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EFFECT OF DIFFERENT APPLICATIONS OF SOIL AMENDMENTS AND SLOW-RELEASE NITROGEN FERTILIZER ON SOIL ENZYMES AND MICROFLORA ACTIVITIES

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ABSTRACT

Populations of microorganisms from soil treated with guanidine thiocyanate, guanylurea sulfate, thiourea, or furfural were compared with those of untreated soil. The materials affected quantitative and/or qualitative changes in composition of the soil microflora depending on the compound used. Guanidine thiocyanate (Gt) significantly increased total fungal populations relative to populations of other treatments. Populations of *Penicillium purpurogenum* were markedly higher in Gt-treated soil. Gt also increased total bacterial populations, and was the only compound that increased actinomycetes populations. The percentage of *Trichoderma harzianum* was significantly higher in soil treated with thiourea more than in the other treatments. Furfural increased the percentage of *P. purpurogenum* with respect to total fungi, and was as effective as guanylurea sulfate in increasing chitinolytic bacteria and those in the *Pseudomonas cepacia*-group. Thiourea most effectively promoted proliferation of coryneform bacteria. Chitinobyotic fungi increased synergistically when Gt and guanylurea sulfate were applied in combination.

Keywords: Slow - release nitrogen fertilizer compounds, total bacterial and fungal population, soil microflorea, soil enzymatic activities, furfural, biological control.

INTRODUCTION

Chemical and physical factors influence the rate of chemicals incorporated into the soil, but soil microorganisms are responsible for the degradation and ultimate dissipation of most compounds. Some organisms are capable of using a chemical as a source of utilizable nutrients and energy for metabolism, while others can transform the compound without deriving energy for growth (Racke, 1990 ; El-Sayed, 1995 and El-Sayed and Abo El-Wafa, 2001). Induction of changes in soil microflora by chemicals known to be active against soilborne plant pathogens provides a useful tool in biological control. In theors it makes possible the selection of ecologically acceptable compounds, such as compounds which could serve as building materials for growth of pupulations and antagonists of the target pathogen (El-Sayed and Abdel-Al, 2002).

Three slow-release nitrogen fertilizers compounds derived from urea : guanidine thiocyanate, guanylurea sulfate , and thiourea were found to be effective in the control of *Sclerotium rolfsii* Sacc. (Canullo and Rodriguez-Kabana, 1991; El-Sayed, 1998 a & b and El-Sayed and Abo El-Wafa 2001). Combined applications of guanidine thiocyanate and guanylurea sulfate

resulted in a synergistic decrease in viable sclerotia of *S. rolfsii* and increased soil urease and chitobiase activity (Canullo and Rodriguez-Kabana, 1990; El-Sayed and Hegab , 2001 a & b and El- Sayed, 1999). Furfural, and aldehyde with a furan ring (furfural-dehyde = furfural), which was reported to control *Rhizoctonia solani* Kuhn on potato tubers (Flor, 1926) stimulatd colonies of *Trichoderma* spp. Pers, in soil and reduced disease produced by *S. rolfsii* on lentil (Lens culinaris).

The purpose of this study was to compare changes induced by guanidine thiocyanate, guanylurea sulfate, thiourea, and furfural on soil microflora associated with reduced viability of sclerotia of *S. rolfsii*, and to evaluate the micro-flora responsible for synergistic decrease in viable sclerotia of *S. rolfsii* when guanidine thiocyanate and guanylurea sulfate are applied in combination.

MATERIALS AND METHODS

Effect of fertilizer compounds and furfural on scleratia of *Sclerotium rolfsii* and on soil microflora:

The experiment consisted of five treatments : guanidine thiocyanate, guanylurea sulfate, or thiourea were used at a dosage of 0.6 g/Kg soil, furfural at 0.6 ml/Kg soil, and control (untreated soil). There are ten replicates per treatment completely randomized.

Soil was a sandy loam collected from the farm of the Faculty of Agriculture in Assiut, Al-Azhar University. It was divided into 500 g fractions, placed in polyethylene bags, and mixed with the tested compounds. After thorough mixing, treated soil was dispensed into plastic pots.

Sclerotia for the experiment were grown in non-sterile soil . A 1-cm thick layer of moist (60 % field capacity) field soil was placed in each of 40 petri dishes. Five lentil seeds infected with virulent isolate of *Sclerotium rolfsii* obtained from soybean (Glycine max L., variety Giza) were distributed equidistantly on the soil surface in each petri dish. Dishes were then placed into polyethylene bags and incubated at 27 + 0.1 C until the sclerotia were darkbrown (approx. 11 days). The sclerotia were hand – picked, dried over filter paper, and kept at room temperature (26-28C) until used.

Ten sclerotia were placed in each of three small nylon bags (5 x 5 cm) inserted 0.6 cm deep into the soil of each pot. Pots were placed in a greenhouse, kept at 28 + SC and watered regularly. Twenty five days later the bago, and a 10g soil sample taken from each pot for microbial enumeration. The experiment was repeated as described, except that an addition 10 g soil sample was taken in order to quantify different bacterial groups.

Evaluation of viable sclerotia:

The bags containing the sclerotia were washed with deionized water to remove adhering soil, blotted , and the sclerotia were removed. Sclerotia from each bag were plated on potato dextrose a gar (PDA) containing 500 PPm of streptomycin sulfate and incubated for 72h at room temperature. Sclerotia- forming colonies (viable sclerotia) were counted, and the percent per replicate was recorded.

Enumeration of total fungal and bacterial populations:

Enumeration of total fungal and bacterial populations was performed with the modified dilution plate procedure (page *et al.*, 1982; Curl and Rodriguez-Kabana, 1986) using the 10 g soil sample taken from each of the ten replicates of each treatment. Culture media were : Ohio a gar fungi and Thornten's standardized a gar for bacteria (Johnson and Curl, 1972). Plates were incubated 96 h at room temperature and the number of colonies was recorded.

Evaluation of changes in soil microflora:

The most frequently occurring fungal species were enumerated from Ohio agar plates used to record total fungal population, and their relative percentage was calculated.

Several different media were used to assess the composition of the bacterial population. Five percent tryptic soy a gar (TSA), which was previously determined to yield population estimates similar to soil extract a gar (Kloepper *et al.*, 1992) was used for enumerating bacteria with low nutrient demands. *Pseudomonas* agar F (PAF), a commercially available king's medium B, was used to enumerate general bvacgteria and *fluorescent pseudomonds. Pseudomonas cepacia* biotypes were enumerated with TB-T (tripton blue - tetracycline) selective medium described by(Hagendorn et al., 1987). *Corynebacterium nebraskense* selective medium (CNS) was used for enumeration of coryneform bacteria (Gross and Vidaver, 1978), starch casein agar (SCA) (Miyashita *et al.*, 1982) for actinomycetes, and colloidal chitin agar (Godoy *et al.*, 1982; Mian et al., 1982) for chitinolytic bacteria.

The 10g soil sample from each of the ten replicates of each treatment was placed into a 250 ml Erlenmeyer flask containing 90 ml of sterile deionized water. Flasks were shaken on an orbital shaker at 150 rpm for 1h, and serial 10 fold dilutions were plated onto the different media using a spiral plater (Spiral System Instruments, Bethesda, MD). tubes contcuning 10–2 dilutions were placed in a water bath at 80 C for 20 min to kill asporogenous bacteria (Norris *et al.*, 1986). The tubes were then cooled to room temperature a nd s piral - p lated o nto T SA t o d etemine n umbers o f s pore – forming bacilli. Plates were incubated at 28 + 0.1 C for 24-96 h, and the number of colonies was counted using a leaser colony counter with Bacterial enumeration software from Spiral System instruments.

Treatment means were calculated by averaging the log cfu g-1 soil (Loper *et al.*, 1984) for each replicate .

Data were analyzed by standard procedures for analysis of variance and means were compared by Duncan's multiple range test (Steel and Torrie, 1980; SAS, 1988) All differences referred to in the text were significant at 5% or lower level of probability unless otherwise noted.

Effect of soil applications of guanidine thiocyanate and guanylurea sulfate singly and in combination on production of sclerotia of *Sclerotium rolfsii* and on soil microflora .

Lentil seeds were infected with the isolate of S. rolfsii and soil were used as the same previous experiment(Epps *et al.*, 1951) . Soil was screened (≤ 1mm mesh) to remove large particles, mixed 50 : 50 (V/V) with fine (<1

mm) sand, and placed into polyethylene bags in 1.5 Kg quantities with a moisture content of 60 % field capacity. This mixture here after will be referred to as soil. Inoculum density was 5 g infected lentil/ Kg soil. The soil was thoroughly mixed in polyethylene bags and transferred to plastic pots (11 cm in diameter x 13 cm long) .

Two days after infestation, it was removed from the pots, placed again into polyethylene bags, and treated with the tested compounds. Treatments were selected based on previously reported data (Canullo and Rodriguez-Kabana, 1990) guanidine thiocyanate 0.06 or .16 g /Kg1 soil, guanylurea sulfate 0.06 or 0.16 g/Kg soil, and a nontreated control. There are 7 replicates per treatment and the replicates were completely randomized in a greenhouse maintained at 28 C $\pm$ 5 C, and watered regularly. Forty days later, soil was removed from the pots and a 10 g sample was taken from each replicate to determine soil microflora. The rest of the soil was allawed to dry on aluminum foil (26-28 C) and stored at 4 C until used to determine sclerotium production by *S. rolfsii*, soil pH, and soil enzymatic activities.

Evaluation of numbers of sclerotia of *Sclerotium rolfsii* ,soil , pH ,and soil enzymatic activities

Numbers of sclerotia of *Sclerotium rolfsii were* determined by the methanol assay procedure (Rodriguez-Kabana *et al.,* 1980).

Soil pH was measured with a corning Model 12 pH meter using a suspension of 10 g air dried soil and 10 ml of de-ionized water. chitobiase (-N -Acelyl B –D-glucosamenidase, E.C 3.2.1.30) and urease (EC 3.5.1.5) activities were determined using methods previously described (Rodriguez-Kabana and King, 1980; Rodriguez-Kabana et al., 1989).

Data were analyzed by standard procedures for analysis of variance and means were compared by or thogonal contrasts (Steel and Torrie, 1980; SAS, 1988). All differences referred to in the text were significant at 5 % or lower level of probability, unleas otherwise noted.

Evaluation of soil microflora :

The total fungal and bacterial populations, *Trichoderma* spp. population, and chitinolytic microorganisms were enumerated using the modified dilution plate technique (Curl and Rodriguez-Kabana, 1986). Culture media were the same as previously mentioned. *Trichoderma* spp. Population was evaluated on *Trichoderma* selective medium (TSM). (Nelson *et al.*, 1988). The most frequently occurring fungal species were enumerated from The Ohio agar plates used to record the total fungal population.

Treatment means were calculated by averaging the log cfu g-1 soil for each replicate. The relative percentage for e ach d ominant s pecies w as calculated.

RESULTS

Effect of fertilizer compounds and furfural on scleratia of *Sclerotium rolfsii* and soil microflora:

Viable sclerotia were reduced by applications of all tested compounds (Table 1). The largest reduction of sclerotial viability was recorded with

reduced viability at the same rate (Table 1). Total fungal populations were increased by applications of all amendments (Table 1). The largest increase occurred with guanidine thiocyanate (Gt), followed by furfural (F), guanylurea sulfate (Gu) and thiourea (T) in decreasing order in both tests (Table 1). Total bacterial populations were increased only by the application of Gt (Test 1), and Gu in the application (Table 1).

Table (1): Mean germination of *Sclerotium rolfsii* sclerotia and mean soil fungal and bacterial populations for soil treated with guanidine thiocyanate, guanylurea sulfate, furfural, or thiourea.

Treatment	Sclerotial germination (%)		Mean log cfu g^{-1} soil on two media *			
			Ohio		Thornton's	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Control	96a	86a	5.33 d	5.33 e	6.69 bc	6.26bc
Guanidine hiocyanate(**)	68b	54b	5.84 a	5.97 a	6.74a	6.54a
Guanylurea sulfate	68b	59b	5.65 bc	5.66 C	6.72 ab	6.43ab
Furfural	45c	46b	5.71 b	5.79 b	6.68 bc	6.27c
Thiourea	73b	61b	5.55 c	5.59 d	6.67 c	6.38 bc

* Ohio a gar was used for enumeration of total fungi, and Thornton's standarized media for total bacteria. Data were recorded 25 days after application of the compounds. There were 10 replications per treatment. Values with the same letter in a column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

**Dosage of guanidine thiocyanate, guanylurea sulfate, or thiourea 0.6 g Kg-1 soil. Dosage of furfural = 0.6 ml/ Kg soil.

The most frequent fungal species were *Trichoderma harzianum* Rifai and *Penicillium purpurogenum*. Stoll. Population of *T. harzianum* increased with all compounds, except with Gu (Table 2). However, the relative percentage was only significantly higher for F and T (Table 2). Populations of *P. purpurogenum* increased with all compounds, except T, but its relative percentage only increased with Gt and F treatments (Table 2).

Table (2): Mean *Trichoderma harzianum* and mean *penicillium purpurogenum* populations for soil treated with guanidine thiocyanate, guanylurea sulfate, furfural, or thiourea.

Treatment	*T. harzianum*		*P. purpurogenum*	
	log cfu g-1 soil (a)	%	log c f u g-1 soil	%
Control	2.33 b	16.5b	2.55c	8.1 b
Guanidine thiocyanate (b)	4.84 a	16.5b	5.30a	36.9 a
Guanylurea sulfate	3.34 b	10.0b	4.39a b	17.1 b
Furfural	4.93 a	40.5a	5.02 a	45.9 a
Thiourea	4.74 a	56.9a	3.35 b c	14.1 b

(a) Data were recorded 25 days after application of the compounds. There were 10 replications per treatment . Values with the same letter in a column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

(b) Dosage of guanidine thiocyanate, guanylurea sulfate, or thiourea = 0.6 g Kg^{-1} soil. Dosage of furfural = 0.6 ml Kg^{-1} soil.

Guanidine thiocyanate was the only compound which stimulated populations of actinomycetes and the general bacterial population enumerated in PAF (Table 3). *Fluorescent pseudomonads* were not present at detectable levels in any of the tested treatments. Coryneform bacteria were increased by all compounds, but thiourea was the most effective (Table 3). Populations of the *Pseudomonas cepacia*-group increased with all compounds, except T. The largest increase was observed for treatment F, which did not differ from Gu (Table 3). Chitinolytic bacterial populations were increased by application of Gu and F and reduced by application T (Table 3). Populations of bacteria with low nutrient demonds and *Bacillus* spp. were not affected significantly by the treatments (Table 3).

Table (3) : Mean soil bacterial populations for soil treated with guanidine thiocyanate, guanylurea sulfate, or thiourea.

Treatment	Mean log c f u g^{-1} soil on various media[(a)]*						
	5% TSA	PAF	TSA-heated	CNS	TB-T	SCA	Chitin a gar
Control	6.70a	4.67b	5.81a	2.62c	3.34c	5.56b	2.80bc
Guanidine thiocyanate**	6.96a	6.14a	5.96a	5.84ab	4.18b	5.82a	3.90ab
Guanylurea sulfate	6.65a	5.17b	5.89a	5.89ab	4.69ab	5.38c	4.61a
Furfural	6.64a	4.90b	5.81a	5.01b	5.20a	5.60b	4.12a
Thiourea	6.78a	4.89b	5.86a	6.14a	2.59d	5.53b	1.92c

***The media were used for enumeration of the following groups of microorganisms 5% TSA for oligotrophic bacteria, PAF for general bacteria, TSA fllowing heat treatment for bacilli, CNS for coryneform bacteria, TB-T for pseudomonas cepacia – group of bacteria, SCA for actinomycetes, and chitina gar for chitinolytic bacteria. Data were recorded 25 days after application of the compounds. There were 10 replications per treatment.Values with the same letter in a column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$**

****Dosage of guanidine thiocyanate , guanylurea sulfate, or thiourea = 0.6 g kg^{-1} soil. Dosage of furfural = 0.6 ml Kg^{-1} soil.**

Effect of soil applications of guanidine thiocyanate and guanylurea sulfate singly and in combination on production of sclerotia of *Sclerotium rolfsii* and on soil microflora.

Total fungal population increased with the application of guanidine thiocyanate (Gt) (Table 4). The highest dosage tested of this compound (0.16 g/ Kg soil) augmented *Trichoderma* spp. and *Trichoderma harzianum* populations, although the relative percent of this species was not affected by the treatment (Table 5). Guanylurea sulfate at 0.16 g /Kg soil sustained a larger population and relative percent of *T. harzianum* (Table 5), and a larger population of chitinolytic fungi than the lowest dosage tested (0.06 g/ Kg soil) (Table 6) . Combinations of Gu and Gt resulted in a synergistic increase in chitinolytic fungi population (Table 6).Total bacterial population increased with

the application of both Gt and Gu (Table 4), The compounds also stimulated chitnolytic bacteria (Table 6). The number of viable sclerotia was reduced by all treatments. Combinations of Gt and Gu resulted in a synergistic reduction of sclerotia viability (Table 6). Soil urease activity increased with application of both compounds, and chitobiase activity increased with application of Gu at 0.16 g Kg-1 soil (Table 7). Soil pH increased in pots treated with Gt and Gu at 0.16g Kg-1 soil. Combinations of Gt and Gu resulted in a synergistic increase in soil PH, and the soil urease and chitobiase activities (Table 7).

Table (4) : Mean total soil fungal and bacterial populations for soil treated with guanidine thiocyanate and guanylurea sulfate singly or in combination.

Treatment	Mean log g-1 soil on two media (a)	
	Ohio	Thornton's
Control	6.40	8.19
Gt 0.06(b)	6.50	8.30
Gt 0.16	6.53	8.33
Gu 0.06	6.39	8.29
Gu 0.16	6.40	8.32
Gt 0.06 + Gu 0.16	6.58	8.35
Gt 0.16 + Gu 0.06	6.60	8.37
Mean squares contrast :		
Control VS treatment	0.05*(c)	0.09*
Control VS Gt 0.06	0.10*	0.03*
Control VS Gt 0.16	0.13	0.07*
Gt 0.06 VS Gt 0.16	0.00	0.02
Control VS Gu 0.06	0.00	0.03*
Control VS Gu 0.16	0.02	0.06*
G 0.06 VS Gu 0.16	0.00	0.00
Interaction (Gt x Gu)	0.02	0.00

(a) Ohio agar was used for enumeration of total fungi, and thornton's standardized medium for total bacteria.

(b) g Kg-1 soil. Gt Guanidine thiocyanate, Gu Guanylurea sulfate . Data were recorded 40 days after application of the compounds. There were 5 replications per treatment.

(c) Numbers followed by * are different at $P \leq 0.05$.

Table (5) : Mean population and relative percentage of *Trichoderma harzianum* and mean *Trichoderma spp.* population for soil treated with guanidine thiocyanate and guanylurea sulfate singly or in combination

Treatment	Ohilo agar (a)		*Trichoderma* selective medium (b) log cfu g^{-1} soil
	T. harzianum		
	log cfu g^{-1} soil	%	
Control	6.12	54	5.81
Gt 0.06 (c)	6.13	51	5.85
Gt 0.16	6.29	63	6.13
Gu 0.06	5.79	41	5.63
Gu 0.16	6.23	73	6.03
Gt 0.06 + Gu 0.16	6.23	54	5.94
Gt 0.16 + Gu 0.06	6.37	63	6.25
Mean squares contrast :			
Control VS treatment	0.03	49	0.07
Control VS Gt 0.06	0.02	524	0.03
Control VS Gt 0.16	0.65*(d	1011	0.92*
Gt 0.06 VS Gt 0.16	0.06	254	0.09
Control VS Gu 0.06	0.08	162	0.00
Control VS Gu 0.16	0.09	529	0.03
G 0.06 VS Gu 0.16	0.50*	2497*	0.14
Interaction (Gt x Gu)	0.20	618	0.11

(a) Ohio agar was used for enumeration of total fungi.
(b) Trichoderona Selective Medium was used for enumeration of trichoderma spp. Gt Guanidine thiocyanate, Gu Guanylurea Sulfate.
(c) g Kg-1 soil. Data were recorded 40 days after application of the compounds. There were 5 replications per treatment.
(d) Numbers followed by * are different at $P \leq 0.05$.

Table (6): Mean number of sclerotia of *Sclerotium rolfsu* and mean soil chitinolytic microorganisms for soil treated with guanidine thiocyanate and guanylurea sulfate singly or in combination.

Treatment	Mean log Cfu g-1 soil Chitin agar (a)		Total sclerotia 500-1 g-1 soil
	Fungi	Bacteria	
Control	6.42	8.08	186
Gt 0.06 (b)	6.38	8.17	108
Gt 0.16	6.44	8.18	51
Gu 0.06	6.00	8.20	83
Gu 0.16	6.22	8.22	21
Gt 0.06 + Gu 0.16	6.64	8.24	3
Gt 0.16 + Gu 0.06	6.69	8.25	10
Mean squares contrast ;			
Control VS treatment	0.02	0.09*	118161*
Control VS Gt 0.06	0.13	0.03*	16177
Control VS Gt 0.16	0.07	0.04*	76651*
Gt 0.06 VS Gt 0.16	0.02	0.00	11486*
Control VS Gu 0.06	0.09	0.05*	36073
Control VS Gu 0.16	0.00	0.06*	128251*
G 0.06 VS Gu 0.16	0.14	0.00	13829*
Interaction (Gt x Gu)	0.28*	0.00	5236*

(a) Chitin a gar selects for chitinoloytic microorganisms.
(b) g Kg-1 soil. Gt Guanidine thiocyanate, Gu Guanylurea sulfate. Data were recorded 40 days after application of the compounds. There were 5 replications per treatment.
(*) Numbers followed by * are different at $\leq$ 0.05 .

Table (7): Mean soil pH and mean soil urease and chitobiasectivities for soil treated with guanidine thiocyanate and guanylurea sulfate singly or in combination.

Treatment	Urease activity (a)	Chitobiase activity (b)	PH
Control	4.9	4.9	7.2
Gt 0.06(c)	5.9	4.5	7.1
Gt 0.16	7.4	5.0	7.5
Gu 0.06	6.9	4.7	7.4
Gu 0.16	8.1	5.1	7.9
Gt 0.06 + Gu 0.16	11.7	6.3	8.7
Gt 0.16 + Gu 0.06	12.9	6.7	8.3
Mean squares contrast			
Control VS treatment	92.3*	3.3	4.3*
Control VS Gt 0.06	38.9*	3.1	2.6
Control VS Gt 0.16	126.0*	9.3	4.8*
Gt 0.06 VS Gt 0.16	9.8	2.8	3.1*
Control VS Gu 0.06	100.5*	6.2	2.7
Control VS Gu 0.16	144.3*	7.8*	10.8*
G 0.06 VS Gu 0.16	7.0	2.5	2.9*
Interaction (Gt x Gu)	16.2*	6.6*	3.0*

(a) Ug NH3 g^{-1} soil 6 h^{-1}
(b) Ug N Acetyle B Δ Glucosamine h^{-1} g^{-1} soil.
(c) g Kg-1 soil. Gt Guanidine thiocyanate, Gu Guanylurea sulfate. Data were recorded 40 days after application of the compounds.
There were 7 replications per treatment.
(*) Numbers followed by * are different at (P) ≤ 0.05 .

DISCUSSION

Nitrogenous fertilizers have long been recognized for suppreasing *S. rolfsii* by changing the microflora in soil and by increasing antagonistic microorganisms (Leach and Davey, 1942; El-Sayed and Hegab, 2001 a and b). Furthermore, bacterial populations and fungal genera like *Trichoderma*, *Penicillium*, *Aspergillus*, and *Fusarium* are known to be stimulated in soils treated with soil fumigants (Sinha *et al.*, 1988; El-Sayed, 1999). The three slow-release nitrogen compounds and the fumigant (furfural) used in this study reduced sclerotial germination and increased total soil fungal population when applied at a dosage of 0.6 g/Kg soil (Table 1). Also, the tested compounds affected differently the populations and relative percentages of *T.harzianum* and *P. purpurogenum*(Table 2). *P. purpurogenum* has not been reported to be an antagonist of *S. rolfsii*. *T.harzianum* has been used as an effective biocontrol agent against the mentioned pathogen and *Rhizoctonia solani* (Elad *et al.*, 1980; El-Sayed and Abdel-Al Abdel-Halem 2002), but

Trichoderma strains differ in their antagonistic capacity (Henis, 1984; El-Sayed, 1995 a and b). Therfore, the assessment of the possible significance of the differential effect of the tested compounds on the populations and relative percentages of *P. purpurogenum* and *T. harzianum* with regard to the reduction in viability of the scleratia of *S. rolfsii* required further investigation.

In contrast to the results obtained for total fungal population, only the application of guanidine thiocyanate consistently increased the total bacterial population (Tables1and 2). However, there was a distinctive effect of the compounds on specific groups of bacteria (Table 3). The effect of guanidine thiocyanate on chitinolytic microorganisms such as actinomycetes and chitinolytic bacteria and the effect on chitinolytic bacteria of guanylurea sulfate and furfural (Table 3) could be related to their effectiveness in reducing sclerotial viability (Table 1), since the hyphal and sclerotial of *S. rolfsii* are composed of B 1,3 - glucan and chitin (Chet *et al.*, 1967). *Pseudomonas cepacia* group of bacteria increased after application of all compounds, except thiourea. This is a large and possibly diverse group of organisms some of which have been studied as biological control agents for certain pathogenic fungi (Elad and Chet, 1987; Kawamoto and Lorbeer, 1976; El-Sayed, 1998 a ,b & c). The potential of the *Pseudomonas cepacia* group as a source of antagonists to *S. Rolfsii* is not known, but should be examined (Table 3).

The same may apply to coryneform bacteria, which increased with all treatments (Table 3).

When guanidine thiocyanate and guanylurea sulfate were applied in combination, chitinolytic fungi was the only group of soil microorganisms whose synergistic increase was concomitant with the decrease in number of viable sclerotia of *S. rolfsii* and with the increase in soil chitobiase activity (Tables 6 and 7). This is a clear indication of the antagonistic activity of these organisms on *S. rolfsii.*

CONCLUSIONS

The synergistic increase in urease activity in soil treated with combined applications of guanidine thiocyanate and guanylurea sulfate indicates an increase in ammonifying microorganisms, some of which may also metabolize chitin. Increased ammonification results in increased release of ammonia, and rise in pH values (Table 7).

The toxicity of ammonia on mycelia and sclerotia of S. rolfsii has been known for a long time (Leach and Davey, 1935; El-Sayed, 1997 and b ; El-Sayed and Salem, 2002). Thus, direct toxicity of ammonia may be enhancing te effect of chitinolytic microflora in reducing sclerotial viability. The significance of the antagonistic activity of chitinolytic fung with respect to ammonia toxicity on sclerotia of S. rolfsii requires further study.

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تأثير التطبيقات المختلفة لمحسنات التربة والأسمدة النيتروجينية بطيئة التحلل على النشاط الإنزيمى وميكروفلورا التربة

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* تم عمل مقارنة بين تعداد الكائنات الدقيقة فى تربة معاملة بثيوسانات الجوانيدين ، كبريتات الجوانيليوريا ، الثيويوريا و الفورفورال مع تربة أخرى غير معاملة بتلك المواد .
* وقد أثرت المواد كميا ونوعيا – فقد غيرت من محتوى التربة من الميكروفلوا وتوقف ذلك على المركب المستعمل.
* ولقد أدى المركب ثيوسيانات الجوانيدين إلى زيادة معنوية في التعداد الكلى للفطريات مقارنة مع بقية المعاملات.
* وكان تعداد الفطر بنيسليوم بوروجينم – أعلى فى التربة المعاملة بثيوسيانات الجوانيدين .. أدى هذا المركب أيضا إلى زيادة التعدادات الكلية للبكتريا وكان المركب الوحيد الذى أدى إلى زيادة تعداد الاكتينوميسيتات . وكانت النسبة المئوية للفطر تريكودرما هارزبانم أعلى بدرجة معنوية فى التربة المعاملة بالثيويوريا مقارنة مع المعاملات الأخرى.
* أدت المعاملة بالفورفورال إلى زيادة النسبة المئوية للفطر بنيسليوم بوربورا جينم بالنسبة لمجموع الفطريات وكانت هذه المعاملة مؤثرة مثلها مثل كبريتات الجوانيليوريا فى زيادة البكتريا المحللة للشيتين ومجاميع البكتريا سيدوموناس سيباسيا. أدت المعاملة بالثيويوريا إلى زيادة الكورينياكتريا. إزداد تعداد الفطريات المحللة لشيتيين بطريقة تعاونية عندما استعملت ثيوسيانات الجوانيدين مع كبريتات الجوانيليوريا فى توليفة بينهما .