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BIOLOGICAL CONTROL OF MAIZE DOWNY MILDEW DISEASE CAUSED BY *Peronosclespora sorghi*, IN EGYPT

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ABSTRACT

This investigation was carried out during 1996 and 1997 maize growing seasons at Gemmeiza Agricultural Research Station to evaluate the efficacy of some biocontrol agents, used as seed treatments, in controlling downy mildew disease of maize caused by *P.sorghi* under Egyptian condition. Field trials results have demonstrated that, downy mildew infection percentages were differed with the different seed treatments . Seed coating with the biocontrol agents was more effective than seed soaking with those agents during the two successive seasons. *Bacillus subtilis* used as seed coating was the most effective in reducing the disease infection percentage compared with the control. It exhibited 86.97 and 82.39% efficacy during 1996 and 1997 seasons, respectively. On the other hand, *Trichoderma harzianum* was the least one in this respect, where it gave only 11.05 and 3.78% efficacy in 1996 and 1997, respectively. No definite trend of infection due to seed soaking was found except with. *Pseudomonas fleuroscence* isolate No. (2) used as seed soaking which was effective with non-significant differences in controlling the disease comparing with the rest of the tested biocontrol agents. This treatment reduced the percentage of disease incidence from 56.8% and 53.20% to 47.07% and 40.36% during 1996 and 1997, respectively. There was no significant difference between treatments with *Bacillus subtilis* as seed coating and the recommended fungicide (Apron), in this respect.

INTRODUCTION

Among the plant diseases, sorghum downy mildew (SDM) caused by *Peronosclerospora sorghi* (Weston and Upal) S.G. Shaw is considered as extremely destructive disease of major significance in reducing crop yield of both maize and sorghum, particularly in tropical and sub-tropical regions of the world (Anahousur, 1980 and Frederiksen 1980).

In view of hazardous impact of pesticides and other agrochemicals on the ecosystem, the biocontrol of plant diseases as an alternative strategy has recieved increasing attention (Papavizas, 1985, Baker 1987 and Lockwood 1988). In this respect, *Trichoderma spp*., *Gliocladium spp., Bacillus spp., Pseudomonas spp.* and *Actinomyces* represent the most thoroughly studied antagonistic micro-organisms. It has proven that, certain species of *Trichoderma* have a promising potential for biological control of plant pathogenic fungi (Papavizas, 1985; Kaur and Mukhopadhyay 1992; Key and Stewart, 1994 and Goutam *et al.,* 1995). In a previous studies it was found that, several species of *Gliocladium* have been explained for their antagonistic potential against a variety of pathogenic fungi (Howell, 1982 and 1991; Papavizas 1985, Zazzerini and Tosi 1985 and Lumsden and Loke 1989).

On the other hand, antagonistic bacteria have been extensively studied as a biocontrol agents effective against soil borne pathogens. Recent investigations have been shown the inhibitory effect of *Bacillus spp*.

against a number of pathogenic fungi (Utkhede and Li 1989, Ferreira *et al.,* 1991). It has been recorded that, *Pseudomonas spp.* suppressed the diseases caused by various pathogens (Weller and Cook 1983, Kropp and Adetuji 1993 . The antagonistic activity of several *streptomyces spp*. against species of *Pythium* and *Phytophthora* was shown by Knauss (1976) and Valais *et al.,* (1993).

This work aimed to evaluate the efficacy of some biocontrol agents used as seed treatments, in controlling SDM under Egyptian condition.

MATERIALS AND METHODS

This study was carried out in the disease nursery prepared for studying sorghum downy mildew (SDM) at Agricultural Research station of Gemmeiza during 1996 and 1997 maize growing season.

The field layout was prepared as follows: The highly susceptible Sudan grass (Sorghum sudanens); pipper variety, was sown in every third row throughout the field at least three weeks prior to the expected date of planting of tested treatments. Three rows of the surrounding border were also planted with the same Sudan grass 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 20-25cm above soil level. This is to increase spore production needed for infection around the tested treatments.

An experiment was carried out to manage the disease by applying biological agents. Some antagonistic fungal, bacterial and actinomycetal isolates, known as biological control agents were used in this work. Two methods of seed applications, i.e. seed coating and seed soaking were used in this experiment.

Fungal isolates were grown for seed treatment on PDA medium for 7 days at 28C. After the elapse of incubation period, fungal spores were harvested by adding 10ml of sterilized coating water to each plate and scraped gently. Number of fungal spores into the aqueous suspension was adjusted by the aid of a haemecytometer to approximately $2\text{-}5x10^8$. Conidial suspension was supplemented with 0.2% (v/v) of an adhesive. Spore suspension was used in treating seeds at the rate of 10ml/Kg. For seed soaking fungal isolates were grown on potato dextrose broth (PDB) for 15 days in shaking incubator at 28C. After incubation period, cultures were filtered through muslin cloth and centrifuged at 5000 rpm for 10 min. Seeds were soaked into the culture filtrates for 6 hours before planting.

Bacterial isolates were grown on nutrient glucose broth (NGB) or King's broth for 3 days at 30C. After incubation period, cultures were centrifuged at 5000 for 10 min. Pellets from each culture were resuspended into 30ml sterile distilled water to be used in seed dressing. Culture filtrates obtained from the centrifugation were used for seed soaking. Seeds were soaked in the filtrates for 6 hours before sowing.

Streptomyces sp. was grown on starch nitrate broth (SNB); the best medium recommended for producing antibiotics (Waksman, 1962) for three days at 30C in a shaking incubator. The same procedure followed for bacterial isolates was followed to prepare the spore suspension or culture filtrate for seed coating or seed soaking, respectively.

Treating seeds with Apron at the rate of 3g/Kg was used as check and seeds without treatment served as control. The highly susceptible TWC 310 was used in this experiment. Three replicate plots were used in this experiment according to the randomized complete blocks methods.

Disease readings were made two times (unless other wise stated); one month after planting and one month later. Number of infected plants for each treatment was recorded and the 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 into arc sine.

RESULTS AND DISCUSSION

Results obtained in 1996 (Table 1 and Fig. 1) showed that most of seed coating treatments by using the bio-agents tested were significant, at 5% level in controlling maize downy mildew infection. Some bio-agents were more effective than the others. *Bacillus subtilis* proved to be the most effective resulting in the lowest downy mildew infection percentage (6.37%) followed by *Gliocladium deliquescens* (12.78%), *Fluorescent pseudomonad* No. 10 (12.8%) and *Pseudomonas fluorescens* No. 2 (15.0%). On the other hand, the highest percentage of downy mildew infection (43.47%) was obtained when seed were treated with *Trichoderma harzianum*. Seed soaking by using the bio-agents tested were not significant in reducing the disease incidence.

In 1997, similar results (Table 2 and Fig. 1) were obtained, since most of bio-agents used as seed coating gave significant reduction of downy mildew disease. *Bacillus subtilis* was the most efficient followed by *Fluorescent Pseudomonad* No. 10 and *Gliocladium deliquescens*. The untreated control showed 49% while seeds dressed with Bacillus subtilis, *Fluorescent pseudomonad* and *Gliocladium deliquescens* resulted in 8.63, 10.82 and 13.75% downy mildew infection, respectively. Also, results presented in table 2 showed that there were no significant differences in the average percentage of downy mildew infection due to the effect of seed soaking with the tested bio-agents used in this study. Agents used in this experiment successfuly controlled many other field crops diseases (Abd El-Moity and Shatla 1981, Well *et al.,* 1972, Backman and Rodiguez 1975 and El-Assiuty *et al.,* 1986).

Some of these biological agents can parasitize their hosts (hyperparasitism), others affect hosts by excreting mycotoxins. Some bacteria grow along the Rhizoplane of roots and appear to form a biological

barrier between roots and many soil borne plant pathogens. Introduction of these types of parasitic organisms into osspore infested fields might provide an effective biological control of SDM. Oospores. of *P. sorghi* are commonly parasitized by naturally occuring chitrid fungi and other micro-organisms. Kenneth (1982), demonstrated that a chitrid (*Phylactochytrium* spp.) is parasitic to oospores of *P. sorghi* and can be increased in vitro.

Table (1) : Effect of seed treatment (coating and soaking) with some biocontrol agents on control of maize downy mildew disease (SDM), at Gemmeiza, during 1996 maize growing season.

Treatments	Downy mildew infection %			
	Seed coating	Efficacy	Seed soaking	Efficacy
1-Trichoderma harzianum	43.47	11.05	60.56	-6.62
2-Trichoderma hamatum	25.77	47.27	47.42	16.51
3-Trichoderma viride	41.73	14.61	47.60	16.20
4-Trichoderma reesei	42.40	13.24	55.24	2.75
5-Trichoderma pseudokoningi	19.37	60.36	51.96	8.52
6-Gliocladium deliquescens	12.78	73.85	55.23	2.76
7- Trichoderma virens	31.47	35.60	51.70	8.98
8- Fluorescent pseudomonad No. (10)	12.80	73.81	59.36	-4.51
9- Fluorescent pseudomonad No. (12)	27.70	43.32	57.90	-1.94
10- Pseudomonas fluorescens	41.20	15.69	56.97	-0.29
11- Streptomyces sp.	26.87	45.02	43.60	24.19
12- Pseudomonas fluorescens No. (2)	15.00	69.31	47.07	17.13
13- Bacillus subtiulis	6.37	86.97	69.74	-22.78
14- Streptomyces sp. 15	40.97	15.98	43.96	26.27
***15-* Control**	48.87	-	56.80	-
***16-* Apron (seed treatment)**	0.00	100.1	0.00	100
L.S.D. 0.05	**12.24**		**27.31**	

Table (2) : Effect of seed treatment (coating and soaking) with some biocontrol agents on control of maize downy mildew disease (SDM), at Gemmeiza, during 1997 maize growing season.

Treatments	Downy mildew infection %			
	Seed coating	Efficacy	Seed soaking	Efficacy
1-Trichoderma harzianum	47.15	3.78	48.23	9.34
2-Trichoderma hamatum	28.14	42.57	56.96	-7.07
3-Trichoderma viride	46.36	5.39	53.37	-0.32
4-Trichoderma reesei	44.83	8.51	51.63	2.95
5-Trichoderma pseudokoningi	18.46	62.33	49.46	7.03
6-Gliocladium deliquescens	13.75	71.94	43.82	17.63
7- Trichoderma virens	31.76	35.18	43.47	18.24
8- Fluorescent pseudomonad No. (10)	10.82	77.92	48.10	9.59
9- Fluorescent pseudomonad No. (12)	30.50	37.76	66.24	-24.51
10- Pseudomonas fluorescens	38.92	20.57	44.18	16.96
11- Streptomyces sp.	28.69	41.15	55.86	-5.00
12- Pseudomonas fluorescens No. (2)	26.44	46.04	40.36	24.14
13- Bacillus subtiulis	8.63	82.39	51.36	2.93
14- Streptomyces sp. 15	34.90	28.78	54.30	-2.07
***15-* Control**	40.00	-	53.30	-
***16-* Apron (seed treatment)**	0.00	100	53.20	100
L.S.D. 0.05	**18.05**			**27.80**

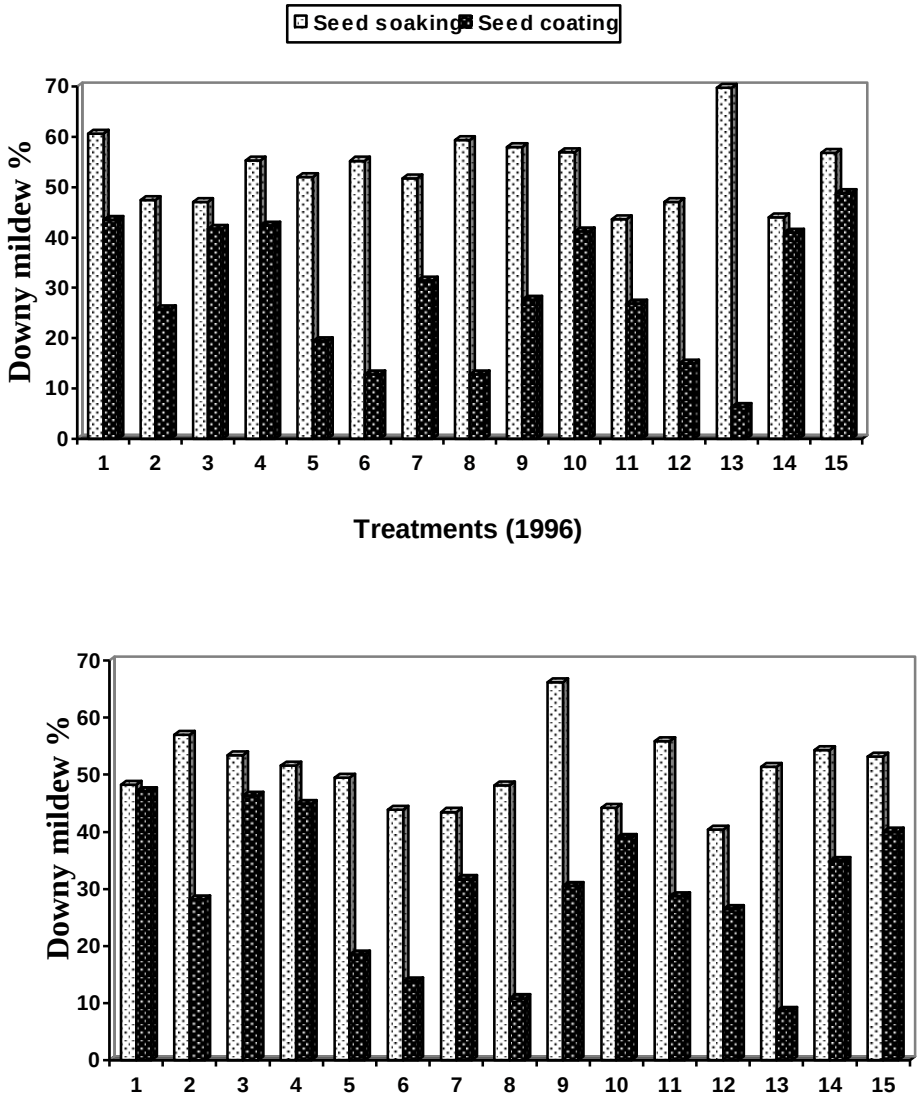

Fig. (1): Effect of some bioagents used as seed soaking and seed coating on the infection with downy mildew disease of maize during 1996 and 1997 .

REFERENCES

Abd-El-Moity, T.H. and Shatla M.N. (1981) . Biological control of white rot of onion *(Sclerotium sepivorum)* by *Trichoderma harzianum*. Phytopathology. Z., 100:29-35.

Anahousur, K.H. (1980) . Current sorghum downy mildew research in all India sorghum project. pp. 200-206. In sorghum Diseases, A World Review (Williams. R.J, Frederiksen R.A., Mughogho, L.K., and Bengston, D.C., eds), International Crops Research Institute for the Semi-Arid Tropics, Patancheru, A.P. India, PP: 469.

Backman, P.A. and Rodiguez-Kabana, R. (1975). A system for the growth and delivery of biological control agents to the soil. Phytopathology. 65:819-821.

Baker, R. (1987). Molecular biology in biological control of soilborne pathogens. Pages. 115-124 in C.J. Arntzen and C. Ryan, eds., Molecular strategies for crop protection. Alan R. Liss, NY.

El-Assiuty, E.M., Abd-El-Moity, T.H. and El-Shafey, H.A. (1986). Mycoparasitic effect of *Trichoderma harzianum* Rifai against *Cephalosporium acremonium* Corda.

Ferreira, J.H.S. F.N. Matthee and A.C. Thomas (1991). Biological control of *Eutypa lota* on grapevine by an antagonistic strain of *Bacillus subtilis.* Phytopathology. 81:283-287.

Frederiksen, R.A. (1980). Sorghum downy mildew in the United States: Overview and outlook. Plant Disease. 64:903-908.

Goutam, M., Srivastava, K.D. and Aggarwal, R. (1995). Antagonistic effect of *Trichoderma* spp. on *Ustilago segetum* var. *tritici* and their compatibility with fungicides and biocides. Indian Phytopath. 48 (4): 466-470.

Howell, C.R. (1982). Effect of *Gliocladium virens* on *Pythium ultimum* and *Rhizoctonia solani* and damping-off of cotton seedings. Phytopathology. 72:496-498.

Howell, C.R. (1991). Biological control of Pythium damping-off of cotton with seed coating preparations of *Gliocladium virens.* Phytopathology. 81:738-741.

Kaur, N.P. and Mukhopadhyay A.N. (1992). Integrated control of chickpea wilt complex by Trichoderma and chemical methods in India. Trop. Pest manag. 38:372-375.

Kenneth, R.G. (1982). Phyletochytrium sp. as a potential biological control agent against oomycetous fungi in soil. Phytoparasitica 10:120.

Key, S.J. and Stewart, A. (1994). Evaluation of fungal antagonists for control of onion white rot in sail box trait. Plant Pathol., 43:371-377.

Knauss J.F. (1976). *In vitro* antagonistic activity of several Streptomyces. spp. against species of *Pythium* and *Phytophthora.* Plant. Dis. Reptr. 60:846-850.

Kropp, B.R., and Adetuji, F.C. (1993). Variation in antagonistic action of soil borne *Pseudomonads* against fusarium culmorum and *helminthosporium sativum*. Sixth Intern. Congr. Plant Pathology, Montreal, Canada, July 28 Aug. 6; 1993, PP 267.

Lockwood, J.L. (1988). Evolution of concepts associated with soil borne plant pathogens. Ann. Rev. Phytopathol. 26:93-121.

Lumsden, R.D. and Loke, J.C. (1989). Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia* with *Gliocladium virens* in soilless mix. Phytopathology, 76:361-366.

Papavizas, G.C. (1985). *Trichoderma* and *Gliocladium*; Biology, Ecology and potential for biocontrol. Ann. Rev. Phytopathology, 23:23-54.

Utkhede, R.S. and T.S.C. Li. (1989). Evaluation of *Bacillus subtilis* for potential control of apple replant Disease. J. Phytopathology,126:305-312.

Valais, D., Brzezinski, R. and Beaulieu, C. (1993). Antagenism between ectinomycetes and *phytophthora* species causing Root Rot of Raspberry. 6th international congress of plant pathology. Montreal, Canada July, 28 August 6; 1993.

Waksman, S.A. (1962): The actinomycetes, III-Antibiotics of Actinomycetes. The Williams and Wilkins Comp, USA.

Well, H.D., Bell, D.K. and Jaworski, C.A. (1972): Efficacy of *Trichoderma harzianum* as biocontrol for *Sclerotium rolfsii,* Phytopathology 62:442-447.

Weller, D.M. and Cook, R.J. (1983): Suppression of take-all of wheat by seed treatments with *Fluorescent pseudomonads*. Phytopathology 73:463-9.

Zazzerini, A. and Tosi, C. (1985): Antagonistic activity of fungi isolated from sclerotia of *Sclerotinia sclerotiorum*. Plant Pathology 34:415-421.

المقاومه البيولوجيه ضد مرض البياض الزغبى فى الذره الشاميه المتسبب عن الفطر بيرونوسكليروسبورا سورجاى فى مصر

عصمت محمد المرساوى

معهد بحوث امراض النباتات – مركز البحوث الزراعيه

أجرى هذا البحث خلال موسم زراعة الذره الشاميه فى الفتره من عام 1996 الى 1997 بمحطة البحوث الزراعيه بالجميزه بغرض تقييم كفاءه بعض الكائنات المقاومه حيويا (والمستخدمه فى معاملة البذره) فى مقاومة مرض البياض الزغبى فى الذره الشاميه تحت الظروف المصريه .

وقد اكدت نتائج التجارب الحقليه على اختلاف نسبة الاصابه بمرض البياض الزغبى باختلاف معاملات البذره تحت هذه الدراسه . وقد كانت معاملات تغليف البذره بالكائنات المقاومه حيويا اكثر فاعليه عن معاملات غمر البذره بنفس هذه الكائنات وذلك خلال موسمى الدراسه .

كانت معاملة تغليف البذره بالبكتريا باسيلس ساتلس هى الاكثر فاعليه فى تقليل نسبة الاصابه المرضيه حيث وصلت كفاءتها مقارنة بالكنترول الى 86,97% و 82,39% خلال 1996 و 1997 على الترتيب .

من ناحيه اخرى كانت المعامله بالتريكودرما هرزيانم الاقل كفاءه فى هذا المجال حيث وصلت كفاءتها الى 11,05% و 3,78% خلال عامى 1996 و 1997 على الترتيب.

تشير النتائج الى عدم وجود تاثير محدد وواضح لمعاملات غمر البذره على نسبة الاصابه بالمرض فيما عدا العزله رقم (2) من البكتريا سيدوموناس فلوروسنس والتى كان لها تاثير غير معنوى فى مقاومة المرض مقارنة بباقى الكائنات المقاومه حيويا المختبره . فقد ادت هذه المعامله الى خفض نسبة الاصابه المرضيه من 56,8% و 53,2% الى 47,07% و 40,36% خلال عامى 1996 و 1997 على الترتيب.

لم يكن هناك فرق معنوى بين معاملة تغليف البذره بالبكتريا باسيلس ساتلس وبين المبيد الفطرى (الابرون) الموصى به فى هذا المجال .