

Effects of Crop Sequence on Pod- and Root-Rot Diseases and Rhizosphere Microbial Activity of Peanut

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Crop sequence is one of those agriculture practices in which allelopathic activities among plant species could be enhanced. In this work, peanut (*Arachis hypogaea* L.) in addition to its pathogens and soil microorganisms were exposed to the allelochemicals derived from its preceding crops during the crop sequence, *i.e.* garlic, onion, barley and canola. Several solvent extracts of preceding crops showed varied antifungal activity against the tested pathogens *in vitro*. For this target field experiment was conducted at Nubaria province, during 2009 summer season, where peanut root- and pod-rots are epidemic. Root- and pod-rot diseases of peanut caused by *Aspergillus niger* Vantighn and *Fusarium oxysporum* Schlecht are the most serious diseases affecting peanut productivity. All preceding crops significantly reduced root- and pod-rots of peanut which effected by increasing of soil phenols content. Garlic was the best preceding crop significantly reduced the two diseases. The combination of garlic as a preceding crop and its residues induced root- and pod-rot incidence reduction by 86.8% and 50.4%, respectively, compared to without residues which gave reduction by 79.8% and 45.2% for both diseases. All preceding crops significantly increased growth characters, microbial counts, N₂ fixation bacteria, phosphate dissolving bacteria, VAM mycorrhiza and yield of peanut, canola was the most effective.

Keywords: Allelopathy, crop rotation, crop sequence, peanut, preceding crops, pod-rot, rhizospheric microflora, root-rot and VAM mycorrhiza.

Peanut (*Arachis hypogaea* L.) is considered as one of the most important export crops in Egypt. Peanut root- and pod-rot are serious worldwide diseases attacking roots and fruits below the ground. The root and pod diseases are subjected to attack with numerous soil borne pathogens such as *Fusarium* spp. and *Aspergillus* spp., *Sclerotium* sp., and *Rhizoctonia solani* in tropical and subtropical regions (Mullen *et al.*, 1995; Sailaja *et al.*, 1998 and Ziedan, 2000).

Using allelopathic phenomenon in suppressing soil-borne pathogens has become an essential need to reduce the environmental pollution and to save the important land cover species, particularly in virgin regions. Conserving the biodiversity of land cover is one of Egyptian government's important targets to combat land degradation following the United Nation Conventions for biodiversity and desertification.

Allelopathy is a common phenomenon under field conditions, occurs direct or indirect biochemical inhibition of one plant or microorganisms on another through the production of toxic compounds or allelochemicals released into the environment (Rice, 1974). Most of these chemicals are secondary metabolites and are produced as primary products of metabolic pathways. These secondary products could be classified into five major categories: phenylpropanes, acetogenins, terpenoids, steroids and alkaloids (Whittaker and Feeny, 1971). The concept suggests that bimolecular (specifically termed allelochemicals) produced by a plant escape into the environment and subsequently influence the growth and development of other neighbouring plants, pathogens and microorganisms (Rizvi and Rizvi, 1992).

Crop rotation is one of the primary tools available for successful farming. It is a crop sequence within a time period in a specific place. It is well known that the agriculture technology applied to re-enrichment soil fertility and controlling pathogens is largely dependent upon enhancing the allelopathic activity of plants including soil microorganisms (Bailey and Lazarovits, 2003 and Chiu-Chung and Chang-Hung, 2003). Dynamic cropping systems, which involve a long-term strategy of annual crop sequencing, require detailed information on management components known to influence crop performance. Considering that proper sequencing of crops is an important component for successful dynamic cropping systems (Kruinsky *et al.*, 2006). Allelopathic potential of the crops can be wisely manipulated by adjusting these crops in the crop sequences as rotational crops, cover crops, or intercrops to reduce the pest pressure and judicious nutrient management (Jabran and Farooq, 2013).

Residue management relies on the decomposition of crop residues to return organic carbon back to the soil. Residue decomposition can vary with depth of placement in the soil, crop type and residue quantity, allelopathic interactions between existing soil biota, and time. Subsequent benefits from partial disease control can be attained using residue management methods such as crop rotation that lower the pathogen's inoculum density in the soil, reduce its ability to survive, deprive the pathogen of its host and create conditions that favour the growth of other microorganisms at the pathogen (Chiu-Chung and Chang-Hung, 2003 and Arshad and Amna, 2013).

Recent studies indicated that crop rotation on west African soils increases in subsequently planted peanut, which can be explained by combination of higher soil nitrogen N refertilized to increase in N fixers population, also higher availability and uptake of phosphates (P) through increased pH and phosphates activity as well as enhanced early colonization with vascular arbuscular mycorrhizae (VAM) and decreased investigation with plant parasite nematodes (Alvey *et al.*, 2001 and Bagayoko *et al.*, 2000). The observed rotation effect might be due to the change in the structure of the rhizosphere microorganisms which could result as the increase in nutrients mobilization as in case of phosphate mobilizing species. The increase in colonization by VAM fungi in rotation soils might be a key factor in such change as it has been shown that VAM colonization can have a strong effect on the rhizosphere microflora (Marschner *et al.*, 2001 and Posta *et al.*, 1994).

Phenolic and other organic molecules from root and seed exudates, leaf lactates, and decaying plant residues, play a major role in soil formation. Because some phenolic metabolites are phytotoxic, their presence in higher concentration can affect soil function (Makoi and Ndakidemi, 2007). Phenolic acids are relatively resistant biochemical species; they undergo transformation in the soil because some microorganisms have the capacity to utilize them as carbon sources. It's well established that several soil bacteria have the ability to oxidized aromatic compounds. Simple phenolic compounds are commonly formed in decaying plant residues which provide alternative carbon sources for some plants and may also serve as precursors for the synthesis of phenolic lipids (Gomez-Roldan *et al.*, 2007). Understanding new roles of phenolic and their functional groups is vital for better rhizosphere fertility, soil pest control and the overall production ecosystems (Makoi and Ndakidemi, 2007).

The target of this study aimed to investigate the advantages and/or disadvantages of previous crop and crop residues for crop sequence to peanut on controlling soil-borne pathogens and rhizosphere microbial activity of peanut.

Materials and Methods

Effect of plant extracts on the in vitro growth of the two tested fungi:

1- Preparation of plant extract:

Powdered air dried of some plants (garlic, onion, barley and canola) were subjected to extract with successive selective organic solvents (petroleum ether b.p. 40-60°C), acetone, chloroform, ethyl alcohol 96%, and ethyl alcohol 70% using Soxhlet apparatus according to Rosenthaler (1930). The residue obtained from each solvent was dried and weighed.

2- Effect of plant extracts on fungal growth

Stock solution of each extract was prepared by dissolving it in the solvents to be 10,000ppm. Different concentrations of each plant extract were prepared according to Zahra (1990) by adding to the potato dextrose agar (PDA) medium to give concentrations of (0, 500, 1000, 2000 and 3000 ppm). The medium was poured into Petri dishes and inoculated with equal discs (9-mm-diam.) of *Fusarium oxysporum* and *Aspergillus niger*. The reduction in colony diameters was measured after the control treatment reached the edges of the plate following the equation of Ismail *et al.* (1989): $\text{Reduction (\%)} = \frac{Y-X}{Y} \times 100$

Whereas: Y= Linear growth of control and X= Linear growth of treatment.

Field experiment:

The field experiment was conducted at El-Nubaria district in 2009 summer season, to investigate the effect of some allelochemicals derived from the preceding crops on peanut root-, pod-rot and rhizosphere microbial activity. The experimental field has a long history as highly infested location with the casuals of peanut root- and pod-rot, *i.e.* *F. oxysporum* Schlecht and *A. niger* Vantighn. Field soil properties are presented in Tables (1 a & b). The complete randomized blocks design in 4 replicates was used as the experimental design, where the plot area was 12m² (3×4m) including 6 rows at 60cm width in hills at 25cm distance.

Table 1a. Physical characteristics (practical size distribution percent) of the used soil

Course to medium sand	>2	2- >1	1- >0.85	035- >0.425	0.425- >0.212	0.212- >0.106	0.106- >0.06	<0.063
	0.00	5.91	5.28	25.35	27.36	19.76	13.19	2.82

Table 1b. Chemical properties of the used soil

pH	Ec.	O.M (%)	T.N (%)	Soluble cations meq/l				Soluble anions			
				K ⁺	Na ⁺	Mg ⁺⁺	Ca ⁺⁺	CO ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻
8.36	1.64	0.079	0.016	0.77	9.06	0.76	1.01	0	3.8	2.2	5.6

Garlic (*Allium sativum* L.), onion (*Allium cepa* L.), barley (*Hordeum vulgare* L.) and canola (*Brassica napus* L.) were sown normally at their sowing dates prior to peanut crop in the plots as allelochemicals source, while the control plot was exposed to fallow.

The application of the allelochemical source plants were done at their harvest time. Two allelochemical treatments were applied in this study, *i.e.* the first (no-tillage system); where residues of these plants were left in the soil (5cm above the soil surface), and completely merged into the soil by hoeing and irrigated intensively then left for one month as decomposition period. The second (tillage system); where the residues of allelochemical source plants were pulled out the plots.

Peanut (var. Giza 6) was sown as two seeds per hill in the experimental plots. The recommended agricultural practices of the area were used including the application of cretin fertilizers as ammonium sulphate and potassium sulphate were added.

Five plants were taken from each replicate as plant samples at 90 and 120 days after sowing date for assess incidence of root- and pod-rot diseases according to Ziedan (2000). Meanwhile, microbial counts, N fixation bacteria, phosphate dissolving bacteria, Vesicular-Arbuscular Mycorrhiza (VAM) as well as fresh and dry weight, No. of pods and yield were also determined at the proper time.

Effect of allelopathic plants on microbial counts:

Rhizosphere soil samples were collected at harvesting stage of peanut plants and analyzed for total microbial count according to Bunt and Rovira (1955). For counting and growing phosphate dissolving bacteria the same medium was used after adding 5ml of 10% K₂HPO₄ as a sterile solution followed by adding 10 ml of sterile solution of 10% CaCl₂ to each 100ml of the medium (Abd El-Hafez, 1966), Azotobacter nitrogen deficient medium (Abd El-Malek and Isac, 1968); Azospirilla on Dobereiner's medium (Dobereiner, 1978).

Testing for the most active Azotobacter and Azospirillum isolates:

- a- Determination of total nitrogen according to modified Kjeldahl method as described by Jakson (1958).
- b- Assay of nitrogenase activity according to the method described by Scholhorn and Burris (1967).

Different colonies from isolates of different treatments were microscopically examined for their morphological shape and tested for gram negative (which were characterized by forming brown pellicle on the inner part of the tube). Azotobacter isolates were tested for their efficiencies in fixing atmospheric nitrogen by grown in liquid medium lacking nitrogen (modified Ashbey's medium by Abd El-Malek and Isac, 1968). After 36 hours of incubation, nitrogen was determined in the liquid medium. Isolates showing high nitrogen fixation, nitrogenase activities were measured in their liquid medium.

Activity of phosphate dissolving bacteria (PDB) isolates:

Several isolates of purified PDB isolates were tested for their efficiencies in solubilising phosphate by having the widest and clearest zone of hydrolysis around bacterial growth on modified medium of Bunt and Rovira (1955).

Extraction of VA mycorrhizal spores:

- a- Spores were collected from rhizosphere and soil samples by wet sieving and decanting technique (Gerdemann and Nicolson, 1963).
- b- Estimation of VA mycorrhizal colonization by using the method described by Trouvelot *et al.* (1986).

Determination of free phenolic compounds:

Phenolic compounds were determined in soil aqueous extract according to the method described by Daniel and George (1972).

Statistical analysis:

Data were subjected to analysis of variance (ANOVA), calculated for mean separation analyzed, subjected to Duncan's multiple range tests and comparison after analysis of variance (Duncan, 1955).

Results

Effect of successive plant extracts on the fungal growth:

The effect of different concentrations of plant extracts, using petroleum ether, acetone, chlorophorm as well as ethyl alcohol 90% and 70% inhibited the growth of *F. oxysporum* and *A. niger in vitro* as shown in Table (2). Ethyl alcohol 90% extracts of garlic and onion are the most effective in reducing radial growth of the two fungi. Canola, acetone, ethyl alcohol 90% and 70% extracts were the most effective once against the two fungi. There were positive correlation between the fungicidal activity (Reduction %) and extract concentration.

Effect of preceding crops on root- and pod-rot diseases:

Data present in (Table3) illustrate the effect of allelopathic activity of some preceding crops to peanut on root and pod diseases. Data show that all preceding crops significantly suppress root- and pod-rot diseases of peanut than in the untreated one. Garlic followed by onion was significantly the best preceding crops in reducing root- and pod-rot infection. The reduction in root- and pod-rot diseases significantly more effective in treatments is having residues from preceding crops than without residues (Table 3). It is also clear that barley is the least effective preceding crop on this concern

Table 2. Effect of different concentrations of some successive plant extracts on the reduction percentages of *F. oxysporum* and *A. niger* growth

Plant extract	Conc. (ppm)	Control		Garlic		Onion		Barley		Canola	
		F*	A**	F	A	F	A	F	A	F	A
Petroleum ether	500	1.1	2.2	5.5	0.2	11.5	12.3	1.5	0	3.5	1.5
	1000	2.0	2.0	9.9	7.5	13.6	15.6	4.3	2.5	11.6	9.2
	2000	2.3	2.5	14.7	11.5	20.7	4.4	4.4	2.8	14.7	12.3
	3000	2.2	2.4	16.7	13.5	22.6	21.7	5.9	3.5	22.6	25
Chloroform	500	0	0	11.3	8.5	8.7	5.7	2.7	3.7	0	0
	1000	1.1	1.2	13.5	10.6	15.6	11.6	6.5	2.8	3.5	4.7
	2000	1.5	1.3	19.5	17.7	22.7	18.8	5.6	2.9	11.6	9.6
	3000	1.7	1.4	22.6	21.6	21.8	20.7	9.7	3.5	15.7	10
Acetone	500	1.2	1.1	9.6	7.3	5.1	4.2	0	0	11.5	8.5
	1000	1.1	1.1	11.7	8.7	7.3	5.7	1.6	0.5	22.7	10.9
	2000	2.2	1.7	17.9	9.8	11.7	9.2	4.2	2.3	29.8	15.8
	3000	2.5	2.0	28.6	12.9	18.9	12.5	5.6	4.3	37.2	31.5
Ethanol 90%	500	1.5	1.2	16.7	11.8	13.5	8.2	1.0	0.9	12.6	8.7
	1000	1.6	1.2	24.3	19.2	15.7	9.5	2.7	1.3	14.7	9.8
	2000	2.3	1.5	31.5	25.4	24.6	15.7	3.1	1.5	21.6	11.9
	3000	2.5	1.6	41.2	33.5	32.7	27.6	7.9	5.1	28.9	19.6
Ethanol 70%	500	0	0	10.9	6.9	8.7	5.9	0	0	19.6	16.7
	1000	1.2	1.2	19.8	11.8	12.6	9.6	1.5	1.2	23.4	19.5
	2000	2.5	1.9	32.9	19.6	18.7	11.3	2.3	1.2	27.2	21.7
	3000	2.9	1.8	32.6	24.7	27.5	19.4	3.5	2.5	38.5	31.8

* F= *F. oxysporum***A= *A. niger***Table 3. Effect of some preceding crops in the crop rotation on root- and pod-rot diseases of peanut**

Preceding crop	With residues				Without residues			
	Root-rot*		Pod-rot**		Root-rot		Pod-rot	
	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
Garlic	5.8 e	0.0e	11.4 b	1.9 b	8.9 e	0.9 e	12.6 b	2.6 b
Onion	9.6 d	0.5 d	10.7 b	1.8 b	12.5 d	1.5 d	15.3 b	2.9 b
Barley	21.5 b	2.1 b	15.0 c	2.2 c	27.9 b	2.8 b	17.0 c	3.5 c
Canola	12.7 c	1.2 c	11.7 b	2.0 b	17.8 c	2.3 c	14.9 b	2.9 b
Control	44.0 a	3.7 a	23.0 a	4.0 a	44.0 a	3.7 a	23.0 a	4.0 a

* Assessed 90 after sowing.

** Assessed 120 days after sowing.

- Values followed by the same letter in each column are not significantly different at p 0.05 according to Duncan's multiple range test.

Effect of preceding crops on densities of total microbial counts, phosphate dissolving bacteria, Azotobacter and Azospirillum rhizosphere and soil apart of peanut:

The densities of the total bacteria (aerobic heterotrophic bacteria) in the rhizosphere and non-rhizosphere soils of peanut plants grown in Nubaria area which preceding planted with garlic, onion, barley and canola are shown in (Table 4a). Counts of the rhizosphere region of canola showed the highest count in both cases, with or without residues, being $130 \text{ cfu} \times 10^5 \text{ g}^{-1}$ and $273 \text{ cfu} \times 10^5 \text{ g}^{-1}$, respectively, while in case of soil apart the highest count was recorded in onion treatment with residues which being $110 \text{ cfu} \times 10^5 \text{ g}^{-1}$ followed by barley treatment with residues. Garlic and onion exhibited the lowest number, being $97 \text{ cfu} \times 10^5 \text{ g}^{-1}$ and $95 \text{ cfu} \times 10^5 \text{ g}^{-1}$ in case rhizospheric soil and $68 \text{ cfu} \times 10^5 \text{ g}^{-1}$ and $75 \text{ cfu} \times 10^5 \text{ g}^{-1}$ in case of soil apart from rhizosphere, respectively. Meanwhile, those of the R/S ratios differ from treatment to another reflecting the highest ratio namely 5.69 in case of canola treatment with residues.

The phosphate dissolving bacteria showed also higher counts in the rhizosphere region than that of soil apart in both canola and barley treatments. The highest number was recorded in canola treatment without residues, being $48 \text{ cfu} \times 10^3 \text{ g}^{-1}$. While the lowest count ($8 \text{ cfu} \times 10^3 \text{ g}^{-1}$) was recorded in garlic treatment without residues. This also refers to the rhizosphere effect that proliferate such microbes in the region adjacent to the roots (Table 4a).

Table 4a. Densities of total microbial counts and phosphate dissolving bacteria (PDB) in rhizosphere and soil apart of peanut plants

Treatment	Total microbial counts ($\text{cfu} \times 10^3 \text{ g}^{-1}$ dry soil)						Densities of PDB ($\text{cfu} \times 10^3 \text{ g}^{-1}$ dry soil)					
	With Residues			Without Residues			With Residues			Without Residues		
	R*	S**	R/S	R	S	R/S	R	S	R/S	R	S	R/S
Garlic	77	68	1.13	110	68	1.62	10	12	5	18	8	2.25
Onion	95	110	0.68	180	75	2.40	10	13	0.8	27	18	1.33
Canola	130	28	4.64	273	48	5.69	22	3	4.5	48	31	1.55
Barley	110	30	3.67	210	84	2.5	18	4	7.3	35	15	2.33
Control	38	30	1.27	41	28	1.48	6	2	3	8	2	4

* R= Rhizosphere and ** S= Soil apart.

Data in Table (4b) show that canola demonstrated the highest count of aerobic N_2 fixers Azotobacter, in the rhizosphere region in both cases with or without residues which being $140 \text{ cfu} \times 10^4 \text{ g}^{-1}$ and $185 \text{ cfu} \times 10^4 \text{ g}^{-1}$, respectively. While, treatments of peanut plants which previously planted with garlic and onion showed the lowest Azotobacter counts. However, Azotobacter counts in all treatments especially in rhizosphere region when plant residues will be remained which being in all treatment still higher than control treatment.

The population of Azospirillum in the rhizosphere and non rhizosphere soils of different treatments (Table 4b) vary from $2 \times 10^4 \text{ g}^{-1}$ to $47 \times 10^4 \text{ cell g}^{-1}$ dry soil) in the rhizosphere region. Counts of Azospirillum were relatively higher than their respective numbers in the non-rhizosphere. This is also evidence by the R/S ratio, which ranged from 0.2 to 5.5.

Table 4b. Densities of Azotobacter and Azospirilla in the rhizosphere and soil apart of peanut plants

Treatment	Azotobacter			Azospirillum		
	Isolate No.	Total N (ppm)	N-ase $\mu\text{C}_2\text{H}_4\text{L}^{-1}\text{h}^{-1}$	Isolate No.	Total N (ppm)	N-ase $\mu\text{C}_2\text{H}_4\text{L}^{-1}\text{h}^{-1}$
Garlic	2S3*	40.8	213	2S4	45.0	718
	2R1**	42.0	216	2R1	45.5	735
Onion	5S2	45.0	218	5S3	41.9	696
	5R3	45.5	222	5R2	44.1	718
Barley	3S1	26.5	151	3S4	28.8	390
	3R2	28.1	173	3R1	26.2	367
Canola	4S4	31.2	190	4S2	38.5	465
	4R1	33.5	198	4R4	39.5	491
Control	1S2	11.2	25	1S1	16.8	000
	1R1	17.0	36	1R3	19.6	000

*, ** As described in footnote of Table (4a).

Effect of proceeding crops on efficiency of the N_2 fixers isolates:

Azotobacter isolates:

Data in Table (5) show that the total nitrogen fixed by different isolates of Azotobacter that are isolated from the rhizosphere region and soil apart from roots nitrogen fixed by different Azotobacter isolates vary in their values according to treatments. Rhizosphere isolates ranged between 17 ppm for isolate 1R1 to 45.5 ppm for isolate 5R3. However, in the non rhizosphere soil it ranged between 11.2 ppm for isolate 1S2 to 45 ppm for isolate 5S2. Azotobacter isolate (5R3) from the rhizosphere of peanut plants in onion treatment fixed the highest amount of nitrogen (45.5 ppm). On the other hand, it is also recorded the highest value of nitrogen fixed (45 ppm) from soil apart. Nitrogenase activity by different isolates ranged between 25 $\mu\text{C}_2\text{H}_4\text{L}^{-1}\text{h}^{-1}$ for isolate (1S2) to 222 $\mu\text{C}_2\text{H}_4\text{L}^{-1}\text{h}^{-1}$ for isolate (5R3).

Table 5. Total nitrogen and nitrogenase activity of Azotobacter and Azospirillum isolates

Treatment	N_2 Fixer densities $\times 10^4\text{g}^{-1}$ dry soil											
	Azotobacter						Azospirillum					
	With residues			Without residues			With residues			Without residues		
	R*	S**	R/S	R	S	R/S	R	S	R/S	R	S	R/S
Garlic	11	40	0.28	13	60	0.21	17	17	1.0	32	19	1.68
Onion	20	19	1.05	16	48	1.65	4	17	0.2	47	18	2.61
Canola	140	92	1.52	185	90	2.06	32	26	1.2	55	15	3.67
Barley	45	24	1.88	80	18	4.44	22	6	3.2	39	19	2.05
Control	9	9	1	13	1.1	11.82	7	2	3.5	11	2	5.5

*, ** As described in footnote of Table (4a).

Azospirillum isolates were also tested for their efficiencies in nitrogen fixation in 100 ml medium and data were represented in Table (5). Values of fixed nitrogen in rhizosphere region ranged from 16.8 ppm for the isolate (1S1) to 45.5 ppm from isolate (2R1). Table (5) show that Azospirillum isolate (2R1) and (2S4) from the rhizosphere of peanut plants in garlic treatment gave the highest value of fixed nitrogen (45.5ppm and 45.0ppm), respectively, and also the highest value of nitrogenase activity ($735 \mu\text{l C}_2\text{H}_4 \text{L}^{-1} \text{h}^{-1}$ and $718 \mu\text{l C}_2\text{H}_4 \text{L}^{-1} \text{h}^{-1}$), respectively, followed by isolates from the rhizosphere of onion treatments (5R2 and 5S3), canola (4R4 and 4S2), respectively. Finally barley treatments (3S4 and 3R1) recorded the lowest values 26.2 and 28.8ppm, respectively.

Effect of preceding crops on activity of phosphate dissolving bacteria isolates:

Several isolates of phosphate solubilisation bacteria which were isolated from the rhizosphere of different treatments tested for their efficiency in solubilising phosphate (Table 6). The diameters of phosphate solubilisation zone induced by the tested PDB were reflecting on the degree of phosphate solubilisation. Table (6) show that zone diameters were ranged from 1.2 to 7.2 cm on the plates. However, phosphate dissolving bacteria isolated from the rhizosphere and soil apart of garlic gave the highest zone diameter (7.2 and 6.4 cm) followed by onion (5.6 and 6.6 cm), canola (5.0 and 5.6 cm) and barley (4.2 and 4.9 cm), respectively.

Table 6. Degree of phosphate solubility induced by the tested phosphate dissolving bacteria

Treatment	Isolate No.	Diameter of clear zone (cm)
Garlic	2S1	6.4
	2R3	7.2
Onion	5S3	5.6
	5R4	6.6
Barley	3S5	4.2
	3R1	4.9
Canola	4S2	5.0
	4R4	5.6
Control	1S2	1.2
	1R3	1.7

Effect of preceding crops on number of mycorrhizal spores and VAM fungal colonization of peanut plants:

Colonization of peanut roots mycorrhizal fungi and spore formation in the rhizosphere region and soil apart were generally higher in treatments where plant residues were left in soil than those without residues (Table 7). In general, there were significant differences in VAM sporulation in all treatment either with or without residues in both rhizosphere region and soil apart. The highest value was recorded in onion treatments (130.5, 110.8, 92 and 42) followed by garlic (107.3, 80.5, 88.0 and 30) while barley (90, 78, 77 and 17) and canola (85, 70.8, 72 and 68) recorded lower values. Root colonization had the same trend as spore numbers. Onion treatment recorded the highest values of VAM fungal colonization (72.8, 56.7, 27 and 18) in both rhizosphere and soil apart regions.

Table 7. Number of mycorrhizal spores and VAM fungal colonization of peanut plants in the rhizosphere and soil apart

Treatment	With residues				Without residues			
	No. of spores/100g soil		Colonization (%)		No. of spores/100g soil		Colonization (%)	
	R*	S**	R	S	R	S	R	S
Garlic	107.3b	80.5 b	47.8 c	31.5 b	88.0 b	30.0bc	28.5 a	12.0 b
Onion	130.5a	110.8a	72.8 a	56.8 a	92.0 a	42.0 b	27.0 a	18.0 a
Barley	90.0 c	78.0db	60.8 b	30.5 b	77.0 c	17.0 c	15.8 b	11.0 b
Canola	88.0 c	70.8 c	33.3 d	29.8 b	72.0 d	68.0 a	16.0 b	13.0 b
Control	71.3 e	65.8 d	22.8 e	21.3 c	68.0 e	66.0 a	11.0 c	10.5 b

*, ** As described in footnote of Table (4a).

- Values followed by the same letter in each column are not significantly different at $p = 0.05$ according to Duncan's multiple range test.

Effect of preceding crops on nodulation and nodules total nitrogen content of peanut plants:

Data presented in Table (8) clearly indicate that nodules number, fresh weight and dry weight were highly affected by plant rotation. It's also noticed that nodulation was not significantly affect in the same treatment (with or without residues). The highest number of nodules, nodules fresh weight and nodules dry weight were recorded in canola treatment followed by garlic, onion and barley. Nitrogen percent of nodules showed that garlic recorded the highest values (2.910 and 3.122) followed by onion and finally barley, this may be referred to the strains which isolated from garlic and onion rhizosphere are very active, which could be survived in the presence of inhibitory substances released by the pervious plant.

Table 8. Nodulation and nodules total nitrogen percent of peanut plants

Treatment	With residues				Without residues			
	No. of nodules	Nodules fresh weight/g	Nodules dry weight/g	N (%) of nodules	No. of nodules	Nodules fresh weight/g	Nodules dry weight/g	N (%) of nodules
Garlic	128.1 b	0.897 b	0.118 c	2.910 a	157.30 b	0.809 b	0.2010 b	3.122 a
Onion	121.0 b	0.781 c	0.113 c	2.170 b	135.30 c	0.788 c	0.1968 c	2.810 b
Barley	63.9 c	0.535 d	0.193 b	1.613 c	65.25 d	0.459 d	0.114 d	2.040 d
Canola	139.0 a	0.982 a	0.237 a	1.829 c	188.50 a	1.120 a	0.2800 a	2.392 c
Control	38.0 d	0.212 e	0.063 d	1.330 d	43.00 e	0.407 e	0.1010 e	1.710 e

- Values followed by the same letter in each column are not significantly different at $p = 0.05$ according to Duncan's multiple range test.

Effect of preceding crops on growth parameters and yield of peanut

Data in Table (9) reveal that all proceeding crops of peanut significantly increased plant fresh weight, number of pods and pod yield than the untreated control. Canola significantly increased fresh weight, number of pods and yield followed by garlic and onion. Canola and garlic were the best and significantly improved morphological characters of peanut plants and increased the yield.

Table 9. Changes in growth parameters and productivity of peanut in response to allelopathic of some preceding crops

Treatment	With residues				Without residues			
	Fresh weight (g)	Dry weight (g)	No. of pods	Pod yield (ardeb / feddan)	Fresh weight (g)	Dry weight (g)	No. of pods	Pod yield (ardeb / feddan)
Garlic	415.07 ab	10.6 ab	35.0 b	27.5 b	400.0 ab	9.8 ab	32.0 b	25.4 b
Onion	365.09 b	8.9 b	33.0 b	26.4 b	347.0 b	7.9 b	30.0 b	24.9 b
Barley	350.05 c	6.8 c	28.0 c	23.0 c	331.0 c	6.1 b	25.0 c	21.8 c
Canola	457.01 a	13.9 a	38.0 a	30.6 a	435.0 a	11.8 a	35.0 a	27.9 a
Control	260.00 d	5.8 d	20.0 d	19.8 d	260.0 d	5.7 c	20.0 d	19.8 d

- Values followed by the same letter in each column are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

Data in Table (3) and (9) confirm that garlic and onion treatment recorded the best results in suppressing soil borne pathogens of peanut. Canola and garlic were the best significantly improved morphological characters of peanut plants and increased the yield. The effect of preceding crops with its residues was the most significantly suppressing soil borne diseases of peanut and significantly increasing growth characters of peanut and yield compared with preceding crops without its residues.

Determination of phenols in soil:

The results of phenols determination illustrated in Fig (1). Crop sequence increased phenol content in both peanut rhizosphere and soil apart; also phenol concentration was attributed to the presence of plant residues while there was a dramatic decrease in phenol concentration in control treatment. The total phenol amount in the soil vary among different treatments, it ranged from 63 to 1135 ppm kg^{-1} soil. The greatest extracted phenol content ration was showed in peanut rhizosphere where garlic residues were left in the soil (1135 ppm kg^{-1} soil) followed by onion (1025 ppm kg^{-1}) and canola (1017 ppm kg^{-1}). Rhizosphere samples gave the highest phenol concentration in comparison to that in soil apart.

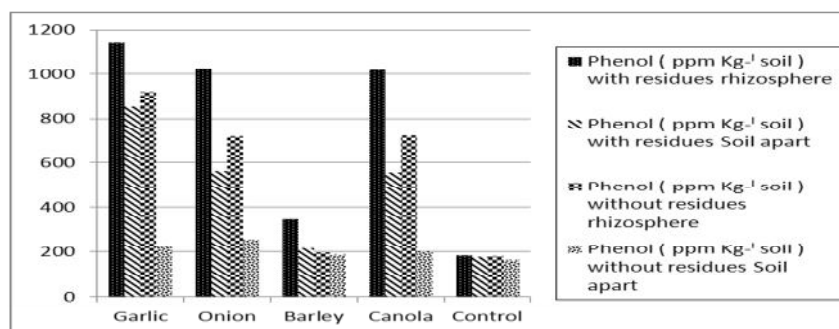


Fig.1. Determination of phenols in peanut soil which previously planted with different crops at Nubaria.

Discussion

Crop selection affects the quality and the quantity of plant residue left in/on the soil. Influential factors include cropping sequence, crop rotation periodicity (short versus long crop cycles including monoculture) and crop management. Both quality and quantity of crop residue from host and non-host crops can influence pathogen growth, sporulation and survival through the release of fungicidal and fungistatic compounds during the residues break down or alternatively by providing feeding substrates for facultative pathogens (Arshad and Amna, 2013).

The results illustrated that residues from preceding crops (no-tillage) enhanced the microbial activity, while suppressed the peanut root- and pod-rot diseases significantly compared to without residues (tillage). It could be concluded that, the use of no-tillage systems greatly increases the benefits of crop sequence and vice-versa. No-till systems increases water conservation by maintain preceding crops residues on the surface. It also reduces the disruption of the soil reducing: soil erosion, water runoff, organic matter oxidation and increases infiltration and all of the benefits of improved organic matter accumulation. Stratification of the soil profile as result of no-till is important for macro invertebrates and soil micro-organisms. On the other hand, tillage leads to unfavourable effects such as: soil erosion, soil compaction, loss of organic matter, degradation of soil aggregates, death or disruption of soil microbes and other organisms including mycorrhizae (O'Neil, 2005).

Continuous no-till needs to be managed very differently in order to maintain or increase crop yields. Soil borne pathogens are not usually a problem in organic farming due to rotations, incorporation of crop residues in the soil and often higher soil microbial activities all leading to the reduction of inoculum. Tillage generally increases the amount and speed of nitrogen mineralization of soil organic matter which may decrease of nitrogen release rate depending on the timing of the subsequent crop's nitrogen needs (O'Neil, 2005). Carbon released from crop residues contributes to increasing soil microbial activity and so increases the likelihood of competition effects in the soil, and may reduce incidence of some borne diseases. The placement of the residue in soil can lead to the displacement of the pathogen form its preferred niche diminishing the pathogen's ability to survive (Bailey and Lazarovits, 2003).

Allelopathic phenomenon in plant disease control is considered to be one of the environmental technologies which exhibit its beneficial effects through crop rotation. This might due to the increasing of interest in cropping system and reduced tillage, either in developed or developing countries, investigation of the interactions among agricultural crops would be of importance (Sarobol and Anderson, 1992; Uremis *et al.*, 2009 and Islam, 2012). Allelochemicals are released from plants either directly, as root exudates, or indirectly as break down products from crop residues during decomposition. These chemical include (simple phenols, phenolic acids derived from benzoic and cinnamic acids, coumarins, flavonoids, isoflavonoids, tannins and a variety of phenolic conjugates) has a profound influence on root zone-micro- and macro-flora (Makoi and Ndakidemi, 2007).

Presented results of this study demonstrated that root- and pod-rot diseases reduction were more significant and effective in treatments that having residues from preceding crops, *i.e.* garlic, onion, canola and barley, than those without residues. This resembles the incorporation of some residues of former crops to the soil before the next agriculture season (Gamlial and Stapleton, 1993).

Canola, as a preceding crop, expressed a significant suppression to peanut root and pod diseases. The acetone and ethyl alcohol 70% canola extracts were the most effective against *F. oxysporum* and *A. niger in vitro*, due to the release of biocidal compounds. The beneficial effects that observed from canola residues are most likely derived from fungicidal compounds, isothiocyanates released from brassica crops. Crucifer crops are harvested and the plant residue is reinvest into the soil, the decomposing of the broccoli stems and leaves released nature chemicals that could significantly reduce the number of *Verticillium dahliae* microsclerotia, *Pythium* sp. and *Rhizoctonia solani* (Cox *et al.*, 2005). Rape (canola) as one of the cruciferous plants used in this work has highest content of sulphur compounds, oils glycosides, glucosinolates as well as indoles, which might play an important role as allelopathic substances during crop rotation systems. They also produced biocidal compounds, principally isothiocyanates, which produced during the decomposition of glucosinolates in Cruciferae residues (Kirkegard *et al.*, 1996).

Obtained results demonstrated that ethanol 90% extracts of garlic and onion were the most effective in reducing the *in vitro* radial growth of *F. oxysporum* and *A. niger*. Garlic and onion were the best preceding crops to suppress peanut root- and pod-rot incidence. The biocidal properties of garlic and onion are attributed to sulphur volatiles produced during degradation of garlic (*Allium* spp.) tissues show fungicidal effects (Auger *et al.*, 2002 and Auger *et al.*, 2004). The volatile antimicrobial substance allicin (diallyl thiosulphinat) is produced in garlic and onion when the tissues are damaged and the substrate alliin (S-allyl-l- cysteine sulphoxide) mixes with the enzyme alliin-lyase (Lancaster and Collin, 1981 and Slusarenko *et al.*, 2008). Allicin effectively controlled seed borne *Alternaria* spp., *Phytophthora* leaf blight of tomato and tuber blight of potato (Slusarenko *et al.*, 2008). Sulphur compounds called Zuriebelanes (Zw) are produced during degradation onion residues (Block *et al.*, 1992; Ferary and Auger, 1996 and Auger *et al.*, 2004). The secondary substances of *Allium* have been extensively studied for their pesticidal effects. Available data have shown that sulphur compounds in *Allium* can be classified not only as insecticides, nematicides, herbicides, fungicides and bactericides. Even so, each sequence strategy will affect the pathogen complexes formed, as well as the antagonistic potential for microbial residents in the soil agro system (Cook, 1993).

Root nodules are a unique and highly organized structure developed as a result of the symbiotic relationship between leguminous plants and bacteria of the genus *Rhizobium*. The highest densities of rhizosphere region of the microbial flora of canola and barley can be attributed to the root excretions containing sugar, amino acids and organic acids (Namdari *et al.*, 2012). The establishment of the symbiosis requires signalling and recognition by both the partners. Various signalling molecules are exchanged between the plant and the infecting bacteria to regulate

nodule initiation, differentiation and functioning. Recent studies indicated that some phenolic compounds from the symbiotic legumes are known to promote growth of rhizobial bacteria in the rhizosphere (Hartwig *et al.*, 1990) and also to serve as chemo attractants that guide rhizobial cells to legume root hairs (Fox *et al.*, 2001). Serevirtne and Jayasingheach (2003) suggested a possible mechanistic explanation for the effects of phenolic acids on the protein profiles of Rhizobium. Results showed that the strains are initially induced to produce specific enzymes that are capable of degrading the phenolic acid and interaction of phenolic acids with the rhizobial nod gene. Kapustka and Rice (1976) and Rice *et al.* (1980) found that seven phenolic acids, previously identified as allelochemicals from pioneer weeds, promote growth of free-living N₂-fixers *Azotobacter chroococcum* and *Azospirilla* sp.

Mycorrhizae are formed in symbiotic interaction between plants and fungi. Growth promotion in plants can be attributed to mycorrhizal fungi. Considerable increases in phenolic compound as a result of mycorrhizal inoculation have been reported. Earlier evidence showed that exudates from host roots elicit growth stimulation in VAM in contrast to non-host exudates. Plant phenolic compounds are potential candidates as signals during mycorrhizal formation. Some reports showed that exogenous application of flavonoids exerts a positive effect on hyphal growth during symbiosis. These effects range from increased spore germination to enhance hyphal growth, hyphal branching and formation of secondary spores (Giovannetti *et al.*, 1996 and Namdari *et al.*, 2012).

Crop sequence increased phenol content in both peanut rhizosphere and soil apart; also phenol concentration was attributed to the presence of plant residues. Imas *et al.* (1997) show that *Brassica napus* were capable of releasing large amounts of phenolic into the rhizosphere. Phenolic, such as syringic, caffeic and protocatechuic acids released into the soil from seeds, roots or residues decomposition, these molecules can act against soilborne pathogens (Ndakidemi and Dakora, 2003). It increases the microbial activity when used as carbon source (Fierer *et al.*, 2001). Phenolic are known to play a dominant role in rhizosphere mineral elements and organic matter dynamics by affecting organic matter degradation, humus formation, alteration of microbial activities, mineralization of nitrogen and its availability (Kraus *et al.*, 2003 and Makoi and Ndakidemi, 2007) direct inhibition of nitrification (Rice, 1984) and solubilise and accumulate inorganic phosphorous (Imas *et al.*, 1997). The greatest extracted phenol content ration were showed in peanut rhizosphere where garlic residues were left in the soil (1135 ppm kg⁻¹ soil) followed by onion (1025ppm kg⁻¹), and canola (1017ppm kg⁻¹) it's due to the quantity and quality of phenolics released by plants can differ from species to species (Waller, 1987).

Conclusion

Crop sequence is the most important management tool available to farmers in both organic and conventional agriculture. Organic farming without crop rotation is impossible on the long term because of detrimental effects on soil fertility and plant health. In addition, rotations and cover crops may significantly contribute to erosion

control and other important agriculture problem. Especially canola and barley play crucial role in crop rotation with respect to nutrient management, soil organic matter accumulation and microbial activity. In addition, the role of garlic, onion and canola crops for the effective management of peanut root- and pod-rot diseases under organic farming.

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تأثيرات التعاقب المحصولي على أمراض أعفان
القرون والجذور ونشاط ميكروبات الريزوسفير
للفول السوداني

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** قسم وقاية النبات -

القاهرة.

ي تعاقب المحاصيل العمليات الزراعية التي يمكن من خلالها تشجيع
ظاهرة الأليلوباثى بين النباتات المختلفة. تناولت هذه الدراسة مدى إستجابة نبات
الفول السودانى لهذه الظاهرة وتأثير المركبات الأليلوكيميائية المستخلصة من
(- - الشعير-)

الدقيقة. لوحظ أن مستخلصات هذه النباتات لها قدرة تثبيطية لنشاط الفطريات
الممرضة معمليا. أجريت تجربة حقلية لتطبيق هذا الغرض فى منطقة النوبارية
حيث تنتشر الإصابة بفطريات *Aspergillus niger*

Fusarium oxysporum

حيث تؤثر هـ الأمراض تأثير مباشر على إنتاجية الفول السودانى.
الدراسة أن جميع المحاصيل السابقة لزراعة الفول السودانى لها تأثير معنوى
زيادة نسبة الفينولات فى منطقة الريزوسفير وقد
أحدثت جميع المحاصيل

المنزرعة قبل الفول السودانى زيادة معنوية فى أعداد الكائنات الدقيقة المثبتة
للنيتروجين المتواجدة بصورة حرة والمسببة للعقد الجذرية وأيضا البكتريا المذبذبة
للفوسفات بالإضافة لأعداد فطريات الميكوريزا الداخلية بالريزوسفير ونسبة إصابة
جذور الفول السودانى بها وقياسات النمو والمحصول وقد كانت أفضلها معاملة