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Effectiveness of Antagonistic Bacterial Isolates for Control *Rhizoctonia Solani* Kuhn on flax and Cotton

Ashour, A. Z. A.[1] and Aida H. Afify[2*]

[1] Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt
[2] Microbiol. Dept., Fac. Agric., Mansoura Univ., Mansoura, Egypt

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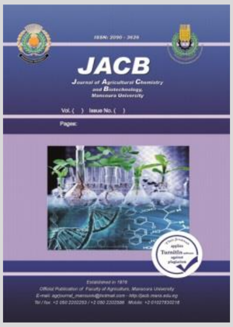

ABSTRACT

Phytopathogenic fungi infections, such as those caused by *Rhizoctonia solani*, result in significant yield losses in several economically important crops. To enhance biological control strategies, three aquatic bacterial strains were isolated from drinking water sources *i.e.* *Pantoea agglomerans* B1, *Serratia plymuthica* B2 and *Proteus mirabilis* B3 were evaluated *in vivo* against *R. solani* in flax and cotton. The infection-inhibition effect of three biocontrol agents was tested in glasshouse and field experiments. In flax, the bacterial strains demonstrated varying effects on seedling survival and technological characteristics. A positive correlation was observed between seedling survival (stand) and straw yield, along with its components. Among the tested strains, *Serratia plymuthica* B2 and *Pantoea agglomerans* B1 showed the highest efficacy under both glasshouse and field conditions compared to the untreated control. In cotton, the greatest disease suppression was achieved by strain B2, followed by strains B1 and B3 in both seasons, compared to the untreated control. The highest yield increases (kentar/feddan) were recorded as 5.342 and5.158 (B2), 5.005 and 4.911 (B1), and 4.644 and 4.394 (B3) during the 2023/2024 seasons, respectively. Finally, in glasshouse experiments, all bacterial biocontrol agents either completely or significantly limited seedling mortality in flax and reduced disease incidence in cotton seedlings caused by *R. solani*. Among them, strain B2 proved to be the most effective biocontrol agent. This study concludes that these bacterial strains, particularly B2, can be recommended as part of an integrated system for controlling *R. solani*, thereby improving straw yield quality in flax and increasing cotton yield.

Keywords: Antagonistic bacteria, Grown flax and cotton, *Rhizoctonia solani*

INTRODUCTION

Several economically crops worldwide are infect by widespread soil-borne pathogen such as *Rhizoctonia solani* kuhn and it causes serious damage of many crops production (Wolf and Verreet 1999). It is well known that *R. solani* attacks wide range of host plants and destroy crops. Under different environmental conditions, fungus of *Rhizoctonia* survives as sclerotia in soil for many years and as mycelium in organic matter. Therefore, control of this fungus is very difficult. Furthermore, another factor that increases problems to control of this fungus that organic matter supported the saprophytic life cycle of *R. solani* (Ogoshi 1987). Biological control is using antagonistic microorganisms biocontrol agents (BCAs) that an environmentally friendly to protect plants from soil-borne pathogens (Weller *et al.* 2002). Many reports have proved microorganisms to suppress diseases by *R. solani* (Ross *et al.* 1998). Additionally, important functional group of beneficial microorganisms for the control of soil-borne plant pathogens are antagonistic plant-associated bacteria (Weller *et al.* 2002). The high potential of bacteria which isolated from sources of drinking water to be used as a biocontrol agents for *R. solani in vitro* (Afify 2024). The strain *Serratia plymuthica* was most high effect for *Rhizoctonia* suppress *in vivo* experiments (Grosch *et al.* 2005). *Serratia marcescens* used for growth inhibition of phytopathogens through antibiosis (Tharmila Sivanantham *et al.* 2013). *Linum usitatismum* L. (Flax) plant is very important sources for fiber and oil. Fungal diseases such as seedling blight in flax caused by *R. solani* kuhn . Under favorable conditions, the fungus of *Rhizoctonia* attack the flax at seedling stage (Nyvall 1981). Cotton (*Gossypium barbadense* L.) is an important raw material for the textile industry and a source of oil for food production (Mayee *et al.* 2002). However, cotton is susceptible to many diseases, including damping-off caused by *R. solani* (Singh and Verma 1988). In recent years, biocontrol agents have been increasingly used for the biological control of soil-borne plant diseases (Saravanakumar *et al.* 2007). Flax seeds treated with biocontrol agents germinated faster and produced more seedlings compared to untreated controls (Afify and Ashour 2018). In cotton plants, studies by Ashour and Afify (1999) & Afify and Ashour (2024) reported that bacterial strains were effective in increasing seedling survival rates and dry weight, as well as improving plant stand and yield. Additionally, various bacterial strains have been shown to significantly enhance cotton yield (Aly *et al.* 2021). This approach also provides an opportunity to analyze the impact of field-applied bacteria on the indigenous plant microbiome (Bonaterra *et al.* 2022). The aim of this study was to evaluate the *in vivo* effectiveness of three bacterial strains—*Pantoea agglomerans* B1, *Serratia plymuthica* B2 and *Proteus mirabilis* B3—isolated from drinking water sources, against *Rhizoctonia solani* infections in flax and cotton under both glasshouse and field conditions.

MATERIALS AND METHODS

Bacterial strains

Bacterial strains are *Pantoea agglomerans* B1, *Serratia plymuthica* B2 and *Proteus mirabilis* B3. These

*** Corresponding author.**
E-mail address: aidaafify@yahoo.com
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strains were obtained from previous studies according to Afify *et al.* (2016) & Afify and AbdAllah (2023). The strains used as biocontrol agents (BCAs) when tested for production antagonistic metabolites can be reduced growth *Rhizoctonia solani* (Afify, 2024).

Pathogens

Inoculation of seed flax by *R. solani* (isolate R 1) isolated from rhizosphere of flax was used as well as seed cotton were inoculated with *R. solani* (isolate R 2) isolated from field. Identificaton of *Rhizoctonia* isolates were carried out at Plant Pathology Lab. Agriculture Research Center (ARC) Giza, Egypt.

Preparation inoculum of pathogens

Substrate for growth of fungi was prepared in bottles contained sorghum grains. Fungal of each inoculum was aseptically introduced into the bottles and mixed throughout with soil at rate of 0.1 g/kg of soil weight according to Schneider *et al*. (1997).

Bacterial seed treatment

1.5 ml of a bacterial suspension was obtained from each bacterial strain inoculum in nutrient broth medium and mixed with 5 g of each plant seeds (flax or cotton) in a small plastic bag and sown in potted soil of glasshouse and/or field experiment (Mew and Rosales 1986).

Experiments of flax

A glasshouse study was conducted using clay pots with a diameter of 20 cm. In the field experiment, treatments were sown in 1.5 × 4 m plots. The seed rate was 85.7 g/plot (equivalent to 60 kg/feddan). The soil used in both experiments was naturally clayey, with a pH of 7.5, clay content of 62.1%, and electrical conductivity (E.C.) of 1.4 mmhos/cm. Both trials followed a randomized complete block design (RCBD) with four replications. The glasshouse and field experiments were conducted during the 2022/2023 and 2023/2024 growing seasons. For the glasshouse study, 30 flax seeds (Sakha 1 cultivar) treated with bacterial suspensions were planted in each pot one week after soil infestation. The percentage of surviving seedlings was recorded 40 days after sowing in both the glasshouse and field experiments. Yield, yield components, and technological characteristics were recorded at the end of the growing seasons in the field trials.

Experiments of cotton

The three bacterial strains were tested against *R.solani*. The glasshouse study was conducted by using clay pots of 20 cm in diameter. Infested soils were planted with 10 cotton seeds per pot (cultivar Giza 89). In the field experiment, treatments were sown in 5.0 x2.4 m plots having 4(5.0 x 0.6 m rows). The soil used was naturally clay black soil (pH7.6 clay 67.0 %, E.C. 1.2 mmhos/cm). The design of layout of both trials was randomized complete block design with four replications. The experiments were carried out during 2022/2023 and 2023/2024 growing seasons. After one week of soils infection, soils were planted with the antagonistic coated seeds and kept under the decided conditions for 5-6 weeks. The percentage of surviving seedlings were recorded. Each treatment for each tested bio-controller was replicated 5 times among twice attempts in each season. Finally the produced cotton yield (kentar/fed) was recorded at the end of growth seasons (Henis *et al.* 1978).

Statistical analysis

Data from experiments were transformed into arc sine angles before carrying out analysis of variance (ANOVA) to produce approximately constant variance. Management and Analysis of Agronomic Research Experiments (MATAT-C, Michigan State Univ., USA).

RESULTS AND DISCUSSION

The three bacterial strains: *Pantoea agglomerans* B1, *Serratia plymuthica* B2 and *Proteus mirabilis* B3 in previous reports indicated suppressed fungal growth *in vitro* by the production of one or more antifungal materials (Afify, 2024). Therefore, the goal of this study was to prove the effect of three bacterial BACs (B1, B2 and B3) against *Rhizoctonia* disease in flax and cotton under glasshouse and field conditions. Inoculation with these microorganisms showed activity affect the growth of plants (Timofeeva *et al.* 2022).

Flax

Data in Tables (1) and (2) showed that all three bacterial strains were effective in increasing the seedlings surviving whether they were applied under glasshouse and field conditions however, their efficiency was always most high means with two strains of *Pantoea agglomerans* B1, *Serratia plymuthica* B2 under glasshouse (71.6 ; 71.4%) and field conditions (68.8 ; 73%) respectivelly. Also, the same of both strains were more effective in reducing disease incidence that control (38.8 ; 43.1) under glasshouse and field conditions.

Table 1. Effect of three bacterial strains isolated from sources of drinking water on seedling survival% of flax under glasshouse experiments

Treatment	seedlings survival%		Mean
	R. solani	Non infested soil[c]	
Pantoea agglomerans B1	65.2[a] (53.85)[b]	78.0 (62.05)	71.6 (57.95)
Serratia plymuthica B2	68.2 (55.56)	74.5 (59.68)	71.4 (57.62)
Proteus mirabilis B3	58.6 (49.97)	72.2 (58.19)	65.4 (54.08)
Nutrient broth	27.7 (31.76)	52.9 (46.67)	40.3 (39.21)
Control	24.9 (29.96)	52.2 (46.27)	38.6 (38.11)
L.S.D (p=0.05)	3.71		

[a] Percentage of surviving seedlings ; [b] Arc sine-transformed data ;
[c] Soil non infested with *R. solani*

Table 2. Effect of three bacterial strains isolated from sources of drinking water on seedling survival % of flax under field experiments

Treatment	seedling survival%		Mean
	2023	2024	
Pantoea agglomerans B1	69.5[a] (56.47)[b]	68.1 (55.60)	68.8 (56.03)
Serratia plymuthica B2	73.0 (58.71)	73.0 (58.71)	73.0 (58.71)
Proteus mirabilis B3	56.6 (48.77)	64.3 (53.33)	60.4 (51.05)
Nutrient broth	49.9 (44.98)	48.6 (44.18)	49.2 (44.58)
Control	43.5 (41.29)	42.8 (40.83)	43.1 (41.06)
L.S.D. (p= 0.05)	5.60	5.66	

[a] surviving seedlings, was calculated as Percentage of the planted seeds in a random row; ; [b] Arc sine-transformed data.

Data in Table (3) show that bacterial strains significantly increased straw yield per feddan as compared with untreated control. *Serratia plymuthica* B2 and *Pantoea agglomerans* B1 strains were effective in increasing straw yield per feddan and its components. While, the lowest value obtained from the untreated treatment. Data in Table (4) indicated that *Serratia plymuthica* B2 strain significantly increased fiber percentage, fiber length (cms), fiber yield per feddan (kgs) and fiber fineness under field experiments. These results are in agreement with previously reported results by other workers (Fukui *et al.* 1994; Pierson *et al.* 1995 and Yeom-Ju, Rip *et al.* 1995). The microorganisms can be found decrease the deleteious effects of pathogens on crop yield (Larkin 2020 and Mohammed *et al.* 2020).

Table 3. Mean values of yield and yield components of flax from analysis over seasons

Characters / Treatment	1	2	3	4	5	6	7	8	9	10
Pantoea agglomerans B1	90.81	2.02	2.78	3.728	10.03	14.28	117.81	1.148	9.12	0.584
Serratia plymuthica B2	92.83	2.01	2.88	3.890	9.87	12.79	106.27	1.038	9.11	0.591
Proteus mirabilis B3	76.78	2.11	2.17	3.187	18.51	25.86	213.35	2.021	9.16	0.786
Nutrient broth	72.78	2.13	2.00	2.986	17.22	38.78	319.94	2.979	9.21	0.987
Control	82.78	2.08	2.49	2.662	13.07	20.07	165.58	1.599	9.14	0.682
L.S.D. (0.05)	2.81	NS	0.09	0.622	2.85	0.21	0.02	0.209	0.12	0.123

1.Technical stem length (cm); 2. Stem diameter (mm); 3. Straw yield/plant (gm); 4. Straw yield/fed.(tons);
5. Fruiting zone length(cms); 6. Number of capsules/plant; 7. Number of seeds/plant; 8. Seed yield/plant (gm);
9. Seed index (weight 1000 seed); 10. Seed yield/fed. (tons).

Table 4. Mean values of technological characters of flax from analysis over seasons

Characters / Treatments	Fiber %	Fiber length(cms)	Fiber yield/fed. (kgs)	Fiber fineness
Pantoea agglomerans B1	11.76	81.50	443.28	260.78
Serratia plymuthica B2	12.78	85.55	485.14	271.82
Proteus mirabilis B3	10.58	74.52	356.23	242.71
Nutrient broth	10.12	67.53	310.78	220.81
Control	09.98	65.50	298.00	217.18
L.S.D. (0.05)	0.20	1.06	11.52	4.44

A positive correlation was observed between flax seedling survival (stand), straw yield and its components. The correlation was negative between stand and each of flax seed yield and its components (Table 5). These correlations are in agreement with the results of Abul-Dahab (2002) and Kineber (2003). These results suggest that the increase in seedling stand achieved by bacterial strains led to a quantitative increase in straw yield and a qualitative improvement in its technological traits. Additionally, these microorganisms can mitigate the deleterious effects of pathogens on crop yield (Larkin 2020; Mohammed *et al.* 2020). The biological control agents are bacteria can be use multiple mechanisms in the limitation of plant disease development and several bacterial-based products have been marketed as biopesticides (Bonaterra *et al.* 2022).

Table 5. Correlation coefficient (r) between flax seedling survival and each of yield , yield components , technological characters under field conditions

Seedlings survival% / Characters	r
Yield and yield components:	
1. Technical stem length (cm)	0.850**
2. Stem diameter (mm)	-0.800**
3. Straw yield/plant (gm)	0.688*
4. Straw yield/fed. (tons)	0.980**
5. Fruiting zone length (cms)	-0.685*
6. Number of capsules/plant	-0.785**
7. Number of seeds/plant	-0.781**
8. Seed yield/plant (gm)	-0.790**
9. Seed index (weight 1000 seed)	-0.811**
10. Seed yield/fed. (tons)	-0.762**
Technological characters:	
1.Fiber percentage	0.909**
2.Fiber length(cms)	0.451 NS
3.Fiber yield/fed. (kgs)	0.983**
4.Fiber fineness	0.990**

Linear correlation coefficient (r) is significant at p<0.01(**) or p<0.05 (*)

Cotton

Glasshouse the mean of data in both seasons (Table 6) showed highly significant effect of each biocontrol agents in 2023 and 2024 seasons. Biocontrol agents were found to represent the first important source of variation in disease incidence. The strain *Serratia plymuthica* B2 was the explained (model) variation in percentage of infection in both seasons. The strain *Pantoea agglomerans* B1 follwed by the strain *Proteus mirabilis* B3 gave the next importance respectivelly, in both seasons . Also, the mean of data in Table 7 indicated that, bacteria as a bioagents significantly reduced the disease incidence by *R.solani* in both seasons as compared with the untreated control. Among from them, *Serratia plymuthica* B2 and *Pantoea agglomerans* B1 reduced the incidence of disease to the lowest values while untreated treatment exhibited the maximum values. Bacterial strains enhanced the resistance in plants through the induction of defence enzymes in cotton plants (Rajendran and Samiyappan 2008). This work approach will allow to analyze the impact of field application bacteria on the indigenous microbiome of plants (Bonaterra *et al.* 2022).

Table 6. Effect of three bacterial strains isolated from sources of drinking water on the incidence of cotton seedlings disease under glasshouse conditions

Treatments	Deseased- Seedling%[a]	
	R. solani	**Non infested soil[b]**
Pantoea agglomerans B1	56.00 (48.46)	78.0 (62.05)
Serratia plymuthica B2	66.00 (54.49)	74.5 (59.68)
Proteus mirabilis B3	64.00 (53.14)	72.2 (58.19)
Nutrient broth	27.70 (31.76)	52.9 (46.67)
Control	81.63 (64.60)	52.2 (46.27)
L.S.D. (p=0.05)	3.294	

[a] **Percentage data were transformed into arc-sineangles before carying out the analysis of variance. Transformed means are shown in parentheses**
[b] **Soil non infested with *R. solani***

Data in Table (7) indicated that the bacterial strains as a whole decreased the incidence of disease as compared to the untreated control. Hence, these results are in full agreement with those reported by Kaur and Mukhopadhyay (1992). The successful application controlling cotton seedling damping-off under field conditions by strains of bacteria (Afify and Ashour 1995). This BCA could be a candidate for producing a antifungal materials against *Rhizoctonia* disease under field conditions (Grosch *et al.* 2005). Also, bacteria introduced the development of growth in plants seedlings (Toribio *et al.* 2020).

Table 7. Effect of three bacterial strains isolated from sources of drinking water on the incidence of cotton seedling diseased under field conditions

Treatment	Deseased- Seedling%	
	2023	2024
Pantoea agglomerans B1	54.44[a] (47.59)[b]	44.89 (42.05)
Serratia plymuthica B2	67.49 (55.36)	51.49 (45.86)
Proteus mirabilis B3	64.61 (53.63)	52.45 (46.44)
Nutrient broth	49.90 (44.98)	48.60 (44.18)
Control	74.69 (59.94)	63.36 (53.15)
L.S.D. (p= 0.05)	2.26	3.234

[a] surviving seedlings, was calculated as Percentage of the planted seeds in a random row ; [b] Arc sine-transformed data.

Among the strain *Serratia plymuthica* B2 increased seed cotton yield significantly up to the highest values i.e., 5.342 and 5.158 kentar/fed for 2023 and 2024 seasons (Table 8). Kloepper *et al.* (1980) reported that plants grown from seeds treated with gram- negative produced yield significantly higher than that obtained from untreated seeds. Weller and Cook (1986) reported that wheat damping-off disease caused by *Pythium* spp. was found to be controlled by bacteria applied as a seed treatment and to increase wheat yield by 26%. These bacteria as *Pantoea* and others strains leading to increase plant productivity (cotton yield and fiber quality) (Timofeeva *et al.* 2022).

Table 8. Effect of three bacterial strains isolated from sources of drinking water on cotton yield (kentar*/feddan) under field conditions

Treatment	Deseased- Seedling%	
	2023	2024
Pantoea agglomerans B1	5.005	4.911
Serratia plymuthica B2	5.342	5.158
Proteus mirabilis B3	4.644	4.394
Control	4.174	4.139
L.S.D. (p= 0.05)	0.4556	0.3101

CONCLUSIONS

This study, for the first time, illustrated the interaction between plants, *Rhizoctonia* and bacterial strains, highlighting the significance of this tripartite relationship in a bioprotection strategy against *Rhizoctonia* diseases. This approach is considered a promising method for delivering a wide range of biocontrol agents to the soil. The significant yield improvements observed in flax and cotton plants treated with *Pantoea agglomerans*, *Serratia plymuthica*, and *Proteus mirabilis*, formulated from strains isolated from drinking water sources, represent a novel strategy. This approach can be integrated as an environmentally friendly component of sustainable agricultural practices.

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فعالية العزلات البكتيرية المضادة لمقاومة فطرالريزوكتونيا سولانى الممرض فى الكتان والقطن

عبد الودود زكى عبدالله عاشور[1] و عايده حافظ عفيفى[2]

[1]معهد امراض النباتات- مركز البحوث الزراعية- الجيزه- مصر
[2]قسم الميكروبيولوجيا الزراعية- كلية الزراعة- جامعة المنصوره- المنصوره- مصر

الملخص

يسبب فطر الريزوكتونيا سولانى خسارة كبيرة فى المحصول لكثير من المحاصيل الزراعية الإقتصادية الهامة. ولكى يتم تطوير إستراتجية المقاومه الحيوية تم تقييم عزلات بكتيرية موجودة فى مصادر مياه للشرب هى: بنتويا أجلوميرانس و سيراتيا بلميثكيا و بروتيس ميرابلس فى مقاومة فطر الريزوكتونيا سولانى وذلك على محصولين هما الكتان والقطن , وقد تمت الدراسة فى تجارب زراعة للمحصولين فى البيوت الزجاجية وكذلك فى الحقل فى تربة ملوثة بفطر الريزوكتونيا سولانى وسجلت سلالات البكتيريا الثلاثة فعاليتها فى زيادة النسبة المئوية للبادرات الباقية على قيد الحياة لكل من الكتان والقطن.فى نبات الكتان سجلت السلالات البكتيرية الثلاثة مستويات مختلفة من التأثير على النسبة المئوية للبادرات الباقية على قيد الحياة , وعلى المحصول ومكوناتة وكذلك الصفات التكنولوجية للكتان. أظهرت العزلتان سيراتيا بلميثكيا و بنتويا أجلوميرانس أفضل تأثير تحت ظروف البيوت الزجاجية وكذلك فى الحقل بالمقارنة بمعاملة الكنترول (بدون إضافة السلالات البكتيرية).فى نبات القطن أظهرت النتائج أعلى القيم مع السلالة الثانية من البكتيريا سيراتيا بلميثكيا و تبعتها السلالة الأولى بنتويا أجلوميرانس وذلك فى موسمى زراعة القطن بالمقارنة بمعاملة الكنترول حيث كانت كمية محصول القطن (قنطار / فدان) نتيجة المعاملة بسلالة البكتيريا الثانية 5,342 و 5,158 أما فى حالة المعاملة بسلالة البكتيريا الأولى 5,005 و 4,911 وفى حالة المعاملة بسلالة البكتيريا الثالثة 4,644 و 4,394 هذا خلال موسمى زراعة القطن 2023/ 2024 بالمقارنة بمعاملة الكنترول.وبصفه عامة أظهرت نتائج البيوت الزجاجية أن جميع السلالات البكتيرية لها تأثير معنوى واضح فى النسبة المئوية للبادرات الباقية على قيد الحياة للكتان كما لوحظ إرتباط موجب بين نسبة الإنبات وكل من محصول القش ومكوناته, كما أن للسلالات البكتيرية تأثير معنوى فى تقليل بادرات القطن المصابة بفطر الريزوكتونيا سولانى.من هذه النتائج نجد أن فى كل التجارب السلالة البكتيرية الثانية سيراتيا بلميثكيا أظهرت أفضل تأثير فى المقاومة الحيوية لفطر الريزكتونيا سولانى. ولهذا يمكن القول أن هذه السلالات البكتيرية المستخدمة فى المقاومة الحيوية أدت إلى تحسين خواص الكتان التكنولوجية وزيادة فى محصول القطن الزهر وتثبيط نمو الفطر الممرض ريزوكتونيا سولانى.