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Screening of Some Biotic and Abiotic Agents for Controlling Maize Late Wilt Disease Caused by *Magnaporthiopsis maydis*

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Abstract: Current work was conducted to evaluate 4 biotic and 8 abiotic agents, as seed soaking, against maize late wilt disease caused by *Magnaporthiopsis maydis*. It was performed *in vitro* and/or *in vivo* to achieve satisfied degree of disease control. Obtained results revealed that all tested fungicides in all doses were *in vitro* effective and completely inhibited *M. maydis* growth. Used fungicides herein ranked the first for antifungal activity followed by *Pseudomonas fluorescens*, sodium and potassium silicate. Greenhouse and field experiments showed that, Strong-X fungicide exhibited stability in its activity against the disease incidence with significant differences compared with the control. However, yield parameters obtained following the tested fungicides, even with the high application dose, were insignificantly different from the control. Meanwhile, potassium and sodium silicate as well as *P. fluorescens* were equivalent or superior the tested fungicides in protecting maize plants from wilt. Furthermore, sodium silicate significantly enhanced the 100-kernel weight (100KW) and net grains weight of ear (NGWE) per plant, whereas potassium silicate increased NGWE/plant only. On the other hand, soaked seeds in the suspension of *Enteromorpha flexuosa* and *Ulva fasciate* exhibited high performance for disease reduction with both application doses in the field and significantly reflected only on NGWE/plant. It could be concluded that the ecofriendly agents; potassium silicate, sodium silicate, *P. fluorescens*, *E. flexuosa* and *U. fasciate* are promising for control the disease. Further studies are needed to test other application methods of these promising materials against maize late wilt disease to maximize the obtained benefit.

Keywords: Corn, Soil Borne Fungi, Disease Control, *M. maydis*

1. Introduction

Late wilt caused by *Magnaporthiopsis maydis* [1] is the most serious disease affects Egyptian maize (*Zea mays* L.) production [2, 3]. *M. maydis* reported also to attack maize cultivars grown in many countries such as India [4], Israel [5], Portugal [6], and Spain [7]. This vascular disease was firstly recorded on Egyptian maize cultivars in 1960. The causal fungus colonizes the plant xylem resulting in late wilt and poorly or no develops cub particularly in the severe infection [8].

It has been reported to cause severe damage in the Egyptian maize hybrids [9, 10]. However, negative correlation had been previously found between disease incidence and grain yield [11, 12]. Additionally, Yield loss might reach 40 - 70% depending on time of symptoms appearance and degree of cultivars susceptibility [10, 11, 13]. The sudden appearance of disease symptoms with rapid wilting [14] make the management more difficult and magnify the yield loss.

Due to the economic importance of late wilt disease of maize [11, 15], an approach of management strategies should be followed to control this disease or at least minimize its impact on the plant performance and final grain yield. However, various control efforts have frequently been directed toward late wilt disease in Egypt. Fungicidal [16, 17] and biological control [18-21] as well as the use of organic acids, organic salts and essential oils [22] were employed.

Despite of those efforts, until now no clear recommended strategy or commercial product was existed for late wilt control in Egypt. Therefore, this study aimed to screen some biotic and abiotic agents against late wilt disease and their impact on maize yield.

2. Materials and Methods

2.1. M. maydis Source

M. maydis isolate Mm-36 was chosen from the culture collection of Maize and Sugar Crops Diseases Research Section, Plant Pathology Research Institute, A.R.C, for use in this study.

2.2. In Vitro Screening of Tested Materials

This experiment was conducted to examine the activity of 2 bacterial bio agents *i.e. Pseudomonas fluorescens*, and *P. putida* [23] as well as 6 fungicides and 2 silicate compounds (Table 1) for controlling the causal agent of late wilt disease. Tested bacteria were grown on the broth of Kings B medium for two days at 27°C. Serial dilutions were done from the growing bio agents and 3 concentrations (stock, 10^8 and 10^6 CFU. /ml) were adjusted [24] to use in this study.

Potato dextrose agar media (PDA) were amended with different volumes of the tested materials (bacteria, fungicides and silicates) just before pouring in Petri plates to obtain the desired concentrations (Table 1). Treated PDA plates were subsequently inoculated with 10-mm agar plugs from the growing 5- day-old *M. maydis* culture. The inoculated plates were then incubated at 27°C and the radial growth (mm) was measured after 6 days. Three doses of each material were tested (Table 1) and 5 replicates for each treatment were used. A mixture of Sterile distilled water and PDA in other plates served as control. Radial growth values (including the fungal plug diameter, 10 mm) were recorded.

2.3. In Vivo Experiment

2.3.1. Inoculum Preparation of M. maydis

Soil infestation technique was applied to evaluate the activity of the promising materials (obtained from the *in vitro* screening) for reducing the number of wilting plants. Autoclaved glass bottles (500 gm in capacity) each contained about 100 gm of wet sorghum grains were used for inoculum preparation. Prepared bottles were inoculated with pieces (2 cm^2) of 5-days old *M. maydis* culture and incubated at room temperature (27-30°C) for about 3-4 weeks [25] for reaching sufficient growth of the fungus. Obtained fungal mass were poured out and mixed with loam soil at the rate of 100g of *M. maydis* colonized seeds per 10Kg potted soil.

2.3.2. Screening of Tested Materials

Two seaweeds (as dry powder); *Enteromorpha flexuosa* (Wulfen) & *Ulva fasciate* (Delile) along with the above mentioned bacteria, silicate compounds and fungicides (Table 1) were evaluated in the following experiments.

(i). Greenhouse Trial

Ten kernels of the susceptible hybrid SC 3062 [11], free from commercial fungicides, were individually cultivated per pot (35 Cm.) after soaking in the tested material for 2h followed by air drying. Two doses of applications were used in this experiment and kernels soaked in water were served as control (Table 3). Five pots (each contains five plants) per treatment were used and all the recommended cultural practices were carefully followed. Disease incidence was expressed as a percentage of infection and recorded after 90 days of planting. After disease assessment, healthy appearing plants were used for *M. maydis* isolation to determine the effect of the tested materials on its entrance into the plant.

Table 1. *Biotic and abiotic agents tested for controlling M. maydis; the cause of maize late wilt disease and its application doses.*

Tested materials	Application doses		
	Dose 1	Dose 2	Dose 3
1. Folicure fungicide EC, (Tebuconazole 25%)	0.25*	0.5	1.0
2. Leader EC, fungicide (Prochloraze 12.5%)	0.5	1.0	2.0
3. Opera SE, fungicide (Pyraclostrobin 18.3% + Epoxiconazole 18.3%)	0.625	1.25	2.5
4. Score EC, fungicide (Difenconazole 25%)	0.25	0.5	1.0
5. Secons EC, fungicide (Difenconazole 15% + Propiconazole 15%)	0.25	0.5	1.0
6. Strong-x EC, fungicide (Pyraclostrobin 18.7% + Propiconazole 11.7%)	0.125	0.25	0.5
7. Potassium silicate (Solution, LOBA CHEMIE. PVT. LTD)	10g/L**	20g/L	40g/L
8. Sodium Silicate (Sodium metasilicate, Advent Chembio PVT. LTD)	10g/L	20g/L	40g/L
9. *P. fluorescens*	Stock	10^8 CFU	10^6 CFU***
10. *P. putida*	Stock	10^8 CFU	10^6 CFU
11. *E. flexuosa*	33g/L	-	133g/L
12. *U. fasciate*	33g/L	-	133g/L

* All fungicides were applied as ml/L; g/L**=gram/Liter; CFU*** = Colony forming unit/ml.

(ii). Field Trial

The impact of tested materials on the incidence of maize late wilt and the subsequent yield component were investigated in Giza Agricultural Research Station, ARC, Egypt. Triplicate randomized complete block design was conducted in 2020 where the plots consisted of one row (6 m) spaced 70 cm from adjacent one. Toilet paper bags each contained 2 treated kernels (Pioneer SC 3062) as described in the greenhouse trail combined with 10 gm of *M. maydis* inoculum were prepared [11]. All bags were sown in a hill, 3-5 cm depth, spaced 20 cm apart. Bags contained non-soaked kernels and *M. maydis*-colonized sorghum seeds served as control. Growing plants were manually thinned to one, 21 days of emergence and all recommended agricultural treatments (irrigation and fertilization) were performed. Disease incidence as a percentage of infection was recorded after 90 days of planting. Net grains weight of ear (NGWE), 100 kernel weight (100KW) and moisture content (MC%) were recorded after harvest [11]. NGWE and 100KW were adjusted to 15.5% of moisture content [26, 27]. The following modified formula [28] of Carangal *et al.,* [29] was used to express the yield in g/plant:

$$\text{Grain yield (g/plant)} = \frac{\text{Fresh ear weight (g/plant)x(100−mc)x0.8}}{(100-15.5)}$$

Where, mc: moisture content % in grains at harvest, 0.8: shelling coefficient and 100-15.5: standard value of grain moisture at 15.5 %.

2.4. Statistical Analysis

Obtained data from both *in vitro* and *in vivo* experiments were subjected to statistical analysis using Web Based Agricultural Statistics Software Package (WASP). The least significant differences (LSD) were used to compare means.

3. Results

3.1. In Vitro Screening of Tested Materials

Statistical analysis revealed the significance of the interaction (Materials X tested concentrations) indicating that effect of any material is depending on the concentration used. All tested fungicides were significantly effective and completely inhibited the *M. maydis* growth. Most of the other materials were also effective compared with the control but potassium and sodium silicates were more efficient ones for reducing *M. maydis* growth (Table 2).

Table 2. *Effect of biotic and abiotic agents on M. maydis growth on PDA, 6 days after incubation at 27°C.*

Tested materials	Fungal growth (mm)*				Efficacy %			
	Dose1	Dose 2	Dose 3	M	Dose1	Dose 2	Dose 3	Mean
1. Folicure fungicide	10.00	10.00	10.00	10.00	88.68	88.68	88.68	88.68
2. Leader EC, fungicide	10.00	10.00	10.00	10.00	88.68	88.68	88.68	88.68
3. Opera SE, fungicide	10.00	10.00	10.00	10.00	88.68	88.68	88.68	88.68
4. Score EC, fungicide	10.00	10.00	10.00	10.00	88.68	88.68	88.68	88.68
5. Secons EC, fungicide	10.00	10.00	10.00	10.00	88.68	88.68	88.68	88.68
6. Strong-x EC, fungicide	10.00	10.00	10.00	10.00	88.68	88.68	88.68	88.68
7. Potassium silicate	65.00	32.60	52.80	50.13	27.03	63.21	40.53	43.59
8. Sodium Silicate	56.20	54.40	42.40	51.00	36.38	38.21	51.97	42.19
9. *P. fluorescens*	56.80	69.00	78.00	67.93	35.72	21.95	12.26	23.31
10. *P. putida*	80.00	70.20	61.80	70.67	9.41	20.68	30.24	20.11
11. Control	88.40	88.40	88.40	88.40	0.00	0.00	0.00	0.00
Mean	36.95	34.06	34.86		58.24	61.47	60.65	
LSD 0.05 (AxB)				12.340				13.884

* Fungal growth values included the plug diameter used for inoculation (10.00 mm).

Table 3. *Effect of biotic and abiotic agents on M. maydis late wilt incidence, in greenhouse.*

Tested materials	Disease incidence %		
	Dose 1	Dose 3	Mean
1. Folicure fungicide	80.00	60.00	70.00
2. Leader EC, fungicide	0.00	48.33	24.17
3. Opera SE, fungicide	40.00	60.00	50.00
4. Score EC, fungicide	0.00	6.67	3.33
5. Secons EC, fungicide	8.33	20.00	14.17
6. Strong-x EC, fungicide	6.67	6.67	6.67
7. Potassium silicate	0.00	0.00	0.00
8. Sodium Silicate	0.00	0.00	0.00
9. *P. fluorescens*	0.00	0.00	0.00
10. *P. putida*	40.00	48.33	44.17
11. *E. flexuosa*	80.00	40.00	60.00
12. *U. fasciate*	53.33	73.33	63.33
13. Control	80.00	80.00	80.00
Mean	29.87	34.10	
LSD 0.05 (AxB)			29.93

3.2. Effect of Tested Materials on Late Wilt Disease in Greenhouse

Seed soaking in potassium and sodium silicate solutions as well as *P. fluorescens* protected maize plants from wilting (no disease incidence). Disease incidence was also reduced when seeds were treated by Strong-X (6.7%), Score (0-6.7%), Secons (8-20%) and Leader (0-48%). Furthermore, the activity of tested materials for reducing the number of wilting plants was dependent on the used dose, since the interaction (Material X tested concentration) was significant (Table 3).

Table 4 shows that up to 90 days of planting, *M. maydis* was recovered from 5.6% & 7.1% of the plants that appeared healthy in Leader and strong-X fungicides treatments respectively for the two tested doses. However, plants emerged from kernels that soaked in the Score and Secons fungicides, potassium and sodium silicates as well as *P. fluorescens* were internally free from *M. maydis*.

Table 4. *Effect of biotic and abiotic agents on the entrance of M. maydis into maize plants.*

Tested materials	**Application doses**	**Number of plants**	
		appeared healthy.	**contained fungus**
Leader EC, fungicide	0.50 Cm/L	11	0
	1.00 Cm/L	7	1
Score EC, fungicide	0.25 Cm/L	15	0
	0.50 Cm/L	14	0
Secons EC, fungicide	0.25 Cm/L	10	0
	0.50 Cm/L	9	0
Strong-x EC, fungicide	0.125 Cm/L	14	2
	0.25 Cm/L	14	0
Potassium silicate	10g/L	15	0
	40g/L	15	0
Sodium Silicate	10g/L	14	0
	40g/L	8	0
P. fluorescens	Stock	14	0
	10^8 CFU	15	0
Control	Infested	14*	14
	Fungal free	15	0

* Plants showed late wilt symptoms.

3.3. Effect of Tested Materials on Late Wilt Disease in the Field

All tested materials were varied in reducing the wilting plants compared with the control (Table 5). Statistical analysis (ANOVA) showed the significance of the interaction (Materials X concentrations) also. Sodium and potassium silicates were the most effective materials provided the lowest percentages of wilting plants (0-5 and 0-11.7% respectively), followed by *U. fasciate* (12.33%) under the two doses of applications. Meanwhile, Folicure, Opera, Strong-X and Score fungicides as well as *E. flexuosa* and *P. fluorescens* were promising under only one of the two tested doses.

Table 5. *Effect of biotic and abiotic agents on M. maydis late wilt incidence, in the field.*

Tested materials	**Dose 1**	**Dose 3**	**Mean**
1. Folicure fungicide	9.00	39.00	24.00
2. Leader EC, fungicide	26.67	24.00	25.33
3. Opera SE, fungicide	0.00	20.00	10.00
4. Score EC, fungicide	31.67	12.67	22.17
5. Secons EC, fungicide	16.67	26.67	21.67
6. Strong-x EC, fungicide	13.33	0.00	6.67
7. Potassium silicate	0.00	11.67	5.83
8. Sodium Silicate	5.00	0.00	2.83
9. *P. fluorescens*	4.33	26.67	15.50
10. *P. putida*	21.00	27.67	24.33
11. *E. flexuosa*	12.33	15.00	13.67
12. *U. fasciate*	12.67	12.33	12.50
13. Control	44.33	44.33	44.33
Mean	15.21	20.00	
LSD 0.05 for interaction (AxB)			24.06

***Table 6.** Effect of biotic and abiotic agents on maize yield components in M. maydis infested field after 120 days of sowing.*

Tested materials	Weight of 100 Kernel/plant			Grain net weight/plant		
	Dose 1	Dose 3	Mean	Dose 1	Dose 3	Mean
1. Folicure fungicide	24.18	21.69	22.94	134.40	126.94	130.67
2. Leader EC, fungicide	22.36	23.27	22.81	127.61	140.38	133.99
3. Opera SE, fungicide	27.22	22.82	25.02	150.92	126.02	138.47
4. Score EC, fungicide	21.86	22.80	22.33	119.12	122.33	120.73
5. Secons EC, fungicide	22.82	24.57	23.69	106.35	136.72	121.54
6. Strong-x EC, fungicide	24.58	26.91	25.88	139.60	143.44	141.52
7. Potassium silicate	27.01	24.10	25.56	168.95	150.57	159.76
8. Sodium Silicate	26.98	29.37	28.18	163.37	175.78	169.58
9. *P. fluorescens*	24.49	22.72	23.61	142.70	123.50	133.10
10. *P. putida*	24.32	24.26	24.29	137.31	131.76	134.53
11. *E. flexuosa*	25.68	25.42	25.55	160.49	158.74	159.62
12. *U. fasciate*	25.96	24.31	25.14	148.20	152.84	150.52
13. Control with fungus	22.75	22.76	22.76	112.94	112.94	112.94
Mean	24.65	24.23		139.38	138.61	
LSD 0.05 for materials (A)			4.014			32.021

Table 6 shows the impact of tested materials on the maize yield component in the field. Generally, most of the tested materials improved both of the 100 KW and NGWE per plant. Treatment with sodium silicate proved the highest values; 28.18 g and 169.58 g for both yield components respectively. On the other hand, kernel soaked with *U. fasciate*, *E. flexuosa* and potassium silicate increased NGWE only (150.52, 159.6 and 159.8 g respectively) compared with the control (112.94 g/ear).

4. Discussion

Maize late wilt caused by *M. maydis* is very serious and economically important disease in Egypt. The risk of this disease has attributed to its sudden occurs at the flowering time and the wilt upwardly rapid progress in the sensitive hybrids [3, 30]. It causes considerable yield loss depending on the time of symptoms appearance [11] and any treatment at this time is not useful. Therefore, the present study screened some biotic and abiotic materials for their activity to suppress maize late wilt causal agent *in vitro* and the disease incidence in greenhouse. Then, the tested materials were applied in the inoculated field aiming to protect the grown plants early from infection and improve their productivity.

Fungicides have been considered the second strategy after resistant or tolerant hybrids (or varieties) for successful management of most soil borne diseases [31, 32]. Obtained results revealed that all tested fungicides in all doses were *in vitro* effective and completely inhibited *M. maydis* growth and ranked the first for antifungal activity compared with other tested materials. On contrary, in the greenhouse and field trails, tested fungicides were mostly equal or followed potassium and sodium silicate as well as *P. fluorescens* for their ability to protect maize plants from wilt. Furthermore, Strong-X fungicide exhibited stability in its activity against the fungal growth and the disease incidence with significant differences compared with the control. Pyraclostrobin and Propiconazole, the active ingredients of Strong-X fungicide, were previously reported to be active against maize late wilt disease [31]. Furthermore, Propiconazole belongs to Triazole family and demethylation inhibiting group (DMI) fungicides. Such group previously reported to suppress C14-demethylase enzyme (which involved in sterols production as well as structure and function of fungal cell membrane) causing abnormal growth and death of fungi [33, 34]. Meanwhile, Strobilurin based fungicides (Pyraclostrobin) reported to disrupt the production of ATPs through the inhibition of electron transportation between b and c1 cytochromes in the mitochondrial respiratory chain [35]. These findings may explain the efficacy of Triazole and Strobilurin based fungicides in the *in vitro* suppression of fungal growth and *in vivo* disease reduction [17, 31, 36]. Additionally, variation in the efficacy level of the same or different fungicide group (s) against *M. maydis* obtained in this study has previously been observed [24, 37]. This result may be attributed to the variation in the level of intrinsic activity of each fungicide against the same pathogen [38]. Factors such as physical or chemical properties of the active ingredient, plant characters, prevalent conditions and application technique may also affect the biological activity of fungicides under field conditions [39-41]. On the other hand, although the promising effect of most fungicides used for reducing the fungal growth and the disease incidence, especially strong-X, there were no significant differences appeared on both of 100 KW & NGWE/plant compared with the control. This result reflects some inconsistent with that obtained by Degani and Cernica [42]. They reported that, Azoxystrobin (AS) when injected into a drip irrigation line assigned for each row reduced *M. maydis* infected plants in the field, furthermore, seed coating provided an additional layer of protection. On the other hand, Degani *et al.,* 2018 [31] reported that seed coating by mixture of Azoxystrobin (AS) + Difenoconazole (DC) fungicides combined with its addition with drip irrigation, minimized wilt symptoms by 41% and increased the yield from 58 to 75%. The inconsistent of our result with that obtained by Degani and Cernica, 2014 and Degani *et al.,* 2018 [31, 42] may be due to the excessive doses received by plants before and after planting (seed coating and in drip irrigation line respectively) in their studies.

Regarding the biotic agents; *P. fluorescens* showed high performance for reducing the wilting plants *in vivo* but its impact on yield components was insignificant compared with the control. *P. fluorescens* activity may be attributed to its capability of producing chitinase and cellulase enzymes that explained its successful usage in the management of sugar beet root-infecting *Fusarium oxysporum* f. sp. *betae* [23, 43]. Furthermore, it may also be due to the secretion of cyclic lipopeptide (CLP) which inhibited the growth of *Gaeumannomyces graminis var. tritici* and *Rhizoctonia solani in vitro* [44]. Meanwhile, seed soaking in the suspension of *E. flexuosa* and *U. fasciate* exhibited high performance for disease reduction with both application doses used in the field experiment and significantly reflected only on NGWE/plant. Marine algae are rich source of nutrients and bioactive compounds, which can improve the cellular metabolism, growth and also disease tolerance in plants [45-49].

Potassium and sodium silicate showed moderate activity against the *in vitro* growth of fungal pathogen regardless the rat of use. Whereas, in the greenhouse and field trails, seed soaking in each of the silicate solutions exhibited high activity and stability for reducing wilted maize plants with the two doses used. Furthermore, sodium silicate treatment significantly enhanced the 100KW and NGWE/ plant, whereas potassium silicate increased NGWE/plant only. These results were agreed with the finding of Farahat, 2019 [50], who reported that late wilt incidence was reduced, maize yield was enhanced and peroxidase enzyme activity was significantly increased following sodium silicate treatment. In addition, silicon was reported to; stimulate the growth of maize, either influence the development of casparian bands, suberin lamellae and root vascular tissues [51], or accumulates in epidermal cell wall inhibiting fungal penetration [52-54]. Potassium and sodium silicate also reported to suppress *Rhizoctonia solai*, the causal agent of sugar beet damping-off, and reduced the disease under greenhouse conditions [55].

5. Conclusion

M. maydis the causal agent of maize late wilt could be controlled *in vitro* and *in vivo* by all fungicides used in this study but without yield enhancing. However, potassium and sodium silicates as well as *P. fluorescens* and *E. flexuosa* were *in vivo* equivalent or superior the tested fungicides for protection of maize plants against such disease. Furthermore, potassium and sodium silicates as well as *E. flexuosa* were significantly enhanced one or two of the measured yield parameters. It could be concluded that late wilt disease of maize could be controlled by these promising ecofriendly fungicide alternatives.

Conflicts of Interest

The authors declare no conflicts of interest.

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