ROLE OF CERTAIN CROPS ROTATED AND INTERCROPPED WITH GARLIC ON THE REDUCTION OF GARLIC WHITE ROT DISEASE INFECTION

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Abstract

Growing some summer crops - preceding garlic – in *Sclerotium cepivorum* heavily infested potted soil significantly reduced white rot disease of garlic. Reductions in disease incidence obtained with sugarcane, roquette and sorghum were 80.0, 73.3 and 66.7 % of control treatment, respectively. Reductions in the disease exhibited with planting corn, squash, pepper, soybean, cotton, sesame, cowpea and roselle ranged between 53.3 and 40.0 % .

Cauliflower, alfalfa, broad bean, nigella, Egyptian clover, coriander and roquette grown in pots, as winter crops preceding garlic, exhibited 46.7 – 26.7 % reduction in white rot disease.

Intercropping cauliflower, coriander and roquette with garlic reduced the disease by 46.0- 53.8 % .

Seedling root exudates of cauliflower, coriander and sorghum significantly reduced mycelial growth of *S. cepivorum* on PDA plates more than did radish and sugarcane. Cauliflower exhibited the highest reduction in mycelial growth followed by sorghum and coriander. Ascending rates of cauliflower root exudates resulted in a higher reduction in fungus growth.

Counts of fungi were significantly higher in soil of sorghum and roquette compared with sugarcane and coriander , while coriander rhizosphere yielded the highest density of fungi. Actinomycetes were detected in the rhizosphere at a highest count with coriander but were completely absent in the soil. Bacteria , in contrast, existed only in the soil where actinomycetes were completely absent. The highest count of bacteria was detected in sorghum soil. Actinomycetes inhabited soil only while bacteria and fungi existed in soil and rhizosphere of cauliflower, radish and garlic intercropped with the two crops. Cauliflower significantly increased counts of fungi and bacteria in rhizosphere when intercropped with garlic compared with those detected with garlic alone. Some recommendations are made.

INTRODUCTION

White rot caused by *Sclerotium cepivorum* (Berk) is the most destructive disease to onion and garlic in highly infested fields. Many of onion growers replaced garlic by other crops in their infested farms. Search for human a reduction in the disease

incidence apart from fungicide treatments is highly desired for human health at the present time.

Crop rotation can reduce plant diseases by the sequence of different types of microbiota associated with each cultivated crop (Baker and Cook, 1974). Investigations on crop rotation resulted in reductions in disease incidence such as : red rot disease of sugarcane caused by *Colletotricum folcatum* (Rehman *et al.*, 1985) ; Fusarium rot of wheat (Seif El-Yasal *et al.*, 1988); stem rot of wheat caused by *Pseudocercosporella herpotrichoides* , *Fusarium* spp., *Gaemnomyces graminis* and *Rizoctonia cerialis* (Vilich, 1993) and potato black scurf caused by *R . solani* (Mole and Sholte , 1996).

Some intercropping systems also exhibited satisfactory levels in the reduction of plant diseases such as : intercropping clover, lupine , chickpea , lentil, maize, sorghum and soybean with sugarcane to reduce smut disease (Abdou *et al.*, 1994); rye , rye-vetch mixture , mustard and phacelia with hop plantation to reduce infection with *Verticillium albo - atrum* (Solarska *et al.*, 1996); sorghum with peanut to control rosette virus infection (Alegbejjo, 1997) and clover – cereal cropping to control wheat leaf spot caused by *Mycosphaerella graminicola* (Deadman and Cooke,1997).

The present research was designed to screen many crops rotated and intercropped with garlic for their effect on the reduction of white rot disease. Effect of root exudates and counts of microbial content of some disease inhibitory crops were also considered.

MATERIALS AND METHODS

1-Isolation of the causal pathogen. Sclerotia of *S. cepivorum* collected from white-rotted onion plants grown at Mallawy Experimental Research Station, were surface sterilized by 10 % clorax for 5 minutes, aseptically air dried on sterilized filter paper, plated on PDA and incubated at 18 °C for 10 days. The isolate was subcultured on PDA slant using hyphal tip technique.The culture was pathogenic to garlic when 0.5 cm diam. fungal mycelium discs were placed on surface sterilized garlic cloves which exhibited clear rot 3 weeks after incubation at 18 °C .

Potted soil (25 cm diam. pots) was infested with one month-old sorghum grains culture of *S. cepivorum* at the rate of 1 % (w/w), to be used in subsequent experiments.

2-Evaluation of crops preceding and intercropped with garlic on the percentage of white rot infection. Infested pots were supplemented with 15 % cultivated field soil w/w to enhance microbial antagonism against *S. cepivorum* . Summer crops were grown for 6 months (planted on May 12 , 1996 and removed on

Nov. 13 , 1996), while winter crops remained for 45 days in pots (from Nov. 30 , 1996 to Jan. 15, 1996) at 5 seeds/pot before planting garlic (5 cloves / pot) to assess their effect on white rot infection.

Summer crops included sugarcane, roquette, sorghum, corn, squash, pepper, soybean, cotton, sesame, cowpea, roselle, eggplants, peanut, radish, tomato and sunflower. While winter crops tested were cauliflower, alfalfa, broad bean, nigella, Egyptian clover, coriander, roquette, caraway, cabbage, fennel, dill, radish, wheat, bean, pea, turnip, barley, garlic, lentil, spinach and onion. Control treatments included infested pots planted with garlic only.

Cauliflower and radish were separately intercropped with garlic at the rate of 5 seeds each / pot. Control treatment was pots planted with 5 garlic cloves for each.

All treatments were replicated 4 times. Percentage of white-rotted garlic plants were recorded at the end of experiments.

3–Effect of root exudates of some disease reductive crops on mycelial growth of S. cepivorum : Seeds of cauliflower, coriander, sorghum, radish and buds of sugarcane were surface sterilized with 5 % clorax for 4 minutes, planted on sterile filter papers wetted with 10 ml sterile water in sterile capped jars (300 ml each) and left to grow at room temperature (18 – 26 °C) for 2 weeks. Liquids in jars were added to PDA before pouring at the rate of 0.2, 1.0 , 2.5, 5.0 % v/v. Plates were inoculated with 0.5 cm. discs of 10 day-old S. cepivorum cultured on PDA culture and incubated at 18 °C. Control treatment was PDA plates without any root exudates. Growth diameters were recorded when fungus growth covered the surface of a plate regardless of the treatment.

4- Isolation of microorganisms from soil and rhizosphere of some crops: Cauliflower , sugarcane, sorghum, coriander , roquette, garlic and radish were grown for 3 months in *S. cepivorum* infested pots amended with 15 % w/w cultured field soil to enhance microbial antagonism against the pathogen. Rhizosphere and soil of these crops were sampled to count microorganisms /1 gm dry weight for each. Fresh soil and rhizosphere samples were hand-shaked with 100 ml sterile water for 2 minutes to prepare $1x10^{-2}$ sample dilutions. Ascending dilutions were prepared at 1×10^{-4}, 1×10^{-5} and 1×10^{-6} to isolate fungi (on Ohio medium), actinomycetes (on Jensen medium) and bacteria (on soil extract medium) , respectively.

One ml suspension was spread on the surface of the respective media in 9 cm Petri dishes and left for 12 hours at room temperature. Plates (3 each treatment) were inverted and incubated at 25 °C. Colonies of bacteria, fungi and actinomycetes were counted after 3,6and 10 days incubation, respectively. Counts of microorganisms were calculated per one gram dry soil or rhizosphere.

5. Used media :

Ohio medium (OAES) consisted of g / l : 5.0 glucose, 2.0 yeast extract , 1.0 Na NO_3 , 0.5 Mg SO_4.7 H_2 O, 1.0 K H_2 PO_4, 0.05 streptomycin sulfate , 0.05 chloromycetin, 1.0 oxgal , 1.0 sodium propionate and 20.0 agar agar. The medium was autoclaved at 11 lb for 15 minutes.

Jensen medium consisted of g / l : 2.0 dextrose , 0.2 casein (dissolved in 10 ml 0.1 Na OH) , 0.5 K_2 H PO_4 , 0.2 Mg S O_4 . 7 H_2 O , trace Fe Cl_3 . 7 H_2 O and 15.0 agar agar (pH adjusted at 6.5 – 6.6).

Soil extract medium consisted of g / l : 15.0 glucose, 0.5 K H_2 PO_4 , 100 ml soil extract and 15.0 agar agar. Soil extract was prepared by boiling 1 Kg garden soil + 1 l tap water + small quantity of calcium carbonate for 30 minutes and filtered two times (Tuite,1969).

RESULTS AND DISCUSSION

The population size and activity of soil micro-organisms are usually affected by the plant species grown. Such an effect may lead to an increase or a decrease of plant pathogenic organisms.

Growing sugarcane , roquette, sorghum and corn in *S. cepivorum* infested potted soil followed by garlic significantly reduced white-rot disease exhibiting 80,73.3, 66.7 , 53.3 % reduction in disease incidence, respectively (Table 1).

Table 1. Effect of some summer crops growing in pots preceding garlic on % of white rot incidence .

Crop	% white rot	% reduction in white rot
Sugarcane	15.0 *	80.0
Roquette	20.0 *	73.33
Sorghum	25.0 *	66.66
Corn	35.0 *	53.33
Squash	35.0 *	53.33
Pepper	35.0 *	53.33
Soybean	40.0 *	46.66
Cotton	45.0 *	40.0
Sesame	45.0 *	40.0
Cowpea	45.0 *	40.0
Roselle	45.0 *	40.0
Eggplant	55.0	26.66
Peanut	60.0	20.0
Radish	60.0	20.0
Tomato	75.0	0.0
Sunflower	80.0	(- 6.66)
Control	75.0	0.0
L.S.D. p = 0.05	23.17	

* Significant treatments.

Squash and pepper exhibited the same level of inhibition as in corn. A reduction in disease incidence ranging between 46.7 and 40.0 was detected with soybean, cotton , sesame, cowpea and roselle. Other crops were not significantly effective in reducing white rot.

Growing cauliflower, alfalfa, broad bean, nigella, Egyptian clover, coriander and roquette as winter crops in rotation with garlic in pots, significantly reduced percentage of white rot compared to control treatment (Table 2). Reduction in the disease incidence obtained with these crops ranged from 46.7 to 26.7 %. Other crops were not significantly effective for controlling the disease. The reduction in disease incidence by crop rotation was previously reported (Goss and Afanasiev, 1938, Rehman *et al.*, 1985, Vilich, 1993, Mole and Sholte, 1996).

Table 2. Effect of growing some winter crops rotated and intercropped with garlic on percentage of white-rot.

Crop	Crops preceded garlic		Crops bicropped with garlic	
	% white rot	% reduction	% white rot	% reduction
Cauliflower	40.0*	46.66	35.0*	46.1
Alfalfa	45.0*	40.0	50.0	23.1
Broad bean	45.0*	40.0	60.0	7.7
Nigella	45.0*	40.0	45.0	30.8
Egyptian clover	55.0*	26.7	60.0	7.7
Coriander	55.0*	26.7	35.0*	46.1
Roquette	55.0*	26.7	30.0*	53.8
Caraway	65.0	13.3	85.0	30.8
Cabbage	65.0	13.3	50.0	23.1
Fennel	65.0	13.3	60.0	7.7
Dill	65.0	13.3	40.0	38.5
Radish	65.0	13.3	50.0	23.1
Wheat	65.0	13.3	80.0	(-23.1)
Bean	65.0	13.3	65.0	0.0
Pea	65.0	13.3	80.0	(-23.1)
Turnip	65.0	13.3	50.0	23.0
Barley	70.0	6.7	60.0	7.7
Garlic	70.0	6.7	75.0	(-15.4)
Lentil	70.0	6.7	60.0	7.7
Spinach	70.0	6.7	75.0	(-15.4)
Onion	75.0	0.0	75.0	(-15.4)
Control	75.0	0.0	65.0	0.0
L.S.D.'s P=0.5	16.0	-	29.0	-

*Significant treatments.

Intercropping roquette, coriander and cauliflower with garlic in S. cepivorum infested potted soil, exhibited a significant reduction in white-rot disease 53.8 – 46.2 % of control treatment. Other crops were not significant. These results are in agreement with some previous reports (Abdou *et al.*, 1994, Solarska *et al.*, 1996, Alegbejjo , 1997 and Deadman and Cooke, 1997).

The reduction in disease incidence could be attributed to certain by - products in the root exudates of certain effective crops. An experiment was conducted to verify this possibility.

Root exudates of some white rot disease reductive crops were significantly suppressive to mycelial growth of *S. cepivorum* on PDA (Table 3).

Highest reduction in fungus growth was exhibited with cauliflower root exudates followed by sorghum and coriander. The magnitude of fungus inhibition was proportional to the concentration of root exudates. Such a relationship did not hold with other crops. Root exudates of other crops lost their toxicity to the fungus at higher rates. This may due to a changes in fungus physiology at these rates leading to a toxicity reverse , similar to that previously reported on some dithiocarbamates (Horsfall,1956) and other fungicides (El-Shehaby *et al.,* 1997).

Table 3 . Mycelial growth of *S. cepivorum* at levels of root exudates of white rot reductive crops.

Root exudates v/v % Crop	Diam. of mycelial growth in cm.					Means
	0.0	0.2	1.0	2.5	5.0	
Cauliflower	9.0	4.75	4.55	2.67	2.30	4.65
Coriander	9.0	3.65	3.75	9.00	9.00	6.98
Sorghum	9.0	8.57	5.62	4.75	6.97	6.98
Radish	9.0	5.67	9.00	9.00	9.00	8.33
Sugarcane	9.0	8.07	9.00	9.00	9.00	8.81
Means	9.0	6.14	6.38	6.88	6.75	-

L.S.D.'s at P = 0.05 for treatments x crop interaction = 1.60 but for means of rates and crops = 0.71

Microorganisms in soil and rhizosphere of some summer crops preceding garlic were counted (Table 4). Counts of fungi were the highest in soil of sorghum and roquette and also in rhizosphere of coriander. Actinomycetes were completely absent in the soil but were detected in the rhizosphere of these crops in a highest count with sugarcane. Bacteria, in contrast, were completely absent in rhizosphere and existed in soil in a highest count with sorghum. This alternate existence between bacteria and actinomycetes in rhizosphere and soil may due to a strong microbial antagonism associated with a selectivity for an inhabitable sphere with these crops.

Table 4. Counts of microorganisms in soil and rhizosphere of some white rot reductive summer crops preceding garlic.

Crop	Counts per 1gm soil			Counts per 1 gm rhizosphere		
	Fungi $x10^4$	Act. $x10^5$	Bac. $x10^6$	Fungi $x10^4$	Act. $X10^5$	Bac. $x10^6$
Sugarcane	1.2	0.0	1.12	2.90	1.40	0.0
Sorghum	4.5	0.0	33.25	9,90	1.13	0.0
Roquette	4.5	0.0	3.20	1.75	1.11	0.0
Coriander	1.8	0.0	1.55	92.60	1.12	0.0
L.S.D.P=0.05	2.51	-	15.10	4.08	0.20	-

Intercropping garlic with white rot - reductive crops exhibited significant differences in counts of fungi and bacteria in soil and rhizosphere compared to singly planted garlic (Table 5).

Table 5 . Counts of micro-organisms in soil and rhizosphere of mixed garlic and winter crops compared with single garlic panting.

Crop	Counts per 1 gm soil			Counts per 1 gm rhizosphere					
				Participating crops			Intercropped garlic		
	Fungi $x10^4$	Act. $x10^5$	Bac. $x10^6$	Fungi $x10^4$	Act. $x10^5$	Bac. $x10^6$	Fungi $x10^4$	Act. $x10^5$	Bac. $x10^6$
Mixed w/ cauliflower	3.2	1.12	14.25	9.30	0.0	51.25	118.1	0.0	87.75
Mixed w/ radish	3.5	1.13	2.00	26.70	0.0	52.00	13.40	0.0	1.20
Single garlic	1.2	1.13	0.0	-	-	-	6.05	0.0	1.50
L.S.D.at 5%	N.S.	N.S.	3.11	1.30	-	8.79	1.3	-	9.74

Counts of fungi in rhizosphere of garlic intercropped with cauliflower and radish were significantly higher than in garlic monoculture, while fungi were not significantly different in the soil of these crops. Actinomycetes were completely absent in rhizosphere of all mixed crops, but existed in the soil. Bacteria were higher in soil and rhizosphere of garlic intercropped with cauliflower than in singly planted garlic. However, planting radish with garlic resulted in nonsignificant counts of bacteria in soil and rhizosphere of garlic. Absence of actinomycetes in intercropping rhizosphere of garlic and other participating crops could be attributed to strong bacterial antagonism pushing the actinomycetes out of the rhizosphere.

While existence of bacteria and actinomycetes together in the intercropping soil may be due to the deminision of the interactive effect between these micro-organisms.

Rapid growth of bacteria in soil and rhizosphere of garlic intercropped with cauliflower more than radish may due to specific products in cauliflower root exudates.

The high toxicity of cauliflower root exudates to *S. cepivorum* mycelial growth in addition to the increase in bacterial counts in the soil and rhizosphere may explain the progressive role of cauliflower in the reduction of white rot disease.

It could be concluded that white rot reductive crops can reduce the disease by their toxic root exudates or the antagonism of associative microorganisms, or both means.

Accordingly, white rot disease inhibitory crops could be grown in rotation with garlic and other *Allium* sp. as an effective tool for controlling the disease in infested

fields. For instance, sugarcane of 2 – 3 years duration preceding garlic or onion could greatly reduce the disease.

Sugarcane and sorghum could be highly recommended to control the disease in Minia, Assuit, Sohag and Qena governorates , where these two crops are widely grown. Whereas, corn, squash, pepper, soybean, cotton, sesame, cowpea and roselle can be cultivated in infested fields at Beni Suef, Fayoum, Giza, Kalubia and Gharbia

Planting sugarcane in the latter governorates for fresh usage could be encouraged to achieve great reduction in white rot disease This approach can also be considered as one component of an IPM program.

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دور بعض المحاصيل المنزرعة في دورة زراعية أو تحميلاً علي الثوم في خفض الإصابة بمرض العفن الأبيض

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أدت زراعة بعض المحاصيل الصيفية (السابقة لزراعة الثوم) في أصص معداة بالفطر المسبب لمرض العفن الأبيض ، تأثيراً معنوياً علي خفض إصابة الثوم بهذا المرض عند زراعته تالياً لها في نفس الأصص ، وقد بلغ الإنخفاض في نسبة الإصابة بعد زراعة قصب السكر والجرجير والذرة الرفيعة ٨٠ ، ٧٣,٣ ، ٦٦,٧% علي التوالي ، وقد أظهرت الزراعة السابقة للذرة الشامية وقرع الكوسة والفلفل وفول الصويا والقطن والسمسم واللوبيا والكركديه انخفاضاً في نسبة إصابة الثوم تراوحت بين ٥٣,٣ - ٤٠%.

وعندما زرعت بالأصص محاصيل شتوية سابقة لزراعة الثوم كان للقنبيط ، البرسيم الحجازي ، الفول البلدي ، حبة البركة ، البرسيم المصري ، الكزبرة ، الجرجير تأثيراً خافضاً للإصابة بنسبة تراوحت بين ٤٦,٧ – ٢٦,٧%.

وقد أدي تحميل القنبيط والكزبرة والجرجير – كل علي حدة – مع الثوم إلي انخفاض في نسبة إصابة الثوم بالمرض تراوحت بين ٤٦ – ٥٣,٨% .

كان لإفرازات جذور كل من القنبيط والكزبرة والذرة الرفيعة – كل علي حدة – تأثيراً مثبطاً علي النمو الميسليومي للفطر المسبب للمرض ، وذلك عند إضافتها إلي بيئة البطاطس- دكستروز – آجار في أطباق بتري ، وكانت إفرازات القنبيط هي الأعلي تأثيراً تليها إفرازات الذرة الرفيعة ثم إفرازات الكزبرة .

ومن حيث تواجد الكائنات الدقيقة بالتربة والريزوسفير لبعض المحاصيل ، فقد وجدت الفطريات بأعداد متقاربة في تربة وريزوسفير قصب السكر ، الذرة الرفيعة ، الجرجير ولكنها كانت عالية في ريزوسفير نبات الكزبرة ، كما وجدت الأكتينوميسيتات في ريزوسفير هذه المحاصيل – دون التربة – بأعداد لم تختلف معنوياً فيما بينها ، كما وجدت البكتيريا في التربة – دون الريزوسفير – وبأعداد أعلي معنوياً مع الذرة الرفيعة عن باقي المحاصيل.

وفي حالة تحميل الثوم مع بعض المحاصيل ، وجدت الأكتينوميسيتات في التربة فقط ، بينما وجدت البكتيريا والفطريات في تربة وريزوسفير القنبيط والفجل وكذلك الثوم المحمل معهما ، مقارنة بالثوم غير المحمل ، وكان لوجود القنبيط تأثير إيجابي علي زيادة أعداد البكتيريا والفطريات في ريزوسفير الثوم المحمل وقد قدمت بعض المقترحات.