BIOLOGICAL CONTROL OF DOWNY MILDEW DISEASE OF MAIZE CAUSED BY *PERONOSCLEROSPORA SORGHI* USING CERTAIN BIOCONTROL AGENTS ALONE OR IN COMBINATION

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ABSTRACT

In an attempt for biological control of maize downy mildew caused by *Peronosclerospora sorghi* under laboratory and field conditions, crude culture filtrates of some bio-agents and their mixtures were tested. Bio-control agents used in the present investigation include *Trichoderma viride, Trichoderma harzianum, Gliocladium virens* and *Bacillus subtilis*. Under laboratory condition, all bioagents inhibited germination of oospores and conidia of *P. sorghi*.Dual cultures of *T. viride* and *T. harzianum* or *B. subtilus* were the most effective in inhibiting spore germination than the individual one .Under field condition, downy mildew infection percentages were different according to treatments i.e. spray, seed soaking and (spray + seed soaking). Seed soaking and spray treatment with the biocontrol agents and their mixtures was more effective than individual treatment of seed soaking or spray during the two successive seasons. Combination of *T. Viride* with *T. harzianum* or *B. subtilis* was the most effective in reducing downy mildew infection compared with the control. It gave 71.09 and 67.74% efficacy, respectively. On the other hand, *Gliocladium virens* was the least effective one in this respect (37.50% efficacy). The fungicide Apron (3.0 g/K seed) was the most effective one over all treatments (100% efficacy).

key words: downy mildew, biological control, *Peronosclerospora sorghi*, maize and sorghum.

INTRODUCTION

Sorghum downy mildew, caused by *Peronosclerospora sorghi* (Weston and Uppal) C.G. shaw, is considered as extremely a destructive disease of maize (*Zea mays L.*), grain sorghum(*Sorghum bicolor L.*) and forage sorghum (*Sorghum vulgar* L.).

In view of hazardous impact of pesticides and other agrochemicals on the ecosystem, the biocontrol of plant diseases as an alternative strategy has received increasing attention (**Papavizas** 1985, **Baker** 1987 and **lockwood** 1988). Biological control of plant pathogens, based on the management of a natural source to develop the antagonistic activity against harmful organisms is currently accepted as a key practice in sustainable agriculture and as potential alternative to the intensive use of chemical pesticides which are harmful to the environment. Several investigators reported the use of biological control against soil-borne disease (**El-Kazzaz *et al.***, 2000

and **Chincholkal** and **Mukerji**, 2007). In this respect the mixture of *Trichoderma viride, Trichoderma harzianum* and *Bacillus subtilis* represent the most thoroughly studied antagonistic micro-organisms. The mixture of bioagents has been used as a good biocontrol agents for many soil-borne and air-borne diseases such as late-wilt of maize (**El-Assiuty** *et al.*, 1991), leaf blight of maize (**Abd El-Naby** ***et al.***, 2007) and root rot of tomato plants (**Morsy** ***et al.***, 2009).

This work aimed to improve bioactivities of some biocontrol agents by combination and to evaluate their efficacy in controlling Sorghum downy mildew of maize under Egyptian conditions.

MATERIALS AND METHODS

The present study was carried out at Gemmeiza Agricultural Research Station, A.R.C during 2009 and 2010 growing seasons.

Source of the causal organism and bioagents:

To collecte a sexual stage (conidia) of causal organism, the fungal growth on the lower surface of systemically infected leaves was scraping in early morning before sun rise and fixed on glass slides. To collected sexual stage (oospores) of the causal organism, leaves of systemically infected Sudan grass (Sordan 79) at the end of the growing seasons were collected and stored for 6 months. After the elapse of storage period the leaves were air dried again and grinned by a wiley mill according to the method described by **Schuh** ***et al.*** (1987).

Microbial isolates:

Trichoderma viride, T. harzianum and *Gliocladium virens* were isolated from maize plant rhizosphere located in El-Gharbeia governorate and identified at the Plant Pathology Research Institute (ARC. Giza, Egypt). *Bacillus subtilis* strain was isolated from maize plants in Gemmeiza Agricultural Research Station, El-Gharbia , Egypt. *B. subtilis* was streaked on PDA plates which were incubated for 48 hours supplemented with 1% yeast extract, and incubated for 3 days at 25°C. Identification was carried out according to the methods of **Harrigan** and **Mc-Cance** (1976) and **Sneath** (1986).

Preparation culture filtrates of bioagents:

Fungal isolates were grown in liquid PD medium (potato extract 200 gm, dextrose 20 gm and distilled water 1000 ml) for 7 days at 28°C. Bacterial antagonists were grown on nutrient glucose broth (NGB) for 3 days at 30°C (beef extract 3 gm peptone 5 gm, glucose 10 gm and distilled water 1000 ml). After the incubation period, cultures were filtered through filter paper and centrifuged at 8000 rpm for 10 minutes.

In vitro

Culture filtrates of *T. viride, T. harzianum, Gliocladium virens* and *B. subtilis* alone or in combinations (1:1 v/v) were used against spore (oospores and condia) germination of *P. sorghi.*

To determine oospores germination, a few amount of the spore containing powder was dusted on slides. Seeds of Sudan grass (Sordan 79) were put onto filter papers and then on dusted slides together with culture filtrates . To determine condial germination, the fungal growth on the lower surface of systemically infected leaves were scraped in early morning before sun rise and fixed on glass slides and then cultures filtrates were added. The slides were put in Petri-dish on feline heels. Then a few amount of sterilized distilled water was added to the blotting paper at bottom of each Petri-dish and the lid of Petri-dish was lined. Petri-dishes incubated at 23-25°C for 3 days. In two experiments Ridomil plus was used at the rate of 125 p.p.m and sterile distilled water was used as control. Treatments were replicated four times, Oospores and conidial germination were recorded as percentage.

Field experiment:-

Field experiment was carried out in the disease nursery prepared for studying sorghum downy mildew (SDM) at Agricultural Research Station of Gemmeiza during 2009 and 2010 maize growing seasons. The field layout was prepared as follows: The highly susceptible Sudan grass (Sordan 79) was sown in every two rows throughout the field at least three weeks prior the expected date of planting of tested treatments. Three rows of the surrounding border were also planted with the same (Sordan 79) spreading the asexual spores (conidia) to the tested materials and to serve as indicator for the uniform distribution of the disease inoculum throughout the field. After establishing of the infector rows and after the appearance of abundant sporulation of the pathogen by producing downy growth on the leaf surfaces (3-4 weeks), the tested rows were planted. After emergence, seedlings were challenged by conidia needed for infection blown by wind from the infector rows. Sudan grass was cut monthly about 20-25 cm above soil level. This is increase spore production needed for infection around the tested treatments.

An experiment was carried out to manage the disease by applying biological agents. Some antagonists, fungal and bacterial isolates, known as biological control agents were used in this work. Three methods of applications were used as follows:

1) Spraying after 7,14 and 21 days from planting on maize plants .

2) Seed soaked in the filtrates for 8 hours before sowing.

3) Spray and soaking together.

Treating seeds with Apron of 3 g/Kg (soaking treatment) and spraying with Ridomil plus of 1.5 g/litter (spraying treatments) were used as check, seeds without treatment and sterile distilled water were used as control. The highly susceptible cultivar, three way cross of maize (T. W.C 310) was used in this experiments, three replicated plots were used in this experiment according to the randomized complete blocks methods. Disease readings were made two times one month after planting and one month later. Number of infected plants for each treatment was recorded and percentage of infection was calculated. The averages were compared at the 0.05 and 0.01 levels using the least significant difference (LSD) after transforming percentages arcsin. The efficacy of each treatment was calculated according to the formula of **Rewal and Jhooly** (1985) in which:

$$\text{Efficacy\%} = \frac{\text{\% infection in the control - \% infection in the treatment}}{\text{\% infection in the control}}$$

RESULTS AND DISCUSSION

***In vitro* test:**

Data presented in Table (1) showed that, the tested bioagents were able to reduce the spore germination of oospore and conidia of the pathogen compared with control. The combined application of *Trichoderma viride* + *Trichodermoa harzianum* was the most effective bioagents and recorded the highest efficacy on Oospores and conidia germination (76.65 and 85.13%, respectively) followed by *Trichodermoa viride*+ *B. subtilis* (74.55 and 83.46%, respectively). While *Gliocladium virens* alone recorded the lowest efficacy, in this respect (32.77 and 41.38 %, respectively) whereas using culture filtrates alone of *T.virida, T. harzianum* and *Bacilis subtilis* recorded, significant reduction in spore germination (oospore and conidia), their efficacy values were [(73.51 and 80.40%) (71.43 and 78.53%) and (69.33 and 76.15%), respectively]. Generally, culture filtrates of *T. viride, T. harzianum* and *B. subtilis* alone or in combination (1:1 v/v) were the most effective bioagents on spore germination (oospores and conidia). Similar results were reported by **Getha *et al.*** (2005) and **Morsy *et al.*** (2009) who observed that the dual treatment by *T. viride* + *B. subtilis* was effective against *F. oxysorum* than individual one.

Table (1): Effect of crude culture filtrates of some biocontrol agents on oospores / conidial germination.

Treatments	Oospores germination	Efficacy %	Conidial germination %	Efficacy %
Tridoderma viride	15.20 h	73.51 c	11.97 i	80.64 d
Tridoderma harzianum	16.39 g	71.43 d	13.28 g	78.53 e
Gliocladium virens	38.58 b	32.77 i	36.24 b	41.38 j
Bucillis subtilis	17.60 f	69.33 e	14.55 f	76.15 f
T. viride + T. harzianum	13.40 i	76.65 b	9.20 k	85.13 b
T. viride + G. virens	21.0 e	63.40 f	18.02 e	70.86 g
T. viride + B. subtilis	14.60 h	74.55 c	10.23 j	83.46 c
T. harzianum + G. virens	24.0 d	58.17g	21.28 d	65.59 h
T. harzianum + B. subtilis	15.20 h	73.51 c	12.12 h	80.40 d
G. virens + B. subtilis	25.0 c	56.43 h	22.17 c	64.15 i
Ridomil plus (125 ppm}	0.00 j	100 a	0.00 l	100 a
Control	57.38 a	0.00 j	61.84 a	0.00 k
L.S.D. at 5%	0.71	1.10	0.72	1.01

Means followed by the same letter in a column do not differ significantly according Duncan's multiple range test at 5% level

Field experiments:

Results presented in (Table 2) showed that, most of spraying seedlings treatment at 7.14, 21 after planting by using culture filtrates were significant at 0.01 and 0.05 levels in controlling maize downy mildew infection, some bioagents were more effective than the others. The dual treatment using *T. viride + B. subtilis* proved to be the most effective resulting the lowest downy mildew infection percentage (17.03%), followed by dual treatment of *T. viride + T. harzianum* (17.61%). On the other hand, use individual ones of *T. viride*, *T. harzianum* and *B. subtilis* recorded the average percentage of downy mildew infection (20.02%, 21.19 % and 21.72%, respectively). Whereas the highest percentage of downy mildew infection (31.26%) was obtained when seedlings were sprayed by *Gliocladium virens*. Generally *T. viride*, *T. harizianum* and *B. subtilis* were the most effective with dual bio-agent than the individual one. Similar results were reported by **El-Fiki *et al.*(2004)**, **Pertot *et al.*** (2008) and **El-Shehawy** (2009) who observed that the downy mildew disease of grain sorghum could be effectively controlled by foliar spray with bio-agents *(T. viride)* and *T. harzianum*

Table (2): Effect of crude culture filtrates of some biocontrol agents used as spray on control maize downy mildew disease (SDM), at Gemmeiza, during 2009 and 2010 maize growing seasons.

Treatments	Downy mildew infection%			Efficacy%		
	2009	2010	Combined	2009	2010	Combined
Trichoderma viride	20.40 ef	19.63 fg	20.02 e	51.99 cd	55.87 de	53.93 c
Trichoderma harzianum	20.98 e	21.39de	21.19 d	50.59 d	51.94 fg	51.27 d
Gliocladium virens	32.00 b	30.52 b	31.26 b	24.66 g	31.38 i	28.02 g
Bacillus subtilis	21.32 e	22.12 d	21.72 b	49.83 de	50.26 g	50.04 de
T. viride + T. harzianum	17.14 g	18.08 i	17.61 h	59.47 b	59.37 b	59.42 e
T. viride + G. virens	22.82cd	20.63 ef	21.73 d	46.27 f	53.62 ef	49.95 d
T. viride + B. subtilis	17.38 g	16.68 h	17.03 g	59.10 b	62.52 c	60.81 b
T. harzianum + G. virens	22.65 d	21.10de	21.88 d	46.70 ef	49.64 g	48.17 e
T. harzianum + B. subtilis	19.47 f	18.40gh	18.94 f	51.19 c	58.62 cd	54.91c
G. virens + B. subtilis	24.05 c	25.24 c	24.65 c	43.40 f	43.28 h	43.34 f
Ridomil plus 125 P.P.m	0.00 h	0.00 j	0.00 i	100 a	100 a	100 a
Control	43.32 a	44.50 a	43.91a	0.00 h	0.00 j	0.00 h
L. S. D at 0.05	1.78	1.72	0.21	4.61	4.08	2.92

Means followed by the same letter in a column do not differ significantly according Duncan's multiple range test at 5% level

Results presented in (Table 3) show that, most of bio-agents used as seed soaking gave significant reduction of downy mildew disease. The dual treatment using *(T. viride + T. harzianum)* (1:1 v/v) was the most efficient followed by *(T. viride + B. subtilis)*. The untreated control showed 41.98% while seeds dressed with dual of treatments *(T.viride + T. harzianum)* and *(T. viride + B. subtilis)* resulted in 14.44 and 15.74% downy mildew infection, respectively. Also, results presented in Table, 3 showed that, there were no significant differences in the average percentage of downy mildew infection due to the effect of seed soaking with the individual bioagents *T. viride, T.harzianum* and *B.subtilis* which recorded (16.88,17.10 and 18.14%) respectively. On the other hand, *Gliocladium virens* was less effective (27.78%). Agents used in this experiment successfully controlled many other field crops diseases (**Well *et al.*** 1972, **Backman and Rodiguex** 1975, **Abd-El Moity and Shatla** 1981, **El– Assiuty *et al.*,** 1986 and **El-Mersawy,** 2000 who observed that *Bacillus subtilis* used as seed coating was the most effective in reducing the downy mildew infection percentage compared with control.

Data in Table (4) revealed that, the high infection percentage of maize plants recorded with control, whereas, low infection percentage was observed in the dual treatment using *T. viride + T. harzianum* (11.82%) followed by *T. viride + B. subtilis* (13.96%) and *T. harzianum + B. subtilis* (14.61%). Combination between *G. virens* and *T. viride, T. harzianum* and *B. subtilis* gave averge percentage of downy mildew infection ranging between (16.19 – 17.89 %). **El-Assiuty *et al.*** 1991 reported that it could reduce the incidence of the

late-wilt of maize by treating soil with *Bacillus subitis* together with *Verticullum tricorpus.* **Abd El-Naby *et al.*** 2007 reported that the mixture of *Streptomyces sp.* + *Bacillus sp.* at the rate of (1:1 v/v) was an efficient bio-control of leaf blight in maize. Also individual treatment using *T. viride, T. harzianum, B. subtilis* have significantly decreased the percentage of infection. There were no significant differences in the average percentage of downy mildew infection between them; 14.35, 15.19 and 15.67 %, respectively. While, *G. virens* was the least one in this respect (26.03%). Agents used in this experiment successfully controlled many other field crops disease **El-Assiuty *et al.*** (1986) and **El-Mersawy** (2000).

Table (3): Effect of seed soaking with some crude culture filtrates on control maize downy mildew disease (SDM), at Gemmeiza during 2009 and 2010 growing seasons.

Treatments	Downy mildew infection%			Efficacy%		
	2009	2010	Combined	2009	2010	Combined
Trichoderma viride	17.04 de	16.72 ef	16.88 efg	56.53 cd	59.76 c	59.30 d
Trichoderma harzianum	17.24 de	16.96 ef	17.10 efg	58.05 cd	59.21 cd	58.63 de
Gliocladium virens	27.02 b	28.54 b	27.78 b	34.26 f	31.32 f	32.79 h
Bacillus subtilis	17.77 d	18.50c d	18.14 d	56.72 d	55.47d e	56.10 f
T. viride + T. harzianum	14.89 f	13.99 g	14.44 i	63.73 b	66.35 b	65.04 b
T. viride + G. virens	18.10 d	17.44d ef	17.77de	55.96 d	58.71 cde	57.34 ef
T. viride + B. subtilis	15.32 f	16.15 e	15.74 h	62.73 b	61.14 c	61.94 c
T. harzianum + G. virens	18.27d	17.56c de	17.92 de	55.53 d	57.74cde	56.64 f
T. harzianum + B. subtilis	16.11ef	16.71ef	16.41gh	60.75bc	59.81 c	60.28 c
G. virens + B. subtilis	19.79 c	18.91c	19.35 c	51.83 e	54.52 e	53.18 g
Apron (3g/kg seeds)	0.00 g	0.00 h	0.00 j	100 a	100 a	100 a
Control	42.37a	41.59a	41.98a	0.00 g	0.00 g	0.00 i
L.S.D at 0.05	1.81	1.81	4.33	4.33	5.09	2.42

Means followed by the same letter in a column do not differ significantly according Duncan's multiple range test at 5% level

Table (4): Effect of crude culture filtrates of some biocontrol agents used as spray and soaking on control maize downy mildew disease (SDM), at Gemmeiza, during 2009 and 2010 maize growing seasons.

Treatments	Downy mildew infection%			Efficacy%		
	2009	2010	Combined	2009	2010	Combined
Trichoderma viride	14.60 e	14.10 efg	14.35 f	64.77 c	66.36 de	65.57 d
Trichoderma harzianum	14.57 e	15.81 d	15.19 e	64.77 c	62.22 f	63.50 e
Gliocladium virens	25.96 b	26.10 b	26.03 b	37.34 f	37.65 h	37.50 h
Bacillus subtilis	16.19 d	15.14 ef	15.67 d e	60.92 d	63.64 def	62.38 ef
T. viride + T. harzianum	11.82 f	12.24 h	12.03 h	74.66 b	70.68 b	71.09 b
T. viride + G. virens	16.38 d	15.43 de	15.91 de	60.44 d	63.16 ef	61.81 f
T. viride + B. subtilis	13.96 e	12.94 g	13.45 g	66.31 c	69.07 bc	67.74 c
T. harzianum + G. virens	16.25 d	15.80 d	16.03 d	63.82 d	62.28 f	61.52 f
T. harzianum + B. subtilis	14.61 e	13.79 fg	14.20 f	64.76 c	67.08 cd	65.90 d
G. virens + B. subtills	17.89 c	17.36 c	17.63 c	61.21 e	58.57 g	59.89 g
Apron (3.0g/kg seeds) Ridomil plus (3g/litter)	0.00 g	0.00 i	0.00 i	100 a	100 a	100 a
Control	40.0 a	41.18 a	43.24 a	0.00 g	0.00 i	0.00 i
L.S.D at 0.05	1.68	2.07	1.02	4.35	4.90	1.93

Means followed by the same letter in a column do not differ significantly according Duncan's multiple range test at 5% level

In conclusion, results obtained in (Table 2, 3 and4) show that all treatments at all three application (spraying, soaking and spraying and soaking together) affected positively downy mildew disease incidence on TWC 310 plants, with different degrees. The most effective application was seed soaking with spraying the seedling followed by application seed soaking. While spray application alone was the least efficacy in this respect. Results also proved that the mixtures, *(T. viride + T. harzianum)* and *(T. viride + B. subtilis)* at the rate of (1:1 v/v) were efficient to control of downy mildew of maize. Biocontrol agents may have a positive interaction between each other, resulting in enhancement of disease suppression. These results were confirmed on maize downy mildew by, **Badawi *et al.*** 2007**, Abd El-Naby *et al.*** 2007 on maize leaf blight and **Morsy *et al.*** 2009. This positive effect of the bioagents might be attributed to induction of resistance in the host plant by biocontrol agents *Trichoderma* spp. (El-Assiuty *et al.*, 1980) or producing several antibiotics by *B. subtilis* such as subtillin, bacillin , abacillomicin.. etc. which may affect the growth of pathogen (Loeffler *et al.*, 1986).

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الملخص العربي

المكافحة البيولوجية لمرض البياض الزغبى فى الذرة الشامية المتسبب عن الفطر بيرونوسكليروسبورا سورجاى باستخدام بعض العوامل الحيوية ومخاليطها .

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فى محاولة لاستخدام المكافحة الحيوية لمرض البياض الزغبى على الذرة الشامية المتسبب عن الفطر بيرونوسكليروسبورا سورجاى تحت ظروف المعمل والحقل. استخدام، الراشح الناتج من بعض الكائنات الحية لفطر التريكودرما فيردى – تريكودرما هارزيانيم – وجليوكلاديم فيرنس وأيضا بكتريا باسليس سابتليس لاختبار كفاءتها على تثبيط إنبات جراثيم الفطر الجنسية واللاجنسية . حيث أثبتت النتائج تحت الظروف المعملية أن جميع العوامل الحيوية المستخدمة ذات كفاءة فى تثبيط إنبات جراثيم الفطر ولكن بدرجات متفاوتة كان أعلاها كفاءة المعاملة المزدوجة من فطرى التريكودرما فيردى والتريكودرما هارزيانيم معا بالمقارنة بالمعاملة االمنفرده لكل منهما. وأيضا المعاملات الأخرى تحت الدراسة. فى التجارب الحقلية موسمى ٢٠٠٩و ٢٠١٠أوضحت نتائج التجارب اختلاف نسبة الإصابة بمرض البياض الزغبى باختلاف التطبيق المستخدم من رش أو نقع أو رش مع النقع وذلك بالراشح الناتج من الكائنات المستخدمة وقد أكدت نتائج هذه التجارب أن تطبيق الرش والنقع معا أكثر فاعلية من الاستخدام المنفرد للرش أو النقع، حيث كانت معاملة الرش والنقع معا بمخلوط الفطر تريكودرما فيردى مع التريكودرما هارزيانيم أو بكتريا البسلس سابتلس الأكثر فاعلية فى تقليل نسبة الإصابة بمرض البياض الزغبي حيث أعطت أعلي نسبه كفاءه و هي ٧١,٠٩% و ٦٧,٧٤ % على التوالي . ومن ناحية أخرى كانت المعاملة براشح الفطر جليوكولاديم فيرنس الأقل كفاءة (٣٧,٥٠%) فى هذه الدراسة. كما اتضح أيضا من الدراسه أن المعا مله بالمبيد الفطري أبرون بمعدل ٣جم /كجم بذره تفوق علي كل المعاملات المستخدمه حيث أعطي ١٠٠% كفاءه في مقاومه مرض البياض الزغبي قي الذره الشاميه.