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Using Green Chemicals and Biological Control Agents for Controlling the Seed-Borne Pathogen *Fusarium moniliforme* in Sugar Beet

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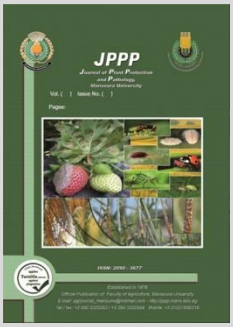

ABSTRACT

Seeds of fifteen sugar beet (*Beta vulgaris* L.) cultivars were collected in 2018 and 2019 in Egypt and screened for their seed-borne mycoflora using deep-freezing blotter method. Among those fungi recovered from sugar beet seeds, a plant pathogen *Fusarium moniliforme* was the most dominant. Thus, it was tested for its pathogenicity and transmission against sugar beet plants. Green chemicals (antioxidants) and biological control agents were used for suppressing *F. moniliforme* in comparison with Fludioxonil/Mefenoxam (Maxim XL 3.5% FS®; a chemical fungicide), Potassium silicate at concentrations (4, 6, 8, 10 and 12 ml/L), Nicotinic acid at concentrations (1, 5, 10, 15 and 20 mM/L), *Trichoderma harzianum*, *T. hamatum* and *Bacillus subtilis* were tested against *F. moniliforme* in vitro, in the greenhouse and in the field. The results assured that nicotinic acid at 5 mM/L, *T. harzianum* and *T. hamatum* and potassium silicate at 12 ml/L were the best treatments compared with the Maxim® fungicide (control). But nicotinic acid at 5 mM/L was the most effective among all treatments.

Keywords: Fusarium moniliforme, green chemicals, biological control agents, sugar beet.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is a relatively new harvest cultivated in temperate regions and spreading widely just in the twentieth century and now developed in 50 countries (James, 2004). Most is grown at latitudes between 30 and 60ºN, as a summer crop in prairie, maritime, semi-tarry and some semi-dry and dried climates and as a summer and/or winter crop in Mediterranean and other semi-dried conditions (Draycott, 1972).

Sugar beet is an perfect crop for production of sugar in Egypt. Its area increases year next year to meet the growing population calls. The total cultivated zone of sugar beet reached around 563422 feddans with 12.11 million ton as total production. However, the total cultivated zone in the world reached around 11.65 million feddans with 301.12 million ton as total production (FAO, 2018).

22 pests (2 bacteria, 14 fungi, 1 nematode, and 5 viruses) are restricted as connected with sugar beet seeds (Agarwal *et al.*, 2006).

The most common sugar beet seed-borne pathogens are *Phoma betae*, *Peronospora farinose*, *Cercospora beticola*, *Ramularia beticola*, *Uromyces betae*, *Alternaria tenuis. Fusarium* spp. and beet yellows virus (BYV) (Mariã and Jevtiã, 2001).

Fusarium yellows in sugar beet is primarily rised by *F. oxysporum* f. sp. *betae* but can be caused by other *Fusarium* spp. including *F avenaceum.*, *F. acuminatum*, *F. moniliforme* and *F. solani* (Hanson and Hill, 2004).

Transmitted fungi on sugar beet seeds inspire significant losses wherever sugar beets are grown. However, in all sugar beet production zone not all these pathogens have been indicated. damages contain reduced sugar recovery white and reduced harvestable tonnage. Also, many of these pathogens cause post-harvest losses in storage piles. Control of diseases caused by these pathogens include avoidance of stresses, planting disease-resistant cultivars, cultural practices such as water management and the use of fungicides (Jacobsen, 2006).

Pesticides cause cancers of the lung, prostate, lymphatic, hematopoietic and childhood cancer. In addition to cancer, there are several other chronic health effects that may be connected to pesticides. The nervous system is particularly vulnerable to many pesticides of sundry distinct chemical classes. It is well known that acute poisoning with organophosphates causes long-term neurobehavioral deficits and depression, but low-dose exposures without clinical poisoning effects on health are less clear (Baker and Wilkinson, 1990).

One of the solutions for sustaining agricultural output and environmental quality known Biofungicides. In order to implement these environmental friendly biofungicide on plant fungal diseases, it is remarcable to pay notice to the way of application and formulation. Biofungicides have many traits over chemical fungicides i.e., they are safe on human health, cheaper, and harmful residues are not detected (Dhiraj *et al.*, 2014).

Thus, the main objective of this study was to control sugar beet seedlings damping off and root rot diseases by using green chemicals (antioxidants) and biological control agents for suppressing the seed-borne pathogen *F. moniliforme* in sugar beet via promoting the plant systemic acquired resistance and / or killing the pathogen.

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MATERIALS AND METHODS

1- Seed health testing and survey for fungi associated with sugar beet seeds

Deep-freezing blotter method was used as described by ISTA (2008). Seeds are placed on moistened filter papers which are set in Petri dishes (25 seeds/dish) for one day at 25+2ºC, then placed in a deep-freezer for 8 hr. Plates were then moved to an incubator at 25 ±2 ˚C with alternating light (12 hr. light/darkness) for 5-7 days. The resulting seed-borne fungi were identified morphologically with a stereo-microscope (Olympus SZ61TR Trinocular Zoom Stereomicroscope, Germany).

2- *Fusarium moniliforme* isolation, purification and identification

The recovered *Fusarium moniliforme* isolates were identified on the basis of morphological and cultural characteristics (shape, color and texture of colony) as well as microscopic features (characteristics of mycelium, and the shape, size and color of conidia, etc.) (Nelson *et al.*, 1983).

1- Preparation of fungal inoculum

Inocula of *F. moniliforme* isolates were intended using Sorghum seeds, coarse sand and water (2:1:2 v/v) medium. The ingredients were mixed, bottled and autoclaved for 1 hour at 121˚C. The sterilized medium was inoculated using agar discs, obtained from the periphery of a seven-day-old colony of the each isolate (El-namla *et al.*, 2018).

2- Pathogenicity test

Fusarium moniliforme isolates that obtained from the survey were examined under greenhouse conditions for their pathogenicity as follows: Black plastic bags (35cm diameter x 30cm height) filled up with soil mix (50% sand and 50% loam) were autoclaved twice in two consequent days at 121˚C for 1 hr. The formerly intended fungal inocula were used to infest soil at a rate of 1% (w/w). The infested soils were watered and left for 7 days before planting to stimulate development and ensure distribution of the pathogen in the soil. The control pots were similarly prepared but without the pathogen. Sugar beet seeds (TORO variety) were surface sterilized by dipping for 3 minutes into 1% Na-hypochlorite solution, then washed many times in sterilized distilled water. Seed sowing was done at rate of (3 seeds / plastic bag). Three replicates were used for each isolate. The percentage of pre-emergence damping off, post-emergence damping off and survived plants were recorded after 15 and 45 days from sowing date. The most aggressive fungal isolate was chosen for further studies (El-namla *et al.*, 2018).

4- In vitro experiments

Chemical, green, and biological materials were tested in vitro for their effects on *F. moniliforme* mycelial growth as follows:

1- Chemical fungicide

Fludioxonil/Mefenoxam (Maxim XL 3.5% FS®; denoted later as Maxim®) was used as a control treatment by adding it to potato dextrose agar (PDA) medium with the recommended concentration (1 ml Maxim® : 7 ml medium). Control plates were made with PDA only without fungicide. Five replicates, each containing 20 ml medium were used for each treatment. All plates were left for 30 minutes to be solidified, then inoculated with 0.5 cm disc from the edge of a 7-day-old culture of the pathogen, which placed in the center of plates. The growth inhibition was calculated after 7 days as the percentage inhibition of redial growth relative to the control (Elmer and McGovern 2004).

2- Potassium silicate solution (PS)

Five concentrations of PS in PDA medium (4, 6, 8, 10 and 12 ml /L) were used. Control plates were made with PDA only without PS. Five replicates were used for each concentration and the control. All plates received 20 ml of the medium and left for 30 minutes to be solidified, then inoculated with 0.5 cm disc from the edge of a 7-day-old culture of the pathogen, which placed in the center of plates. The growth inhibition was calculated after 7 days as the percentage inhibition of redial growth relative to the control (Menzies *et al.*, 1991).

3- Nicotinic acid solution (NA)

Five concentrations of NA in PDA medium (1, 5, 10, 15 and 20 mM (v/v)) were used. Control plates were made with PDA only without NA. Five replicates were used for each concentration and the control. All plates received 20 ml of the medium and left for 30 minutes to be solidified, and then inoculated with 0.5 cm disc from the edge of a 7-day-old culture of the pathogen, which placed in the center of plates. The growth inhibition was calculated after 7 days as the percentage inhibition of redial growth relative to the control (Shahda, 2001).

4- Antagonistic effect of bio-agents

Antagonistic fungi and bacteria were tested for their antifungal activity against *F. moniliforme*. The inhibitory effect of antagonistic fungi and bacteria (*Trichoderma hamatum*, *T. harzianum* and four isolates of *Bacillus subtilis*) against the pathogen fungal growth was assayed by using the dual culture method described by Rajeev and Mukhopadhyay (2001). Petri plates (9-cm diameter), each containing PDA (20 ml) were inoculated with a 0.5cm disc of *F. moniliforme* at 1 cm from the edge of the Petri dish and then a 0. 5 cm disc of the bio-agent was placed at 1 cm from the opposite edge of the Petri dish. The paired cultures were incubated at 25±2 ˚C for 5 days. Plates inoculated with the pathogen only at 1 cm from edge of plate served as a control. The growth of individual and dual cultures was recorded then the inhibition (%) of the pathogen was calculated relative to the control (Pandey *et al*., 2000).

5- In vivo experiments

1- Greenhouse experiment

The most effective treatments resulted from in vitro experiments were used in the greenhouse. Black polyethylene bags (20-cm diameter x 30-cm height) were filled with (5 kg) of soil infested with the pathogen (1 g inoculum : 100 g soil), then irrigated and left for one week to allow the inoculum to grow and produce mycelium and spores (Abdel-Kader *et al.*, 2010). Sugar beet variety that is sensitive to damping off and root rot diseases (Toro variety from Germany) was planted in pots. Before planting, seeds were soaked in treatments solutions or spore suspensions (8 to 12 hr) and treatments were:

1. Control I: Just sterilized distilled water.
2. Control II: Maxim® (1 ml Maxim® : 7 ml water).
3. Potassium silicate (8, 10 and 12 ml/L).
4. Spore suspension of *T. harzianum* with concentration ($1x10^6$ spores/ ml).
5. Spores suspension of *T. hamatum* with concentration ($1x10^6$ spores/ ml).
6. Cell suspension of the most aggressive strain of *B. subtilis* ($1x10^6$ cell/ml).

7. Nicotinic acid (5, 10 and 15 mM/L).

Five replicates (15 seeds/each) were used for each treatment and pots were arranged in a completely randomized setting in the greenhouse.

1-Determination of pre-and post-emergence damping off and survival plants

The number of live seedlings was recorded after 15 days (to calculate the pre-emergence damping off %), and after 45 days (to calculate the post-emergence damping off, and the survival plants %) (Dahiphale, 2006).

2- Field experiment

The experiment was done on sugar beet in two locations in the same season (2018/2019) in winter. The two locations were (MeetNabit Village, Talkha District, Dakahlia Governorate, Egypt) and (Agricultural Research station in Gemmiza village, Alsanta District, Gharbia Governorate, Egypt). The latter belongs to Plant Pathology Research Institute, Agricultural Research Center. Selected districts have been growing sugar beet for at least 5 years. The experiments were set for both locations in a completely randomized design. Seven treatments with three replications were used. Fifteen seeds were planted in each treatment (5 seeds x 3 replicates). Each replicate consisted of 5 seeds sown in a 3.5 m-long row at a 70-cm interspacing. Before planting, seeds were soaked in treatments solutions or spore suspensions for (8-12 hr). The treatments were:

1 - Control I: Just sterilized distilled water.
2 - Control II: Maxim® (1 ml Maxim® : 7 ml water).
3 - Potassium silicate (12 ml/L).
4 - Spore suspension of *T. harzianum* ($1x10^6$ spores/1 ml).
5 - Spores suspension of *T. hamatum* ($1x10^6$ spores/1 ml).
6 - Cell suspension of the most aggressive strain of *B. subtilis* ($1x10^6$ cfu/ml).
7 - Nicotinic acid (5 mM/L).

1- Pre-and post-emergence damping off and survival plants

The number of live seedlings was listed after 15 days (to calculate the pre-emergence damping off %), and after 45 days (to calculate the post-emergence damping off, and the survival plants %) (Dahiphale 2006).

2- Disease severity and disease incidence in the fields

Disease incidence %(DI), survived plants and disease severity (DS) were recorded after 60, 80 and 100 days from the planting date. The disease severity index was determined according to the scale 0-10, whereas 0= Healthy root and 10= complete root damage. Disease severity index was calculated according to the following equation (Grainger, 1949):

$$\textbf{DSI (\%)} = (\sum \textbf{(NPC×CR)/ NIP×MSC)} \times 100,$$

whereas,

DSI = disease severity index.
NPC = number of roots in each class rate.
CR = class rate.
NIP = number of infected plants.
MSC = is the maximum severity class rate.

The percentage of disease incidence was measured according to the following equation (Trapero-Casas and Jimenez Diaz, 1985):

$$\textbf{DI \%} = \textbf{((A-B) / A) x 100}$$

DI = Disease incidence.
A = Number of healthy plants produced from planting in soil free of pathogen
B = Number of healthy plants produced from planting in soil infested with pathogen.

3- Plant growth characters: After 180 days old sugar beet plants, the next characters were measured: Root weight (g)/plant, root length (cm)/plant, root diameter (cm)/plant, foliage length (cm)/plant and foliage weight (g)/plant according to Elwakil *et al.* (2017).

4- Photosynthetic pigments (chlorophyll and carotenoids): After 120 days from sowing, leaf samples were composed from all treatments (the forth leaf on the plant) to determine the photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoids) according to methods described by (Mackinney, 1941).

5- Total phenol: Sugar beet fresh leaves (120-day-old) were collected to define their contents of the total phenols using Foline-ciocalteau reagent according to (Singleton and Rossi, 1965).

6- Determination of total soluble solids (TSS): were determined by Refractometer for roots in each treatment (Hozayen, 2002).

7- Statistical analysis: All data were statistically analyzed using CoStat 6.311 software (2005) for the analysis of variance (ANOVA) (Gomez and Gomez, 1984). All comparisons were first subjected to ANOVA and significant differences among treatments means were determined with Duncan's Multiple Range test at $P \leq 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

Results

1- Seed health testing and survey for fungi associated with sugar beet seeds

Data presented in Table (1) showed that *F. moniliforme* was the most frequent fungus associated with sugar beet seeds of most varieties followed by *Aspergillus* spp., and *Penicillium* spp. *Verticillium* spp., *Alternaria alternata* and *Chaitomiium* sp. moderately occurred, while *Cladosporium* spp. and *Bipolaris* spp. were the least frequent fungi. Since *F. moniliforme* was the most frequent pathogen associated with sugar beet seeds, it was used for further studies.

Table 1. Seed-borne fungi associated with seeds of 15 sugar beet varieties.

	Sugar beet variety*															
Fungi	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**Total**
Alternaria alternata	0	0	0	0	0	0	3	0	3	13	12	0	2	0	0	33
Aspergillus spp.	8	3	15	1	4	3	1	8	8	2	34	11	1	2	4	105
Bipolaris spp.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Botrytis spp.	1	0	1	0	1	0	0	0	8	0	0	0	0	0	0	11
Chaitomium sp.	1	0	3	0	1	0	0	1	7	2	2	9	1	0	0	27
Cladosporium spp.	0	0	0	0	0	0	1	0	0	1	2	0	0	0	0	4
Fusarium moniliforme	6	7	23	1	10	31	1	10	29	5	47	2	4	0	18	194
Penicillium spp.	7	3	14	15	5	3	0	6	10	3	6	10	3	3	7	95
Rhizobus spp.	0	0	0	1	0	2	0	0	0	0	2	0	0	15	1	21
Verticillium spp.	2	0	0	5	1	0	1	1	11	9	0	4	3	0	0	37

*** (1)Athos, (2)Bolat, (3)BTS645, (4)Carola, (5)Classic, (6)Dareah, (7)Heliopolis, (8)Hend, (9)Hosam15, (10)Hosam17, (11)Karam, (12)Natura, (13)Sahar, (14)Top15 and (15)Top17.**

2- Isolation, purification, identification and pathogenicity of *Fusarium moniliforme*

Isolates of *F. moniliforme* (fig. 1) were purified using hyphal tip technique. Pure cultures of all isolates were used to fulfill Koch's postulates. All isolates were pathogenic and caused damping off with various degrees of disease severity. The most virulent isolate (isolate # 25) was used for further studies.

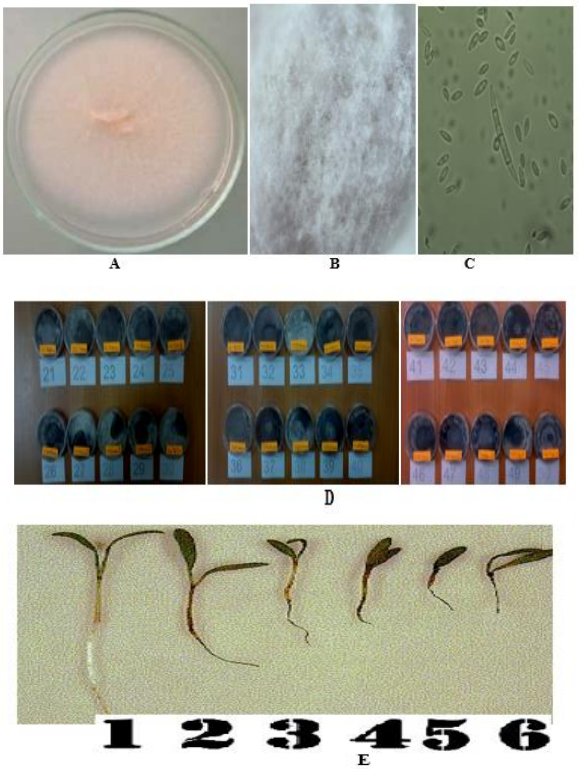

Figure 1. *Fusarium moniliforme* associated with sugar beet seeds, A=a pure culture on PDA, B=habit character (20x) on a sugar beet seed, C=macro-and micro-conidia (400x), D=30 isolates of *F. moniliforme* selected from 194 isolates recovered from 15 imported varieties of sugar beet seeds, E=different levels of post-emergence damping off caused by several isolates of *F. moniliforme* that were different in their virulence, 15 days after sowing, whereas (1)=normal seedling (control) and (2-6)=different levels of damping off.

3- In vitro experiment

Results in Table (2) and Fig. (2-7) show that different concentrations of green chemicals and biological control agents significantly reduced the linear growth of *F. moniliforme* isolated from sugar beet seeds. It was also found that the reduction in the linear growth is positively correlated to the increase in the concentration of the tested green chemicals listed in table (2). A complete growth inhibition was occurred by the Maxim® fungicide as well as the treatments of 5 mM or above of nicotinic acid and 8ml/L or above of potassium silicate. Both species of *Trichoderma* had moderate level of growth inhibition while *B. subtilis* was the least effective against *F. moniliforme* growth.

Table 2. Effect of green chemicals, biological control agents, and a chemical fungicide on radial growth of *F. moniliforme* on PDA.

Treatment	Concentration	Radial growth (cm)	Inhibition (%)
Nicotinic acid	1 mM	2.45H*	72.8B
	5 mM	0I	100A
	10 mM	0I	100A
	15 mM	0I	100A
	20 mM	0I	100A
Potassium silicate	4 ml/L	9A	0J
	6 ml/L	6.2F	31.1E
	8 ml/L	0I	100A
	10 ml/L	0I	100A
	12 ml/L	0I	100A
Maxim® fungicide	143 ml/L	0I	100A
Control (untreated)	PDA	9A	0J
T. harzianum	Dual culture on PDA	2.9G	67.8D
T. hamatum	Dual culture on PDA	2.8G	68.9C
B. subtillis	Strain 1	7.9B	12.2I
	Strain 2	7.1D	21.1G
	Strain 3	6.6E	26.7F
	Strain 4	7.6C	15.6H
Control for biological control agents	PDA	9A	0J

* **Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (*P*=0.05).**

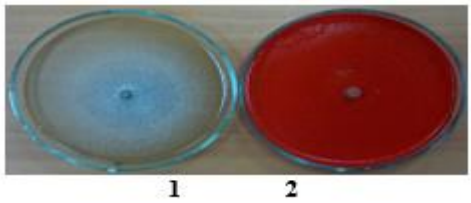

Figure 2. Effect of Maxim® fungicide (143ml/L) on the radial growth of *F. moniliforme*, whereas 1=untreated control (0ml/L) and 2=Maxim® (143ml/L).

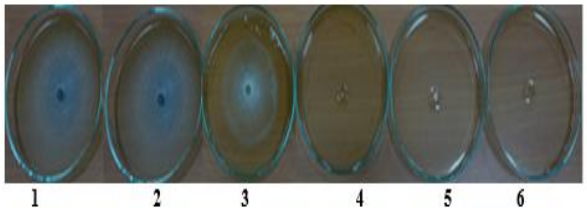

Figure 3. Effect of potassium silicate at different concentrations on the radial growth of *F. moniliforme*, whereas 1=untreated control (0ml/L), 2=4ml/L, 3=6ml/L, 4=8ml/L, 5=10ml/L and 6=12ml/L.

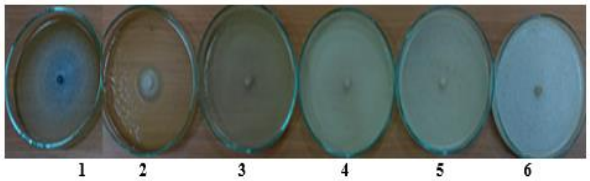

Figure 4. Effect of nicotinic acid at different concentrations on the radial growth of *F. moniliforme*, whereas 1=untreated control (0ml/L), 2=1mM, 3=5mM, 4=10mM, 5=15mM and 6=20mM.

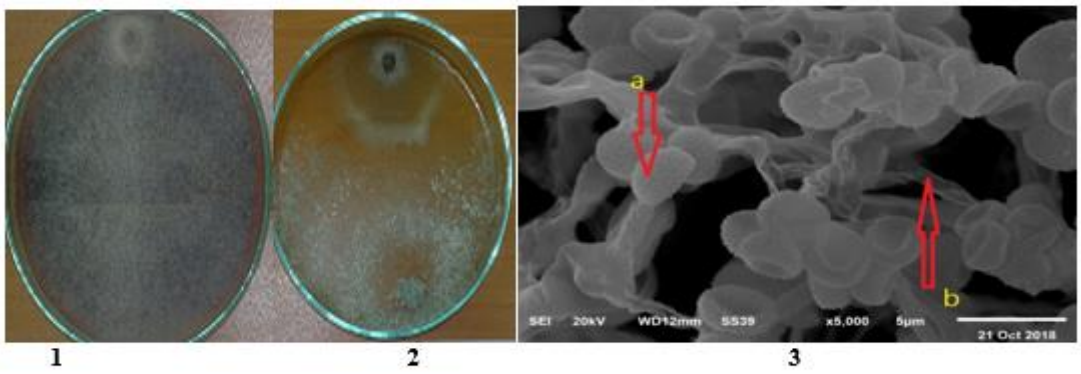

Figure 5. Antagonistic effect of *T. harzianum* against the growth of *F. moniliforme,* whereas 1=monoculture of *F. moniliforme* (control), 2=dual culture of *T. harzianum* and *F. moniliforme,* and 3=scanning of parasitism by electronic microscope (5000x), whereas a=spores of *T. harzianum* and b=damaged mycelium of *F. moniliforme* (parasitism area).

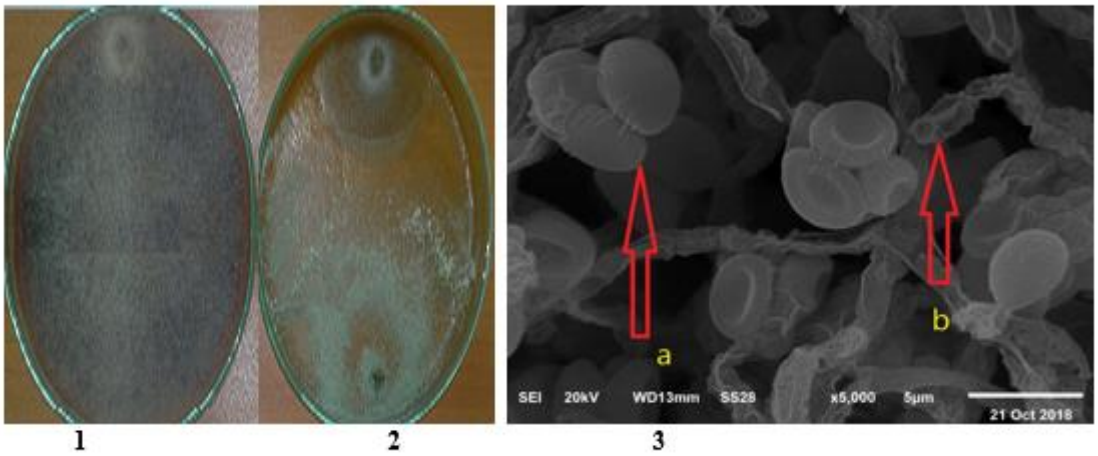

Figure 6. Antagonistic effect of *T. hamatum* against the growth of *F. moniliforme,* whereas 1=monoculture of *F. moniliforme* (control), 2=dual culture of *T. hamatum* and *F. moniliforme,* and 3=scanning of parasitism by electronic microscope (5000x), whereas a=spores of *T. hamatum* and b=damaged mycelium of *F. moniliforme* (parasitism area).

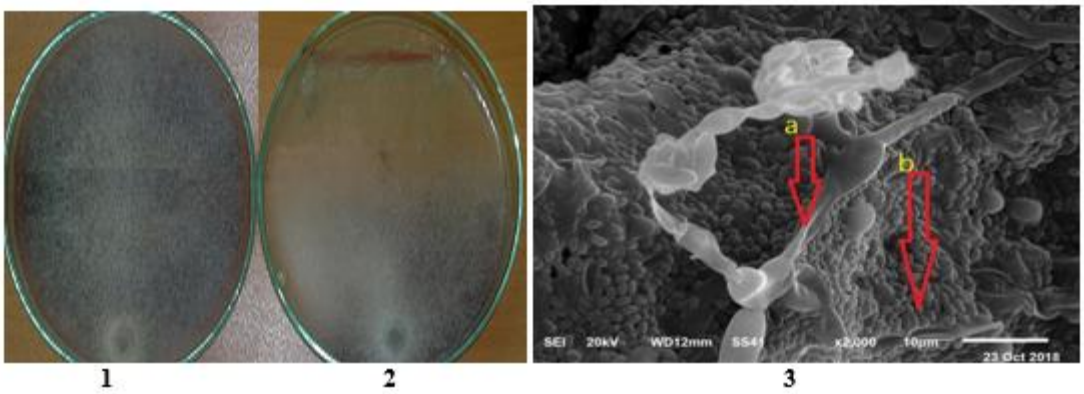

Figure 7. Antagonistic effect of the most aggressive *B. subtilis* isolate against the growth of *F. moniliforme,* whereas 1=monoculture of *F. moniliforme* (control), 2=dual culture of *B. subtilis* (strain 3) and *F. moniliforme,* and 3=scanning of parasitism by electronic microscope (2000x), whereas a=bacterial cells of *B. subtilis* and b=damaged mycelium of *F. moniliforme* (parasitism area).

4- In vivo experiments

1- Effect of green chemicals and bioagents on DI and DSI caused by *F. moniliforme* under greenhouse conditions

Data in Table (3) reveal that soaking sugar beet seeds in green chemicals and spore suspensions of biological control agents for 8-12 hr before sowing significantly reduced the percentage of damping-off disease of sugar beet infected with *F. moniliforme.* Maxim® was the most effective treatment in reducing the disease. It was followed by nicotinic acid (5mM) and *T. harzianum.*

2- Effect of green chemicals and bioagents on DI and DSI caused by *F. moniliforme* under field conditions

Results in Table (4) reveal that soaking sugar beet seeds in green chemicals and spore suspensions of

biological control agents for 8-12 hr before sowing significantly reduced the percentage of damping-off disease of sugar beet in comparison with the untreated seeds. Maxim® and nicotinic acid (5mM) had the highest effect on reducing the percentage of damping-off disease. Both *Trichderma* species and the potassium silicate (12ml/L) were in the second most effective treatment.

Data in Table (5) reveal that soaking sugar beet seeds in green chemicals and spore suspensions of biological control agents for 8-12 hr before sowing significantly reduced the percentage of damping-off disease of sugar beet in comparison with the untreated seed. Maxim® followed by nicotinic acid (5mM) had the highest effect on decreasing the percentage of damping-off disease.

Table 3. Efficacy of green chemicals and bioagents (in reference to a chemical fungicide) on damping off disease caused by *F. moniliforme* on sugar beet under greenhouse conditions.

Treatment	Pre emergence damping off	%	Post emergence damping off	Survival plants	%
Untreated control I (Pathogen only) control II	5j*	6.6j	0	70a	93.4a
Chemical fungicide (Maxim®)	31c	41.3c	0	44h	58.7h
Potassium silicate (8ml/L)	14i	18.7i	0	61b	81.3b
Potassium silicate (10ml/L)	26e	34.7e	0	49f	65.3f
Potassium silicate (12ml/L)	32b	42.7b	0	43i	57.3i
T. harzianum	21f	28f	0	54e	72e
T. hamatum	15h	20h	0	60c	80c
B. subtilis	19g	25.3g	0	56d	74.66d
Nicotinic acid (5mM)	29d	38.6d	0	46g	61.34g
Nicotinic acid (10mM)	15h	20h	0	60c	80c
Nicotinic acid (15mM)	32b	42.7b	0	43i	57.3i
	38a	50.7a	0	37j	49.3j

* **Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (*P*=0.05).**

Table 4. Efficacy of green chemicals and bioagents (in reference to a chemical fungicide) on damping off disease caused by *F. moniliforme* on sugar beet under field conditions (in Gemmeiza).

Treatment	Pre emergence damping off	%	Post emergence damping off	Survival plants	%
Untreated control	8a*	53.3a	0	7e	46.7e
Chemical fungicide (Maxim®)	3e	20e	0	12a	80a
Potassium silicate (12ml/L)	5c	33.3c	0	10c	66.7c
T. harzianum	4d	26.7d	0	11b	73.3b
T. hamatum	4d	26.7d	0	11b	73.3b
B. subtilis	6b	40b	0	9d	60d
Nicotinic acid (5mM)	3e	20e	0	12a	80a

* **Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (*P*=0.05).**

Table 5. Efficacy of green chemicals and bioagents (in reference to chemical fungicide) on damping off disease caused by *F. moniliforme* on sugar beet under field conditions (in Meetnabit).

Treatment	Pre emergence damping off	%	Post emergence damping off	Survival plants	%
Untreated control	6a*	40a	0	9f	60f
Chemical fungicide(Maxim®)	1f	6.7f	0	14a	93.3a
Potassium silicate (12ml/L)	4c	26.7c	0	11d	73.3d
T. harzianum	3d	20d	0	12c	80c
T. hamatum	4c	26.7c	0	11d	73.3d
B. subtilis	5b	33.3b	0	10e	66.7e
Nicotinic acid (5mM)	2e	13.3e	0	13b	86.7b

* **Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test *P*=0.05).**

2- Disease severity and disease incidence in the fields

Data in Table (6) show that soaking sugar beet seeds in green chemicals and spore suspensions of biological control agents for 8-12 hr before sowing significantly decreased the percentage of disease severity index and disease incidence of sugar beet under the natural infection in the field in comparison with the untreated seeds. Maxim® and nicotinic acid (5mM) had the highest effect on reducing the percentage of disease severity index and disease incidence. Then comes *Trichderma* spp. followed by potassium silicate. *B. subtilis* was the least effective in this regard in comparison with the untreated control.

Table 6. Efficacy of green chemicals and bioagents (in reference to a chemical fungicide) on disease incidence and disease severity caused by *F. moniliforme* on sugar beet under field conditions (in Gemmeiza location)

Treatment	Disease severity (%)			Disease incidence (%)		
	60 days	90 days	120 days	60 days	90 days	120 days
Untreated control	24.54a*	29.39a	34.24a	53.30a	53.30a	53.30a
Chemical fungicide (Maxim®)	15.45f	17.77f	19.09g	20.00e	20.00e	20.00e
Potassium silicate (12ml/L)	19.90c	22.12c	25.15c	33.30c	33.30c	33.30c
T. harzianum	16.57e	19.09e	21.12e	26.70d	26.70d	26.70d
T. hamatum	17.97d	19.29d	23.13d	26.70d	26.70d	26.70d
B. subtilis	21.90b	24.54b	28.18b	40.00b	40.00b	40.00b
Nicotinic acid (5mM)	15.45f	17.27g	19.59f	20.00e	20.00e	20.00e

* **Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (*P*=0.05).**

Data in Table (7) illustrate that soaking sugar beet seeds in green chemicals and spore suspensions of biological control agents for 8-12 hr before sowing significantly reduced the percentage of disease severity index and disease incidence of sugar beet under the natural infection in the field of Meetnabit location in comparison with the untreated seeds. Maxim® and then nicotinic acid (5mM) had the highest effect on reducing the percentage of disease severity index and disease incidence. Those were followed by *T. harzianum* and then *T. hamatum* while potassium silicate, then *B. subtilis* were the least effective in this regard.

Table 7. Efficacy of green chemicals and bioagents (in reference to a chemical fungicide) on disease incidence and disease severity caused by F. moniliforme on sugar beet under field conditions (in Meetnabit location)

Treatment	Disease severity%			Disease incidence%		
	60 days	90 days	120 days	60 days	90 days	120 days
Untreated control	24.14a*	29.20a	34.00a	40.00a	40.00a	40.00a
Chemical fungicide (Maxim®)	12.40f	13.20g	15.80g	6.70f	6.70f	6.70f
Potassium silicate (12ml/L)	17.60c	20.80c	22.01c	26.70c	26.70c	26.70c
T. harzianum	13.20e	15.60e	17.23e	20.00d	20.00d	20.00d
T. hamatum	14.60d	16.80d	18.04d	26.70c	26.70c	26.70c
B. subtilis	19.40b	21.10b	23.16b	33.30b	33.30b	33.30b
Nicotinic acid (5mM)	13.01e	15.40f	17.03f	13.30e	13.30e	13.30e

*Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (*P*=0.05).

3- Plant growth characters

The results in Table (8) reveal that soaking seeds of sugar beet in green chemicals and spore suspensions of biological control agents for 8-12 hr before sowing significantly enhanced root length, root weight, root diameter, foliage length and foliage weight when compared with the untreated control. Maxim® and then *Trichoderma harzianum* and nicotinic acid (5mM) had the highest values of root length, root weight, root diameter, foliage length and foliage weight.

Table 8. Effect of green chemicals and bioagents (in reference to a chemical fungicide) on root length, root weight, root diameter, foliage length and foliage weight of sugar beet plants under field conditions (in Gemmeiza location).

Treatment	Root length (cm)	Root weight (g)	Root diameter (cm)	Foliage length (cm)	Foliage weight (g)
Untreated control	32.40e*	0.90e	30.9d	40.30f	0.41c
Chemical fungicide (Maxim®)	41.29a	2.03a	41.66a	59.25a	0.82a
Potassium silicate (12ml/L)	37.30c	1.61c	33c	50.70d	0.69b
T. harzianum	40.90a	1.93a	39.2a	55.90b	0.79a
T. hamatum	39.30b	1.75b	35.61b	54.00c	0.75ab
B. subtilis	35.44d	1.46d	31.4d	46.10e	0.49c
Nicotinic acid (5mM)	39.90ab	1.91a	40a	54.70bc	0.80a

* Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (*P*=0.05).

The results in Table (9) show that soaking seeds of sugar beet in green chemicals and spore suspensions of biological control agents for 8-12 hr before sowing significantly enhanced root length, root weight, root diameter, foliage length and foliage weight when compared with the untreated control. Maxim® and then *Trichoderma harzianum* and nicotinic acid (5mM) had the highest values of root length, root weight, root diameter, foliage length and foliage weight.

4- Photosynthetic pigments

The results in Table (10) reveal that soaking seeds of sugar beet in green chemicals and spore suspensions of biological control agents for 8-12 hr before sowing significantly increased the contents of chlorophyll a, b, total chlorophyll and carotenoids when compared with the untreated control. Maxim® and then nicotinic acid (5mM) had the highest values of chlorophyll a, b, total chlorophyll and carotenoids.

The results in Table (11) show that soaking seeds of sugar beet in green chemicals and spore suspensions of biological control agents for 8-12 hr before sowing significantly increased the contents of chlorophyll a, b, total chlorophyll and carotenoids in comparison with the untreated seeds. Maxim® and then nicotinic acid (5mM) had the highest levels of chlorophyll a, b, total chlorophyll and carotenoids.

Table 9. Effect of green chemicals and bioagents (in reference to a chemical fungicide) on root length, root weight, root diameter, foliage length and foliage weight of sugar beet plants under field conditions (in Meetnabit location)

Treatment	Root length (cm)	Root weight (g)	Root diameter (cm)	Foliage length (cm)	Foliage weight (g)
Untreated control	35.60e*	1.20e	34.90g	43.90f	0.73c
Chemical fungicide (Maxim®)	46.49a	2.73a	47.16a	65.55a	1.51a
Potassium silicate (12ml/L)	41.40c	2.10c	40.10e	55.10d	1.23b
T. harzianum	45.90a	2.50b	45.30c	61.20b	1.45a
T. hamatum	43.10b	2.25c	43.10d	58.40c	1.30ab
B. subtilis	38.14d	1.80d	36.90f	50.13e	0.88c
Nicotinic acid (5mM)	45.00a	2.60ab	45.50b	60.16b	1.47a

* Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (*P*=0.05).

Table 10. Effect of green chemicals and bioagents (in reference to a chemical fungicide) on chlorophyll and carotenoids contents of sugar beet plants under field conditions (in Gemmeiza location)

Treatment	Chlorophyll A (mg/g)	Chlorophyll B (mg/g)	Total chlorophyll (mg/g)	Carotenoids (mg/g)
Untreated control	0.601g*	0.312g	0.913g	0.237f
Chemical fungicide (Maxim®)	1.300a	0.976a	2.276a	0.516a
Potassium silicate (12ml/L)	0.950e	0.654e	1.672e	0.377d
T. harzianum	1.200c	0.869c	2.099c	0.498b
T. hamatum	1.090d	0.762d	1.744d	0.477c
B. subtilis	0.910f	0.643f	1.593f	0.322e
Nicotinic acid (5mM)	1.250b	0.899b	2.119b	0.495b

* Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (*P*=0.05).

Table 11. Effect of green chemicals and bioagents (in reference to a chemical fungicide) on chlorophyll and carotenoids contents of sugar beet plants under field conditions (in Meetnabit location)

Treatment	Chlorophyll A (mg/g)	Chlorophyll B (mg/g)	Total chlorophyll (mg/g)	Carotenoids (mg/g)
Untreated control	0.776g*	0.489g	1.265g	0.221g
Chemical fungicide (Maxim®)	1.520a	0.940a	2.460a	0.601a
Potassium silicate (12ml/L)	0.961e	0.685e	1.595e	0.364e
T. harzianum	1.390c	0.913c	2.303c	0.513c
T. hamatum	1.090d	0.897d	1.987d	0.498d
B. subtilis	0.910f	0.612f	1.573f	0.317f
Nicotinic acid (5mM)	1.430b	0.923b	2.353b	0.565b

* Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (*P*=0.05).

5- Determination of total soluble solids (TSS) and total phenol

The results in Table (12) report that soaking seeds of sugar beet in green chemicals and spore suspensions of biological control agents for 8-12 hr before sowing significantly increased the contents of total phenols (in plants leaves) and TSS (in roots) in comparison with the untreated control. In general, treatments with Maxim®, nicotinic acid (5mM) and *T. harzianum* gave the highest content of total phenols and TSS.

Table 12. Effect of green chemicals and bioagents (in reference to a chemical fungicide) on total phenol and TSS contents of sugar beet plants under field conditions (in Gemmeiza and Meetnabit locations)

Treatment	Gemmeiza		Meetnabit	
	Phenol (mg/100 g)	TSS (%)	Phenol (mg/100g)	TSS (%)
Untreated control	211f*	18.7f	255c	19.6f
Chemical fungicide (Maxim®)	613a	24.2a	666a	25.6a
Potassium silicate (12ml/L)	459d	21.9d	493b	22.7d
T. harzianum	592b	23.1b	623a	23.9c
T. hamatum	570c	22.4c	598a	23d
B. subtilis	297e	20.05e	311c	20.5e
Nicotinic acid (5mM)	597b	23.9a	632a	24.7b

* **Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (*P*=0.05).**

Discussion

During the course of this study, *F. moniliforme* was the most common fungus isolated from sugar beet seeds. Pathogenicity test revealed that this fungus attacks sugar beet seedlings causing damping-off disease which leads to a reduction in the number of healthy plants. These results are similar to what Pethybridge *et al.* (2018) and Abawi *et al.* (1986) found.

This research protocol was carried out using a modern method for reducing the detrimental effect on sugar beet plants caused by *F. moniliforme*. Aqueous solutions of the potassium silicate and nicotinic acid, and the spores suspensions of biological control agents *T. harzianum*, *T. hamatum* and *B. subtilis* besides Maxim® fungicide increased the growth of the tested plants even the plants were grown under the stress of *F. moniliforme*. The in-vitro studies showed a significant inhibition effect of these materials reduction in the growth of the tested fungus.

In parallel to these results, Marschner (2012) resulted that K is a major plant element and plays a major part in photosynthesis, a variety of physiological processes, maintenance of water status and protein synthesis in plant tissues.

The antioxidants × free radicals interaction was demonstrated by a number of researchers; Giridhar and Reddy (2001) hypothesis that the antioxidants may reduce the subsequently alter the membrane permeability and oxygen tension in media of the fungus which leads to scale up the production of mycotoxin in the medium. The antioxidant antimicrobial action could be also due to inhibition of the functions of many enzymes by oxidizing the membrane lipids as they interfere with the membrane functions including proteins, RNA and DNA synthesis (Nesci et al. 2003). Howell (2003) in commercial agriculture found that Trichderma spp. are very strong biocontrol agents against several plant diseases. Vey et al. (2001); Raaijmakers et al. (2009) stated that some toxic metabolites against phytopathogens were produces by Trichoderma, which include alamethicins, harzianic acid, peptaibols, tricholin, 6-penthyl-α-pyrone, antibiotics, massoilactone, gliovirin, viridian, heptelidic acid and glisoprenins.

The results presented here show that the most effective seed treatments for best germination rate of sugar beet were Maxim® fungicide at 143ml/L, nicotinic acid at 5 mM, *T. harzianum* $1x10^6$ spores/ml, and *T. hamatum* $1x10^6$ spores/ml. Similar finding was reported on beetroot by Elwakil *et al.* (2019) realized that the percentage of seed germination was significantly increased with soaking beetroot seeds in aqueous solution of antioxidants for 12 h. Papavizas (1985) reported that plant disease control is considered as a good alternative by application of biocontrol agents. Plant diseases control with *Trichoderma* builds on its metabolic versatility, ability to degrade organic substrates, occupy and tolerance to microbial inhibitors in the soil so the plant seed germination be better.

The in-*vivo* studies insured a good potential of soaking seeds in Maxim® fungicide (143ml/L), nicotinic acid (5mM), *T. harzianum* ($1x10^6$ spores/ml), and *T. hamatum* ($1x10^6$ spores/ml) for suppressing the damping-off disease of sugar beet seedlings and increasing the content of the photosynthetic pigments and total phenols. Phenolic compounds are known as stimulant to the immune system of the plants as described by Taiz and Zeiger (2002) and Ibrahim *et al.* (2015). Elwakil *et al.* (2019) also found that antioxidants formulations significantly reduced the percentage of damping-off disease in beetroot plants.

Our results of our field trials conducted in the two growing locations insured that soaking seeds of sugar beet in aqueous solution of nicotinic acid (5mM) before planting significantly reduced the incidence and severity of damping-off disease in sugar beet, they significantly increased the content of the photosynthetic pigments and the total phenols in plants, improved the root total soluble solids (TSS), and enhanced the growth parameters including root weight, root length, root diameter, foliage weight and foliage length. These results are in agreement with those Kaya *et al.* (2005) and Salwa and Eisa (2011) found that a significant increase was recorded in yield component of sugar beet and sugar yield (ton/feddan) by antioxidants, moreover that antioxidants cause significant increment in many growth aspects as diameter, stem length, leaves/plant and number of formed branches as well as fresh and dry weight of leaves and stems, specific leaf weight and total leaf area/plant.

In the present study, some possibilities can be suggested to explain the positive effect of nicotinic acid (5mM) on the plant quality: First as amino acids can influence the physiological activities of the plant protein synthesis: Proteins have a structural function, metabolic function (enzymes). Water stresses and antioxidants tolerate environmental, activitors of phytohormones or precursors, growth factors and several other important bioconstituents (Xing-Quan Liu and Kyu-Seung Lee 2012). The second rational possibility is that antioxidants

stimulate the respiration rates, increase plant root uptake of P, enhance root and shoot growth on a fresh and dry weight basis, Cu, K, Zn, Ca and Fe, suppress diseases, stimulate plant enzymes and hormones as well as heat stress and frost damage (Seydabadi and Armin 2014). Antioxidants increase phytohormones auxin and gibberellins, provide the plant with hormones such as cytokinins and auxin also, inhibit IAA-oxidase, Thus prevents destruction of plant growth hormone (El-Bassiony *et al.* 2010).

CONCLUSION

Seeds of sugar beet soak treatment in nicotinic acid 5mM, offers protection against the soil and seed borne fungus *F. moniliforme* attacking sugar beet crop as well as significantly scale up the yield and quality of both. The author hopes that such results can alter the traditional means of treating soil and seed borne fungi of sugar beet and approve such antioxidants as a novel application specially in the areas in which sugar beet is grown.

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استخدام بعض الكيماويات الخضراء وعوامل المقاومة الحيوية في مقاومة فطر فيوزيريوم مونيليفورمي في نبات بنجر السكر

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[٢] قسم المحاصيل كلية الزراعة جامعة المنصورة

[٣] قسم أمراض النبات كلية الزراعة جامعة المنصورة

تم جمع بذور خمسة عشر صنفاً من بنجر السكر (Beta vulgaris L.) في ٢٠١٨ و ٢٠١٩ في مصر، وتم فحصها للتعرف على الفطريات المحمولة عليها باستخدام طريقة التفريد علي ورق الترشيح المرطب مع التجميد. بعد الفحص لهذه الأصناف وجد أن فطر فيوزاريوم مونيليفورمي كان هو الأكثر انتشارا في الخمسة عشرة صنفا، لذلك تم اختبار قدرته المرضية للتأكد من قدرته على إحداث الإصابة لنباتات بنجر السكر. وبعد التأكد من قدرته المرضية تم استخدام عدد من الكيماويات الخضراء الآمنة (مضادات الأكسدة) وعدد من عوامل المقاومة الحيوية لتقييم تأثيرها فى تثبيط هذا الفطر الممرض وذلك بالمقارنة بالمبيد الكيماوي مكسيم إكس إلـ ٣,٥% إف إس. وكانت هذه المعاملات كالتالى: سيليكات البوتاسيوم (بتركيزات ٤, ٦, ٨, ١٠, ١٢ ملل/لتر)، وحامض النيكوتينيك (بتركيزات ١, ٥, ١٠, ١٥, ٢٠ ملليمول/لتر)، وفطر ترايكوديرما هارزيانم وفطر ترايكوديرما هاماتام ويكتريا باسيلوس ساتلس في الإختبارات المعملية فى أطباق بترى. وبناء على نتائج الاختبارات المعملية تم اختيار أكثر التركيزات والمعاملات تثبيطا للفطر الممرض لاختبارها في الصوبة والحقل المفتوح. وقد أكدت النتائج أن حمض النيكوتينيك (بتركيز ٥ ملليمول/لتر) وفطر ترايكوديرما هارزيانم وفطر ترايكوديرما هاماتام وسيليكات البوتاسيوم (بتركيز ١٢ ملل/لتر) كانت هي المعاملات الأفضل مقارنة بالمبيد الكيماوى مكسيم وهو الكونترول، ولكن حامض النيكوتينيك (٥ ملليمول/لتر) كانت هي المعاملة الأفضل علي الإطلاق من بين كل المعاملات.