنترول ، أظهرت المعاملة بتركيز 20٪ من مذيب الإيثانول تأثير أباديا للفطر بينما كان للتركيز 5٪ تأثيرا ضعيفا على نمو الفطر المختبر.. أظهرت نتائج تجارب الصوبة أن زراعة البصل المعامل بالمستخلص الإيثانولي للتاكسوديم والجاتروفا بنسبة 20٪ أدى إلى انخفاض معنوي في نسبة الإصابة بمرض العفن الأبيض على البصل مع زيادة معنوية في محصول الأبصال مقارنة بمعاملة الكنترول. هذا التقرير هو واحد من الدراسات القليلة التي استخدمت مستخلص مخاريط التاكسوديم وبذور الجاتروفا كمواد مضادة للفطريات ضد مسببات الأمراض النباتية وخاصة الفطر سكليروشيوم سيبفورم.

الكلمات المفتاحية: جاتروفا كركاس ، وتاكسديوم دستكم، سكليروشيوم سيبفورم، عفن البصل الأبيض (OWR) ، مستخلصات نباتية