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Influence of Seeds and Roots Extracts and Exudates of Bean Plant on Growth of Some Pathogenic Fungi

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Abstract

Extracts and exudates of seeds and roots of plant play a role in plant health and attracted the pathogen to the host. This study was conducted the effect of extract and exudates from bean seeds and roots on fungal growth of *Botrytis cinerea*, *Macrophomina phaseolina* and *Rhizoctonia solani*. Chemical analysis of extract and exudates was also studied. Seed and root extracts and exudates obtained from Libyan cv. gave the most stimulating effect on the mycelia growth of all tested fungi in a descending order, than those extracted and exuded from seeds and roots of the Giza-6 cv. *In vitro* chemical analysis of seed and root extracts and exudates of both cultivars indicated that total amino acids were much higher in Libyan cv. than those in Giza-6 cv., while free and total phenol contents were much greater in the seed and root extracts and exudates of Giza-6 cv. compared with those from Libyan cv.

Keywords

***Phaseolus vulgaris*, Seed, Root, Extracts, Exudates, Linear Growth, Pathogenic Fungi**

Subject Areas: **Plant Science**

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is the most important leguminous crop for human consumption in the world. The common bean comprises 50% of the grain legumes consumed worldwide [1]. Seeds are associated with a range of fungi [2], reflecting negatively on plant growth and the produced yield. *Botrytis cinerea*, *Macrophomina phaseolina* and *Rhizoctonia solani* are three fungal pathogens that heavily infect bean and thus influence growth from germination to all stages of plant development [3]. These species are responsible for plant diseases such as seeds rot, seedlings damping of, root rot and lesions on a part under soil surface [3]-[6]. Most of

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soil microorganisms are attracted to seeds and root of plants under exudates effect [7]. Exudates of organic compounds from germination seeds and plant roots could supply some of energy sources required for the parasites to maintain vegetative growth and spore germination, enhancing infection of the host [3] [7]. These compounds include sugars, amino acids, organic anions (OAs), phenolics and various other secondary metabolites. The seeds and roots exudates may stimulate or inhibit the growth and/or the development of the pathogen and consequently increase or decrease the disease incidence [8]-[10]. Several researchers have reported that interactions between plant roots and soil fungi in the rhizosphere are critical for plant growth. Gowily *et al.* [11] reported that root exudates of susceptible chickpea cv. Giza-2, C-290, C-104 inhibited mycelial growth of *Fusariumsolani* less than Giza-1 resistant cv. Ibrahim [12] found that in case of host root exudates of *M. phaseolina*, normal sclerotia germination, stimulation of mycelia growth with profuse branches was observed. However, in case of non-host root exudates, abnormal sclerotia germination in the form of malformed and restricted mycelium without directional attraction towards the root was observed. Root exudates obtained from the highly susceptible bean Giza-3cv. gave the most stimulating effect on the mycelia growth of either *F. solani*, *F. solani* f. sp. *phaseoli*, *M. phaseolina*, *R. solani* or *S. rolfsii* in a descending order, than those exuded from roots of the less susceptible Bronco cv. *in vitro* [13]. Omer *et al.* [14] studied effect of root exudates of alfalfa cultivars in controlling late wilt disease of maize (Balady cultivar's). He found that the root exudates inhibited significantly mycelia growth of *Cephalosporium maydis*. The root exudates of hairy root cultures of sweet basil (*Ocimum basilicum*) have been shown to have an antimicrobial activity against *Pseudomonas aeruginosa* [15]. Shi [7] studied the effect of radiata pineroot exudates on rhizosphere soil microbial communities. Significant differences in rhizosphere microbial communities were detected. The shifts in microbial communities could have been related to changes in exudate production and composition.

Different plants and cultivars have different exudate compositions [16]-[18]. Radwan *et al.* [19] showed that root exudates of four varieties of tomato differed in their resistance and susceptibility to Fusarium with revealed differences in qualitative and quantitative contents of amino acids. Aspartic acid, serine, glycine, glutamic, theronine, and tryptophane were higher in susceptible varieties than those in resistant ones. On the other hand, the amino acids, alanine, methionine and valine were higher in exudates of resistant than susceptible varieties. Gowily *et al.* [11] noted that the resistant cultivars of chickpea contained fewer amount of free amino acids and free sugars than in the susceptible ones.

Plant defense mechanism or susceptible response to infection by microorganisms depends on biochemical changes in host tissues. The phenol compounds of host plant are one of the best-known factors involved in resistant and/or susceptible response to infection [20]. Root exudates (e.g. phytoalexins) can also be a mechanism of plant defence against soil-borne pathogens and can stimulate or inhibit interactions with other soil organisms [21]-[23]. Gowily *et al.* [11] noted that the amount of phenol compounds in resistant cultivars were more than in the susceptible ones. Free and total phenol contents in the root exudates of less susceptible bean cv. Bronco were much greater than those exuded from the root of highly susceptible Giza 3 cv. [13]. Omar *et al.* [14] showed that the exudates of Ismailia 92 cv. of alfalfa contained high levels of free, conjugated and total phenols than other cultivars.

The main objective of this study was to investigate the effect of seed and root exudates and extracts of bean cultivars (Giza-6, Libyan), on the growth of *B. cinerea*, *M. phaseolina* and *R. solani*. Chemical analysis of bean exudates and extracts for total amino acids and total phenolic compounds was conducted to study natural resistance.

2. Materials and Methods

2.1. Fungal Material

Three isolates of *B. cinerea*, *M. phaseolina* and *R. solani* were used throughout this study. They were isolated from samples of white bean seeds naturally infected with seed pathogens.

2.1.1. Seed Exudate Collection

Exudates of seeds were collected aseptically according to the method described by Singh and Mehrotra [24]. Fifty gm of surface sterilized seeds of each cultivars were placed in 50 ml of sterile glass distilled water in 250 ml flasks containing 100 gm of glass beads with five replicates of each cultivar. The exudates of germinated seeds were collected after 96 hours of incubation at 20˚C ± 2˚C and then sterilized by Zeits filter No. 0.24

µl and used.

2.1.2. Root Exudate Collection

Concerning the roots exudates collection, the method described by Gowily, *et al.* [11] was used. Conical flaks (250 ml) containing glass beads and 20 ml of distilled water were autoclaved. Bean seeds were surface sterilized with sodium hypochlorite (1%) and then washed with sterile distilled water. Seeds were planted at a rate of 10-seeds/flask and each cultivar was replicated five times. Root exudates of the developed seedlings were collected one week after emergence. The exudates were filtrated through Whatman No. 1 filter paper and sterilized by using Zeits filter No. 0.24 µl before use.

2.1.3. Preparation of Seed Extract

The extraction of seeds was obtained according to method described by Hartman, *et al.* [25]. Seeds of each cultivar were washed with sterile distilled water and oven dried at 70˚C for 48 hrs. The dried seeds were finally ground to powder. Eight grams of seed fine powder were blended with 20 ml distilled water for ten minutes in a warring blender. The mixture was filtered through Whatman No. 1 filter paper, then sterilized by Zeits filter (0.24 µl) and used.

2.1.4. Preparation of Root Extract

Root extracts were obtained from 7 days old seedlings, growing in sterilized sandy-loam soil, of the tested cultivars using the following procedures as described by Hartman, *et al.* [25]. Fresh root samples (10 gm) of 7 days old seedlings were washed by distilled water several times and cut into segments (approximately 1 cm each), crushed with mortar and pestle. The crushed tissue was suspended in 20 ml distilled water, then filtered through cheese-cloth, and centrifuged (2500 rpm) to remove the root debris. The extracts were sterilized by Zeits filter (0.24 µl) and tested.

2.2. Effect of Crude Extracts and Exudates on Fungal Growth

The influence of obtained extracts and exudates on the pathogenic fungus was tested by measuring the linear mycelial growth rates on PDA medium containing exudates or extracts. Five milliliters of the crudeexudates or extracts to be tested were thoroughly mixed with 45 ml PDA medium, just before solidification, at 45˚C, then poured in Petri-dishes. Inocula 5 mm in diameter from 7 days old cultures of the tested fungus were used, and incubated at 22˚C ± 2˚C. The increase in diameter of growing culture was daily recorded. Five replicates were used in each case. Like number of replicates free from exudates or extracts were served as check control.

2.3. Chemical Analysis

Determination of Amino Acids in Extracts and Exudates

1) Qualitative determination

Qualitative determination of free amino acids of seed extracts and root extracts and exudates were performed by paper chromatography on Whatman No. 1 filter paper sheets. Ordinarily 30 µl from each sample of seed extracts, and root extracts, and 50 µl in case of seed and root exudates were applied on the base line using a 10 µl pipette. The sheets were then descending run for about 14 hrs in the solvent system containing n-butanol:glacial acetic acid:distilled water (4:1:1, v/v/v), and formic acid at the rate of 1.0%. The chromatograms were then dried and development of amino acid was accomplished by dipping the chromatograms in a solution of 0.2% ninhydrin in absolute ethanol [26]. The chromatograms were then dried at 45˚C for 1 hr. for better color development. The developed amino acid spectra were identified according to their R_f values in comparison with the standard reference compounds.

2) Quantitative determination

For quantitative determination of amino acids, the coloured zones on chromatograms were cut-off and placed in test tubes containing 5.0 ml. Methanol (50 V/V) and shaked. The density of colour extracted was determined at 540 nm. Spectronic colorimeter [27]. Standard curve of leucine was constructed and the amount of each amino acid was extrapolated. Total amounts of amino acids were calculated from sum of detected amount, and data presented as µg leucine/10 ml extracts.

3) Determination of phenol components

Total and free phenols in exudates and extracts were colourimetrically determined as described by Snell and Snell [28].

4) Determination of total phenols

Total phenols were determined by adding 0.5 ml of conc. HCL to 0.03 ml of the sample and boiling in a water bath for 10 minutes. After cooling, 0.5 ml of Folin-Denis reagent and 2.0 ml of $NaCO_3$ (20%) were added. The mixture was completed to 10 ml by distilled water. After 20 minutes the density of colour was determined at 520 nm on spectronic colorimeter. Standard curve for catechol was constructed and total phenols was extrapolated. Data presented as µg catechol/ml root exudates.

5) Determination of free phenols

Free phenols were determined by adding 0.5 ml of Folin-Denis reagent and 2.0 ml $NaCO_3$ (20%) to 0.03 ml of the sample. The mixture was completed to 10 ml with distilled water and left to stand for 20 minutes. The density of color was readed at 520 nm. The amount of free phenols was extrapolated from the standard curve of catechol and data presented as µg catechol/ml root exudates.

6) Determination of conjugated phenols

Conjugated phenols were determined from subtracting free phenols from the total phenols.

2.4. Statistical Analysis

A completely randomized block design with 5 replications were used in the present study. For statistical analysis, data were subjected to the analysis of variance (ANOVA) using Co Stat Program. Least significant difference (LSD) at 5% level of probability was computed.

3. Results and Discussion

3.1. Effect of Crude Extracts and Exudates on Fungal Growth

These experiments were carried out to test the effect of root extracts and exudates, and seed extracts and exudates of the less susceptible and more susceptible bean cultivars Giza-6 and Libyan respectively, on the mycelia growth of *B. cinerea*, *M. phaseolina* and *R. solani*. The results of these experiments were given in **Table 1** and **Table 2**) and **Figure 1** and **Figure 2**). Both root extracts and exudates of the susceptible bean cultivar's (Libyan) stimulated the growth rate of tested fungi (**Figure 1**). While, root extracts and exudates of the less susceptible bean cultivar (Giza-6) inhibited the growth of the pathogenic fungi, (**Figure 2**). Stimulation effect of seed extracts of the more susceptible bean cultivar's (Libyan) was more pronounced than those of root extracts and seed exudates of the same cultivar's. Inhibition effect of seed exudates of the less susceptible bean cultivar's (Giza-6) was more pronounced than of the root extracts and exudates of the same cultivar's. The increase of the mycelia growth rate reached a climax at the fifth day of inoculation. Experimental data (**Table 1** and **Table 2**) also showed that the root exudates induced more fungistatic action on the tested fungi than the seed extracts.

Table 1. The effect of extracts and exudates collected from bean seeds and roots of Libyan cv. on the linear growth of the tested fungi during 5 days of inoculation.

Treatment	% increase in fungal linear growth														
	B. cinerea					*M. phaseolina*					*R. solani*				
	DAI					DAI					DAI				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Seed extracts	2.5	2.8	7.9	9.0	9.0	3.0	4.3	6.4	8.7	9.0	3.0	5.0	7.0	8.0	8.7
Seed exudates	1.9	2.5	3.7	5.3	8.3	1.0	2.4	4.2	5.9	8.0	1.0	1.6	3.0	6.0	7.0
Root extracts	2.5	2.8	6.6	8.4	9.0	2.0	3.0	6.1	7.9	9.0	2.0	4.0	4.7	7.2	8.0
Root exudates	2.2	2.8	5.3	7.2	8.5	1.5	2.6	4.5	6.8	8.7	1.5	3.0	3.0	5.0	7.5

LSD at 0.05 for Fungi (F): 0.259 Days (D): 0.335 Treatment (T): 0.300 F × D: 1.66, D × T: 1.43 F × T: 1.85 F × D × T: 2.14. DAI: Days after incubation. Values are means of 5 replicates % of increase in the linear growth was calculated based on control treatment.

Table 2. The effect of extracts and exudates collected from bean seeds and roots of Giza-6 cv. on the linear growth of the tested fungi during 5 days of inoculation.

Treatment	% decrease in fungal linear growth														
	B. cinerea					*M. phaseolina*					*R. solani*				
	DAI					DAI					DAI				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Seed extracts	14	12.5	10	8.0	6.0	9.0	6.5	5.0	4.0	3.0	18	15.7	11.5	9.3	8.0
Seed exudates	18.7	16	12	8.7	7.2	13	13	10	8.3	6.0	22	17	11	11	8.5
Root extracts	23	18.6	14.7	10	9.7	17	11.9	8.5	8.3	7.5	25	19	18.3	17	18.3
Root exudates	25	20	19.6	19	18	20.4	19.9	18	15	13	27.2	27.3	23	20	18.5

LSD at 0.05 for Fungi (F): 0.224 Days (D): 0.354 Treatment (T): 0.316 F × D: 1.75 D × T: 1.51 F × T: 1.95 F × D × T: 2.90. DAI: Days after incubation. Values are means of 5 replicates % of decrease in the linear growth was calculated based on control treatment.

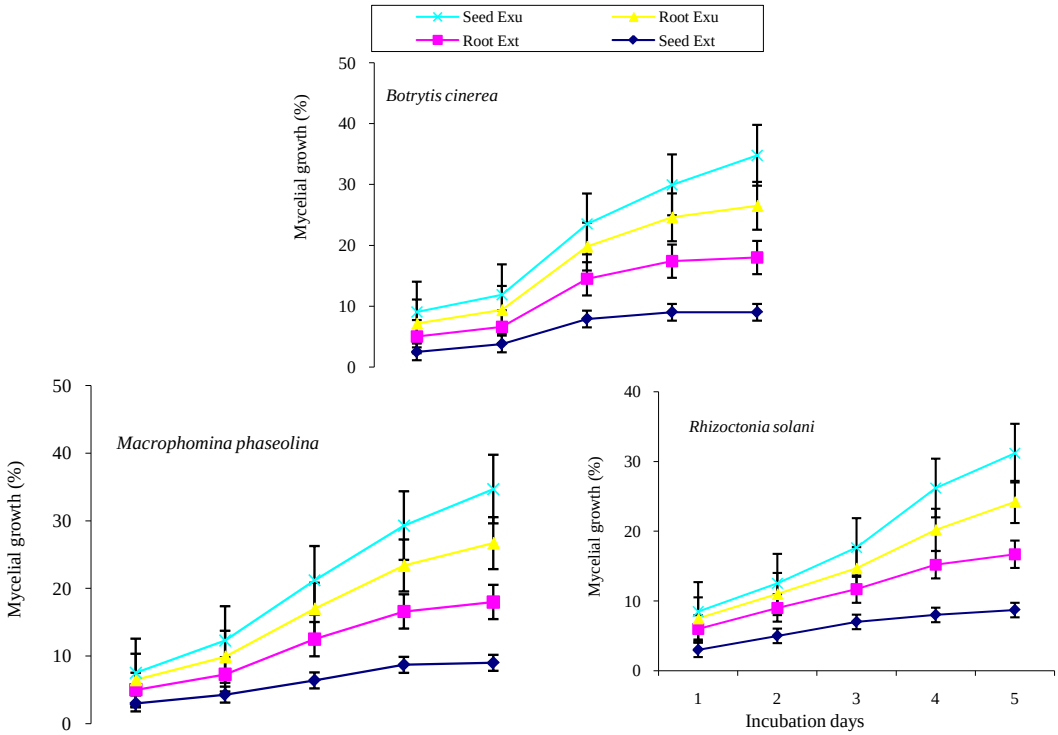


Figure 1. The effect of crud extracts and exudates obtained from bean seeds and roots of Libyan cv. on fungal growth.

Rhizoctonia solani showed the highest of growth rate followed by *B. cinerea*, however *M. phaseolina* exhibited lowest rate of linear growth.

Such observation was recorded by Noaman [29]. He noted that stimulatory effect of the susceptible variety "Contender" exudates was much more pronounced mycelia growth of the *F. solani* than that of the resistant "Swiss blance" bean variety. Exudates obtained from healthy roots of the highly susceptible bean cultivars *i.e.* Giza-3, gave the most stimulating effect on the mycelia growth of either *F. solani* f. sp*phaseoli*, *M. phaseolina*, *S. rolfsii* and *R. solani* causing root rot disease in a descending order than those exudates from healthy roots of the less susceptible cultivar's Bronco *in vitro* [13]. Germination of sclerotia of *Rhizoctonia solani* and *Sclerotiumrolfsii* and subsequent hyphal growth were stimulated by exposure to volatiles from aged but not nonaged pea seeds. Hyphae grew preferentially toward aged seeds. In natural soil, bacterial and fungal populations showed significant increases after exposure to volatiles from aged seed. *Fusarium* spp. and *Pseudomonas* spp. showed

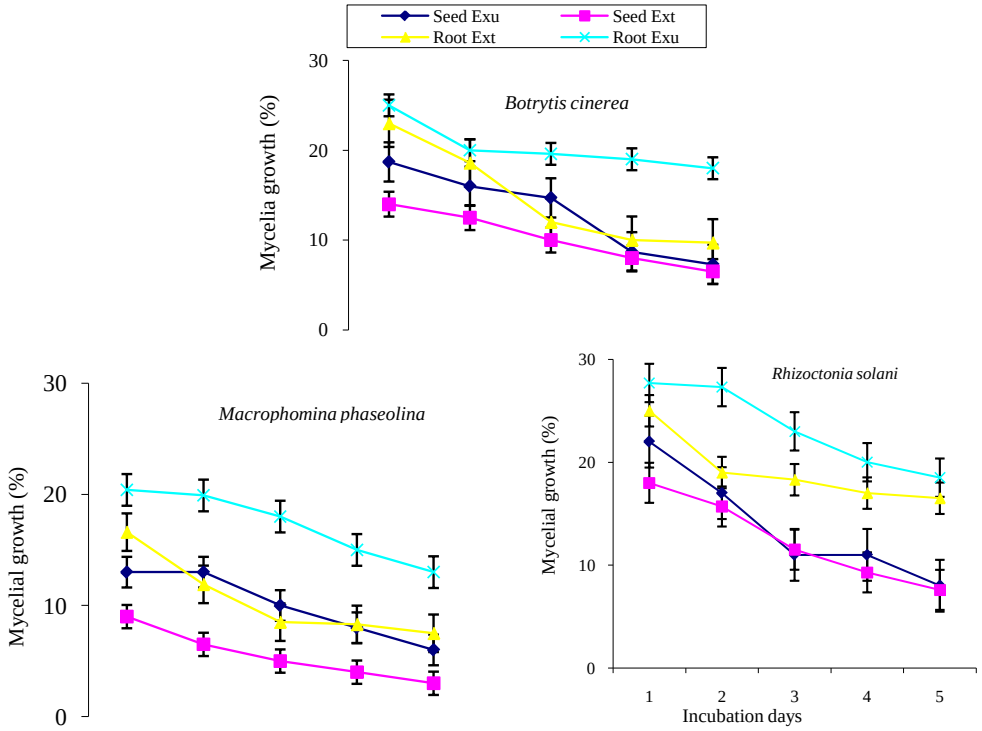


Figure 2. The effect of crud extracts and exudates obtained from bean seeds and roots of Giza-6 cv. on fungal growth.

increases over their original population levels after a 48-h exposure to volatiles. Conversely, *Pythium* populations and associated seed-rotting potential of soil decreased in natural soils exposed to volatiles [30]. Plant roots release a broad variety of chemical compounds to attract and select microorganisms in the rhizosphere that influence plant health and growth [31].

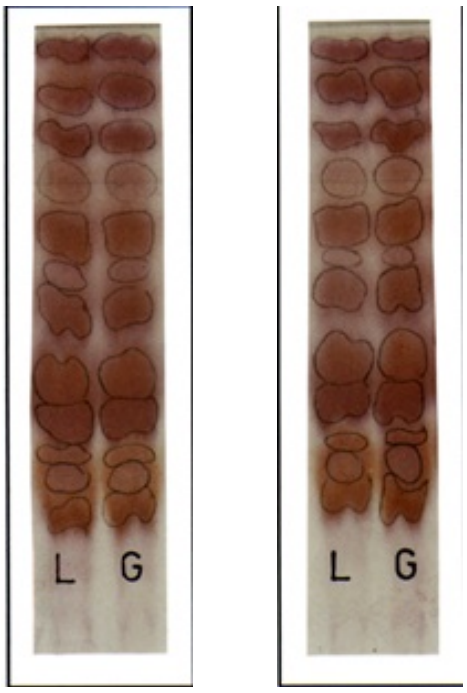
3.2. Chemical Analysis of Crud Extracts and Exudates

3.2.1. Qualitative and Quantitative Determination of Amino Acids in Extracts and Exudates

Amino acids present in seed and root extracts and both exudates of Libyan and Giza-6 bean cultivars are presented in **Table 3**, and illustrated in **Figure 3** and **Figure 4**. Results showed that twelve amino acids were found in seed and root extracts of both cultivars while the seed and root exudates contained eleven amino acids except root exudates of Giza-6 cultivar's that contained ten amino acids. The detectable amino acids were arginine, glutamine, aspartic acid, serine, glycine, hydroxyproline, alanine, threonine, glutamic acid, methionine, valine, tyrosine, phenylalanine, tryptophan, isoleucine, and leucine. These all amino acids were presented in seed extracts of both the less susceptible (Libyan) and the more susceptible (Giza-6) bean cultivars (**Table 3** and **Figure 3(a)**). In construct these amino acids, also had be found in root extracts of both cultivars (**Figure 3(b)**).

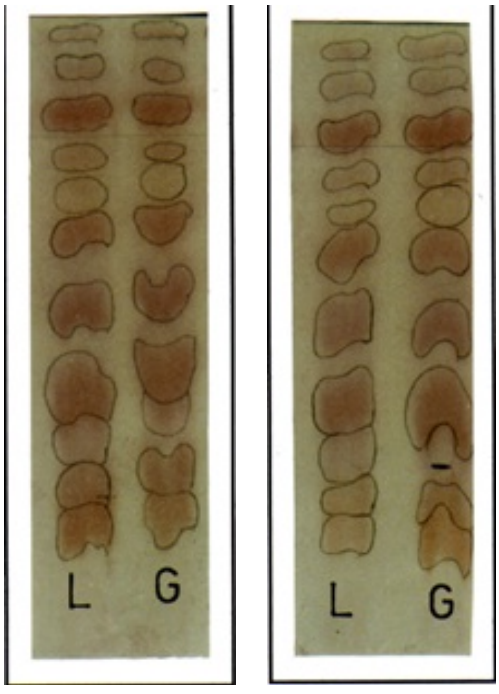
The seed and root exudates of the tested bean cultivars exuded about eleven amino acids. The exuded amino acids were, arginine, glutamine, aspartic acid, serine, glycine, hydroxyproline, alanine threoinine, glutamic acid, methionine, valine, tyrosine, phenylalanine, and tryptophan. The seed and root exudates of both cultivars were free found from leucine and isoleucine amino acids (**Figure 4(a)**). While root extracts of Giza-6 was free from aspartic acid (**Figure 4(b)**). Several metabolites, including organic and amino acids, were determined in root exudates by Tawaraya, *et al.* [32].

Quantity of twelve amino acids recovered in seed extracts, root extracts and both exudates of seed and root of Libyan and Giza-6 cultivars were estimated and the data presented in **Table 3**. From results obtained it can be observed that the total amount of amino acids was greater in seed extracts of Libyan (2.383 µg/gm dry matter) than the total amount of Giza-6 (1.702 µg/gm dry matter). The amounts of amino acids presented in root extracts showed a great variation between the less susceptible cultivar Giza-6 and the less resistant one Libyan. Quantity of amino acids exuded from seed and root exudates of the two bean cultivars Libyan and Giza-6 are given in **Table 3**. The obtained results indicated that the amount of amino acids exuded by seed and root exudates of



(a) (b)

Figure 3. Paper chromatograph showed separation of free amino acids from seeds from seeds extracts (a) and root extracts (b) of bean cultivars. L: Libyan, G: Giza-6.



(a) (b)

Figure 4. Paper chromatograph showed separation of free amino acids from seeds exudates (a) and root exudates (b) of bean cultivars. L: Libyan, G: Giza-6.

Libyan cultivar were more than those of Giza-6 (0.342, 0.504 and 0.309, 0.450) respectively. In general, the concentration of each amino acid varied between Libyan and Giza-6 varieties. Tabulated data indicated that

Table 3. Determination of amino acids in seed and root extracts and exudates of bean Libyan and Giza-6 cvs.

Amino acids	Free amino acids µg/10 ml seed and root extracts and exudates							
	Libyan				Giza-6			
	Seed extract	Seed exudate	Root extract	Root exudate	Seed extract	Seed exudate	Root extract	Root exudate
Arginine	0.110	0.046	0.092	0.112	0.123	0.015	0.101	0.017
Glutamine	0.106	0.044	0.110	0.037	0.115	0.013	0.105	0.031
Aspartic acid	0.99	0.022	0.105	0.055	0.223	0.083	0.108	-
Serine and glycine	0.127	0.016	0.112	0.032	0.155	0.057	0.105	0.050
Hydroxyproline	0.156	0.035	0.103	0.082	0.206	0.023	0.110	0.080
Alanine and threonine	0.166	0.030	0.186	0.020	0.099	0.010	0.147	0.020
Glutamic acid	0.111	0.055	0.120	0.022	0.128	0.012	0.095	0.025
Methionine and valine	0.134	0.039	0.098	0.097	0.098	0.021	0.103	0.054
Tyrosine	0.107	0.011	0.200	0.012	0.131	0.040	0.101	0.072
Phenylalanine	0.137	0.025	0.059	0.018	0.102	0.020	0.103	0.068
Tryptophan	0.106	0.019	0.076	0.017	0.183	0.015	0.054	0.033
Leucine and isoleucine	0.133	-	0.066	-	0.139	-	0.086	-
Total mean	2.383	0.342	1.327	0.504	1.702	0.309	1.218	0.450

Aspartic acid was recorded (0.99, 0.223) in seed extract of both cultivars and also it recorded (0.083) in seed exudate of Giza-6 cv. The greatest amount of amino acids were noticed in of Arginine (0.112), Glutamic acid (0.055) and Tyrosine (0.200) in root, seed exudate and root extract of Libyan cv. While Alanine and Threonine (0.147) and Hydroxyproline (0.080) were recorded in root extract and exudate of Giza-6 cv. Also, El-Tony [33] found that total free amino acids were increased in pea plants of susceptible variety to *F. oxysporum* or *F. solani* than in resistant ones.

3.2.2. Phenols Content

Total, free and conjugated phenol compounds were quantitatively determined in seed, root extracts and exudates of two bean cultivars, Libyan and Giza-6 to explain their role in infection with tested fungi. These contents were evaluated as µg catechol/ml extracts or exudates. The obtained data are presented in **Table 4** which indicated that seed extracts and root extracts and both exudates of two tested bean cultivars differed in their phenol contents, in which seed extracts and root extracts and exudates of Giza-6 cultivar's had high quantity of free and conjugated phenols as compared with extracts and exudates of Libyan cultivar's. Generally the concentration of phenols were higher in seed extracts, while root exudates showed the least quantity of phenols.

Phenols have been used as indicators for resistance to several diseases. In this study, free and total phenol contents (**Table 4**) in the root exudates of less susceptible bean cultivar Giza-6 were much greater than those exuded from the roots of highly susceptible cultivar (Libyan). Therefore, it may be suggested that phenol contents of root exudates are major chemical constituents that could determine the reaction of bean cultivars to root rot disease. Reeves [34] explained that resistance to beans root rot caused by *F. solani* f. sp. *phaseoli* seemed more closely correlated with the production named substance II than phaseolin who found that resistant lines could develop phytoalexins more rapidly in the 3 days after inoculation than susceptible lines. Abdelal, *et al.* [35] concluded that resistant soybean varieties to *M. phaseolina* showed higher amount of phenol content in their root exudates when compared with root exudates of susceptible varieties. Also, Zayed, *et al.* [36] mentioned that less infected varieties of soybean by *Stemphlium vesicorum* possessed higher amounts of phenols than the susceptible ones.

Table 4. Phenolic contents, free, conjugated and total, in seed and root extracts and exudates of two bean cultivars, Libyan and Giza-6.

Cultivars	Treatment	µg catecol/ml extract or exudate		
		Free phenols	Conjugated phenols	Total phenols
Libyan	Seed extracts	2.69	2.51	5.20
	Seed exudates	2.50	0.915	3.40
	Root extracts	2.16	1.19	3.35
	Root exudates	1.80	0.60	2.40
Giza-6	Seed extracts	5.00	2.80	7.80
	Seed exudates	3.37	1.10	4.47
	Root extracts	4.85	1.83	6.68
	Root exudates	2.53	0.69	3.22

Values are means of 5 replicates.

4. Conclusion

To summarize, my study clearly shows that the extracts and exudates of bean plants (seeds and roots) express a different bioactive effect on bean pathogen *Botrytis cinerea*, *Macrophomina phaseolina* and *Rhizoctonia solani*, thus, indicating that alterations of fungal growth either inhibition or stimulation. Chemical analysis of extracts and exudates from bean plant reported the difference in amino acids and phenols content in both cultivars.

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