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EFFECT OF HERBICIDES AND MICROELEMENTS ON RHIZOSPHERE MICROFLORA IN RELATION TO COTTON SEEDLING DISEASE CAUSED BY *RHIZOCTONIA SOLANI* (AG-4)

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Abstract

Greenhouse experiments with naturally infested field soil indicated that there was no interaction between the herbicides pendimethalin, fluometuron or norflurazon and the microelements manganese sulfate, sodium molybdate or sodium chloride on cotton stand and on the qualitative or quantitative status of rhizosphere microflora 14 and 28 days after planting (DAP). However, a significant interaction was found between herbicides and microelements on the emergence percentage 4 DAP and on the percentage of plants forming the first true leaf 11 DAP. Herbicides or microelements singly showed some influence on cotton stand and population of rhizosphere microflora depending on the treatment and the group of microorganisms (fungi, bacteria or actinomycetes). The qualitative identification of rhizosphere was not affected within treatments. The herbicides pendimethalin and fluometuron decreased cotton stand 14 and 28 DAR but all the three herbicides increased percentage of moderately discolored roots and decreased the population of *R.Solani* (AG-4) in soil samples as compared with no herbicide treatment.

INTRODUCTION

Sore-shin disease caused by *Rhizoctonia solani* kuhn belonging to anastomosis group (AG-4) presents an annual problem reducing the number of standing cotton plants. The behavior of plant pathogens and occurrence of root diseases are distinctly related to certain rhizosphere phenomena (Curl 1982). Ecology and dynamics of rhizosphere microflora are greatly affected by agricultural practices that stimulate or restrict root growth (Bowen and Rovira 1991). In this study, the effect of herbicides and microelements; singly or combined, on the population of rhizosphere microflora of cotton seedlings were studied in relation to the incidence of disease caused by *R.Solani* (AG-4) in naturally infested soil.

MATERIALS AND METHODS

The experiments were carried out to investigate the effect of 3 herbicides and 3 microelements (separately or in combinations) on the disease incidence, population of rhizosphere microflora and the host plant. The experiments were established in the greenhouse (20-26 C) during the winter of 1991, in Tifton loamy sand soil (fine loamy, siliceous, thermic, plinthic, paleudult; pH 6.0-6.4 ; 2.5 % organic matter) at the department of plant pathology, Coastal Plain Experiment station, University of Georgia, Tifton, GA (USA). Soil collected from fields previously cropped with cotton after peanut and naturally infested with *R.Solani* AG-4 was dispensed in plastic pots (22 cm in diameter, 1.8 L soil). Cotton seeds of the cultivar Delta pine 90 (*Gossypium hirsutum* L) delinted and treated with the fungicide combination carboxin + PCNB , were planted at 20 seeds /pot (2 seed/hole in 10 holes, 2 cm deep and 5 cm apart). Four replicates were used in each experiment and each replicate consisted of 2 pots (one for rhizosphere samples and the other for stand counting at several intervals) . Samples of rhizosphere soil were taken (5 plants/replicate) 14 and 28 days after planting (DAP). Rhizosphere suspensions were prepared by shaking the root system of 5 plants in 100 ml sterile, demineralized water for 5 minutes. These suspensions were used to determine the population of microflora (fungi, bacteria and actinomycetes per gram of dry weight of rhizospher soil) by the dilution plating technique (Wollum 1982). Then , roots were rated for disease severity (Sumner 1985), and the total chlorophyll content of the green leaves was measured and total chlorophyll content of the green leaves was measured and calculated (Procotor 1981). Also the relative percentage of the root system area was measured (Carley and Watson 1966), and then the 5 plants were air dried for 7 days at 20 C and

weighed. The populations of *R.solani* from soil samples (the excess soil after shaking the plants) were assayed with a multiple-pellet soil sampler (Henis *et al.* 1978) on tannic acid-benomyl agar media (TABA) as reported by Flowers (1976) and modified by Sumner and Bell (1982) .

Preliminary studies of several agar media indicated that (OAES) Ohio state Agar (Schmithenner and Williams 1958) was satisfactory for identifying fungi; Peptone-Dextrose-Rose Bengal Agar (modified by Johnson 1957) was superior for assaying populations of fungi, and soil Extract Agar (Allen 1975) was superior for assaying populations of both bacteria and actinomycetes. Identification of fungi to genus or species was based on gross and microscopic colony and fruiting characteristics on OAES plates using the unaided eye and the light microscope. Some colonies were identified to genus and others (which were rarely isolated) were not identified. Actinomycetes and bacterial colonies were enumerated quantitatively using a binocular miroscope.

1 . Effect of herbicides :

Three herbicides commonly used in cotton fields for weed control (norflurazon, fluometuron and pendimethalin) were applied to soil in pots at the recommended rates (1.1 , 1.7 and 1 kg a.i. / ha , respectively), just after planting and before watering. Control pots were sprayed with 200 ml water/pot. In this experiment, a completely randomized design was used. Data were recorded weekly as percentage of standing plants and rhizosphere samples were collected 14 and 28 DAP.

2 . Effect of microelements :

Three microelements were tested separately to investigate their effect on the disease incidence, rhizosphere microflora and the host plant in a completely randomized design. Forty grams of cotton seeds were soaked for 4 hr. in 0.1% solutions of manganese sulfate monohydrate ($MnSo_4$ $H2O$, 32 . 51% Mn), sodium molybdate ($NaMoO_4.2$ $H2O$,39.6% MO) , sodium chloride (NaCl, 60.66 % cl) or sterilized demineralized water (as a control treatment). Seeds of all treatments were planted immediately after soaking. Stand counts were recorded weekly.

1 . Interaction between herbicides and microelements :

In this experiment, microelements and herbicides were tested in combinations. Cotton seeds treated with microelements were planted immediately after soaking and the herbicides were sprayed then at the recommended rates on the soil just after planting. Stand counts were recorded 4,6,10,14,19,28,33 and 40 DAP. Plants forming the first true leaf were observed daily until 14 DAP. A split-plot design was used with herbicides as main plots and microelements as sub-plots.

RESULTS

Identification of soil fungi from the experimental samples :

Penicillium funiculosum was the only species of Penicillia that could be identified. *Aspergillus fumigatus*, *A.terreus* and *A.niger* were the most commonly identified Aspergilli. *Fusarium tricinatum*, *F.solani* and *F.oxysporum* were the only identified Fusaria. Other fungi not identified to species were *Rhizopus* spp., *Trichoderma* spp., *Mucor* spp., *Gliocladium* spp., *Neocosmospora* spp., *Chaetomium* spp., *Diplodia* spp., *Phoma* spp., *curvularia* spp. and *Helminthosporium* spp. .The identified colonies from soil pellet plates were mostly *Rhizoctonia solani*, *R.zeae* and *Laetisaria arvalis*. Most of these species were frequently isolated from rhizosphere soil of cotton seedlings in all experiments.

Influence of herbicides on the disease incidence, quantification of rhizosphere microflora and the host plants :

The herbicide pendimethalin and fluometuron significantly decreased cotton stand when herbicides were applied separately, and all herbicides significantly increased the percentage of moderately discolored roots (Root index 3,4) and decreased the population of *R.solani* AG-4 in soil pellet samples compared with the no-herbicide treatment (Table 1-A). The relative percentage of the root system area was greatly decreased by all herbicides 28 DAP and the number of fungal CFU were significantly increased 14 DAP (Table 1-B). In contrast, the population of actinomycetes and bacteria were not affected by herbicidal treatments 14 or 28 DAP. Bacterial CFU at 28 DAP, although increased to about 3 folds, yet the change was not statistically different.

The restricted growth of cotton roots by all herbicides, particularly by pendimethalin followed by fluometuron, was visually observed but there was no differences in dry weights among treatments . However, using the amount of absorbed or adhered solution (of 6 parts calcium nitrate and one part water) by the root system as a parameter confirmed the effect of the herbicides on the growth of cotton roots (Table 1-C) . The total chlorophyll content of leaves was not affected by the herbicides as calculated in mg/m2 (Table 1-C).

The statistical analysis, using T-test, showed a significant correlation between the herbicidal effect on percent stand 28 DAP and the percentage of moderately infected roots, population of R.solani AG-4 in soil, the root system absorption and total chlorophyll content. The correlation between standing plants and the quantification of rhizosphere microflora was not significant.

Influence of microelements:

The influence of microelements on the different parameters are shown in Tables (2-A), (2-B) and (2-C). Soaking cotton seeds in a solution of manganese sulfate significantly increased the percentage of stand 14 DAP and decreased the percentage of plants with moderate root disease after 28 DAP compared with the control treatment. None of the microelement treatments had a significant effect on percent stand 28 DAP, plants with severe root disease 14 or 28 DAP, or the populations of R.Solani G-4 14 or 28 DAP. However, the population of R.solani AG-4 28 DAP was agreater than 14 DAP (Table 2-A). Results in Table (2-B) and (2-C) indicate non-significant influences of microelements on the host plant parameters or the rhizosphere microflora except the population of actinomycetes 28 DAP.

Table 1-A.* Effect of herbicide on standing plants, disease severity, and *Rhizoctonia solani* AG-4 (28 DAP).

		Root Diisease			
	% Stand 28	*Severity*[Y]		*Soil Pellet plates*	
	days afte	%	%	Number	CFU[2]
Herbicide	Planting	Moderate	Severe	of colonies	R.Solani AG-4
Pendimethalin	36.5 B	38.3 A	8.3 A	6.6 A	3.4 BC
Norflurazon	42.0 A	25.0 S	6.7 A	6.9 A	2.0C
Fluometuron	36.1 B	33.3 A	5.0 A	7.7 A	3.9 B
No-Herbicide	40.3 A	10.0 B	0.0 A	6.7A	6.1 A
LSD (P-0.05)	3.5	14.5	12.1	2.5	1.4

[X] Means followed by the same letter in each colum or sub-table are not significantly different (P-0.05).

[Y] Root disease severity : 1-<2. 2 -10 , 3-11 - 50,4 - > 50% discolorntion or decay, and 5- dead or dying plants. % moderate - % of plants rated 3 or 4 in the root disease index or 5 % severe - % of plants rated 4 or 5 in the root disease severity index.

[X] CFU - Colony forming units per 100 g of oven - dried soil.

Influence of herbicides and microelements:

In general, there was no interaction between herbicides and microelements except in the percent emergence 4 DAP and the percentage of plants forming the first true leaf 11 DAP. Figure (1) presents the interaction between herbicides and microelements on the perectage of stand 4 DAP (early emergence), where distilled water increased the percent stand in the nonherbicide treatment compared to treat-

Table 1-B. Effect of herbicide on relative size of the root system and the population of microflora in 1 g oven dried rhizosphere soil of cotton seedlings.

Herbicides	Relative Size of Root System (%) 28 DAP[X]	CFU[Y] *Fungi* 14 Days	28 Days1	CFU[Y] *Bacteria* 4 Days	28 Days	CFU[Y] *Actinomycetes* 14 Days	28 Days
Pendimethalin	40.7 B	0.20 A	0.9A	102.3A	182.6A	0.05A	0.3A
Norflurazon	33.6 B	0.19 A	0.6A	44.3A	237.6 A	0.03A	0.14A
Fluometuron	20.4 C	0.19 A	0.5A	57.8 A	247.3A	0.3A	0.1A
No-Herbicide	90.9 A	0.07 B	0.4A	63.7 A	75.0A	0.04A	0.2A
LSD (P-0.05)	9.4	0.04	1.4	75.2	205.0	0.024	0.3

X Relative percentage of absorbed solution (calcium nitrante) compared to 607 mg absorbed by root system of non-treateds plants (Replicate 1).

Y CFU - Colony forming units per 1 g oven dried soil rhizosphere in millions.

Table 1-C.* Effect of herbicide on cotton plant.[X]

Herbicides	Root Absorptiony (mg)	Dry weight / Plant (mg)	Total chlorophyll contentx in mg / m2
Pendimethalin	439 B	423 A	5.573 B
Norflurazon	362 B	422A	5.212 B
Fluometuron	220 C	392 A	7.731 A
No-Herbicide	657 A	437A	6.334AB
LSD (P-0.05)	102	82	3.2

X Means of 2 time intervals (14 and 28 DAP)

Y Weight of absorbed solution by root system.

X Number are divided by E-10.

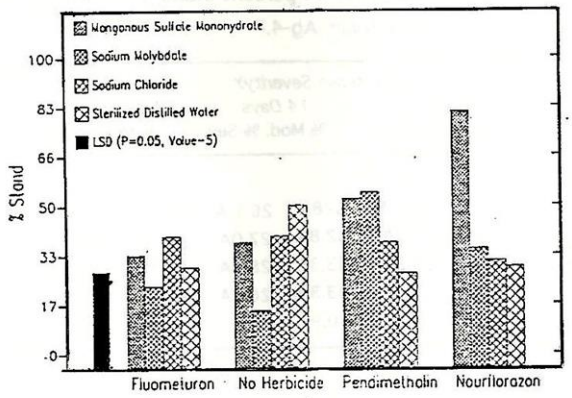

Fig.1. Interaction between Herbicides and Microelements as affecting Percentageof Stand of Contton at 4 DAP.

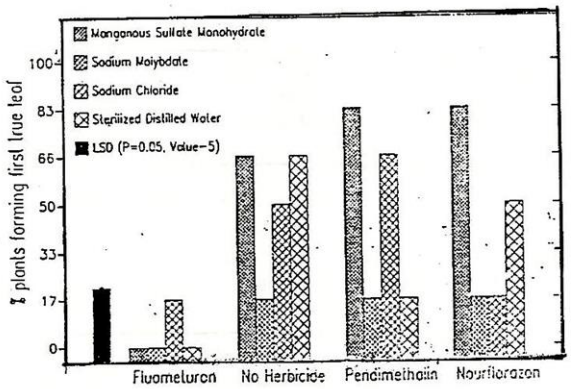

Fig. 2 . Interaction between Herbicides and Microelements as affecting Percentageof of Plants forming the 1st True Leaf at 11 DAP.

Table 2-A. Effect of microelements on percent stand, root disease seveity, and population of *Rhizoctonia solani* Ag-4.X

Microelements	*Root Disease SeverityY* *% Stand* 14 Days	28 Days	*14 Days* % Mod.	% Sev.	*28 Days* % Mod.	% Sev.	*CFU/100 & D.Soi* 14 Days	28 Days
Manganese sulfate monohydrate	62.5 A	55.5 A	328A	26.1 A	64.1B	43.8A	1.3A	3.4A
Sodium molybdate dihydrate	42.2 B	48.2A	32.8A	27.0A	87.5A	57.8A	1.2A	2.4A
Sodium Chloride (Deionized water)	53.2AB	39.8B	33.3A	28.1A	82.3A	54.7A	2.6A	2.5A
	46.9AB	38.3A	33.3A	26.6A	82.8A	50.0A	2.2A	4.6A
LDS (P-0.05)	18.9	19.2	0.9	6.5	14.3	24.9	1.6	2.5

X Means of herbicide treatments.

Y Root disease severtiy : 1-<2, 2-2-10, 3-11-50, 4 - > 50% discoloration or decay, and 5- dead or dying plants. Percentage Mob. - Plants with root index of 3.4. or 5. Percentage Sev. - Percentage plants with a root index of 4 or 5.

Table 2-B.* Effect of microelements on the populations of microflora in 1 g oven dried rhizosphere soil of cotton seedlings.

Microelements	Relative %x CFUY of root are 14 Days	28 Days	CFUY Fungl % Mod.	% Sev.	CFUY Bancterin % Mod.	% Sev.	CFUY Actinomycetes 14 Days	28 Days
Manganese sulfate monohydrate	48.5 A	37.9A	0.53A	0.57A	2.94A	4.31A	0.010A	0.03B
Sodium molybdate dihydrate	49.7A	40.3A	0.13A	0.85A	2.21A	4.17A	0.006A	0.06AB
Sodium Chloride (Deionized water)	59.9A	38.5A	0.12A	0.72A	1.92A	5.44A	0.005A	0.07AB
	57.5A	38.9A	0.20A	0.99A	2.12A	4.63A	0.009A	0.10A
LDS (P-0.05)	27.7	19.6	0.42	0.5	1.53	2.31	0.0063	0.06

X Relative percentage of absorbed solution (calcium nitrate compared to 449 mg absorbed by the root system of 5 non-treated plants at 14 days and to 959 mg of the same treatment at 28 days.

Y CFU - colony forming units per 1 g oven dried soil rhizosphere in millions .

Table 2-C. Effect of microelements on the host plant.[X]

Microelements	Root Area [Y] (mg) 14 Days	28 Days	Dry Weight / plant (mg) % Mod.	% Sev.	Total chlorophylll Contents[X](mg/m2) % Mod.	% Sev.	14 Days	28 Days
Manganese sulfate monohydrate	479A	928A	383A		956A	4.75A	6.56A	
Sodium molybdate dihydrate	491A	988A	268A		813A	8.75A	5.94A	
Sodium Chloride (Deionized water)	591A	943A	436A		774A	7.62A	5.87A	
	567A	950A	467A		7628A	3.88A	6.33A	
LDS (P-0.05)	173	179	220		265	5.84	1.51	

X Means across herbicide treatments.

Y Weight of absorbed solution by root system.

X Numbers are divided by E-10.

* Means followed by the same letter in each column of each sub table are not significantly different (P-0.05)..

ments with microelements, and had the lowest percentage of early emergence in the presence of the herbicides norflurazon and pendimethalin. Soaking cotton seeds in sodium molybdate showed the opposite trend. The microelement treatment of manganese sulfate significantly increased percent stand in presence of the herbicide norflurazon compared with all other treatments.

The effect of interaction between herbicides and microelements on percentage of plants forming the first true leaf is shown in Fig. (2). The combination of microelements and the herbicide fluometuron significantly delayed the formation of the rst true leaf. Soaking cotton seeds in sodium molybdate reduced the formation of the first true leaf in all herbicide treatments. Sodium chloride delayed formation of the first true leaf compared with distilled water in the no-herbicide treatment but induced a significant increase in formation of the first true leaf when pendimethalin or norflurazon were applied (Fig. 2).

DISCUSSION

Data indicate that herbicides have a significant influence on the percent stand, percent of plants with moderate or greater degree of root discoloration , population of R.solani AG-4 in soil, and the relative percentage of the root area. Also, herbicides increased populations of soil fungi 14 DAP. Also, herbicides increased popula-

tions of soil funggi 14 DAP. Populations of *R.solani* AG-4 was decreased by approximately 50 % in the presence of herbicides 28 DAP compared with 14 DAP, but such changes were not observed in the control. The decreases in population of *R.solani* with the herbicides contrasted with the deleterious effects of the herbicides on plant stand, but the in *vivo* inhibition of the pathogen by herbicides (Ragab *et al* 1987 and Youssef *et al* 1987) may explain the reduction of R.solani AG-4 population in soil. Also, the population of fungal colonies 14 DAP was significantly increased by herbicides compared with no-herbicide treatment. Such increase in the fungal and the pathogen populations was reflected by a significant increase of plants with infected roots or a decrease in plant stand. The non-significant effects of the herbicides on the quantification of rhizosphere microflora herein was also reported by several researchers (Lewis et al 1978 and Neweigy et al 1983). This lack of effects has been related to the huge variation among treatments and environmental factors (Rovira, 1991 and Bowen and Rovira 1991). Nevertheless, certain herbicides influence the microflora of the rehizosphere of cotton seedlings (McInroy and Kloepper 1991).

Regarding the microelement effects, it is well known that nutrient ions move in the soil towards plant roots by mass flow with soil water, or by diffusion. As for the agricultural soils, mass flow supplies Ca^{++}, Mg^{++}, CL^{-}, Mn^{++}, $SO4^{--}$, Mo^{+}, but other nutrients are supplied by diffusion . Plant genetics determine nutrients supplied by diffusion while environmental factors usually affect root extension and consequently the rhizosphere components (Drew 1990). Thus , the influence of the micronutrients Mn , Mo , and Cl were selected to investigate their influence on the host plants, disease incidence caused by *R.solani* and the rhizosphere microflora, separately or in the presence of the herbicides. Our results indicate that emergence 4 DAP or the formation of the first true leaf 11 DAP were the only two parameters affected by the interaction between herbicides and microelements. The microelements greatly affected stand percent during the first 11 DAP, while the herbicides expressed their maximum effects 11-32 DAP.

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تأثير مبيدات الحشائش وبعض العناصر الصغرى على الكائنات الدقيقه بالمجال الجذرى وعلاقتها بإصابة بادرات القطن بالفطر رايزوكتونياسولانى

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أستخدمت مبيدات الحشائش ستوب (بندا ميثيلين) وكوتوران (فلوميترون) وزويال (نورفلورازون) بالمعدلات الموصى بها رشا على سطح التربه ، عقب الزراعه وقبل الرى تحت ظروف الصوبه الزجاجيه. وباستخدام تربه طينيه رمليه غير معقمه من حقول زراعية ملوثة طبيعيا بالفطر رايزوكتونيا سولانى (AG-4) ، ونتج عن ذلك نقصاً معنوياً فى عدد بادرات القطن فى الاصص المعاملة بمبيدى الحشائش ستوب وكوتوران مقارنه بمبيد الحشائش زورياال أو بالأصص غير المعاملة بأى مبيدات حشائش (المقارنه)، أدت مبيدات الحشائش الثلاث الى زيادة معنوية فى تلون جذور البادرات الناتج عن أصابات متوسطه الشدة ، والى نقص معنوى فى عدد وحدات الكائن الممرض فى ١ جرام من عينات تربه المجال الجذرى (الريزوسفير) وذلك مقارنة بغير المعاملة ، وبرغم أن مساحة المجموع الجذرى فى بادرات القطن المعاملة بأي من مبيدات الحشائش الثلاثة كانت أقل ظاهريا ومعنويا منها في بادرات المقارنة بعد ١٤ يوما و ١٨ يوما من الزراعه الا أنه لم تظهر أى فروق معنوية فى عدد مستعمرات البكتريا أو الاكتينوميسينس فى تربة المجال الجذرى (الريزوسفير) فى جميع المعاملات بما فيها المقارنة ، على عكس ذلك كانت هناك فروقا معنوية بين المعاملات المختلفة فى عدد الوحدات القادرة على تكوين مستعمرات فطريه بعد ١٤ يوما وليس بعد ٢٨ يوما من الزراعة واشارت النتائج الى عدم وجود تأثيرات تداخلية لمبيدات الحشائش والعناصر الصغرى على عدد بادرات القطن أو على عدد الكائنات الدقيقة فى تربه الريزوسفير أو على أجناس الفطريات المعزولة من منطقة الريزوسفير ، ومع ذلك فقد كان هناك تأثيرا تداخليا لكل من مبيدات الحشائش والعناصر الصغرى أدى الى فروق معنويه بين المعاملات فى نسبة الانبات بعد أربعة أيام من الزراعه أو فى النسبة المئوية للنباتات التى نجحت فى تكوين الورقة الحقيقية الأولى بعد ١١ يوما من الزراعة.

كان لبعض العناصر الصغرى المختبرة والتى شملت سلفات المنجنيز ومولبيدات الصوديوم وكلوريد الصوديوم تأثيرات سلبية أو ايجابية على عدد النباتات الناتجه أو على عدد كائنات ميكروفلورا الريزوسفير عندما أستخدمت بمفردها فى غير وجود مبيدات الحشائش ، وأختلف التأثير سلبا أو ايجاباً بأختلاف المعاملة وبأختلاف مجموعة الكائنات الدقيقة (بكتريا – فطريات – اكتينوميسيس) ، الا أن أى من هذه المعاملات لم يكن لها تأثير يذكر على أجناس الفطريات المعزوله من ريزوسفير جذور نباتات القطن بعد ١٤ أو ٢٨ يوما من الزراعه مقارنة بنباتات المقارنة (الكونترول).