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Allelopathic Effect of Some Cruciferous Seeds on *Rhizoctonia solani* kuhn and *Gossypium barbadense* L.

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Abstract: Phenolic substances, lipoidal matters and fatty acid contents of some cruciferous powdered seeds, *Raphanus sativus* L., *Brassica oleracea* L. var *capitata, Sinapis alba* L., *Brassica nigra* Koch, *Eruca sativa* Mill, *Brassica napus* L. and *Lipidium sativum* L., were determined. The results indicated that *R. sativus* L., *B. napus* L. and *B. oleracea* L. var *capitata* contained higher percentage of phenolic compounds, lipoidal matters and unsaturated fatty acids, respectively, compared to other tested plants. The aqueous and ethanolic extracts of the above seeds were prepared. The alcoholic extract was fractionated with petroleum ether 60-80°C, chloroform and ethyl acetate, respectively. The prepared extracts and fractions were subjected to phytochemical screening and the results indicated the presence of various active constituents. Their allelopathic effect on *Rhizoctonia solani* Kuhn was tested *in vitro*. The tested extracts have variable inhibitory effect on *R. solani* Kuhn. The effect depends on the concentration of the extracts, plant species and the active constituents. *Rhizoctania solani* infested and non-infested soil sowed with cotton (*Gossypium barbadense* L) seeds were treated with the above powdered seeds. All treatments reduced cotton seed germination, decreased significantly root length but increased seedling shoot height. The results indicated that application of powdered seeds of *R. sativus* L., *E. sativa* MILL and *S. alba* L., to the soil infested with *R. solani* Kuhn, enhanced the germination percentage of cotton, reduced damping off precentage and improved the growth criteria of the cotton seedlings. Fatty acids composition and mineral contents of the untreated cotton and infested cotton seedlings treated with seed powders were also investigated.

Key words: Allelopathy, craciferous seeds, damping-off disease

INTRODUCTION

Allelopathy is any direct or indirect harmful or beneficial effect by one plant, including microorganisms, on another through production of allelochemical substances that escape into the environment[1-4]. Allelochemicals are secondary plant metabolites or waste products of metabolism[5]. Simple water-soluble and insoluble organic acids; fatty and phenolic acids; straight chain alcohols; aliphatic aldehydes and ketones; simple unsaturated lactones; acetylenic compounds naphtho-quinones; anthraquinones, complex quinones; simple phenols, flavonoids and tannins; terpenoids of many categories; alkaloids and saponins are groups of secondary metabolites that have been encountered in allelopathic interactions[6-16]

It was reported that the mode of action for allelochemicals are inhibition or modification of plant growth and development. Some cause inhibition of cell division (coumarins, many alkaloids); modification of cell wall construction, membrane permeability, modification of active transport, inhibition of specific enzymes as indole acetic acid oxidase[4].

Others affacted the germination of pollens, spores and seeds, minerals uptake, pigment synthesis, photosynthesis (many flavonoids); protein synthesis (many phenolics and alkaloids); electron transport involving cytochrome (saponins) and changes in the frequency of other pathogenic organisms[3,4,17-19]. They are also suppress plant growth and regulate species diversity (like herbicides) in the habitat of the producer plant[20,13,21]. Allelochemicals may be selective in their action, or plant may be selective in their responses[19]. Allelochemicals are rapidly degraded into non-toxic compounds, so they are safe in comparing with commercial herbicides. The adoption of allelopathic strategies in farming are essential to provide clean environment for the future generations.

Cruciferous seeds contain active constituents of important interst. Therefore, in this study, the phenolic substances, lipoidal matters, fatty acids as well as the

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group composition of the active constituents of these seeds were outlined by chemical determination and phytochemical screening. The allelopathic potential of different seed extracts on the growth of *Rhizoctonia solani* kuhn, the causal pathogen of cotton damping off were investigated in *in vitro*. The *in vivo* effects of tested powdered seeds, when they applied either alone as a soil additives or in combination with *R. solani* on cotton seedling growth criteria, damping-off disease incidence, fatty acids composition and mineral contents of cotton seedlings were also studied.

MATERIALS AND METHODS

Fungal isolate and host plant: An isolate of *Rhizoctonia solani* kuhn was isolated from diseased cotton plants grown in El-Gharbia Governorate, Egypt. The isolate was maintained on potato-dextrose agar (PDA).

Seeds of susceptible cotton plant (*Gossypium barbadense* L.) cultivare Giza 67, were obtained from the Ministry of Agriculture, Giza, Egypt.

Tested seeds: Seeds of seven plants belonging to family Crucifereae including, *Raphanus sativus* L., *Brassica oleracea* L. var *capitata, Sinapis alba* L., *Brassica nigra* KOCH, *Eruca sativa* MILL, *Brassica napus* L. and *Lipidium sativum* L. were obtained from the local market and ground to fine powders.

Determination of total phenols: Quantitative determination of total soluble phenols in the tested seeds were estimated colormetrically according to Folin-Denis method[22].

Determination of lipids and fatty acids: The fatty acids of the tested seeds were isolated from the corresponding lipoidal matter and analysed by GLC according to Bligh and Dyer,[23] and Flood,[24] using GC-Shimadzu-4CM (PEE) equipped with PID detector and glass column 2.5 mm X 3 MM (i.d), packed with 5% DEGS on 80/100 chromo Q. Column temperature, 180°C isothermal, N_2 carrier gas flow rate 20 ml min^{-1}, detector temperature 270°C with helium flow rate 75 ml min^{-1}.

Preparation of seed extracts

Aqueous extract: Ten grams of each tested powdered seeds were infused in distilled water until exhaustion. The residues were dried and weighed

Organic solvent extracts: Ten grams of powdered seeds were separately defatted by cold maceration with petroleum ether (60-80°C) then exhausted by cold maceration with ethanol. The concentrated ethanolic extract was diluted with H_2O and extracted by fractionation with chloroform and ethyl acetate, respectively. Solvents were distilled off and residues were determined.

Chemical screening of the prepared extracts: The aqueous and organic solvent extracts and fractions of the tested seeds were separately screened for their constituents according to the procedures mentioned by Kapoor *et al.*[25], Segelman[26], Trease and Evans[27], Ahmed *et al.*[28], Rizk[29], El-Tawil[30] and Afifi[31].

Bioassay of different seed extracts: *Rhizoctonia solani* kuhn discs, (5 mm diameter) were put on the surface of potato dextrose agar media plates supplemented with the following concentrations (0, 125, 250, 500, 1000, 1500 and 2000 ppm) of the seed extracts. The plates were incubated in the dark at 28°C for 7 days and the reduction percentage in colony diameter was calculated.

***In vivo* test:** Two experiments were conducted out to investigate the allelopathic effect of the tested powdered seeds. Sixteen pots (15x20 cm diameter), each one filled with sterilized sandy loam soil (1 kg pot^{-1}) and sowed with 1% NaOCl pre-sterilized 10 cotton seeds, were used for each experiment. One experiment was used as non-infested and the other as infested. Two pots in each experiment were used as control, while the other pots were treated with the tested powdered seeds (5 g pot^{-1}). The infested pots were treated by the same powdered seeds plus 20 ml washed mycelia of *Rhizoctonia solani* kuhn as described by El-Khadem and Papavizas[32] before sowing the cotton seeds. Germination percentage was calculated after 3 days of sowing. Pre and post emergence damping off were recorded after 10 and 30 days of planting, respectively. Seedling length and dry weight were also measured (samples were dried in an oven at 60°C until constant weight).

Fatty acid composition: Fatty acids composition of the uninfected cotton seedlings as well as infected seedlings which were separately treated with seed powders of *R. sativus* L., *E. sativa* MILL and *S. alba* L., was determined

Mineral contents: Cation estimation was carried out for the untreated cotton seedlings as well as the selected treatments as describe by Allen *et al.*[33]. K^+, Na^+ and Ca^{2+} were determined using flame photometer (Clinical Flame Photometer), while Mg^{2+} and Mn^{2+} were assayed using atomic absorption (Flame Emission Spectrophotometer, Shimadzu Model A.A-640-12).

Table 1: Total phenolic and lipids extracted from the the tested powdered seeds

Tested seeds species	Contents mg g^{-1} Total Phenolics	Total Lipids	Total phenolics and lipids
Raphanus sativus L.	31.75±0.15	12.89±2.3	44.64±0.75
Brassica oleracea l. *var capitata*	12.33±0.30	18.79±1.3	31.12±0.86
Sinapis alba L.	27.0±0.00	6.3±0.05	33.3±0.4
Brassica nigra Koch	18.62±0.40	9.79±0.80	28.41±2.1
Eruca sativa Mill	28.80±0.23	10.77±1.80	39.57±0.6
Brassica napus L.	2.41 ±0.01	20.13±1.10	22.54±1.2
Lipidium sativum L.	8.29±0.02	6.40±0.70	14.69±1.4

Each value represent the mean of 3 replica

The enrichment ratio of each cation was calculated as follows :

$$ER = \frac{\text{concentration of cations in plant (mg g}^{-1})}{\text{concentration of cations in soil (mg 100 g}^{-1})}$$

Statistical analysis: The obtained results were statistically treated with ANOVA, correlation coefficient and regression equations according to Snedecor and Cochran[34].

RESULTS

Determination of phenolic compounds, lipids and fatty acid compositions: Table 1 revealed that the tested seeds contained different concentrations of total soluble phenolics and lipids. Total phenolics were detected in higher concentration in *Raphanus sativus* L., followed by *Eruca sativa* MILL and *Sinapis alba* L. in decreasing order while *Brassica napus* L. seeds exhibited the lowest concentration. On the other hand, the highest value of lipids content was recorded in *B. napus* L., followed by *Brassica oleracea* L. var *capitata*. The lowest concentrations of lipids were detected in *S. alba* L. and *L. sativum* L. However, *R. sativus* L. showed highest value in the total phenolics and lipids content followed by *E sativa* Mill. These values were nearly equal in *S. alba* L., *B. oleracea* L. var *capitata* and *B. nigra* Koch. The lowest concentration was aquired by *L. sativum* L.

Table 2 showed the individual fatty acids components in each of the investigated seeds. The saturated fatty acids proportion ranged between 15.36-63.22% of the total fatty acids in the different tested seeds. The highest value was detected in *L. sativum* L. Palmatic acid $C_{16.0}$ was the most abundant saturated fatty acid specialy in *R. sativus* L. However, unsaturated fatty acids ranged between 36.78-84.64% of the total fatty acids. The highest percentage was detected in *B.oleracea* L. var *capitata*. Erucic $C_{21.1}$, Linolenic $C_{18.3}$ and Oleic $C_{18.1}$ represent the abundant unsaturated fatty acids specialy in *B. napus* L., *L. sativum* L. and *B. oleracea* L. var. *capitata*, respectively.

Chemical screening of the tested seeds extracts: The preliminary chemical screening of powdered seeds extracts revealed the presence of different types of chemical groups (Table 3).

Aqueous extracts of all tested seeds gave positive tests for carbohydrates, tannins and saponins except *B. nigra* Koch and *L. sativum* L. which gave negative test for the latter. Ethanolic extract for all tested seeds proved the presence of carbohydrates and/or glycosides and flavonoids. Alkaloids were detected in chloroformic as well as the ethanolic extracts of *R. sativus* L., *E. sativa* Mill and *L. sativum* L.. Ethyl acetate extracts of the tested seeds revealed the presence of flavonoids. Unsaturated sterols and/or triterpenes were detected in petroleum ether extracts of all tested seeds.

Table 2: Relative percentages of fatty acids in the tested powdered seeds

Fatty acids Type	Relative % of fatty acids *R. sativus L.*	*B.oleracea L.* var *capilata*	*S. alba L.*	*B. nigra Koch*	*E. sativa Mill*	*B. napus L.*	*L. sativum* L.
Myristic $C_{14:0}$	0.228	0.365	0.135	0.421	0.841	----	0.840
Palmitic $C_{16:0}$	10.294	3.311	4.989	4.542	5.257	4.335	8.739
Palmitoleic $C_{16:1}$	0.609	---	0.405	----	0.631	----	----
Stearic $C_{18:0}$	0.670	0.974	0.445	0.591	1.051	1.529	1.261
Oleic $C_{18:1}$	23.877	34.088	25.724	8.636	19.428	16.318	22.409
Linoleic $C_{18:2}$	14.497	12.710	15.370	19.136	8.074	13.463	9.748
Linolenic $C_{18:3}$	17.085	10.713	17.878	24.057	19.302	14.023	52.380
Arachidic $C_{20:0}$	Tr. *	Tr.	Tr.	Tr.	Tr.	Tr.	1.681
Eicosenic $C_{20:1}$	----	----	----	----	----	----	----
Behemic $C_{22:0}$	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
Erucic $C_{22:1}$	32.739	37.838	35.054	42.647	45.419	50.331	2.942
Lignoceric $C_{24:0}$	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	----
Total saturated	28.277	15.363	23.447	29.581	26.451	19.887	63.220
Total unsaturated	71.722	84.636	76.553	70.419	73.549	80.112	36.780

* Tr = traces, -- = not detected

Table 3: Phytochemical screening for the different extracts of the tested seeds

Extract	Chemical groups	*R. sativus* L.	*B.oleracea* var *capitata*	*S. alba L.*	*B. nigra* Koch	*E. sativa* Mill	*B. napus* L.	*L. sativum* L.
Hot water	Saponins	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	- ve
	Carbohydrates and/or glycosides	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
	Tannins	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Ethanol	Tannins	± ve	- ve	- ve	- ve	+ ve	- ve	+ ve
	Carbohydrates and/or glycoside rearrangement	± ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
	Alkaloids	± ve	- ve	- ve	- ve	± ve	- ve	± ve
	Flavonoids	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Petroleum ether	Unsaturated sterols and / or triterpenes	+ve	+ ve	+ ve	+ve	+ve	+ ve	+ve
Chloroform	1-Alkaloids	+ve	+ve	-ve	-ve	+ve	-ve	+ve
	2-Glycosides	+ve	+ve	+ve	-ve	-ve	+ve	-ve
Ethyl acetate	Flavonoids	+ve	+ve	+ve	+ve	+ve	+ve	+ve

(±) = present in traces, (+) = present, (-) = absent

Effect of different extracts of the tested seeds on *Rhizoctonia solani*, Kuhn: The radial growth of *Rhizoctonia solani* was inhibited by aqueous and organic solvent extracts of the different tested species. Table 4 showed significant variations in inhibitory effect of the extracts on *R. solani* Kuhn. The inhibition was affected by the plant species (active constituents) and concentrations of the extracting solvents. The highest percentage inhibition (86.66 and 85.55, respectively) were noticed at concentration of 1500 ppm of chloroformic extracts of *R. sativus* L. and *E. sativa* Mill, respectively. However petroleum ether extract of *S. alba* L. at concentration of 1500 ppm recorded (83.33%) inhibition. Higher values of inhibition (80.0 and 76.66, respectively) were recorded also with ethanolic and aqueous extracts of *R. sativus* L. at concentration of 500 ppm. On the other hand, *B. nigra* Koch ethyl acetate extract and *E. sativa* Mill petroleum ether extract, recorded the same inhibition value (74.44) at concentration of 1500 ppm of each solvent. Both of *S. alba* L. and *L. sativum* L. aqueous and ethanolic extracts, respectively showed the inhibition value (61.11%) at concentration of 500 ppm.

The relationships between radial fungal growth and concentrations of the extracted materials from the tested seeds by different solvents were significant and acquired linear regression lines (Table 5).

Regression equations of these relations indicated that the lethal concentration, which gives rise to zero growth was depending on the tested seeds and solvents used. Table 5 indicates that the lethal concentration was greater for ethyl acetate extract of *R. sativum* L., *L. sativum* L., *B. oleracea* var *capitata* L. and *E. sativum* MILL in a descending order, compared with the other solvents. The lowest lethal concentrations were noticed with both ethanolic and aqueous extracts of *B. oleracea* var. *capitata*, *R. sativum* L., *S. alba* L., *B. nigra* Koch, *L. sativum* L. and *B. napus* L. in an ascending order.

Allelopathic effects of tested powdered seeds on *Gossypium barbadense* L.

Germination percentage: All the tested powdered seeds showed great variation in their ability to suppress germination of *Gossypium barbadense* L. seeds (Table 6). The highest germination percentage of cotton seeds was 94,91 and 90%, respectively for the non-infested soil treated with *R. sativus* L., *S. alba* L and *E. sativa* Mill respectively. However, *B. oleracea* L. var *capitata* exerted the lowest germination (52%).

Inoculation of soil with *R. solani* Kuhn decreased the percentage of germination to 43% compared with the non-infested control. Treatment the infested soil with the powdered seeds increased the percentage of germination. The most effective treatments were noticed with *R. sativus* L., *S. alba* L and *E. sativa* which achieved (69,66 and 65% germination, respectively), while *B. oleracea* L. var *capitata* treatment showed the same effect as control (43%).

Growth criteria: Data presented in Table 6 demonstrated the influence of different treatments on growth criteria of 10 days old non-infected cotton seedlings. Root length was markedly decreased by all treatment, the highest decrease was detected with *B.oleracea* L. var *capitata* treatment. Table 6 also further showed significant increase in the shoot height for all treatments. Some treatments showed a significant increase in the dry weight compared to the control. The highest values were recorded with *S. alba* L., *R. sativus* L. and *E. sativa* Mill (0.457, 0.412 and 0.400 g/seedling, respectively).

The infection of cotton seedlings with *R. solani* Kuhn caused a reduction in root length. However, the pre-addition of the tested powdered seed enhanced shoot growth, except in case of *B. oleracea* L. var *capitata* and *B. napus* L. (Table 6). Cotton seedlings dry weights decreased in all treatments except with *S. alba* L. *E. sativa*

Table 4: Percent inhibition of different seed extracts on radial growth of *R. solani* Kuhn

Tested seeds	% Inhibition at concentration (ppm) 125	250	500	1000	1500	2000
	Hot water extract					
R. sativus L.	45.55	65.5	76.66	76.66	76.66	76.66
B. oleracea var *capitata* L.	46.66	50.0	56.66	56.66	56.66	56.66
S. alba L.	31.11	57.77	61.11	61.11	61.11	61.11
B. nigra Koch	35.55	46.66	67.77	70.00	70.00	70.00
E . sativa Mill	15.15	32.22	68.88	68.88	68.88	68.88
B. napus L.	24.44	27.77	54.44	64.44	68.88	68.88
L. sativum L.	31.11	42.22	57.77	61.11	61.11	61.11
Control	0.0	0.0	0.0	0.0	0.0	0.0
	Ethanolic extract					
R. sativus L.	48.88	65.55	80.00	80.00	80.00	80.00
B. oleracea var *capitata* L.	43.33	48.22	50.00	50.00	50.00	50.00
S. alba L.	33.33	63.33	71.11	73.33	73.33	73.33
B .nigra Koch	41.11	48.88	74.44	75.55	75.55	75.55
E. sativa Mill	27.77	35.55	65.55	75.55	75.55	75.55
B. napus L.	25.55	34.44	57.77	65.55	65.55	65.55
L. sativum L.	44.44	54.44	61.11	61.11	61.11	61.11
Control	0.0	0.0	0.0	0.0	0.0	0.0
	Chloroform extract					
R. sativus L,	18.88	24.44	46.66	54.44	86.66	86.66
B. oleracea var *capitata* L.	13.33	31.11	36.66	45.55	67.77	67.77
S. alba L.	14.40	20.00	40.00	65.55	72.22	76.66
B. nigra Koch	11.11	26.66	32.22	64.44	70.55	78.88
E.sativa Mill	2.20	12.22	23.33	47.77	85.55	85.55
B.napus L.	12.22	30.00	34.44	50.00	57.77	57.77
L.sativum L.	6.66	21.11	44.44	50.00	57.77	57.77
Control	0.0	0.0	0.0	0.0	0.0	0.0
	Ethyl acetate extract					
R.sativus L.	13.33	32.22	38.88	51.11	63.33	67.77
B. oleracea var *capitata* L.	13.33	27.77	33.33	53.33	57.77	57.77
S.alba L.	12.22	17.77	35.55	61.11	65.55	65.55
B. nigra Koch	7.77	23.33	28.88	62.22	74.44	74.44
E. sativa Mill	2.22	21.11	28.88	52.22	60.00	71.11
B. napus L.	18.88	26.66	31.11	54.44	59.00	59.00
L. sativum L.	12.22	24.44	41.11	56.66	56.66	56.66
Control	0.0	0.0	0.0	0.0	0.0	0.0
	Petroleum ether (60-80°C) extract					
R. sativus L.	12.22	20.00	34.44	53.33	65.55	65.55
B. oleracea var *capitata* L.	12.22	24.44	41.11	46.66	54.44	54.44
S. alba L.	8.88	15.55	42.22	74.44	83.33	83.33
B. nigra Koch	13.33	16.66	26.66	47.77	67.77	67.77
E. sativa Mill	11.11	13.33	48.88	60.00	74.44	74.44
B. napus L.	6.66	12.22	24.44	48.88	58.88	58.88
L. sativum L.	7.77	13.33	23.33	53.33	56.66	56.66
Control	0.0	0.0	0.0	0.0	0.0	0.0

Mill and *R. sativus* L. (0.425, 0.330 and 0.290 g/seedling, respectively).

Cotton damping-off disease incidence: Data presented in Table 7 revealed that all the screened powdered seeds were effective in reducing damping-off of cotton, expressed as the survived seedlings after 30 days of planting. *R. sativus* L. was the highest effective, followed by *E. sativa* Mill and *S. alba* L. respectively, in controlling the disease, compared with untreated control.

Fatty acids analysis of cotton seedlings: Fatty acids analysis revealed the presence of different saturated and unsaturated acids in the infected cotton seedlings

Table 5: Regression equations, R^2 value for the relationships between concentrations of the extracted materials of the tested seeds (X) and growth of *Rhizoctonia solani* (Y) and the calculated lethal concentrations (ppm)

Tested plant species	Regression equation	R^2	Lethal con. (ppm)
	Hot water extract		
R. sativus L.	$Y=3.790 - 1.245 \times 10^{-6} x^2$	0.586	1744.25
B. oleracea var *capitata* L.	$Y=2.738 \_ 9.013 \times 10^{-6} x^2$	0.530	1743.03
S. alba L.	$Y=3.825 - 1.208 \times 10^{-6} x^2$	0.630	1779.58
B. nigra Koch	$Y=4.161 - 1.302 \times 10^{-6} x^2$	0.718	1787.53
E.sativa Mill	$Y=4.858 - 1.510 \times 10^{-6} x^2$	0.695	1793.25
B. napus L.	$Y=5.474 - 1.378 \times 10^{-6} x^2$	0.860	1992.58
L. sativum L.	$Y=4.536 - 1.374 \times 10^{-6} x^2$	0.840	1816.56
	Ethanol extract		
R.sativus L.	$Y=3.843 - 1.254 \times 10^{-6} x^2$	0.613	1748.19
B. oleracea var *capitata* L.	$Y=2.580 - 8.555 \times 10^{-6} x^2$	0.451	1736.74
S.alba L.	$Y=3.485 - 1.109 \times 10^{-6} x^2$	0.579	1772.02
B. nigra Koch	$Y=3.739 - 1.178x\ 10^{-6} x^2$	0.675	1788.85
E. sativa Mill	$Y=4.852 - 1.519 \times 10^{-6} x^2$	0.699	1786.92
B. napus L.	$Y=5.467 - 1.373 \times 10^{-6} x^2$	0.829	1995.47
L. sativum L.	$Y=3.742 - 1.142 \times 10^{-6} x^2$	0.771	1810.09
	Chloroform extract		
R. sativus L.	$Y=6.248 - 1.753 \times 10^{-6} x^2$	0.894	1887.47
B. oleracea var *capitata* L.	$Y=6.961 - 1.829x\ 10^{-6} x^2$	0.961	1950.43
S.alba L.	$Y=6.468 - 1.835 \times 10^{-6} x^2$	0.867	1877.12
B. nigra Koch	$Y=6.618 - 1.835 \times 10^{-6} x^2$	0.887	1898.90
E. sativa Mill	$Y=7.687 - 1.771 \times 10^{-6} x^2$	0.916	2083.20
B. napus L.	$Y=6.623 - 1.734 \times 10^{-6} x^2$	0.945	1954.24
L. sativum L.	$Y=6.901 - 1.782 \times 10^{-6} x^2$	0.889	1967.54
	Ethyl acetate extract		
Raphanus sativus L,	$Y=6.259 - 1.010 \times 10^{-6} x^2$	0.740	2489.16
B. oleracea var *capitata* L.	$Y=6.586 - 1.190 \times 10^{-6} x^2$	0.848	2352.58
S. alba L.	$Y=6.809 - 1.868 \times 10^{-6} x^2$	0.900	1909.15
B. nigra Koch	$Y=6.938 - 1.890 \times 10^{-6} x^2$	0.890	1915.56
E.sativa Mill	$Y=7.120 - 1.309 \times 10^{-6} x^2$	0.777	2331.79
B.napus L.	$Y=6.668 - 1.729 \times 10^{-6} x^2$	0.968	1963.34
Lipidium sativum L.	$Y=6.307 - 1.048 \times 10^{-6} x^2$	0.665	2452.79
	Petroleum ether (60-80°C) extract		
R. sativus L.	$Y=6.603 - 1.467 \times 10^{-6} x^2$	0.819	2126.82
B. oleracea var *capitata* L.	$Y=6.801 - 1.742 \times 10^{-6} x^2$	0.930	1975.45
S. alba L.	$Y=6.503 - 1.906 \times 10^{-6} x^2$	0.784	1847.56
B.nigra Koch	$Y=6.766 - 2.084 \times 10^{-6} x^2$	0.820	1817.28
E.sativa Mill	$Y=6.625 - 1.801 \times 10^{-6} x^2$	0.826	1917.60
B. napus L.	$Y=7.908 - 1.933 \times 10^{-6} x^2$	0.976	2022.56
L. sativum L.	$Y=7.289 - 1.297 \times 10^{-6} x^2$	0.798	2369.83

Table 8. $C_{14.0}$ (Myristic) and $C_{18.0}$ (stearic) and $C_{18.1}$ Oleic fatty acids were the predominant acids, representing 36.25% and 26.9% and 25.5% of the total fatty acids, respectively. However, treatment of infected cotton seedlings separately with of *R. sativus* L., *E. sativa* MILL and *S. alba* L. had resulted in alteration of the fatty acids distribution. In general all treatments induced a noticeable increase in the percentage of $C_{16.0}$ (Palmitic) and $C_{22.0}$ (Behemic) fatty acids compared with control plants. Treatment with *E. sativa* Mill had led to the disappearance of several saturated fatty acids from C_8:C_{14} and increase % of other fatty acids $C_{18.0}$ (Stearic) and $C_{20.0}$ (Arachidic). In addition unsaturated fatty acids as $C_{18.3}$ (Linolenic) disappeared of. In contrast, treatment with *S. alba* L. had led to the increase of the percentage of $C_{18.1}$ (Oleic) and $C_{18.3}$ (Linolenic) fatty acids and the disappearance of $C_{14.0}$ (Myristic) fatty acids.

Table 6: Effect of the tested powdered seeds treatments on germination percentage and some growth criteria of *Gossypium barbadense* L. in non infested and infested soil

	Non infested soil				Infested soil			
Treatment	Germi-nation %	Shoot height	Root length	Dry weight g/seedling	Germi-nation %	Shoot height (cm)	Root length (cm)	Dry weight g/seedling
Control (Soil without seed additives)	100	6.25±0.9	7.90±1.94	0.321±0.027	43	5.65±0.472	5.32±1.940	0.260±0.0027
Soil + *R. sativus* (S.P)	94	10.75±1.44	4.80±1.04	0.412±0.0062	69	11.86±1.300	4.80±1.045	0.290±0.00270
Soil + *B. oleracea* L. var *capitata*	52	9.00±2.16	3.35±0.47	0.222±0.0045	43	5.00±0.816	3.35±0.472	0.126±0.00215
Soil + *S. alba* L.	91	10.65±0.94	4.15±0.19	0.457±0.0050	66	8.80±0.400	4.15±0.191	0.425±0.00957
Soil + *B. nigra* Koch	85	10.25±0.86	4.60±0.71	0.286±0.0025	60	11.77±2.090	4.60±0.711	0.225±0.00208
Soil + *E. sativa* Mill	90	11.90±0.98	4.80±0.54	0.400±0.0040	65	8.10±0.840	4.80±0.541	0.330±0.00816
Soil + *B. napus* L.	60	12.25±1.70	3.65±0.47	0.230±0.0047	50	5.55±1.360	3.65±0.472	0.214±0.00902
Soil + E.*sativum* L.	63	9.25±0.95	4.00±0.40	0.250±0.0040	52	6.72±0.480	4.00±0.408	0.142±0.0018
F-value	---	8.268	10.112	17.393	---	23.871	10.112	7.343
L.S.D at 0.05	---	1.043	0.470	0.00115	---	0.741	0.470	0.0133
at 0.01	---	0.712	0.637	0.00156	---	1.005	0.637	0.0180

The mean difference is significant at 0.05 and 0.01 level, (S.P) = Seed powder

Table 7: Effect of the tested powdered seeds treatments on the incidence of cotton damping off caused by *R. salani* Kuhn

	Damping-off		
Treatment	Pre-emergence % * (10 DAS)	Post-emergence % * (30 DAS)	Surviving plants
Control : Uninfested	0.0a	0.0a	100.00g
Control : (C)treated with	77.79f	14.81c	7.40a
R. sativus L.	5.00b	29.63e	65.37f
B. oleracea var *capitata* L.	62.97e	7.41b	29.62b
S. alba L.	40.74d	0.00	59.26e
B. nigra Koch	33.35c	13.70c	52.95de
E. sativa Mill	33.33c	6.33b	60.34e
B. napus L.	48.15d	18.52d	33.33c
L. sativum L.	59.26e	0.00	40.74d

Mean followed by the same letter are not significantly different at P≥ 0.5, * DAS = Days after sowing

Table 8: Relative percentage of fatty acids in cotton seedlings grown in infested soil with *R. solani* Kuhn as influenced by the application of powdered seeds of *R. sativus* L., *E. sativa* MIL and *S. alba* L.

		Seedlings treated with			
Fatty acid	Symbol	Control	*R. sativus*	*E. sativa Mill*	*S. alba L.*
Capoic	$C_{6.0}$	---	-----	0.9	0.11
Caprilic	$C_{8.0}$	0.70	-----	---	----
Carpric	$C_{10.0}$	0.12	----	---	0.80
Lauric	$C_{12.0}$	0.35	3.56	---	3.20
Myristic	$C_{14.0}$	36.25	29.90	---	----
Palmitic	$C_{16.0}$	9.95	15.33	18.15	17.85
Palmitoleic	$C_{16.1}$	17.10	10.30	14.20	---
Stearic	$C_{18.0}$	26.90	18.75	32.54	27.15
Oleic	$C_{18.1}$	25.50	16.00	39.40	45.15
Linoleic	$C_{18:2}$	21.19	7.00	20.35	14.30
Linolenic	$C_{18.3}$	24.20	30.40	----	35.35
Arachidic	$C_{20.0}$	-----	----	5.55	----
Behimic	$C_{22.0}$	----	3.39	2.90	7.49
Total Sat. %	----	74.27	48.79	60.04	56.60
Total Unsat %	---	87.99	63.70	73.95	94.80
Unsat/sat	---	1.18	1.30	1.23	1.67

Control = infected, --- = not detected

Generally all the treatment of infected cotton seedling with *R. sativus* L., *E. sativa* Mill and *S. alba* L. enhanced the unsaturated/ saturated fatty acids ratio in comparing with the control by 1.30, 1.23 and 1.67, respectively.

Mineral content: The effect of the tested powdered seeds treatments on the enrichment ratio (ER) of some cations Na^+, K^+, Ca^{2+}, Mg^{2+} and Mn^{2+} in 10 days-old cotton seedlings infected with *R. solani* kuhn is presented in Table 9. ER is an indicator of ion uptake from the soil.

Generally all the treatments increased the ER of Ca^{2+}. *R. sativus* L. treatment led to 23% increase in ER of Na^+ with concomitant decrease in ER of K^+ (54%) comparing with the control that resulting in a noticeable increase in Na^+/K^+ ratio. On the other hand a marked increase was observed in ER of, Mg^{2+} and Mn^{2+} (26 and 43%, respectively with this treatment). *E. sativa* Mill treatment decreased ER of Na^+ and K^+, (5 and 16%, respectively) compared with control. This decrease of ER of Na^+ and K^+ was accompanied by an increase of ER of Ca^+, Mg^{2+} and Mn^{2+} (85, 39 and 31%, respectively). *S. alba* L. treatment had also led to an appreciable decrease in ER of Na^+ and K^+ (21 and 49%, respectively). One the contrary, this treatment resulted in an increase of other cations ER, especially Mg^{2+} (53% increase of the control).

DISCUSSION

In the present study the highest inhibitory effect of *Rhizoctonia solani* kuhn growth was achieved with aqueous, ethanolic and chloroformic extracts of the tested seeds. This result was mainly due to the presence saponins, carbohydrates, phenolic compounds, flavonoids and lipids in these extracts. Allelopathic inhibition of the tested extracts to *R. solani* Kuhn typically resulted from the combined action of allelochemicals group, which collectively interfere with several physiological processes[19]. Carbohydrates and phenolic compounds especially flavonoids may be responsible for the most fungicidal activity. The deleterious actions of phenolic allelochemicals has been proposed as a reduction in the nutrients concentrations, alteration in the membrane potential, callular disruption and reduction of protein synthesis[19,35-38]. The antifungal activity of phenolic compounds was reported by Martin[39]

Table 9: Enrichment ratio (ER) of some mineral ions detected in 10 days old cotton seedlings infected with *R. solani* and treated with seed powders of *R. sativus* L., *E. sativa* Milland *S. alba* L.

ER	Na^+	K^+	Na^+/K^+	Ca^{2+}	Mg^{2+}	Mn^{2+}
Control (C)	229.88±0.00	645.90±2.24	0.355±0.00	67.52±0.68	41.36±0.80	15.19±0.10
C treated with *R. sativus* L.	283.00±3.04**	353.67±4.57**	0.801±7.60*	143.80±1.04**	52.15±0.72**	21.67±0.90**
C treated with *E. sativa* Mill	217.30±8.24*	539.45±0.72**	0.402±0.02**	125.0±1.49**	57.47±0.36**	19.84±0.57**
C treated with *S. alba L.*	181.00±1.82**	327.05±0.00**	0.553±0.00**	93.27±0.73**	63.22±0.55**	18.72±1.02**
L.S.D at 0.05	5.46	8.62	0.035	1.90	1.05	0.35
at 0.01	9.34	12.26	0.052	2.51	1.21	0.47

Values represent mean±standard deviation, Each value represent the mean of 3 replica, * Significant at probability≤ 0.05
** Significant at probability≤ 0.01

and El-Sayed *et al.*[40]. Saponins is another group of potentially allelopathic compounds. Saponins comprise a triterpenoid lipophilic component combined with a polyglycosidic moiety which by conferring hydrophilicity at the part of the molecule, gives an overall surfactant effect. By disrupting membranes in the plant, saponins can act as herbicidal[14,41].The inhibitory effect of lipids and fatty acids detected in the present study may be attributed to the presence of long-chain fatty acids. That was in agreement with the evidence of the reported allelopathic effect[15,42].

In the present study, germination reduction through allelopathic activity was observed. These results are in agreement with Mandava[43]; Rice[18]; Weston[44] and Assawah and Elhaak[45]. They stated that secondary plant products, including the water-soluble phytotoxic compounds, were released into the soil environment and inhibit germination and growth of several plant species.

Significant inhibitory effect of the tested powdered seeds, was observed on roots than shoots, because roots were present in direct contact with inhibitors[3]. The reduction in root length may be due to block of gibberellins and indol acetic acid functions[43,13]. Soil infested with *R. solani* kuhn caused a greater inhibition of seed germination (43%). Application of *R. sativus* L., *E. sativa* MILL and *S. alba* L. counteracted the fungal inhibition partially and enchanced the germination that may be correlated with the high phenolic contents of the tested seeds. Harborne and Band Boxter[36] reported that most of the phenolic compounds hinder plant disease incidence and cause synergistic interactions which is established in several series of known and unknown bioactive compounds.

Treatment of the infested soil with powdered seed of *R. sativus* L., *E. sativa* Mill and *S. alba* L.had increased Na^+/K^+ ratio, marked increase in ER of Ca^{2+} (112%) specially in case of *R. sativus* L. This increase may be a defense mechanism for resistance of high Na^+/K^+ ratio, which has been found to lead to a metabolic damage. Calcium plays an important role to the cell wall and membranes structure. It reduces ion diffusion and maintain membrane integrity and selectivity[46]. The observed increase of ER of Mg^{2+} and Mn^{2+} accounts that due to the greatest growth parameters of seedling shoot. Mg^{2+} is an essential constituent of chlorophyll. The role of Mn^{2+} in water oxidations comes from physical evidence for redox changes in Mn^{2+} associated with water oxidation[47].

Also in our study, the application of *R. sativus* L., *E. sativa* MILL and *S. alba* L. powdered seeds to the infested soil has led to alteration of fatty acid distribution. This could be explained as cellular response.

The substitution of saturated fatty acids to polyunsaturated fatty acids (18:3) in membrane lipids was explained by Niki[48] which stated that this change minimized lipid peroxidation. Cellular response seems to have a stimulatory effect on fatty acid syntheses (elongase enzyme) resulting in the appearance of $C_{20.0}$ and $C_{22.0}$ fatty acids, which are absent in cotton seedling control.

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