J. Agric. Sci. Mansoura Univ., 28 (2): 1029 - 1038, 2003

EFFECT OF SEED TREATMENT WITH AN ANTAGONISTIC STRAIN OF *Pseudomonas fluorescens* ON SEED MYCOFLORA, GERMINATION,AND VIGOR OF SORGHUM (*Sorghum bicolor*) SEEDLINGS.

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ABSTRACT

A talc formulation of an antagonistic strain of *Pseudomonas fluorescens (Pf-1)* isolated from roots of rice plants was tested for its use as a seed dressing agent. Standard blotter test ,germination test ,vigor test and growing in water agar and peat soil were employed to test the effect of the talc formulation on sorghum seed mycoflora ,germination,seed vigor and rate of growth of seedlings.The formulation when applied to seeds inhibited seed-borne pathogenic fungi like *Fusarium moniliforme ,F.semitectum,Curvularia lunata and Phoma spp.* Also ,saprophtic fungi *like Aspergillus flavus ,Cladosporium cladosporoides* and *curvularia lunata* were inhibited on seeds .The treated seeds showed higher germination and the seedlings exhibited increased vigor .The possibility of using powdered talc formulation of *Pf-1* strain of *P.fluorescens* in routine seed treatments is recommended.

Keywords: *Pseudomonas fluorescens* ,sorghum, seed dressing ,seed vigor.

INTRODUCTION

Biological control is emerging as a promising control strategy for management of plant diseases.Some bacteria have shown potential to control plant diseases. Among them fluorescent pseudomonads are currently considered effective for control of root and foliar diseases.Strains of *Pseudomonas fluorescens* have been reported to be antagonistic to several disease causing pathogens (Howell and Stipanovic, 1979; Keel *et al.*,1991; Ownley *et al.*, 1992).Fungi like *Fusarium moniliforme ,Curvularia lunata* and *Phoma sorghina* are known to be frequntly associated with grain mold problem in sorghum (Mathur *et al.*,1975;Rao and Williams,1977; Castor,1977;Garud,*et al.*,1994 and Usha *et al.*,1994).While they reduce the marketing and nutritional value on sorghum grains on seed crops they reduce viability and vigor of seeds (Narashiman and Rangaswamy,1969;Tripathi,1974 and Mathur *et al.*,1975).Infected seeds can produce blighted seedlings upon sowing (Bhatnagar,1971).In addition to the above pathogenic fungi ,other fungi like *Aspergilis flavus, A.niger*,and *Cladosporium cladosporides* are known to affect seed quality during storage. They often reduce seed germination (Garud *et al.*,1994).In some instances *P.fluorescens* has been used as a seed dresser (Weeler and Cook,1983; Alstrom, 1991;Dilip Kumar and Dubey, 1992 ;Fukui *et al* ., 1994 and Vidyasekaran and Muthaimilan,1995) .The antagonistic strain of *P.fluorescens* used in this investigation was isolated from rhizosphere of rice plants cultivated in the Sabahya research station ,Alexandria ,Egypt.Out of 10 strains of *P.fluorescens* isolated strain *(Pf-1)* showed anatgonistic properties on pathogenic fungi like *Rhizoctonia solani* on rice and *Fusarium oxysporum*

f.sp. ciceris on chickpea (Dilip Kumar and Dubey,1992) In an earlier studies Rosales *et al.*,1995 observed that *P.fluorescens* talc formulation when applied to seeds reduced the incidence of *Bipolaris oryzae* and *Altarnaria padwickii* on rice seeds and contributed for increased germination of seeds and vigor of seedlings. Promoted by such results the present investigations were conducted to explore the possibilities of using this formulation to contorol the grain molds on sorghum seeds and to study its effect on seed mycoflora ,germination and vigor of sorghum seedlings.

MATERIALS AND METHODS

Preparation of talc formulation and seed treatment.

P.fluorescens bacterium was cultured on King *et al.*,`s B agar (KB) medium for 24 h at 25°C(Booth,1971).The inoculum suspension of the bacterium was prepared by incubation at 25 °C in darkness for 36 hours . The concentration of the bacterium suspension was adjusted to10 cfu/ml.Subsequently the culture was mixed with 500 g of sterile talc powder at pH 7 along with 1 % carboxymethylcellulose.Calcium carbonate was added When necessary to the talc powder before mixing bacterial suspension to adjust the pH of talc to 7 ±0.2.The talc formulation was then kept in polyethelen bags in the laboratory at 22± 2 °C.

Population of bacterium in the sample was estimated by serial dilution plating . The concentration of Pf-1 in the talc formulation was adjusted to 2 x 10 cfu /g . For seed treatment ,seeds were mixed with known volume of talc formulation and sterile water and shaken on a mechanical shaker . Untreated seeds were treated with talc powder and sterile water only . Treated seeds were allowed to air dry on laboratory benches and kept at room temperature until use.

Seed source

Sorghum seed varities used in this paper were collected from Nubaria research station ,Beheira Govovnorate,Egypt.

Effect of Pf 1 talc formulation on seed infection by several grain molds.

The health of the seeds was evaluated by blotter test (ISTA,1993) with eight replicates of 25 seeds each .Seeds were incubated for 7 days at 22 °C in alternating cycles of 12 hours of darkness and 12 hours under NUV light. Observations were made on the occurrence of fungi on seeds, more specifically of *Fusarium moniliforme, F. semitectum ,Curvularia lunata* and *Phoma sorghina* .

In vitro study of antagonism

F.moniliforme, P. sorghina ,A. tennuis and *C. lunata* were isolated from infected seeds under blotter tests . Pure culture of *P.fluorescens* (Pf-1) was maintained on King`s B(KB) medium Fifty µl of water suspension of Pf-1 (2x 10 cfu / ml) were streaked on PDA ,KB and on plates with two layers of

KB and PDA media (sandwich layers of 2mm thick) plates. Discs of mycelial mat (2 mm diameter) were taken from the growing edge of fresh and pure cultures of tested fungi and placed in a central well on the plate . The plates were incubated under alternating cycles of 12 h of near ultraviolet light (NUV)and 12 h of darkness then observed after 6 days .The growth of the tested fungus was estimated by measuring the length of the mycelial growth from the edge of the disc .

Germination test

The seeds were subjected to germination tests (ISTA, 1993). Fifty seeds in four replicates were germinated between tow rolled layers of moist paper towel .The rolled papers were wrapped in plastic bag then incubated in greminator cabinet at 25 °C in darkness .For evaluating germination first counting was made after 4 days of incubation .Fresh seeds and abnormal seedlings were counted after 10 days .The seedlings were classified into normal ,fresh ,abnormal and rot/dead for evaluations .The results of germination test was computed as the average of the replicates and expressed as a percentage of normal and abnormal seedlings and fresh and rot/dead seeds .

Vigor test

Effect of pf-1 talc formulation on vigor of sorghum seeds was tested by the method of Perry (1987) with few modifications. Stiff water absorbent blotter papers, 30 x 25 cm were used. A line was drawen at the center of the long axis of the parallel lines at I cm interval were drawen to one side of the central line .The central line was marked with 25 points at I cm interval to plote seeds .The seeds were gluted to the central line with a non toxic adhesive so that plumule pointed at right angles to the parallel lines and embryo facing away from the paper . Two additional sheets of paper were placed on top and one below the paper bearing seeds and the whole set was immeresed in water for a while to make the filter paper wet . Excess of water was allowed to drain from the papers and basal 2 cm was folded upwards . They were lossely rolled to form a tube of approximately 4 cm diameter and held with a rubber band The rolls were placed in metal boxes in upright position and incubated in a seed germinator at 25 °C in darkness for 5 days .At the end of 5 days the number of plumule tips which lied between each of the parallel lines were counted . Successive pairs of lines were given the value of the distance of the mid point from the centre line , ie , 1,2,3,4,.. and the numbers of plumule tips lying within each pair of line was multiplied by corresponding mid –point length and summed . The total length was divided by the number of seeds as follows :

$$L = \frac{(nx\,1 + nx2 \ldots + nx8)}{25}$$

where " L" is mean plumule length in cm , " n" is the number of plumule tips within the pair of parallel lines , " x" is the mid point distance from the central line. While Perry (1987) excluded the abnormal seedlings for calculation of

length we have included them for computation of seed vigor . Infected seedlings (whose germination was affected on account of infection)were abnormal seedlings and if they were left out of computation of seed vigor they fails to bring about such relation between seed infection and seed vigor , we have adopted this modification .

Germination in water agar

Fifty seeds were individually sown in16 mm diameter test tubes with 7 ml of 1 % water agar. The tubes were incubated for 7 days under alternating cycles of 12 hours of artificial day light and darkness . On the eighth day observation was made on germination , shoot length and cause of infection if any .

Germination in peat soil

Fifty seeds were sown in plastic pots containing peat soil , at the rate of one seed per pot .The pots were incubated for 7 days under alternating cycles of 12 hours of artificial day light and darkness. On the eighth day observation was made on germination and height of the seedlings .

All experiments were laid out in randomized block design and results were analysed stastistically using the software COSTAT.

RESULTS

Effect of Pf- 1 talc formulation on the grain molds incidence.

Seed treatment with Pf 1 talc formulation reduced the incidence of grain molds.Seed samples were infected by *C. lunata ,F.moniliforme, F.semitectum, P.sorghina ,Alternaria tennuis , Exhirohilum turcicum, Aspergillus niger, A.flavus ,Penicillium spp., Cladosporium cladosporoides* .The suppression of fungi and increased growth of seedlings raised from treated seeds was clearly evident in blotter testsTable(1) .Data on the supression of pathogenic fungi on sorghum seeds like *F.moniliforme* , *F.semitectum* and *C.lunata* is given in table 1. The highest reduction of these fungi was observed more at the high dose of 10 g/kg . The suppression was not complete .However , the growth of pathogenic fungi on treated seeds was less profuse in comparison to the untreated controls .

In vitro evaluation of antagonism

Data on the growth of *F.moniliforme* ,*C.lunata* and *P.fluorescens* smeared surface of agar media is presented in table 2. The growth of the two tested fungi was inhibited to a great extent by *P.fluorescens* . The assay medium did influence the expression of antagonistic relationship . On Kb medium *P.fluorescens* expressed the highest level of antagonism .However on **PDA** which is known to be favourable for growth of the tested fungi,the antagonistic activity was less pronounced .

Effect of *Pf1* on germination

The effect of *Pf-1* talc formulation on germination is presented in table 3.Higher germination was observed in treated seeds than in untreated

seeds. There was a positive correlation between the reduction in incidence of seed infection by *F.moniliforme* , *C.lunata* , *P.sorghina* , *A.tennuis* and other fungi and increase in seedlings germination . The number of abnormal seedlings and dead seeds were significantly low in treated seeds than in untreated controls.Higher germination was recorded at high dose of 10 g/kg than at 5 g/kg .

Effect of *Pf -1* on seed vigor

The talc formulation significantly increased the vigor of seeds,(table 4).

Effect of *Pf-1* on germination in water agar and peat soil

The effects of *Pf-1* talc formulation on germination in water agar and peat soil are presented in table 5 . The average growth indicated by the shoot length was higher in seedligs raised from treated seeds than in those from untreated controls. This was true for all the seed samples . Germination and average shoot length was increased at the highest doses .The treated seeds germinated earlier than untreated seeds . Some plants were allowed to grow further in growth chambers and after 30 days a remarkable significantly difference in the seedling height was observed in plants raised from treated seeds .

Table 1: Effect of talc formulation of *Pseudomonas fluorescene* on the incidence of *Curvularia lunata, Fusarium moniliforme,* and *Phoma sorghina* on sorghum seeds (evaluated in blotter tests).

Cultivars	Dose of Pf 1 talc formulation g/kg	*Curvularia lunata%*	*Fusarium moniliforme%*	*Phoma sorghina%*
Dorado	0	10	7.5	7.5
	5	4	2	3
	10	2	1	2
Giza 15	0	15.5	12.5	8
	5	2	2	1.5
	10	2	1.5	2
Giza 113	0	8.5	12	11.5
	5	1	1.5	4.5
	10	.5	1	1
SX1	0	11	5.5	0
	5	2	2	0
	10	1	3	0

Significant at P = 0.05

Table 2: Growth of tested fungi on *Pseudomonas fluorescens* (Pf-1) seeded media.

Tested fungi	Treatment (2x10 cfu/ml)	Average colony radius from the edge of the mycelial disc (cm)			
		KB(Kings mediumB)	PDA(Potato dextrose agar)	PDAtop and KB lower sandwich medium	KB top and PDA lower sandwich medium
Phoma sorghina	With Pf-1	0.08	1.06	0.82	0 83
	Control	2.77	3 91	3.86	3.45
Fusarium moniliforme	With Pf-1	0.15	1.35	1.33	1.30
	Control	3.33	4.28	4.30	4.21
Curvularia lunata	With Pf-1	0.09	1.64	1.33	1.05
	Control	2.82	3.91	4.21	3.23

Significant at p = 0.05

Table 3: Germination test results of sorghum seeds treated with different dose levels of talc formulation of *Pseudomonas fluorescens*.

Cultivars	Talc formulation (g)	%Germination			
		Normal seedlings	Abnormal seedlings	Dead and rot seeds	Fresh seeds
Dorado	0	68.0	6.0	16.5	9.5
	5	72.0	10.0	4.5	13.5
	10	81.5	2.0	9.5	7.0
Giza 15	0	60.5	6.0	24.5	9.0
	5	72.0	1.0	20.0	7.0
	10	67.5	4.5	13.0	6.5
Giza 113	0	53.0	8.0	30.0	8.5
	5	70.0	6.0	17.5	6.5
	10	73.0	11.5	9.5	6.0
SX1	0	65.0	16.5	19.0	0.0
	5	71.0	12.5	16.5	0.0
	10	85.5	7.5	7.0	0.0

Table 4: Effect of seed treatment with *Pseudomonas fluoescens* (talc formulation) on seedling vigor.

Cultivars	Talc formulation (g/kg) seeds		
	0 g/kg	5 g/kg	10 g/kg
Dorado	4.16a	4.83ab	5.64b
Giza 15	3.97a	4.59ab	5.39b
Giza 113	4.14a	4.74ab	5.50b
SX 1	3.05a	3.79a	4.12a

LSD.05=1.3

Data expressed as mean plumule length(cm).

Table 5: Effect of seed treatment with *Pseudomonas fluorescens* (talc formulation) on shoot length of plants rised in peat soil and water agar.

Cultivars	Average seedling height in water agar (cm).			Average seedling height in peat soil (cm).		
	0 g/kg	5 g/kg	10 g/kg	0 g/kg	5 g/kg	10 g/kg
Dorado	4.54a	4.91a	6.01b	4.42a	5.05ab	5.47b
Giza 15	4.57a	5.27ab	5.97b	4.72a	4.96a	5.82b
Giza 113	4.57a	5.08ab	5.59b	4.61a	5.09a	6.14b
SX 1	5.05a	6.39ab	6.50b	5.08a	6.22b	6.93b

LSD 0.05 = 0.75

DISCUSSION

Present results showed the efficacy of powdered talc based formulation of *P.fluorescens* as a seed dressing agent in reducing seed mycoflora , increasing germination and vigour of seeds The results indicate that seed treatment with talc fomulation of Pf 1 strain of *P. fluorescens* could be an effective strategy to manage seed–borne pathogens of sorghum and to reduce the use of seed dressing chemical fungicides .

Weeler and Cook (1983) , Alstrom (1991) , Ownly (1992) and other research workers have demonstrated the antagonistic activity of *P.fluorescens* and have shown the possibilities of using such strains for the management of soil–borne pathogens .Vidyasekaran and Muthiamilan (1995) identified the inhibitory action of Pf 1 strain of *P.fluorescens* on fungi like *Rhizoctonia solani* and *Fusarium oxysporium* .Our earlier investigations on rice have indicated the inhibitory action of Pf 1 strain of *P.fluoresces* towards important seed-borne pathogens like *A.padwickii* and *B.oryzae* . In conformity with the earlier observations in this paper we confirm the inhibitory effect of talc formulation of Pf 1 on seed-borne fungi of sorghum .

Grain molds on sorghum were not considered as major constraints to sorghum production programmes during 1960s (Taar, 1962) . As a result of continued research on sorghum in the semi arid tropical countries during 1970s high yielding , short-statured , short-growth period and fertilizer responsive hybrid sorghum were released.While these hybrids made sorghum production more profitable , new disease problems arose The International Workshop on Sorghum held at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Hydrabad in 1977 placed the grain mold problem as a major constrain in sorghum improvement programmes (Williams and Rao,1978). Fungi like *Fusarium moniliforme* ,*Curvularia lunata* and *Phoma sorghina* are known to be frequently associated with grain mold problem in sorghum (Mathur et al., 1975; Rao and Williams, 1977;Castor, 1977;Garud,*et al.*, 1994 and Usha *et al.*, 1994). While they reduce the marketing and nutritional value on sorghum grains on seed crops they reduce

viability and vigour of seeds (Narashiman and Rangaswamy, 1969; Tripathi, 1974 and Mathur *et al.*, 1975). Infected seeds can produce blighted seedlings upon sowing (Bhatnagar, 1971). In addition to the above pathogenic fungi , other fungi like *Aspergillus flavus* , *A.niger* ,and *Cladosporium cladosporoides* are known to affect seed quality during storage. They often reduce seed germination (Garud *et al.*, 1994). The present investigations indicate that Pf –1 talc formulation is effective not only against the pathogenic seed-borne fungi but also on the so called "saprophytic fungi" .Further research is perhaps is needed to study the utility talc formulation as a pre-storage seed dressing agent for seeds.

The germination of seeds and growth of seedlings was higher in Pf-1 treated seeds in comparison to the untreated controls . This was a consistant observation in germination tests , water agar germination and seedling symptom tests and germination in peat soil .Fluorescent pseudomonads are known to improve plant growth either through direct stimulation of the plant or suppression of pathogens(Leeman *et al.*,1995). The observed improvement in germination and growth of seedlings treated with Pf-1 talc formulation during the present investigations may be attributed to both direct stimulation of growth by *P.fluorescens* and supression of fungi on seeds, which could have interfered in seed germination.

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تأثير معاملة بذور الذرة الرفيعة بسلالات سيدوموناس فلورسنس المثبطة لميكوفلورا البذور على الانبات حيوية البادرات

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مركز البحوث الزراعية-معهد بحوث أمراض النباتات- الجيزة -مصر

تم أختبار مستحضر سلالات بكتريا سيدوموناس فلورسنس مع بودرة التلك بغرض أستخدامة كعامل مغلف لبذور الذرة الرفيعة وذلك بعد عزل هذة السلالات من جذور نباتات الارز . وأجري أختبار ورق الترشيح المبلل (البلوتر)وأختبار الانبات والحيوية وأختبار الانماء على الأجار المائى بالأضافة الى أختبار النمو فى تربة البيت لدراسة تأثير مخلوط البكتريا والتلك على نمو الميكوفلورا المصاحبة لبذور الذرة الرفيعة وأيضا تأثيرها على حيوية البذور ومعدل نمو البادرات . أظهرت نتائج البحث أنة بمعاملة البذور بهذا الخليط قد تم تثبيط بعض الفطريات الممرضة والمصاحبة للبذرة مثل فيوزاريوم مونيليفورم وفيوزاريوم سميتكتم وكرفيولاريا لوناتا وجنس الفوما وأيضا بعض الفطريات المترممة على البذرة مثل اسبرجلس فلافس وكلادوسبوريم كلادوسبوريدس قد تم تثبيطها تماما . كما أظهرت البذور المعاملة بالمخلوط نسب أعلى فى انباتها ونمو أقوى لبادراتها ومن خلال ذلك فيمكن التوصية باستخدام هذا المخلوط فى الاختبارات الروتينية لصحة وسلامة البذور .