ROLE OF VACOMIL-PLUS AND SOME PLANT EXTRACTS FOR SUPPRESSING DOWNY MILDEW DISEASE IN PROMISING MAIZE HYBRIDS

BY

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ABSTRACT

Effect of a new synthetic fungicide vacomil-plus and aqueous extracts of both onion and jimsonweed plants on a set of 30 maize hybrids were investigated for suppressing sorghum downy mildew, caused by *Peronosclerospora sorghi*. Due to foliar spraying with vacomil-plus, controlling of SDM disease on all studied hybrids was improved. With disease incidence less than 5 %, number of 27 and 26 hybrids became highly resistant during 2004 and 2005 seasons, respectively. So, lowest disease incidence of 2.21 and 1.87 % during two successive seasons leading to a greater resistance of 97.96 % were obtained and, accordingly the disease incidence was suppressed for about 85.55 %. Therefore, vacomil-plus, 1.5 g L^{-1} was recommended in the National Campaign Report of Maize Yield Improvement, for controlling SDM in Egypt. On the other hand, resistant degrees of 13 and 15 hybrids were improved during 2004 and 2005 seasons, respectively, due to immersing maize grains in onion extract. Due to use of seed treatment technique, mean of disease incidence reached 9.39 % using onion extract, leading to suppress SDM for about 35.86 %. For jimsonweed extract, poorest anti-mildew effect of 1.23 % only was observed in this study.

Keywords:*P. sorghi*, SDM, vacomil-plus, plant extracts, *Zea mays*

INTRODUCTION

Peronosclerospora sorghi (Weston & Uppal) C. G. Shaw, (syns. *Sclerospora sorghi* W. Weston & Uppal and *S. graminicola* var. *andropogenis-sorghi* Kulkarni), is an oomycete obligate pathogen caused downy mildew of sorghum and maize (SDM) . As for all obligate parasites, the pathogen cannot be cultured in a laboratory. *P. sorghi* has both a long-surviving oospore stage in the soil and a rapid repeating asexual reproduction cycle that can create epidemics from limited initial inoculums. It can also survive in debris's of perennial grasses (Williams, 1984). SDM, the most destructive disease of maize, has been reported in China, India, Indonesia, Japan, Nepal, Pakistan, Philippines, Thailand and Vietnam (Sharma, *et al.* 1993). In Egypt, SDM has been considered as a major disease of maize in areas, where sorghum is grown as a forage crop, which *P. sorghi* produces large quantities of oospores (Nazim *et al.* 1995). Conidia produced on leaves as a result of systemic infection are windblown to nearby plants and provide the inoculums for secondary (local) infection. Infection occurs when conidia germinate and enter leaf tissue via stomata (Sabry, 2003). Heavy losses in maize (as high as 100%) have been recorded due to downy mildew disease in different countries of the world (Rifin, 1983). Linear relationship has been demonstrated between disease incidence of SDM and yield loss (Craig *et al.* 1989).

For management SDM on maize, host resistance is an effective technique identified by exposing plants to the pathogen in field nurseries (Craig *et al.* 1977) or by conidial inoculation of seedlings in the greenhouse (Craig, 1976). But, to develop a variety with a stable and durable resistance needs a long and expensive process (De Leon, 1970). Durability, however, can be enhanced by using other control practices to reduce inoculum's pressure. For chemical control, metalaxyl is an effective fungicide used against growth of *Peronosclerospora sorghi* when applied to foliage (Odvedy and Frederiksen, 1984b) or to grains (Frederiksen, 1980) of sorghum and corn cultivars. Anaso, *et al.* (1989) stated that metalaxyl (as apron 35SD) was highly effective in controlling *P. sorghi*. Sabry (1996), revealed that spraying maize or sorghum plants with ridomyl-m or dressing seeds with apron (1g. a. i. /kg) effectively controlled SDM. In 2002, a report suggests that the *P. sorghi* became resistant to the apron/allegiance seed treatment. Therefore, suggestion of using new fungicides was taken in consideration. Vacomil-plus is a new synthetic fungicide, including metalaxyl, recently made available for controlling downy mildew diseases. Instead of chemical control, natural extracts have highly performance to control several plant diseases. Wilson *et al.* (1997) reported that, among 345 plant extracts, species of *Allium* were the most effective showing antifungal activity. Malhotra and Rai (1990) investigated effect of *Datura metal* extract in controlling some fungal pathogens.

This study was aimed to investigate efficiency of a new synthetic fungicide comparing with safer effective extracts for controlling sorghum downy mildew on maize (SDM). To create this purpose, a comparison between the curative activity of foliar spraying using vacomil-plus and the seed treatment of onion and jimsonweed extracts were carried out on different maize hybrids.

MATERIALS AND METHODS

1. Control agents:

For controlling sorghum downy mildew (SDM) on maize (*Zea mays* L.), foliar spraying with synthetic fungicide and seed treatment with some plant extracts were two techniques applied in this study. For foliar spraying, vacomil-plus is a new fungicide selected for this purpose. It consists, chemically, of Cupper oxychloride 35%, metalaxyl 15%, TENS-BCZ 7%, Sipernate-22S 2% and Ca-Carbonate E 41%. The recommended dose of vacomil-plus was 1.5 g L^{-1} produced by Vabco Company. For seed treatment, 25% concentration of two aqueous extracts of onion bulbs (*Allium cepa*) and jimsonweed leaves (*Datura stramonium*) were used and compared with vacomil-plus for controlling SDM. Preparation of the required concentration of both onion and jimsonweed extracts was carried out according to (EL-Moghazy and Shalaby, 2006).

2. Field experiments:

Field experiments were conducted in oospores disease nursery of Sakha Agricultural Research Station during 2004 and 2005 seasons. Maize grains were sown in plots of 9.6 m^2 in area. Each plot contains two rows with 6 m. long and 80 cm. apart. Three replicates (plots) were used in these trials. For seed treatment, maize grains of 30 hybrids were soaked in both extracts for eight hours before sowing. Grains soaked in sterilized distilled water were acted as control. 60 grains were used for each row. For foliar spraying, other untreated grains of the previous 30 hybrids were planted in the disease nursery. Using vacomil-plus, maize plants were sprayed two times after two and three weeks from planting.

3. Disease parameters:

After 35 days from planting, numbers of infected plants were recorded and, percentage of SDM disease incidence was calculated according to El-Shafey, *et al.* (1988):

$$\text{Disease incidence (DI \%)} = \frac{\text{No. of infected plants}}{\text{No. of total plants}} \times 100$$

Data obtained from disease incidence were reused to calculate percentages of disease inhibition of each treatment as follow:

$$\text{Inhibition \%} = \frac{\text{DI \% of control} - \text{DI \% of treatment}}{\text{DI \% of control}} \times 100$$

To assess the resistant degree, values were integrated with the percentages of disease incidence according to this ratio:

$$\text{Resistant \%} = 100 - \text{Disease incidence \%}$$

A complete randomize design was used in this study. Least significant differences (LSD) and Duncan's multiple range tests were applied for comparing means (Duncan, 1955).

RESULTS AND DISCUSSION

To avoid its expected harmful effect against activity of the seed embryo, foliar spraying was the selected technique suitable for vacomil-plus. This selection was supported by Anahosur (1986), who found lower effectiveness of metalaxyl, considerably the main active ingredient of vacomil-plus, when used as seed treatment for controlling SDM caused by *Peronosclerospora sorghi*. To offer cheaper and environmentally safer alternative instead of the synthetic fungicide, a technique of seed treatment with onion and jimsonweed extracts was applied to suppress SDM. To guarantee wide and successful application of the selected treatments, thirty promising maize hybrids were used and their resistance categories were also evaluated in this study. Therefore, mean percentages of disease incidence for each hybrid were calculated and grouped according to the scale adopted by Gowda *et al.* (1989) during 2004 (Table 1) and during 2005 (Table 2) seasons.

Table (1): Percentages of SDM disease incidence on 30 maize hybrids treated with different control agents during 2004 season.

Hybrids	Untreated		Vacomil-plus		Onion		Jimsonweed	
	%	cat	%	cat	%	cat	%	cat
Gm1001x Sk5001-1	27.25	MS	0.00	HR	16.67	MR	16.07	MR
Gm1001x Sk5001-2	44.62	S	7.69	R	0.00	HR	47.62	S
Gm1001x Sk5001-3	29.17	MS	0.00	HR	27.78	MS	25.00	MS
Gm1001x Sk5001-4	31.04	S	6.82	HR	14.29	MR	37.50	S
Gm1001x Sk5001-5	2.89	HR	0.00	HR	0.00	HR	0.00	HR
Gm1001x Sk5001-6	10.56	MR	9.75	R	25.00	MS	4.55	HR
Gm1001x Sk5001-8	11.36	MR	0.00	HR	0.00	HR	0.00	HR
Gm1001x Sk5001-9	13.04	MR	7.90	HR	12.70	MR	8.33	R
Gm1001x Sk5001-10	2.38	HR	4.35	HR	0.00	HR	7.50	R
Gm1001x Sk5001-11	13.66	MR	0.00	HR	20.00	MR	9.22	R
Gm1001x Sk5001-12	20.19	MS	0.00	HR	6.82	R	8.70	R
Gm1001x Sk5001-13	6.45	R	4.11	HR	0.00	HR	29.17	MS
Gm1001x Sk5001-14	1.92	HR	1.67	HR	9.26	R	20.77	MS
Gm1001x Sk5001-15	20.17	MS	0.00	HR	31.25	S	0.00	HR
Sk6241x Sk5001-1	19.49	MR	4.59	HR	0.00	HR	0.00	HR
Sk6241x Sk5001-2	10.00	R	6.67	R	5.00	HR	18.68	MR
Sk6241x Sk5001-3	35.68	S	1.56	HR	10.00	R	38.67	S
Sk6241x Sk5001-4	7.14	R	4.00	HR	15.83	MR	23.19	MS
Sk6241x Sk5001-5	10.35	MR	0.00	HR	16.67	MR	3.85	HR
Sk6241x Sk5001-6	16.52	MR	0.00	HR	8.82	R	13.86	MR
Sk6241x Sk5001-8	4.55	HR	0.00	HR	7.14	R	0.00	HR
Sk6241x Sk5001-9	1.69	HR	0.00	HR	0.00	HR	0.00	HR
Sk6241x Sk5001-10	2.28	HR	0.00	HR	0.00	HR	12.00	MR
Sk6241x Sk5001-11	2.17	HR	0.00	HR	0.00	HR	0.00	HR
Sk6241x Sk5001-12	6.08	R	1.52	HR	4.17	HR	7.50	R
Sk6241x Sk5001-13	1.52	HR	0.00	HR	0.00	HR	3.33	HR
Sk6241x Sk5001-14	20.00	MR	3.33	HR	32.38	S	15.34	MR
Sk6241x Sk5001-15	32.14	S	0.00	HR	2.27	HR	0.00	HR
SC 155	20.00	MR	2.38	HR	8.33	R	39.00	S
SC 3080	2.38	HR	0.00	HR	0.00	HR	0.00	HR

Where: S=Susceptible; MS=Moderately Susceptible; MR=Moderately Resistant; R = Resistant; HR = Highly Resistant; Cat = Categories.

Data represented in Table (1) show that, the chosen maize hybrids included 9 highly resistant, 4 resistant, 9 moderate resistant, 4 moderate susceptible and 4 susceptible hybrids. Due to foliar spraying by vacomil-plus, resistance level of all maize hybrids was improved to suppress SDM. Out of them, 27 hybrids became highly resistant, with percentages of disease incidence less than 5 %. Three hybrids of Gm1001xSk5001-2, 6 and Sk6241xSk5001-2 were resistant with disease incidence of 7.69, 9.75 and 6.67 %, respectively. These results are in agreement with the findings of Anaso, *et al.* (1989), who reported that metalaxyl has strongly effect in controlling *P. sorghi* causing SDM.

Due to seed treatment using onion extract, Gm1001xSk5001-2, 5, 8, 10, 13, Sk6241xSk5001-1, 2, 9, 10, 11, 12, 13, 15 and SC3080 became highly resistant hybrids. Moreover, resistant categories of Gm1001xSk5001-12, 14, Sk6241xSk5001-3, 6, 8 and SC155 were improved to resistant levels. On the other hand, disease incidence of six hybrids was increased as a result of onion treatment, leading to lower resistance categories. However, plant protection of most hybrids against the oospores infection in the soil using onion extract was done (Odvody and Frederiksen, 1984a).

As well as, resistance cases of 10 hybrids were improved by jimsonweed extract. To highly resistant, 6 hybrids of Gm1001xSk5001-6, 8, 15, Sk6241xSk5001-1, 5 and 15 were transformed. Three hybrids of Gm1001xSk5001-9, 11 and 12 became resistant and only one of Gm1001xSk5001-1 became moderate resistant. Moreover, resistance levels of thirteen hybrids stayed constant. In contrast, disease incidence of seven hybrids of Gm1001xSk5001-10, 13, 14, Sk6241xSk5001-2, 4, 10 and SC155 were increased and their resistance to SDM was reduced.

During 2005, results in Table (2) show further improvement in the resistant levels of 22 hybrids by using vacomil-plus, but efficiency of 8 hybrids stayed constant. On the other hand, no hybrids became less resistant after treatment with vacomil-plus, indicating high efficiency for controlling SDM. These results are in agreement with the findings of Odvody and Frederiksen (1984b), who reported that sporulation of *Peronosclerospora sorghi* was inhibited strongly by spraying of metalaxyl on sorghum and corn leaves. Therefore, anti-sporulant activity was done, but via inhibition of either spore germination or spore production. So, further study is necessary.

Due to onion extract, resistance levels of 15 hybrids were changed to better. Out of them, five hybrids of Gm1001xSk5001-2, 5, 10, 14 and Sk6241xSk5001-1 were improved to highly resistant. Six hybrids of Gm1001xSk5001-3, 11, 15, Sk6241xSk5001-2, 5 and 6 became resistant, in addition to two moderate resistant and two moderate susceptible. As well as, resistance categories of 12 hybrids stayed constant. On the other hand, disease incidence of three hybrids of Gm1001xSk5001-1, 6 and Sk6241xSk5001-8 were increased and their resistance to SDM was reduced in this season.

For jimsonweed extract, 7 hybrids were transformed into more resistant level, 13 hybrids stayed constant and 10 hybrids became less resistant to SDM. It indicates that, fewer efficiency of jimsonweed extract against SDM on the tested maize hybrids, in comparison with onion, was observed.

Table (2): Percentages of SDM disease incidence on 30 maize hybrids treated with different control agents during 2005 season.

Hybrids	Untreated		Vacomil-plus		Onion		Jimsonweed	
	%	cat	%	cat	%	cat	%	cat
Gm1001x Sk5001-1	19.44	MR	9.38	R	30.03	S	20.56	MS
Gm1001x Sk5001-2	10.64	MR	3.57	HR	0.00	HR	12.50	MR
Gm1001x Sk5001-3	14.59	MR	2.63	HR	5.56	R	14.29	MR
Gm1001x Sk5001-4	36.49	S	1.92	HR	29.55	MS	37.98	S
Gm1001x Sk5001-5	5.77	R	2.50	HR	0.00	HR	0.00	HR
Gm1001x Sk5001-6	8.70	R	0.00	HR	35.63	S	21.47	MS
Gm1001x Sk5001-8	5.68	R	1.79	HR	7.14	R	14.29	MR
Gm1001x Sk5001-9	19.64	MR	2.00	HR	15.00	MR	17.93	MR
Gm1001x Sk5001-10	18.81	MR	6.52	R	0.00	HR	15.14	MR
Gm1001x Sk5001-11	22.67	MS	0.00	HR	10.00	R	19.23	MR
Gm1001x Sk5001-12	8.07	R	8.18	R	7.50	R	15.22	MR
Gm1001x Sk5001-13	14.50	MR	0.00	HR	14.56	MR	10.00	R
Gm1001x Sk5001-14	11.62	MR	1.92	HR	4.35	HR	12.70	MR
Gm1001x Sk5001-15	10.94	MR	5.36	R	9.79	R	0.00	HR
Sk6241x Sk5001-1	25.08	MS	0.00	HR	4.00	HR	24.77	MS
Sk6241x Sk5001-2	17.56	MS	0.00	HR	6.82	R	34.76	S
Sk6241x Sk5001-3	43.80	S	3.23	HR	24.76	MS	30.15	S
Sk6241x Sk5001-4	18.18	MR	0.00	HR	12.50	MR	19.07	MR
Sk6241x Sk5001-5	18.51	MR	0.00	HR	8.33	R	15.26	MR
Sk6241x Sk5001-6	13.64	MR	0.00	HR	5.36	R	11.69	MR
Sk6241x Sk5001-8	3.18	HR	0.00	HR	7.14	R	6.67	R
Sk6241x Sk5001-9	3.37	HR	0.00	HR	0.00	HR	34.90	S
Sk6241x Sk5001-10	4.55	HR	0.00	HR	2.00	HR	5.36	R
Sk6241x Sk5001-11	3.03	HR	3.03	HR	0.00	HR	3.57	HR
Sk6241x Sk5001-12	20.31	MS	0.00	HR	14.52	MR	8.82	R
Sk6241x Sk5001-13	3.03	HR	1.92	HR	0.00	HR	0.00	HR
Sk6241x Sk5001-14	19.39	MR	2.17	HR	14.76	MR	35.29	S
Sk6241x Sk5001-15	4.84	HR	0.00	HR	4.06	HR	6.06	R
SC 155	40.92	S	0.00	HR	15.48	MR	19.19	MR
SC 3080	4.76	HR	0.00	HR	0.00	HR	10.71	MR

To overcome the discrepancy between efficiency of the evaluated hybrids for controlling SDM, averages of all data obtained by each treatment during 2004 and 2005 seasons were compared in Table (3).

Table (3): Effect of vacomil-plus, onion and jimsonweed extracts on disease incidence and inhibition of SDM during two seasons.

Treatment	% Disease incidence		Mean %	Inhibition %
	2004	2005		
Untreated (control)	14.22 a	15.06 a	14.64 a	0.0
Vacomil-plus	02.21 c	01.87 c	02.04 c	85.55
Onion	09.15 b	09.63 b	09.39 b	35.86
Jimsonweed	13.00 a	15.92 a	14.46 a	01.23
LSD at 5 %	1.88	1.88	1.12	
LSD at 1 %	2.74	2.74	1.63	

The results indicate that, vacomil-plus was the most effective fungicide suppressing SDM in comparison with the other agents. Using vacomil-plus, lowest percentages of SDM disease incidence of 2.21 and 1.87% during 2004 and 2005 were obtained, respectively, leading to higher SDM inhibition reached 85.55%. It means that, plant protection against infection of the conidiospores in the plant leaves by the foliar spraying of vacomil-plus was done. On the other hand, mean of the disease incidence reached to 9.39% for onion extract when applied as seed treatment in comparison with 14.64 and 14.46% for untreated and jimsonweed extract, respectively. Fungitoxicity of onion extract, leading to inhibition of SDM reached 35.36%, may be due to presence of some antibiotics, in the form of phenolic substances, (Dubey and Dwivedi, 1991). Wilson *et al.* (1997) reported that, among 345 plant extracts, species of *Allium* were the most effective showing antifungal activity. *Allium* spp. may have an antifungal compounds play an important role in controlling several diseases (Kurucheve *et al.* 1997). In contrast, lowest disease inhibition reached 1.23% with *Datura stramonium* extract was obtained, indicating poorest effectiveness for controlling SDM. It was supported by Malhotra and Rai (1990), who found low effect of *Datura metal* extract against some fungi. Using 25 % aqueous extract of onion and jimsonweed, great suppression of late wilt disease on maize caused by *Cephalosporium maydis* was successfully obtained (EL-Moghazy and Shalaby, 2006).

To summarize these findings, mean percentages of SDM resistant for all treatments were plotted in Fig. (1). It demonstrates that, the new synthetic fungicide of vacomil-plus was the most effective treatment controlling SDM on maize with resistant percentage of 97.96%, followed by 90.61 and 85.54% for onion and jimsonweed extracts, respectively.

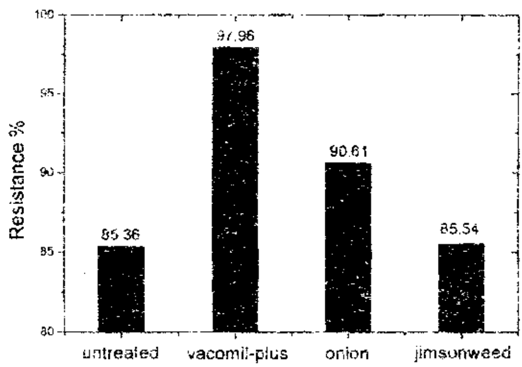

Fig. (1): Mean percentages of SDM resistant resulting from spraying with vacomil-plus or soaking the hybrid seeds in onion or jimsonweed extracts.

Due to its realized toxicity against conidiospores activity, vacomil-plus inhibits local infection of SDM strongly in this study. It exhibited remarkable antisporulant effect even at concentration of 1.5 g L^{-1}. Deepak *et al.* (2006) stated that inhibition of asexual spores of *Sclerospora graminicola* causing Pearl millet downy mildew by metalaxyl is important, because these provide the greatest opportunity for rapid built up in the number of infective propagates and subsequently, the high potential for infection of new plants. Metalaxyl also reduced disease severity and sporulation of *Plasmopara viticola* causing grapevine downy mildew disease when used in protecting mode (Wong and Wilcox, 2001).

Therefore, foliar spraying using 1.5 g L^{-1} of a new fungicide vacomil-plus was recommended by the National Campaign Report of Maize Yield Improvement, for controlling SDM in Egypt. On the other hand, soaking of maize grains in onion extract can prevent, or at least reduce germination of oospores in the soil and offer environmentally safer and effective technique toward systemic infection of SDM. Although the screening activity against an obligate endoparasite is complicated, Deepak *et al.* (2005) investigated the antisporulation activity of *Sclerospora graminicola* by 40 plant extract and genus *Allium* was of them.

To evaluate efficiency of SDM control techniques obtained in this study, the presented results were compared with the biological control data previously cited in literatures. El-Moghazy (2003) found that *Bacillus subtilis* was the most effective bio-agent reducing SDM on maize comparing with *Trichoderma viride* and *T. hamatum*. This is in agreement with El-Mersawy (2000), who found that *Bacillus subtilis* suppressed SDM strongly. Due to synthesis of a specific antibiotic, namely, subtilin or bacillin, most *B. subtilis* strains possess high potentiality to inhibit activity of different pathogens *in vitro* and *in vivo* (De, 1982 and Utkhede and Sholberg, 1986).

Recently, a set of 42 tropical/sub-tropical maize inbred lines developed in different countries in Asia (India, Thailand and Philippines) and Mexico were evaluated for resistance to *P. sorghi* causing SDM (Yen *et al.* 2004). In conclusion, this study is an initial attempt for successful evaluation of a set of 30 cross hybrids of maize cultivars originated in Egypt for resistance to sorghum downy mildew caused by *P. sorghi*. A foliar spraying of vacomil-plus and soaking grains in onion extract can be suggested for integrated suppression of SDM in this study. Additionally, ideal SDM control must include resistant hybrids, biological control and crop rotation to reduce spore populations over several years. To avoid SDM on maize cultivars and to minimize soil infestation with the pathogen, no-cultivation of susceptible forage sorghum in maize growing region must be taken into consideration.

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الملخص العربى

دور فاكوميل- بلس وبعض المستخلصات النباتية فى مكافحة مرض البياض الزغبى فى هجن الذرة المبشرة

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درس تأثير المبيد الفطرى النخليقى الجديد فاكوميل- بلس وكذا مستخلصات كل من البصل والداتورة على مجموعة مكونة من ٣٠ من هجن الذرة الشامية لمكافحة مرض البياض الزغبى المتسبب عن فطر *Peronosclerospora sorghi*. ولقد أدى إستخدام فاكوميل-بلس رشا على المجموع الخضرى إلى تحسين مستوى المقاومة لكل الهجن موضع الدراسة. وبنسبة إصابة أقل من ٥ % ، فقد أصبح عدد ٢٧ و ٢٦ من هجن الذرة هجناً عالية المقاومة خلال موسمى ٢٠٠٤ و ٢٠٠٥ على التوالى. حيث تحققت نسبة إصابة دنيا بلغت ٢,٢١ و ١,٨% خلال موسمين متعاقبين أدت إلى مقاومة فائقة بلغت ٩٧,٩٦% ، مع كبح نسبة حدوث المرض بحوالى ٨٥,٥٥ % . لذلك فقد تمت التوصية باستخدام فاكوميل- بلس بمعدل ١,٥ جم/ لتر فى مقاومة مرض البياض الزغبى فى الذرة من خلال نشرة الحملة القومية للنهوض بمحصول الذرة فى مصر. على الجانب الأخر، تحسنت درجات المقاومة لعدد ١٣ و ١٥ من الهجن خلال موسمى ٢٠٠٤ و ٢٠٠٥ على التوالى نتيجة لنقع حبوب الذرة فى مستخلص البصل. وبسبب إستخدام تكنيك معاملة البذور، فان متوسط نسبة الأصابة بلغت ٩,٣٩ % باستخدام مستخلص البصل مما أدى إلى كبح معدل المرض بحوالى ٣٥,٨٦ %. وبالنسبة لمستخلص نبات الداتورة، فقد تضائل التأثير المضاد للبياض الزغبى حيث بلغ ١,٢٣ % فقط فى هذه الدراسة.