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IN VIVO ANTAGONISTIC CAPABILITIES OF VARIOUS *Bacillus subtilis* AND *Streptomyces* SPP. FORMULATIONS AGAINST *Acremonium strictum*.

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ABSTRACT

Greenhouse and field experiments were conducted to identify the most effective and applicable formulation of *B. subtilis* and *Sterptomyces* sp. either singly or in combination for controlling Acremonium wilt in sorghum cv. Giza 113 .The single and combined (1:1) inocula of *B. subtilis* (isolate No. 9), *Streptomyces* sp. (isolate No. 3) as well as the fungicide Occidor Plus (carbendazim 50 % S.C.) significantly ($p<0.05$) reduced Acremonium wilt incidence in grain sorghum plants grown in pots. The tested carriers *i.e.*, vermiculite, peat-moss, kaolinite, bentonite, wheat bran and talc powder favoured the antagonistic capabilities of single and combined inocula bioagents. Data of the field experiment showed no significant difference between a combination of the two bioagents and the fungicide with respect to average percentage of infection and average grain yield. Vermiculite-entrapped cells were superior in reducing infection to19.83 % and increasing grain yield to 127.6 $g.plant^{-1}$ compared to 25.75 % and 113.0 $g.plant^{-1}$ for control, respectively.The data suggested the use of a combined inoculum of the examined bioagents entrapped on vermiculite for control of Acremonium wilt in grain sorghum plants.

Keywords: *Acremonium strictum*, sorghum, antagonistic fomulations.

INTRODUCTION

Due to the steadily increasing in the area cultivated with sorghum in Egypt and all over the world, grain sorghum diseases have become a serious problem particularly Acremonium wilt caused by *Acremonium strictum*. Biological control of such disease has received increasing interest in the racent years as an alternative strategy for reducing the hazardous impact of pesticides on the ecosystem (Lockwood, 1988 ; Abd El-Moity *et al.*, 1991). Incorporation of specific microbial antagonists into soil or to planting materials is a direct approach of the strategy (Deacon, 1994 and Yates *et al.*, 1999). In this regard, several strains of *Bacillus* and *Streptomyces* have received special attention as biocontrol agents for plant protection against seed and soil-borne pathogenic fungi (Cook and Baker, 1983; Kim *et al.*, 1997).

Different materials such as peat moss, vermiculite, talc powder and bentonite are widely used as carriers or seed coating materials for introducing antagonistic formulations in the area of microbial control of some plant diseases. In the majoirity of cases, such formullations succefully improved plant growth and reduced the incidence of the disease (Gasoni *et al.*, 1998; Rodham *et al.*, 1999; Ibrahim and Zein El-Abedeen, 2000). However, the antagonistic potentiality of a biocontrol agent varies with the environment, biocontrol agent, formulation of the antagonist, type of the pathogen and the host genotype (Ferrira *et al.*, 1991). The future of biocontrol of soil-borne plant pathogens probably lies in integrated control system, that combines, the developments of crop cultivars and cropping practices in a way that is intended to exploit both nature and inoculated biocontrol agents not alone but in concert (Deacon and Berry 1992).

The present work was designed to evaluate the efficacy of some formulations of *B. subtilis*, *Streptomyces* spp. and their mixture in controlling Acremonium wilt disease in grain sorghum cv. Giza 113 under greenhouse and field conditions in order to select the supperior candidate for application as a grain sorghum-cropping- practice.

MATERIALS AND METHODS

Microorganisms

Acremonium wilt causing fungus, *Acremonium strictum* (isolate No.9) isolated by Ali *et.al.*(2004a) from Tameya, El Fayoum , was used for soil infestation. The superiority of *Bacillus subtilis* (isolate No. 9) and *Streptomyces* spp. (isolate No. 3) among variuos antagonistic microorganisms was proven in previous work (Ali *et.al.*,2004b); therefore, they were selected to carry out the present study.

Sorghum grains

Grains of the Acremonium wilt-susceptible sorghum cultivar Giza-113 were obtained from Sorghum Res. Dept., Field Crops Res. Inst., ARC, Giza, Egypt and used in both greenhouse and field experiments.

Carriers

Peat-moss, vermiculate, kaolinite and bentonite were kindly provided by Soils, Water and Environment Res. Inst., ARC, Giza, Egypt (Amendments Project). Talc powder and wheat bran were purchased from local market.

Fungicide

Occidor Plus [carbendazim (methybenzimidazole-2-ylcarbamate) 50% S.C.] was purchased from El-Wattania Chemical Company, Cairo, Egypt.

Greenhouse experiment

The experiment was conducted in the greenhouse of Maize, Sugar and Forage Crops Res. Dis. Dept., Plant Pathol. Res. Inst., ARC. Giza, during summer season of 2002. Talc powder, peat-moss, wheat bran, vermiculite, kaolinite and bentonite were examined as carriers using gum Arabic as adhesive agent.

Soil was infested prior to sowing in pots (25cm and 40cm height) filled with loamy sand soil from Giza Exp. Stat., ARC. Broth culture of *Bacillus subtilis* (isolate No. 9) and a spore suspension of *Streptomyces* sp. (isolate No. 3) were prepared as previously described by Ali *et al.* (2004b) containing 10^8 cfu ml^{-1} and used for preparing the various antagonistic formulations. One liter of a culture suspension was added to 1 kg carrier material for preparing a single formulation and 0.5 liter from each suspension were mixed with 1 kg of carrier to obtain the two-membered antagonistic formulation.

Eight grams of a formulation was thoroughly mixed with 20 g of gum Arabic coated-seeds and left overnight to dry. Five treated seeds were sown in each pot and 3 sets of pots were included for comparison.The first was sown with seeds coated with the carrier materials without antagonist, the second was sown with seeds treated with Occidor Plus at the rate of 3 g. kg^{-1} grains while the third was sown with seeds recieved the antagonists without a carrier . Pots were arranged in a complete randomised design with 4 replicates for each treatment. The temperature in the greenhouse was adjusted to 28 ± 2 °C. Plants were fertilized 21 days after sowing at a rate of 3 g urea (46 % N) pot^{-1} and watered regularly using tap water. Percentages of infected plants were recorded 90 days after sowing and transferred to arc sine.

Field experiment

Two field trials were excuted at Giza and Sids Experimental Stations, Agric. Res. Center during 2002's cultivation season.The same materials, treatments and techniques mentioned in the pot experiment were applied in both fields in a split-plot design.

Acremonium strictum inoculum was distributed in hills, at a rate of 50 g fresh weight $hill^{-1}$, just before sowing. Four treated seeds were sown in a hill and thinned 21 days later to one seedling.Treatments were replicated four times each replicate of two rows, each contained 20 hills at 30 cm apart.. Plants were fertilized at a rate of 150 kg.$fed.^{1}$ ammonium nitrate (33.5% N), 21 days after sowing. Disease readings were recorded as percentages of infected plants, and transferred to arc sine. Dry grain yield was recorded after 120 days of sward establishment.

Data were subjected to statistical analysis according to the procedures reported by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Antibiosis in greenhouse experiment

Data in Table (1) show that the percentage of infected plants was significantly ($P<0.05$) reduced as a result of applying the antagonistic isolates. All the tested carriers supported the antagonistic effect of the applied biocontrol agents. These results are in harmony with those of El-Khateeb *et al.* (1990) and Ibrahim and Zein El-Abedeen (2000). Very pronounced effect of carrier type on Acremonium wilt incidence in sorghum plants was recorded. The infection percentages caused by *A. strictum* in presence of entrapped antagonists to various carriers ranged between 0.0 to 27.3 being the highest with vermiculite and the lowest with bentonite. As the most favourable carrier for both single or the two-membered antagonistic formulation, vermiculite showed the least severity of Acremonium wilt disease with mean infection of 11.52% followed by talc powder (14.93%), peat-moss (16.28%), wheat bran

(17.54), kaolinite (18.36%) and bentonite (19.03%). All carrier-free treatments were the least effective exhibiting infection rate of 24.48%.

The superiority of vermiculite as a carrier was previously recorded by Abdel-Azeem (1998) who found that vermiculite, as a carrier for *Bacillus megatherium*, was better than wheat bran, peat-moss, ground corn cobs and dried clover meal. Moreover, Steinmetz and Schonbeck (1992) found that both *T. harzianum* and *Gliocladium roseum* grew actively in vermiculite much better than other carriers and their formulations were used with great success in controlling damping-off of lettuce caused by *Rhizoctinia solani*.

Table (1) : Effect of various formulations of *B. subtilis* (isolate No.9) (A), *Streptomyces* spp. (isolate No. 3) (B), their mixture (A+B) and Occider Plus(O) on the incidence of Acremonium wilt disease in grain sorghum plants (Giza 113 cv.) under greenhouse conditions

Treatments	Carriers							
	Bento- nite	Peat-moss	Vermi culite	Talc powder	Wheat bran	Kaol- inite	Without carrier	Mean
A	9.28*	9.28	0.00	8.33	16.43	19.46	22.46	12.18
B	27.28	22.22	7.71	8.72	21.39	22.46	30.13	19.99
A + B	8.72	0.00	0.00	7.71	0.00	0.00	19.91	5.19
O	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contro l	49.89	49.89	49.89	49.89	49.89	49.89	49.89	49.89
Mean	19.03	16.28	11.52	14.93	17.54	18.36	24.48	17.45

L.S.D.($p<0.05$) : Treatment(T)= 2.88, Carrier (C)= 2.12 , T x C= 5.92

* infection rate.

With respect to the effect of type of antagonist, it appears that the two-membered antagonists grew rapidly and colonized efficiently in four of the tested carriers namely vermiculite, peat-moss, wheat bran and kaolinite. Acremonium wilt was never detected with these formulations. Such higher performances compared to single formulations might be explained by a synergistic relationship between the two candidates as described by Baker and Cook (1974) and Deacon (1994) and/or a combined antagonistic effect caused by a complementary set of antifungal metabolites (Novikova *et al.*, 1995) which might not be the case with single antagonist formulations.The interaction between carriers and antagonists confirmed the antagonistic potentiality of the two-membered formulation with significant differences compared to other formulations (Table 1). Their formulation displayed the lowest mean percentage of infection (5.19), followed by the single formulation of *B. subtilis* (12.8) and finally *Streptomyces* sp. (49.89). Again, treatment with the fungicide Occider Plus resulted in complete suppression to growth of the causal pathogen (*A. strictum*).

Effect of various antagonistic formulations on Acremonium wilt disease incidence under field conditions

Table (2) presents the results with respect to disease incidence as affected by type of antagonist, nature of carrier and locality of application. Surprisingly, the two-membered antagonist performed extraordinarily, when formulated with either vermiculite or talc powder, as carrier, achieving complete elimination of Acremonium wilt (0.0% infection) in plants grown at Giza Station. This was never happened even with the fungicide Occidor Plus which displayed an infection percentage of 10.78 at both farms. However, when the mean values of infection percentages for the various treatments were considered, different picture was seen as such influence was masked.

Table(2): Effect of various formulations of *B. subtilis* (isolate No.9)(A) *Streptomyces* sp. (isolate No.3)(B), their mixture (A+B) and Occider Plus(O) on the incidence of Acremonium wilt disease in grain sorghum under field conditions.

Location	Treatments	Carriers							Mean
		Bentonite	Peat-moss	Vermiculite	Talc powder	Wheat bran	Kaolinite	Without carrier	
Giza	A	21.81*	17.56	9.28	17.16	18.81	20.96	25.92	18.79
	B	22.46	18.44	17.95	21.81	22.46	22.06	25.40	21.51
	A + B	17.56	17.16	0.00	0.00	9.28	17.56	20.44	11.77
	O	10.78	10.78	10.78	10.78	10.78	10.78	10.78	10.78
	Control	49.14	49.14	49.14	49.14	49.14	49.14	49.14	49.14
	Mean	24.35	22.62	17.43	19.78	22.09	24.10	26.34	22.39
Sids	A	17.56	12.66	17.56	16.74	19.55	17.95	20.96	17.57
	B	21.39	18.44	21.81	17.16	22.46	25.92	22.46	21.38
	A + B	9.81	9.81	9.28	8.91	18.91	17.16	18.05	13.13
	O	10.31	10.31	10.31	10.31	10.31	10.31	10.31	10.31
	Control	52.18	52.18	52.18	52.18	52.18	52.18	52.18	52.18
	Mean	22.25	20.68	22.23	21.06	24.68	24.70	24.79	22.91
Mean	A	19.69	15.11	13.42	16.95	19.18	19.46	23.44	18.18
	B	21.93	18.44	19.88	19.49	22.46	23.99	23.93	21.45
	A + B	13.69	13.49	4.64	4.46	14.10	17.36	19.25	12.43
	O	10.55	10.55	10.55	10.55	10.55	10.55	10.55	10.55
	Control	50.66	50.66	50.66	50.66	50.66	50.66	50.66	50.66
	Average	23.30	21.65	19.83	20.42	23.39	24.40	25.57	22.65

L.S.D. 5: Location (L)= 0.4, Treatment (T) = 3.26, T x C= 6.22, Carrier = 2.72, L x T x C= 5.37, L x T = 4.47 , L x C= Not significant

*infection percentage

Generally, all the control treatments showed significant differences and all carriers favoured the biocontrol agents either singly or in combination. As expected, the best disease control was obtained by the chemical fungicide treatment with mean infection rate of only 10.55 % followed by the two-membered formulation (12.43 %), *B. subtilis* (18.18 %) and *Streptomyces* sp. (21.45 %). These results are in conformity with those obtained from the pot experiment.

Regarding the influence of carriers and similar to the results obtained from the greenhouse experiment, vermiculite deemed the most favourable carrier in accommodating and maintaining the activities of all antagonists, singly or in combination, with an average infection rate of 19.83 % followed in a descending order by talc powder (20.42 %), peat-moss (21.65 %), bentonite (23.30 %), wheat bran (23.39 %) and kaolinite (24.40 %).

Results in the two sites, Giza and Sids Stations showed significant differences.At6 Giza Station, lower infection rate compared to Sids was reported, this is most probably due to geographical and environmental differences. For instance, an infection rate of 9.28% was recovered at Sids with the vermiculite entrapped two-membered formulation being in use, compared with infection absence at Giza using the same microbial preparation.

On the other hand, the interaction between the two locations and the various treatments resulted in significant differences on the disease incidence. However, the interaction between locations and carriers showed no significant ($P<0.05$) effect on disease occurrence.

The interaction between locations, treatments and carriers showed significant ($P<0.05$) differences, as biocontrol treatments significantly affected by environmental conditions of both locations. This confirms the findings of Ferrira *et al.* (1991) and Deacon and Berry (1992) that the antagonistic potentiality of biocontrol agents varies with environments, biocontrol agents, formulation of the antagonist, the genotype of either pathogen or the host plant.

Grain yield was obviously influenced by the various treatments. The results shown in Table (3) revealed that all treatments resulted in significant increases in grain yield in both locations compared to control, but with higher yields achieved at Giza. Among tested treatments, the two-membered antagonist demonstrated superiority with all types of carriers compared to cell suspension, without carrier, even in mixture. In this respect, and regardless of carrier, the entrapped two-membered antagonist achieved a very comparable grain yield of 133.14 $g.plant^{-1}$ with no significant difference from the highest yield (137.0 $g.plant^{-1}$) obtained by the chemical fungicide Occidor Plus. However, there were significant differences in grain yield between entrapped single antagonist and the fungicide. Grain yield of 123.47 and 116.64 $g.plant^{-1}$ were recorded for entrapped cells of *B. subtilis* (isolate No. 9) and *Streptomyces* sp. (isolate No. 3), respectively. The grain yield for the control was 73 $g.plant^{-1}$. The high efficiencies. in both reducing disease incidence and increasing grain yield, achieved by two-membered antagonistic formulation compared to singly applied formulations have been reported by several investigators (Kundu and Nandi, 1993; Novikova *et al.*, 1995 ; Ahmed, 2004).

On the other hand, when various carriers were compared, the maximum grain yield was obtained when vermiculite was used with an average of 127.6 g grains plant^{-1} which represents an increase of >20 % over non-entrapped formulations. This was followed in a descending order by talc powder (119.6), peat-moss (118.1), bentonite (117.7), wheat bran (114.4) and kaolinite (113.0). Non entrapped cells displayed an average grain yield of 106.16 g grain plant^{-1}. An increase in maize yield by 3.8–12 % was reported in China (Wang, 2000) when coated seeds with various antagonists were cultivated compared to untreated seeds. This was also recorded with carrot seeds treated with *Bacillus amyloliquificans* entrapped onto vermiculite (Chen and Wu, 1999) and in tomato and cucumber yields through bacterization of their seeds with the bacterium *B. subtilis* (Grosch et al.,1999) .

Table (3): Effect of various formulations of *B. subtilis* (isolate No.9) (A), *Streptomyces* sp. (isolate No.3) (B), their mixture(A+B) and Occider Plus(O) on grain yield of sorghum (Giza 113 cv.) under field conditions

Location	Treatments	Carriers							Mean
		Bentonite	Peat-moss	Vermiculite	Talc powder	Wheat bran	Kaolinite	Without carrier	
Giza	A	128.00*	126.00	145.00	125.00	128.00	125.00	112.60	127.09
	B	125.00	125.00	137.00	125.00	112.00	117.00	100.00	120.14
	A + B	135.00	135.00	155.00	144.00	130.00	133.00	125.00	136.71
	O	140.00	140.00	140.00	140.00	140.00	140.00	140.00	140.00
	Control	84.00	84.00	84.00	84.00	84.00	84.00	84.00	84.00
	Mean	122.40	122.00	132.20	123.60	118.80	119.80	112.30	121.59
ids	A	121.00	124.00	138.00	122.00	120.00	110.00	104.00	119.86
	B	118.00	121.00	135.00	121.00	112.00	100.00	85.00	113.14
	A + B	130.00	130.00	146.00	139.00	122.00	125.00	115.00	129.57
	O	134.00	134.00	134.00	134.00	134.00	134.00	134.00	134.00
	Control	62.00	62.00	62.00	62.00	62.00	62.00	62.00	62.00
	Mean	113.00	114.20	123.00	115.60	110.00	106.20	100.00	111.71
Mean	A	124.50	125.00	141.50	123.50	124.00	117.50	108.30	123.47
	B	121.50	123.00	136.00	123.00	112.00	108.50	92.50	116.64
	A + B	132.50	132.50	150.50	141.50	126.00	129.00	120.00	133.14
	O	137.00	137.00	137.00	137.00	137.00	137.00	137.00	137.00
	Control	73.00	73.00	73.00	73.00	73.00	73.00	73.00	73.00
	Average	117.70	118.10	127.60	119.60	114.40	113.00	106.16	116.65

L.S.D.($p<0.05$%): Location (L)= 3.64 , L x T= 8.14, Treatment (T)= 6.81
Carrier=5.73, T x C = 15.23,L x T x C= 12.67, L x C= Not significant
*g/plant

In conclusion, the developed two-membered vermiculite entrapped formulation, in particular, has many advantages as a biocontrol agent for grain sorghum cultivation under Egyptian conditions. It reduced significantly the disease incidence , increased markedly crop yield. These together with being economical, health and environmentally safe are of great importance when such formulation is to be considered for large-scale commercial application. Work is in progress concerning the influence of such antagonistic microorganisms on the incidence of sorghum grain-associated fungi. The possible N_2-fixing capabilities, growth promoting activities of these microorganisms and their possible interactions with mycorhizae and rhizobacteria and diazotrophes in a multi-membered formulation for biological control of Acremonium wilt and biofertilization of sorghum grain should be considered .

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كفاءة بعض اللقاحات المضادة لفطر اكريمونيم ستريكتم المسبب لمرض الذ بـول الاكريمونى فى الذرة الرفيعة

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١- قسم الميكروبيولوجيا الزراعية- كلية الزراعة جامعة القاهرة - الجيزة.
٢- قسم بحوث أمراض الذرة والمحاصيل السكرية والأعلاف- معهد بحوث أمراض النبات - مركز البحوث الزراعية - الجيزة.

أجريت تجربتين لمقاومة فطر أكريمونيوم ستريكتم المسبب للذبول فى الذرة الرفيعة حيويا أحداهما فى الصوبة والاخرى حقلية بمحطتى بحوث الجيزة وسدس.وقدأدى تغليف حبوب الذرة الرفيعةفى تجربة الأصص تحت ظروف الزراعة المحمية بالباسيللس ساتلس (رقم ٩) ،ستربتوميسيس (رقم ٣) منفردين أو مختلطين بنسبة ١:١ وكذا معاملتها بمبيد الفطريات أوكسيدور بلس (كاربندازيم ٥٠%) إلى خفض نسبة الإصابة بمرض الذبول الأكريمونيومى بدرجة ملموسة مقارنة بالبذور غير المعاملة وكانت المعاملة بالمبيد أكثر هذه المعاملات فعالية فى خفض نسبة الإصابة بالمرض. كانت جميع المواد التى إستخدمت فى تحميل العوامل الحيوية (فيرميكوليت - بيت موس - كاؤولينيت - بنتونيت - نخالة القمح - مسحوق التلك) ملائمة لتحميل كل من باسيللس ساتلس (رقم ٩) وستربتوميسيس (رقم ٣) منفردين أو مختلطين بنسبة ١:١ وكان الفيرميكوليت أكثرهذه المواد فعالية. أيضا دلت نتائج التجربة الحقلية على أن الفيرميكوليت أكثر مواد التحميل ملائمة لكلا العاملين الحيويين منفردين أو مخلوطين بنسبة ١:١ حيث إنخفض معدل الإصابة بالمرض إلى ١٩,٨٣% بينما زاد معدل المحصول/نبات إلى ١٢٧,٦ جرام مقارنة بالحبوب غير المعاملة (٢٥,٧٥%و ١٠٦,١٦ جرام على التوالى).