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MINIMIZING OF *Fusarium oxysporum f.sp. NIVEUM* INFECTED WATERMELON SEEDS USING BIOCONTROL AGENTS

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ABSTRACT

Five different cultivars of watermelon seeds infected with a varied degrees of *Fusarium oxysporum f.sp. niveum* were treated with biocontrol agents. *Pseudomonas fluorescens, Trichoderma harzianum* and *Chaetomium globosum* at the rate of $1x10^8$ cfu g^{-1} and talcum based formulations of (28×10^7 $cfug^{-1}$), (19×10^7 $cfug^{-1}$) and (4×10^6 $cfug^{-}$) at the rate of 6 g kg^{-1} and 10g kg^{-1} of seeds were used, respectively. The treated seeds were evaluated for percent reduction of *F. oxysporum f.sp. niveum,* seed germination, vigour index and field emergence. It was found that *P. fluorescens* was more effective in reducing *F. oxysporum f.sp. niveum* infection followed by *T. harzianum* and *C. globosum* than vitavax 200 treated and untreated seeds. the formulations of *P. fluorescens* were effective in reducing *F. oxysporum f.sp. niveum* infection and also increasing seed germination, vigour index and field emergence, followed by *T. harzianum* and *C. globosum* treatments compared to control.
Keywords: Watermelon; *Fusarium oxysporum f.sp. niveum;* seed quality; field emergence; biocontrol agents

INTRODUCTION

Fusarium wilt of watermelon (Citrullus lanatus L.) occurs throughout the world and is often a limiting factor in watermelon production in Egypt. *Fusarium oxysporum f.sp. niveum* (E.F. Sm.) Snyder & H.N. Hansen [anamorph], causes wilt of watermelon, damping off, cortical rot ,stunting of seedlings and sudden progressive wilt of older plants. (Michail *et al.,*1989). *F. oxysporum f.sp. niveum* most important route of transmission is through seed, which can transport the pathogen into new watermelon-growing areas (Chen *et al.*, 1993).

F. oxysporum f.sp. niveum is currently controlled by seed dressing with fungicides, which polute the enviroment that has promoted a search for non-chemical seed treatments(Burgess and Hopworth ,1997) . However, application of a biocontrol agent to seed may provide a more convenient method for disease suppression. An isolate of *Trichoderma harzianum* from the rhizosphere of a cotton plant was found to be an effective biocontrol agent of *F. oxysporum f.sp. niveum* on watermelon. Application of the antagonist under field conditions as a seed coating decreased the incidence of the disease and increased yield (Sivan and Chet, 1986). *F. oxysporum f.sp. niveum* can be controlled by using bacterial antagonists such as *Pseudomonas fluorescens* and *P. putida* (Shim *et al.*, 1995). Three isolates of *Bacillus subtilis* from the rhizosphere of watermelon were also found to control seedling disease by *F. oxysporum f.sp. niveum* through seed bacterization (Lin *et al.*, 1997). *Trichoderma viride* and *Trichoderma harzianum* are fungal antagonists that can live in or colonize the rhizosphere of watermelon and inhibit *F. oxysporum f.sp. niveum* through : the production of antagonistic substances, nutrient competition and/or hyper-parasitic action (Zhao *et al.*, 1998). In Egypt, Michail *et al.*, (1989) reported that Fusarium wilt of watermelon could be controlled by cross protection i.e., Prior inoculation of plants with *F. oxysporum f.sp. cucumerinum,* which causes cucumber wilt disease, followed by the pathogen 5 days later resulted in no apparent wilt symptoms. In *in vitro* tests proved that the cucumber pathogen was antagonistic to *F. oxysporum f.sp. niveum.*

The objective of the present investigation was, therefore, to use bioagents instead of fungicides, to reduce the *F. oxysporum f.sp. niveum* incidence and to improve the seed quality.

MATERIALS AND METHODS

Source of seed sample

Watermelon seeds of five different cultivars, namely Giza 1, Dexli, Bikouk 60 , Crimson Sweet and Aswan were collected from West delta, (El-banger region).

Preparation of biocontrol agents

The antagonistic strains of *Pseudomonas fluorescens, Trichoderma harzianum* and *Chaetomium globosum* were isolated from the native soil, maintained on nutrient medium and then used as biocontrol agents. *P. fluorescens* was mass multiplied on kings 'B' medium and incubated at 26±1°C. After 48h of incubation, culture broth was centrifuged at 10 000 x g for five minutes. The pellet was suspended in sterile distilled water and used for seed treatment. *P fluorescens* formulation (28 x 10^7 $cfug^{-1}$) was prepared by mixing 100ml of *P fluorescens* suspension and 25g of talcum powder under sterile conditions. Carboxyl methyl cellulose (2.5g) was also added to 250g of formulation and stored in the form of talc and packed in polyethylene bags under ambient conditions (Rabindran *et al.*,1996). Bioagents, *T. harzianum* and *C. globosum* were mass multiplied on potato dextrose agar (PDA) petri plates and incubated at 22±2°C under12/12h/h cycles of a dark and NUV for 10 days. Conidial mass was suspended in sterile distilled water were used for seed treatment. Formulations were prepared by mixing the conidial mass with talcum powder (1:10w/w)and packed in polyethylene bags and stored under ambient conditions of 23±2°C and used as and when required.

Seed treatment with biocontrol agents

Watermelon seeds were treated with suspensions of either *P. fluorescens*, or *T. harzianum* and *C. globosum* at the rate of 1 x 10^8 $cfug^{-1}$ by mixing 400 seeds with 5ml of colonis/conidial suspension. Formulations of *P. fluorescens* (28 x 10^7 $cfug^{-1}$), *T. hazianum* (19 x 10^7 $cfug^{-1}$) and *C. globosum* (4 x 10^6 $cfug^{-1}$)in the form of slurry were applied for treatment of watermelon seeds at the rate of 6g kg^{-1} and 10g
kg^{-1} of seeds, respectively. After 24h the seeds were air dried and then infected with *F. oxysporum f.sp. niveum* , wilt incidence, germination, vigour index and field emergence were estimated.

Seed treatment with Vitavax 200

Commercially available fungicide Vitavax [carboxin] 200 was used,. Watermelon seeds were treated at the rate of 2g kg^{-1} of seed as slurry.

Screening for *F. oxysporum f.sp. niveum* incidence in the seed

400 seeds of each cultivar were screened to record the percent incidence of *F. oxysporum f.sp. niveum* by Standard Blotter Method (ISTA 1993) .

The germination test

Treated seeds (400) were placed between paper rolls in four replicates of 100 seeds each for germination. The rolls were kept at 23±2°C in a seed germinator. First count of normal seedlings was taken on the fourth day and the second count on the seventh day.

Vigour index (VI)

The root and shoot lengths of the normal seedlings were measured and vigour index (vi) was calculated using the formula of Abdul Baki *et al.*1973 :

VI =(mean root length + mean shoot length) x percentage germination.

Field emergence test

Field were ploughed well and the soil was leveled , fertiliser NPK was added at the rate of 33:15:48kgF^{-1} . Watermelon seeds treated with bioagents,Vitavax 200 and untreated seeds were performed in three replicates of 100 seeds each , sown in three rows (5m) randomly distributed, with an isolated distance of 2m between rows and 30cm between plants. The treatments were irrigated immediately after sowing. Seedling emergence was recorded seven days after sowing.

Statistical analysis

Data obtained with each cultivar was taken as replicate and values obtained to arcsin transformed and then subjected to analysis of variance.

RESULTS

Effect of biocontrol agents on incidence of *F. oxysporum f.sp. niveum:*

The effect of biocontrol agents on percent reduction of *F. oxysporum f.sp. niveum* incidence in treated seeds over control are presented in (Table 1). *P. fluorescens* at the rate of 1 x 10^8 cfug decrease the *F. oxysporum f.sp. niveum* incidence by 76%. On the other hand, 70% and 68% reduction was recorded for the talcum based formulations at the rate of 6g kg^{-1} and 10g kg^{-1} of seed, respectively. Pure culture of *T. harzianum* at the rate of 1 x 10^8 $cfug^{-1}$ decrease incidence of *F. oxysporum f.sp. niveum* by 67%, whereas talcum based formulation of the rate of 6g kg^{-1} and 10g kg^{-1} seeds reduced the incidence by 66% and 63%, respectively. *C. globosum* decrease the incidence of *F. oxysporum f.sp. niveum* by 65%.The talcum based formulation of the same reduce the incidence by 62% and 63%, respectively. Vitavax 200 treatment decreased the incidence by 57% .

Table 1 : Effect of different biocontrol agents on the incidence of seed-borne *F. oxysporum f.sp. niveum* in five tested cultivars of watermelon on standard blotter method.

Treatments	Incidence of *F. oxysporum f.sp. niveum* (%)					
	Cultivars					Mean[1]
	Giza1	Dexli	Bikouk 60	Crimson Sweet	Aswan	
Control	53.31	57.73	54.76	52.12	53.31	54.24±0.96h
Pseudomonas fluorescens ($1x10^8$cfug^{-1})	9.97	12.52	13.81	13.56	13.31	12.63±0.70a
Formulation of P. *fluorescens* (6gKg^{-1})	15.18	15.34	15.46	16.74	16.43	15.83±0.57b
Formulation of P. *fluorescens* (10gKg^{-1})	14.92	14.14	15.89	15.89	15.34	16.84±0.48bc
Trichoderma harzianum(10^8cfug^{-1})	16.11	16.95	18.91	18.15	17.75	17.57±0.53bcd
Formulation of *T. harzianum* (6gKg^{-1})	16.74	18.43	20.00	18.91	19.09	18.13±0.55bcd
Formulation of *T. harzianum* (10gKg^{-1})	17.43	17.46	19.64	18.43	18.72	19.89±0.73bcd
Chaetomium Globosum (10^8cfug^{-1})	20.70	16.43	20.00	18.43	18.91	18.89±0.73bcd
Formulation of *C. Globosum* (6gKg^{-1})	22.14	18.43	21.72	20.27	20.27	20.56±0.65bcdef
Formulation of *C. Globosum* (10gKg^{-1})	21.56	17.95	21.13	19.37	19.37	19.87±0.65bcde
Vitavax 200(2gKg^{-1})	24.88	24.58	19.37	23.17	24.88	23.37±1.05g

[1]Values given are means +SE. Figures followed by different letters in rows differ significantly when subjected to DMRT (P?0.05).

Effect of biocontrol agents on seed germination

Data presented in (Table 2) show that treatment of watermelon seeds with *P. fluorescens* at the rate of 1 x 10^8 cfug^{-1} increased the germination by 25% whereas the talcum based formulations increased the germination by 19% and 14%, respectively. *T. harzianum* increased the germination by 17%. Formulations of the same at 6g kg^{-1} and 10g kg^{-1} ed the germinations by 14% and 16%. Pure culture *C. globosum* increased the germination by 13%. However,the talcum based formulations of *C. globosum* at the rate of 6g kg^{-1} and 10g kg^{-1} seeds increased the germination by 11% and 12%, respectively. Vitavax 200 increased the germination by 5%.

Effect of biocontrol agents on seedling vigour

Data Table 3 show that followings : *P. fluorescens* (1 x 10^8 cfug^{-1}) increased seedling vigour by 59% while the talcum based formulations of *P. fluorescens* increased the vigour by 39% and 44%,respectively. *T. harzianum* increased the vigour by 28% and talcum based formulation increased the vigour by 28% and 26%. *C. globosum* increased the seedling vigour by 25% whereas formulations of *C. globosum* increased the vigour by 17% and 21%, respectively. Treatment with Vitavax 200 increased the seedling vigour by 20% .

Table 2 : Effect of different biocontrol agents on seed germination of the five watermelon tested cultivars.

Treatments	Germination (%)					Mean[1]
	Cultivars					
	Giza1	Dexli	Bikouk 60	Crimson Sweet	Aswan	
Control	57.61	57.73	57.42	58.24	58.05	57.81±0.14a
Pseudomonas fluorescens ($1x10^8 cfug^{-1}$)	73.26	72.85	72.24	73.05	71.57	72.59±0.30h
Formulation of P. *fluorescens* ($6gKg^{-1}$)	68.44	70.00	68.87	69.47	67.86	68.92±0.73cdefg
Formulation of P. *fluorescens* ($10gKg^{-1}$)	69.47	64.16	69.73	63.43	63.94	66.12±1.42cd
Trichoderma harzianum ($10^8 cfug^{-1}$)	69.12	69.47	66.42	66.25	67.21	67.69±0.67cdef
Formulation of *T. harzianum* ($6gKg^{-1}$)	65.65	67.78	66.82	66.12	66.42	66.35±0.46cd
Formulation of *T. harzianum* ($10gKg^{-1}$)	68.03	68.87	67.62	65.88	66.65	67.12±0.52cde
Chaetomium Globosum ($10^8 cfug^{-1}$)	64.90	64.90	66.03	65.12	66.03	65.39±0.26cd
Formulation of *C. Globosum* ($6gKg^{-1}$)	64.53	65.27	64.33	63.65	64.23	64.40±0.26c
Formulation of *C. Globosum* ($10gKg^{-1}$)	64.97	65.73	64.97	65.05	64.97	65.13±0.14c
Vitavax 200 ($2gKg^{-1}$)	59.34	61.00	65.42	58.89	60.20	60.97±1.17b

[1]Values given are means ±SE. Figures followed by different letters in rows differ significantly when subjected to DMRT (P?0.05).

Table 3 : Effect of different biocontrol agents on the seedling vigour index (VI) of watermelon tested cultivars.

Treatments	Vigour index (VI)					Mean[1]
	Cultivars					
	Giza1	Dexli	Bikouk 60	Crimson Sweet	Aswan	
Control	711.75	785.75	772.00	784.50	830.25	776.73± 19.14a
Pseudomonas fluorescens ($1x10^8 cfug^{-1}$)	1201.75	1316.25	1225.75	1191.00	1260.00	1238.95± 22.65f
Formulation of P. *fluorescens* ($6gKg^{-1}$)	1137.00	1120.00	1021.00	1041.25	1100.00	1083.85± 22.53bcde
Formulation of P. *fluorescens* ($10gKg^{-1}$)	1157.75	1227.75	1061.50	1078.25	1099.50	1124.95± 30.41bcde
Trichoderma harzianum ($10^8 cfug^{-1}$)	997.00	993.75	992.11	1022.50	984.25	997.92± 6.49bcd
Formulation of *T. harzianum* ($6gKg^{-1}$)	917.75	955.00	962.00	967.50	973.75	995.20± 9.85b
Formulation of *T. harzianum* ($10gKg^{-1}$)	987.75	980.00	991.75	987.00	980.00	985.20± 2.28bc
Chaetomium Globosum ($10^8 cfug^{-1}$)	980.10	970.00	985.75	960.00	965.00	972.71± 4.75bc
Formulation of *C. Globosum* ($6gKg^{-1}$)	870.50	863.25	959.50	940.50	910.25	908.80± 18.86b
Formulation of *C. Globosum* ($10gKg^{-1}$)	888.75	905.00	982.75	987.75	931.25	939.10± 20.03b
Vitavax 200 ($2gKg^{-1}$)	890.50	897.50	948.75	971.25	978.75	937.35± 18.40b

[1]Values given are means ±SE. Figures followed by different letters significantly differ when subjected to DMRT (P?0.05).

Effect of biocontrol agents on field emergence

Data Table 4 show the following : *P. flurescens* increased surviving seedlings by 22% while, it was 19% and 18%, respectively in case of its formular.. *T. harzianum* at the rate of 1 x 10^8cfug^{-1} increased the field emergence by 15% and it was 12&13% with it formulation. *C. globosum* increased the field emergence by 10% whereas the talcum based formulation of *C. globosum increased* field emergence by 9% and 10%, respectively Vitavax 200 increased the surviving seedlings by 6%.

Table 4 : Effect of different biocontrol agents on field emergence of watermelon in tested cultivars.

Treatments	Cultivars					Mean[1]
	Giza1	Dexli	Bikouk 60	Crimson Sweet	Aswan	
Control	55.73	60.33	60.67	58.23	59.34	58.86±0.89a
Pseudomonas fluorescens (1x10^8cfug^{-1})	72.85	70.63	70.63	58.25	73.05	72.04±0.57a
Formulation of P. *fluorescens* (6gKg^{-1})	70.36	69.30	69.30	73.05	70.00	69.99±0.76bcd
Formulation of P. *fluorescens* (10gKg^{-1})	70.36	65.73	69.73	66.03	71.57	69.47±o.93bcd
Trichoderma harzianum (10^8cfug^{-1})	68.03	70.00	66.82	65.97	70.36	67.91±1.09bcd
Formulation of *T. harzianum* (6gKg^{-1})	66.82	66.03	65.88	64.38	66.24	65.96±0.36bc
Formulation of *T. harzianum* (10gKg^{-1})	66.65	66.03	65.88	64.67	70.36	66.7±0.96bc
Chaetomium Globosum (10^8cfug^{-1})	63.58	63.22	65.80	65.42	67.78	65.16±0.86bc
Formulation of *C. Globosum* (6gKg^{-1})	63.22	61.82	64.38	64.53	67.78	64.30±0.98b
Formulation of *C. Globosum* (10gKg^{-1})	65.12	62.24	64.53	65.12	68.03	685.00±0.92bc
Vitavax 200 (2gKg^{-1})	63.58	60.67	60.87	61.14	64.38	62.16±0.77b

[1]Values given are means ±SE. Figures followed by different letters in rows differ significantly when subjected to DMRT (P?0.05).

DISCUSSION

In the present investigation, five different watermelon cultivars having different levels of *Fusarium oxysporum f.sp. niveum* infected seeds were treated, with *P. flurescens, T. harzianum* and *C. globosum* and evaluated for the reduction of *F. oxysporum f.sp. niveum* incidence, effect on the seed germination, seedling vigour, field emergence and grain yield. All the tested antagonistic organisms significantly, reduced *F. oxysporum f.sp. niveum* incidence increased seed germination, seedling vigour and field emergence, although the obtained results varied with different biocontrol treatments. High populations of actinomycetes, fluorescent *pseudomonads* and other bacteria occurred with successive plantings of susceptible cultivars. The obtained results suggeste that cultivar differences were responsible for the promotion of differences in rhizosphere microflora populations associated with soil suppressiveness (Hopkins et al., 1987; Larkin *et al.*, 1993). *T. harzianum* is known to control many fungal diseases , an isolate of *T. harzianum* from the rhizosphere of a cotton plant was found to be an effective biocontrol agent of *F. oxysporum f.sp. niveum* on watermelon (Sivan and Chet, 1986). Huang and Sun, (1991) reported that bacterial non-pathogenic isolates associated with watermelon roots inhibited chlamydospores germination and caused germ tube lysis of *F. oxysporum f.sp. niveum.* The culture filtrate of *C. globosum* reduced downy mildew incidence in pearl millet (Shishupala and Shetty, 1990) All the tested antagonists reduced *F. oxysporum f.sp. niveum* incidence in all the watermelon tested cultivars and the results indicated that the biological control agents are more effective than the recommended vitavax 200 to reduce *F. oxysporum f.sp. niveum* in watermelon seeds. Among the antagonists, *P. flurescens* was more effective in reducing seed-borne infection of *F. oxysporum f.sp. niveum* in watermelon seeds. All the three antagonists used were more effective in reducing *F. oxysporum f.sp. niveum* infection than the talcum based formulations. This may be due to the loss of viability of spores in the formulation (Sankar *et al.*,1996). All the three antagonists significantly increased seed germination. Vitavax 200 treated seeds showed higher seed germination than the untreated ones, and the antagonist treated seeds also showed higher percent of seed germination. This may be due to the reduction of *F. oxysporum f.sp. niveum* incidence. *P. flurescens* have been reported to increase the germination in treated seeds of radish in commercial greenhouses (Leeman *et al.*,1995). *T. harzianum* increased seed germination and seedling vigour of lettuce seeds (Gopinath *and Shetty,*1992). It has been suggested that some *Pseudomonas* have the ability to synthesise hydrogen cyanide which is known to inhibit the expression of pathogenic fungi, (Voisard *et al.*,1989), and also the ability to hydrolyse fusaric acid produce by some *Fusarium spp.*(Mauch *et al.*,1988). *P. flurescens* is known to produce the plant growth regulators like gibberellins, cytokinins and indole acetic acide (Dubeikovsky *et al.*,1993).The ability of *P. flurescens* to increase the field emergence, seedling vigour and germination was attributed to the plant growth promoting substances produced by the bacteria that could act to enhance various stages of plant growth (Brown,1974 and Davison, 1988). *T. harzianum* is known to produce chemical compounds such as chytinolytic enzymes, glucanase and proteases (Haran and Schickler,1996). The antagonistic nature of *C. globosum* was not studied well but the secondary metabolites of *C. globosum* have reduced the disease incidence in pearl millet (Shishupala and Shetty,1990). All the five different varieties of watermelon have shown a much increased vigour when treated with biocontrol agents than when treated with vitavax. The reason for the increase in vigour may be certain chemicals produced by *P. flurescens* , *T. harzianum* and *C. globosum* which are known to have increased growth rate as reported by(Lynch and Hobbie ,1991 and Kimura *et al.*, 1992). A similar type of higher field emergence was also observed under field conditions in all the tested cultivars of watermelon. In recent years much attention has been given to non-chemical systems for seed treatment as well as to protect them against seed-borne pathogens. The present study has shown that biological control agents like *P. flurescens T. harzianum* and *C. globosum,* which are eco-friendly and much more effective, can be used instead of fungicides.

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أستخدام العوامل الأحيائية فى خفض الأصابة بالفطر *Fusarium oxysporum f.sp. niveum* المصاحب لبذور البطيخ.

محمد رفعت رسمى
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عوملت بذور خمس أصناف من البطيخ مصابة بدرجات متفاوتة بالفطر فيوزاريوم أوكسيسبورم فورماسبيشيالس نيفيوم *Fusarium oxysporum f.sp. niveum* بالعوامل الحيوية، وهى البكتيرة سودوموناس فلوريسينس والفطريات تريكوديرما هارزيانيم و فطر كيتوميم جلوبوزم بمعدل ١٠×١^{٨} مستعمرة / جم بذرة لاختبار فاعليتها فى تقليل لقاح الفطر فيوزاريوم أوكسيسبورم فورماسبيشيالس نيفيوم *Fusarium oxysporum f.sp. niveum* و عوملت بها البذور بطريقتين الأولى إضافة معلقات مزارع العوامل الأحيائية مباشرة إلى البذور والثانية خلط مزارع العوامل الأحيائية بأحد المواد الحاملة مثل بودرة التلك لتكوين مسحوق مركب حيوي بمعدل ١٠×٢٨^{٧} مستعمرة / جم بذرة , ١٠×٢٩^{٧} جرثومة / جم بذرة , ١٠×٤^{٦} جرثومة / جم بذرة على التولى وبمعدلى أضافة هما ٦جم / كجم بذرة و ١٠جم / كجم بذرة. تم تقييم للبذور المعاملة من حيث درجة الإصابة ونسبة الإنبات وحيوية وقوة نمو البادرات الطبيعية vigour index ونسبة ظهور النباتات فى الحقل field emergence. وقد وجد أن البكتيرة سودوموناس فلوريسينس كانت أشد تأثيرا فى تقليل الإصابة بالفطر فيوزاريوم أوكسيسبورم فورماسبيشيالس نيفيوم *Fusarium oxysporum f.sp. niveum* يليها الفطر تريكوديرما هارزيانيم ثم الفطر كيتوميم جلوبوزم مقارنة بالمبيد الفطرى فيتافاكس ٢٠٠ وذلك فى حالة البذور المعاملة و الغير معاملة. أدى المركب الحيوى للبكتيرة سودوموناس فلوريسينس ألى تقليل نسبة الإصابة بالفطر فيوزاريوم أوكسيسبورم فورماسبيشيالس نيفيوم كما أدى الى زيادة نسبة إنبات بذور البطيخ وإنتاج بادرات قوية ذات شكل طبيعى مع أرتفاع نسبة ظهور النباتات فى الحقل، تلى ذلك الفطرين تريكوديرما هارزيانيم و كيتوميم جلوبوزم وذلك مقارنة بالكنترول.