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BIOLOGICAL CONTROL OF *RHIZOCTONIA SOLANI* (AG-4) IN COTTON SEEDLINGS

S. M. MOUSTAFA MAHMOUD[1], MONA M. RAGAB[2], D. R. SUMNER[3] AND M. M. RAGAB[2]

1 Institute of Plant Pathology, Agricultural Research Center, Giza, Egypt.
2 Department of Plant Pathology, Fac. of Agric. Cairo Univ. Giza, Egypt.
3 Univ. of Georgia, Coastal Plain Experiment Staation, Tifton, GA 31793.

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Abstract

A significant interaction between antagonistic soil micro-organisms and cotton cultivars influenced the development of disease symptoms by *R. solani* AG-4 *in vivo*. Two isolates of each of *Trichoderma* spp. and G*liocladium* spp were selected from 50 different micro-organisms using a fast assay. *Rhizoctonia solani* (AG-4) was controlled successfully in the greenhouse when the biological agents were applied on autoclaved organic manure, but wheat bran or gel suspensions were not as efficacious. In field experiments, no significant differences were observed between the biological agents and nontreated control. Several other soil borne pathogens were isolated from diseased seedlings from the field in addition to *R. solani* AG-4.

INTRODUCTION

Rhizoctonia solani Kuhn anastomosis group (AG)-4 (teleomorph : *Thanatephorus cucumeris* (A. B. Frank), Donk) is one of the most important pathogens in cotton (*Gossypium* spp.) wherever the crop is grown. Bio-agents can

control root and hypocotyl diseases caused by *R. solani* in the greenhouse (Howell 1982) but their efficacy in the field is usually inconsistent (Sumner *et al.*, 1992). Performance of bio-control agents might be more consistent if indigenous antagonistic micro-organisms were used (Weller 1988). Cotton farmers have practiced biological control of plant pathogens since ancient times through the use of organic manure amendments (Sterling *et al.* 1989). One objective of this research was to select some indigenous micro-organisms having antagonistic potential against *R. solani* AG-4. Another objective was to compare the effectiveness of three biological agents when applied as a seed treatment (with gel suspension) or as a soil treatment (with wheat bran or autoclaved cow manure).

MATERIALS AND METHODS

The antagonistic potential of soil micro-organisms was tested first on PDA. Antagonistic cultures were evaluated again by the fast pre-screening assay. The most efficient bio-agents were then tested in the greenhouse. Finally, four of the best isolates were tested for efficacy in the field.

1. Testing the antagonistic potential of soil micro-organisms :

The method reported by Johnson and Curl (1972) was considered where more than 600 colonies of micro-organisms representing the total population on dilution plates from the rhizosphere of cotton seedlings were obtained randomly and transferred individually to 3 cm diameter plates of PDA. Preliminary assays were always done on 8 cm diameter plates of PDA where a 4-mm plug of actively growing hyphae of *R. solani* AG-4 was placed 4 cm from tester colonies of bacteria or 6 cm from 4 mm plugs of tester fungi. The antagonistic potential of all cultures was tested in the presence of a highly virulent isolate of *R. solani* belonging to AG-4 which was isolated from diseased cotton seedlings collected from the experimental fields in the previous season. Growth rates of both the pathogen and test micro-organism were determined 3-5 days after incubation at 26°C. Micro-organisms that had no antagonistic reaction against *R. solani* AG-4 were discarded and the remaining cultures were used in the succeeding experiments.

2. Selection of efficient biological agents by the fast pre-screening assay :

A fast pre-screening assay (Kloepper 1991) dependent on rating the development of symptoms in cotton radicles and hypocotyls was used to test the efficacy of 50 micro-organisms that had visible antagonistic potential against *R. solani* AG-4. A plug of each fungal culture was placed 2 cm from one side of the radicle or hypocotyl and a plug of *R. solani* was placed 2 cm from the other side. For bacteria and actinomycetes, the cotton radicle or hypocotyl was immersed in suspensions and placed in the middle of a plate of water agar (WA) and the plug of *R. solani* was placed 2 cm from the tissues. The assay with each micro-organism was conducted with five replications of each of five cotton cultivars (Tamcot CAB-CS, Taamcot CAMD-E, Taamcot SP-37, Lankart 57, and Delta Pina 90) to investigate the interaction between micro-organisms and cultivars. All pllates of each replication were incubated at 26^{o}C on the same shelf. Development of symptoms was rated every 24 hours for 6 days. The experimental design was a split-split-plot where micro-organisms were main plots, cultivars were sub-plots and dates of recording data were sub-sub-plots. In the statistical analysis a rating of 4 or less in radicle or hypocotyls was considered a survived plant and counted as one and ratings of 5-7 were considered dead and counted as zero. Ten micro-organisms were selected according to symptoms of necrosis caused by *R. solani* AG-4 on radicles, survival precentage and a disease index on hypocotyls in descending order.

3. Greenhouse experiments :

Two experiments were conducted in the greenhouse. One objective is to confirm the *in vitro* efficacy of selected cultures for biological control of *R. solani* AG-4 in steamed soil infested with the pathogen at 0.05% (w/w) and the other is to test their efficacy in naturally infested soil. In the first experiment, all of the isolates of *Trichoderma* spp. (7 cultures) and *Gliocladium* spp. (6 cultures) including two selected cultures of each genus showing the most efficacy in the laboratory, were tested in comparison to two standard isolates of *T. harzianum* Rifai [*T. harzianum* from the American Type Culture Collection ATCC 224243 (standard 1) and *T. harzianum* T-12, Dr. Gary Harman, New York State Agricultural Experiment Station, Geneva, NY, U.S.A. (Standard 2)]. Each culture was cultivated on 100ml autoclaved manure or wheat bran. The experimental disgn was a split-split-plot with four replications where the carriers were main plots, bio-agent were sub-plots and

date of counting plant stand was sub-sub plots.

In the second experiment, four selected micro-organisms (T_1, T_7, G_3, and G_4) were used as seed treatments for cv. Delta Pine 90 (not treated with fungicides), planted in natural soil compared with a seed treatment of the fungicide carboxin + thiram (applied commercially). Cotton seed not treated with fungicides and coated with a gel suspension was used as a control treatment. Ten ml. of conidial suspension (containing 3.4×10^8 conidia / ml) were applied to 20g of seeds and left to dry in a hood 2 hours before sowing (Moustaafa-Mahmoud 1993). A completely randomized experimental design with four replicates was used in this experiment.

4. Field evaluation of selected biological agents :

Two experiments were conducted in parallel in the field to evaluate the efficacy of two indigenous cultures of *Trichoderma* spp. and two of *Gliocladium* spp. (T_1, T_7, G_3, and G_4 respectively). In the first experiment, biological agents were delivered as a suspension of the conidia in a 2% aqueous gel sprayed on cotton seed not treated with fungicides. In the second experiment, biological agents grown on autocclaved cow manure were applied on Delta Pine 90 cotton seed commercially treated with the fungicide carboxin 17% : thiram 17% (Moustafa-Mahmoud, 1993). A completely randomized design was used in each experiment and T-test was used for statistical comparison in both experriments.

RESULTS

1. Selection of soil micro-organisms antaagonistic to R. solani AAG-4

A virulent isolate of R. solani AG-4 was used to test the antagonistic potential of more than 600 colonies of soil micro-organisms isolated randomly from the rhizosphere of cotton plants. Fifty micro-organisms having visible antagonistic potential against this virulent isolate on PDA, were selected according to their parasitism, rate of inhibition and morphological characters (Figure 1). These tester bio-agents included 10 bacteria, 7 actinomycetes and 33 fungi. The cultures of bacteria or actionomycetes were not identified, but the fungi were primarily

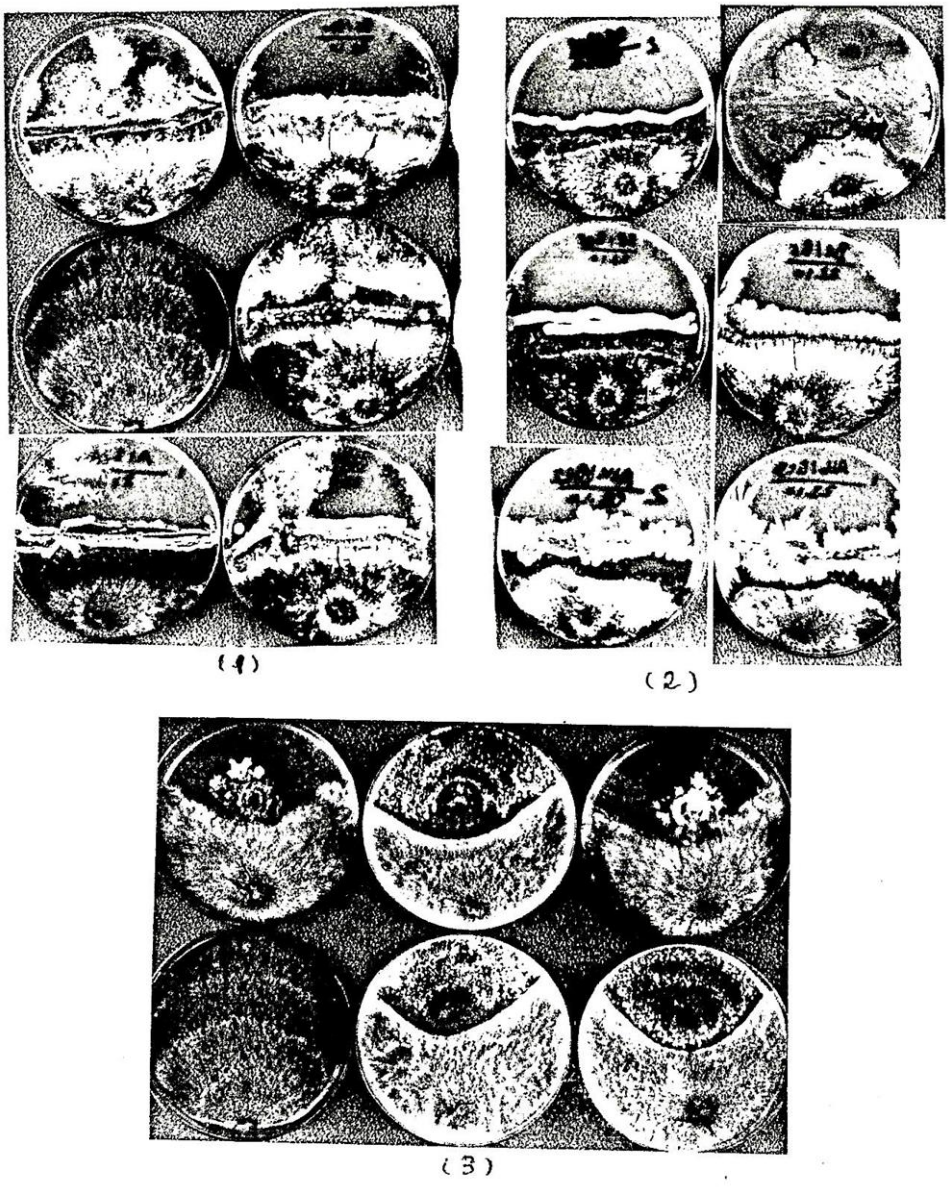

Fig. 1. Antagonistic potential of some indigenous soil microorganisms to *R. solani* AG-4 on PAD : (1) Actinomycetes, (2) Bacteria, (3) Fungi (*Trichoderma* spp. & *Laetisaria arvalis*).

Table 1. Analysis of variance of the fast prescreening assay for antagonism of 50 soil microorganisms against *R. solani* AG-4 in five cotton cultivars.

Source of Variance	df	ANOVA SS	F Value	PR>F	ANOVA SS	F Value	PR>F
		Radicle Assay			Hypocotyl Assay		
Soil microorganisms[y]	49	432.59	2.9	0.0001	177.91	4.3	0.0001
Cotton cultivarsy	4	144.93	11.8	0.0001	6.74	2.1	0.0025
Microorganisms x cultivars	196	599.8	4.9	0.0001	159.47	2.0	0.0001
Days of rating symptoms	5	4063.96	1288.5	0.0000	9813.92	4823.2	0.0000
Microorganisms x day	245	357.81	2.3	0.0001	333.72	3.35	0.0001
Cultivars x day	20	74.98	5.9	0.0001	35.39	4.35	0.0001

[y] Soil microorganisms and cotton cultivars were tested using microorganisms x cultivars as source of error.

Table 2. Fast prescreening assay for susceptibility of cotton cultivars to *R. solani* AG-4 in presence of soil micro-organisms.

Cotton cultivars [x]	Radicle Assay[z]		Hypocotyl Assay[z]	
	Mean of symptoms scale	Mean of survival	Mean of symptoms scale	Mean of survival
Tamcot CAB-CS	1.82c	0.80a	3.96b	0.55a
Tamcot CAMD-E	2.10c	0.73ab	3.95b	0.56a
Tamcot SP-37	2.02c	0.75a	3.93b	0.55a
Lankart 57	2.41b	0.66bc	4.00ab	0.52b
Delta Pine 90	2.71a	0.60c	4.12a	0.52b
L. S. D. (.05)	.29	.07	.15	.025

x Means of cotton cultivars across micro-organisms and time of rating symptoms development.

z Means followed by the same letter are not significantly different at P - .05 according to the T-test mean separation test.

Table 3. Biological control of *R. solani* AG-4 in cotton seedlings with *Trichoderma* spp. and *Gliocladium* spp. applied on autoclaved manure or wheat bran.

Treatment [x]	Stand [y] with manure (%)	Stand [y] with bran (%)
G1	89.2 a[z]	67.5 bcde
G2	80.0 b	65.0 def
G3	92.5 a	72.5 bc
G4	79.2 b	60.8 ef
G5	80.8 b	61.7 ef
G6	74.2 bc	70.0 bcd
T1	81.7 b	39.2 h
T2	78.3 bc	58.3 f
T3	90.8 a	50.0 g
T4	95.8 a	65.8 cde
T5	80.0 b	40.8 h
T6	76.7 bc	66.7 bcde
T7	94.2 a	61.7 ef
Standard 1	72.5 c	79.3 a
Standard 2	78.3 bc	39.2 h
Carrier	80.0 b	64.2 def
Control	52.5 e	57.8 f

[x] G - *Gliocladium* ssp., T - *Trichoderma* spp., standard 1 is ACCT24743 and standard 2 is T-12 YP, and carrier is autoclaved manure or wheat bran.

[y] 28 days after planting in pots with steamed soil. Percentage calculated from the number of seed planted.

[z] Mean followed by the same letter within column are not significantly different at P = 0.05 according to Waller Duncan separation test.

Table 4. Effectiveness of selected cultures of *Trichoderma* spp. and *Gliocladium* spp. applied to cotton seeds in a gel suspension on plant stand in pots with natural soil compared with a fungicide treatment with carboxinthiram.

Seed treatments of cotton	Stand [z] 28 days after planting (%)
Trichoderma spp. (T1)	47.5 b
Trichoderma spp. (T7)	31.5 cd
Gliocladium spp. (G3)	36.5 c
Gliocladium spp. (G4)	28.0 cd
Gel suspension (control)	20.5 d
Fungicide treatment (carboxin + thiram)	84.5 a

[z] Mean followed by the same letters are not significantly different at P = 0.05 according to Waller Duncan separation test.

Trichoderma spp., *Gliocladium* spp., or *Penicillium* spp. .

2. Selection of indigenous bio-agents by the fast prescreening assay:

The statistical analysis showed highly significant differences among soil micro-organisms and cotton cultivars and significant interactions between micro-organisms x cultivars, micro-organisms x day and cultivars x day in both the radicle and hypocotyl assays (Table 1). Separation test using means of cultivars across micro-organisms proved the relative resistance among the multi-adversity resistant (MAR) cultivars (Tamcot CAB-CS, Tamcot CAMD-E, and Tamcot SP-37) to a virulent isolate of *R. solani* AG-4 compared with the cultivars Lankart 57 and Delta Pine 90 in the presence of 50 tested micro-organisms (Table 2). The maximum development of disease symptoms occurred after 5 days in the hypocotyl assay and after 6 days in the radicle assay.

However, data that were used to select micro-oragnisms were based statistically on means of the cultivars Tamcot CAB-CS and Tamcot CAMD-E to avoid the interaction between micro-organisms and cultivars. For selecting indigenous bio-agents from the 50 antagonistic micro-organisms, micro-organisms that had a higher rating of symptoms in the radicle assay index were avoided in the first step of selection, and those that showed a lower rate of survival (in radicle assay) were avoided in the second step. Then, selected micro-organisms that had a higher rating of symptoms in the hypocotyl assay index were dropped (Moustafa-Mahmoud, 1993), and ten micro-organisms were selected for further studies in the greenhouse, including four cultures of *Trichoderma* spp. and two bacterial cultures. The *Penicillium* spp. and the bacteria were dropped because they showed phytotoxicity to cotton seedlings.

3. Efficiency of selected bioagents for controlling *R. solani* AG-4 in the greenhouse :

In the greenhouse experiment with soil infested with *R. solani* AG-4, all of the tested cultures of the bioagents *Trichoderma* spp. and *Gliocladium* spp. significantly controlled the disease incidence and increased percentage of stand 28 DAP, when applied on autoclaved cow manure as carrier compared to the control treatment (Table 3). The standard isolate ATCC 24243 (Standard 1) followed by *Gliocladium* spp. isolate G_3 showed the best control of *R. solani* AG-4 with wheat bran (Table 3).

In contrast, the selected isolates of *Trichoderma* spp. (T_1 and T_7) or *Gliocladium* spp. (G_3 and G_4) were not effective in imporoving plant stand in natural soil compared with the fungicide treatment of carboxin + thiram (Table 4). However, the biological treatment T_1 followed by G_3 increased plant stand significantly 28 DAP when compared with the gel suspension control (Table 4).

4. Field evaluation of selected biological control agents :

In field experiments during the spring of 1992, the selected indigenous cultures of *Trichoderma* spp. (T1 ,T7) and a culture of *Gliocladium* spp . (G3) had no significant effect on percentage of plant stand either when applied on autoclaved organic manure to seed treated with carboxin-thiram or applied as conidia suspended in a 2% gel solution to seed not treated with fungicides , compared with the control treatments . In addition to *R . solani* AG-4, *Pythium* spp ., *Fusarium spp.* and *Sclerotium rolfsii* were isolated from lesions on diseased seedlings 1-3 weeks after planting .

DISCUSSION

Bacteria , fungi and actinomycetes that naturally colonize the root surface of cotton were hypothesized to have a major role in resistance of MAR cultivars to cotton seedling diseases , and it was further hypothesized that their colonization of root systems is under the genetic control of the host (Bird 1982) . This hypothesis was confirmed in this study. Our results confirm the possibility of using the fast pre-screening assay to evaluate the micro-organisms antagonistic against *R . solani* AG-4 in the presence of the host plant (Kloepper 1991) . The indigenous micro -organisms that showed antagonistic potential against *R . solani* AG-4 on PDA plates also restricted the development of disease symptoms in the assay .

The selected bioagents of *Trichoderma* ssp . (T1 and T7) or *Gliocladium* ssp . (G3 and G4) successfully colonized the autoclaved cow manure and increased plant stand compared with the control treatment (52.5%) when delivered with autoclaved manure to soil infested with *R.solani* AG-4 in the greenhouse. In contrast , biocontrol treatments were ineffective in the field experiment . This may have been because several fungi were associated with the root disease complex .

Therefore , selection of a biological agent should depend on the antagonistic or parasitic potential of the organism against a wide range of pathogens without ignoring the role of the host plant .

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المقاومه الحيويه للفطر رايزوكتونيا سولاني AG-4

الذى يسبب مرض البادرات فى القطن

سامى محمد مصطفى محمود١ ، منى محمود ماهر رجب٢
دونالد روى سمنر٣ ، ماهر رجب٢

١ – معهد بحوث أمراض النبات – مركز البحوث الزراعيه بالجيزه .
٢ – قسم أمراض النبات بكلية الزراعه – جامعة القاهره .
٣ – جامعة جورجيا – محطة البحوث الزراعيه بتيفتون – جورجيا الولايات المتحده .

أظهرت الدراسات وجود تأثيرات تداخليه بين أربعه أصناف من القطن الأمريكى وعديد من كائنات التربه التى لها خاصيه التضاد للفطر رايزوكتونيا سولانى AG-4 على تكثيف وتطور ظهور الأعراض المرضيه على نباتات القطن فى أختبار معملى سريع على أطباق بترى باستخدام بيئه الآجار لتحديد القدره المرضيه لعزلات الكائن الممرض فى وجود النبات العائل ودلت النتائج أن عزلات الجنس ترايكود يرما أو الجنس جليوكلاد يوم كانت أكثر العزلات فعاليه فى الحد من تكشف وتطور الأعراض المرضيه للفطر رايزوكتونيا سولانى من بين ٥٠ عزله لكائنات المجموع الجذرى المضاده والتى تضم ١٠ عزلات بكتيريه ، ٧ عزلات أكتنوميستس ، ٣٣ عزله فطريه تتبع الأجناس ترايكود يرما وجليوكلاد يوم وبنسيليوم وأربعه أجناس أخرى لم يتم تعريفها.

نجحت عزلات الفطر تريكود يرما والفطر جليوكلاد يوم فى تقليل النسبه المئويه للإصابه تحت ظروف الصوبه الزجاجيه بإستخدام تربه زراعيه معقمه ومعديه صناعيا بالفطر رايزوكتونيا سولانى عندما أستخدم السماد العضوى المعقم كماده حامله للكائنات المضاده . الا أن المقاومه الحيويه للفطر كانت فعاله بدرجات متفاوته مقارنة بالأصص غير المعامله (المقارنه).

لم تكن هناك أى فروق معنويه فى تجارب الحقل بين المقاومه الحيويه بهذه العزلات المضاده وبين القطع التجريبيه غير المعامله سواء بأستخدام السماد العضوى المعقم كماده حامله أو بإستخدام المعلق الجيلاتينى ، الا أن عديدا من فطريات التربه الأخرى المسببه لمرض سقوط البادرات مثل سكليروشيوم رولفزياى وفيوزار يوم سولانى وفطريات الجنس بيثيوم قد أمكن عزلها من بادرات القطن المصابه فى تجارب الحقل . بالأضافه للفطر رايزوكتونيا سولانى .