Evaluation Of The Efficiency Of Some Bio-Fertilizers And Different Silicon Sources For Controlling Bulb Rot Of *Lilium* spp. In Egypt

Taghreed F. M. Abdel-Rahman

Ornamental, Medicinal & Aromatic Plants Dis. Res. Dept., Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

E-mail: ttaghreedfekry@gmail.com https://orcid.org/0000-0003-3668-7337

DOI: 10.21608/jalexu.2022.115736.1041

Article Information

Received:December 23rd 2021

Revised: December 27th 2021

Accepted:December 28th 2021

Published: December 31st 2021

ABSTRACT: Six fungal species were isolated and identified from lily plants showing bulb rot collected from greenhouses and fields in Egypt. They were *Fusarium oxysporum, F. proliferatum, F. semitectum, F. verticillioides, Pythium splendens,* and *Rhizoctonia solani*. *F. oxysporum* showed the highest occurrence percentage being 44.77% of the total isolates recovered (370). Significant differences for pathogenicity were found where *F. oxysporum* showed the highest mean percentages of disease severity being 93.33% over the two tested cvs. (*L. corleone* and *L. litouwen)*, while *R. solani* exhibited the lowest rates of disease severity 29.83%. The molecular study for the recovered *F. oxysporum* AUMC15124 strain showed 100% identity and 100% coverage with strains of *F. oxysporum* f. sp. *gladioli* accessed from GenBank.

Under greenhouse conditions, the efficacy of three biofertilizers (Biogen®, Phosphorine®, Rhizobactrien®) and silicate compounds (calcium silicate, aluminum silicate, magnesium silicate), in comparison with the fungicide Topsin-M® against *F. oxysporum* AUMC15124 were evaluated over two tested cvs. (*L. corleone* and *L. litouwen)*. All the tested treatments significantly reduced the incidence of the bulb rot disease, compared to the untreated control. Calcium silicate was the most effective and was not significantly different from Topsin-M®.

The field trial confirmed these results and a trend similar to the greenhouse test but at a bit lower effect. This was accompanied with an enhancement in '*L. litouwen*' growth parameters (plant height, number of flowers, and bulbs weight). Topsin-M® gave the highest significant mean percentage of efficiency in controlling bulb rot, 68.83%, followed by calcium silicate 62.19% compared with control.

Keywords: *Lilium* spp., calcium silicate, bio-fertilizers, *F. oxysporum* AUMC15124 (GB: MZ715094)

INTRODUCTION

Lilium spp. is a perennial bulb that comes from the Liliaceae family of flowering plants. Lilies are in high demand in the flower market as cut flowers, gardens/parks, landscaping, pot plants, greenhouses, and highly scented **(Bakhshaie *et al*., 2016 and Lakshman *et al.*, 2017).** It is one of the most important bulbous flowers grown for cut flowers in Egypt and worldwide. This crop has recently gained popularity in several Egyptian governorates because their shapes possess natural beauty, fragrance, and different exquisite colors of their flowers. It is the source of the major alluring to buying (**Mohamed *et al*., 2017)**. Diseases pose a major threat to the productivity and quality of lily bulbs and flowers production wherever they are grown. Because *Lilium* spp. was proved to be sensitive to different diseases as their bulbs contain much moisture, and their scales are thick and succulent, making pathogens easier to penetrate causing bulb rot disease (**Sirin, 2011 and Mohamed *et al*., 2017).**

Serious below-ground diseases infecting lily bulbs were obtained by *Fusarium oxysporum, F. moniliforme, F. solani, F. semitectum, F. roseum, Rhizoctonia solani,* and *Pythium* spp., resulting in major losses of lily bulbs **(Lakshman *et al.*, 2017, Zhou *et al*., 2018 and Hua *et al*., 2019).**

Even though chemicals are available to control fungal diseases in the field and on harvested bulbs. However, for safety and environmental concerns, there is now a movement to find alternatives that reduce chemical applications and fungicides to control pathogens where the use of silicon sources is an important disease control alternative to lily bulbs **(Khalifa *et al*., 2013, Mohamed *et al*., 2017, and**

Tsegaye *et al.*, 2018). Silicon plays a vital role in activating disease resistance by improving plant growth and physiological characteristics of the plant cell. Accumulation of silica beneath cuticles fortifies the cell wall against pathogen attack **(Jayawardana *et al.*, 2014 and Elsharkawy *et al.*, 2015).** The use of silicon and or silica as a control method for soilborne pathogens, such as *F. oxysporum* and *Pythium* spp., (**Sakr, 2016 and Khalifa *et al.*, 2017).**

Meanwhile, Bio-fertilizers are biologically active products or microbial inoculants, which are formulations containing one or more living microorganisms, such as beneficial bacteria, that when applied to soil colonize the rhizosphere or interior of the host plant and promote growth by increasing the availability of primary nutrients to the host plant **(Eid *et al.*, 2021 and Fasusi *et al.*, 2021)**. So, it is considered an important component of organic farming because they help to maintain long-term soil fertility and sustainability by fixing atmospheric di-nitrogen, mobilizing fixed macro and micronutrients, and converting insoluble phosphorus in the soil into plant-available forms. Hence, they have important and long-term environmental implications, negating the adverse effects of chemicals **(Nosheen *et al.*, 2021 and Pirttilä *et al.*, 2021).**

Therefore, the present investigation aims to investigate environmentally alternative safer methods by evaluating the efficacy of some bio-fertilizers and sources of silicon for controlling bulb rot infecting *Lilium* spp. in Egypt.

MATERIALS AND METHODS

Laboratory and greenhouse experiments were carried out in "Research Branch, Plant Pathology Research Institute, Ornamental, Medicinal, and Aromatic Plant Diseases Research Department, El-Sabihia Agricultural Research Station, Alexandria", while a field experiment was conducted in El-Qanater El-Khairia, Qalyubia governorate" during an average of two seasons of 2019 and 2020.

Plant materials: Two cultivars of bulbs of *Lilium* are lightly scented ('*L. corleone*' pinkish-red blooms, and '*L. litouwen*' white), were obtained from Egypt Holland International CO., Elmenofia, Ashrnon, Egypt. It was purchased of uniform size from Ostensibly healthy bulbs (bulb sizes 18/20, diameter 2.5- 3.2 cm, and fresh weight 34.73 gm).

Chemicals: Three biofertilizers and silicate (silicon sources), compared to the Topsin-M® 70% fungicide, were tested, shown in **Table 1,** noting that 5 grams of each of the biofertilizer powdered treatments were soaked in one liter of tap water for 72 hours, then continued mixing for 10 minutes to ensure complete mixing before treating.

Table 1: Bio-fertilizers, silicates and fungicide, their commercial name, composition, concentrations tested and source

Commercial name	Composition	Concentration (g/l)	Source
	Bio-fertilizers		
Biogen® (10^6 - 10^8cfu/g)	(*Azotobacter vinaudit* + *A. chroococum*)	5	General Organization for Agric. Equalization Fund, Agricultural Research Center, Egypt
Phosphorine® (10^8 cfu/g)	(*Bacillus megaterium* var. *phosphaticum*)	5	
Rhizobactrien® (10^{10} cfu/g)	(*A. chroococcum*)	5	
	Silicate (sources of silicon)		
Aluminum silicate	(Al_2O_3 SiO_2)	5	Central Drug House (P) LTD Post Bon No. 7138, New Delhi-110002
Calcium silicate	(Ca_2 SiO_4)	5	
Magnesium silicate	(MgO SiO_2 xH_2O)	5	
	Fungicides		
Topsin-M®70% wp	(70% Thiophanate-methyl) [dimethyl 4,4`-(*o*-phenylene) bis(3-thioallophanate)]	3	Nippon Soda CO., LTD Japan, Imported by Cairo Company for Chemicals- Giza- Egypt, Sumotomo corporation -Egypt
Control	Tap water only		

I. Isolation, identification and frequency of fungi associated with bulb rot of lilies

The diseased lily bulbs, showing symptoms of bulbs rot, were sampled, were collected from different greenhouses and fields (in Alexandria, Giza and Qalyubia) in Egypt. The sample was thoroughly washed several times under tap water, air dried, cut infected parts into small pieces, each piece containing between healthy and rotten tissue. surface sterilized by immersing for 2 minutes in (1% active chlorine) sodium hypochlorite, washed in several changes of sterile distilled water (**Muhanna 2020**). Next dried between two sterilized filter papers then aseptically transferred into PDA at the rate of 4 pieces/plate and incubated for 4-7 days in the dark at 27±1°C (**Abdel-Rahman *et al.*, 2020)**. Microscopically, the growing fungal colonies

were examined. The number of fungi was counted, and the frequency of each fungus was calculated according to **Mohamed *et al.* (2017)** formula as follows:

$$\text{Fungus frequency (\%)} = \frac{\text{No. of detected fungal colonies}}{\text{Total No. of fungal isolates}} \text{ X } 100$$

The growing fungi were purified using hyphal tips or the single spore method (**Riker and Riker, 1936**) and (**Dhingra and Sinclair, 1995**). The isolate fungi were first identified according to their morphological and cultural characteristics as described by **Domsch *et al.* (1980**), **Nelson *et al.* (1983)**, **Nirenberg and O'Donnell (1998), Klich (2002)**, and **Leslie and Summerell (2006).** Identifications were then confirmed in the Mycological Center, Assiut University [AUMC], Egypt.

II. Pathogenicity tests

Pathogenicity was conducted as described by **Mohamed *et al.* (2017)** and **Gálvez and Palmero (2021)** using six fungal taken randomly to represent the six isolated species, one isolate for each fungal species, and tested on two ostensibly healthy *Lilium* cultivars *i.e., 'L. corleone'* and *'L. litouwen'*. Bulbs were washed with tap water, surface-sterilized by dipping in 1.5% NaOCl solution for 1 min. rinsed with sterile distilled water. A sterile cork borer (10 mm diameter) was used to make a cavity near the basal part of each bulb (5 mm in deep). Then the tissues plug was pulled out. The mycelial disc of the equivalent diameter obtained from the edge of actively growing pure cultures (PDA medium) 7 days old was inserted into the cavities and the bulb was plugged, placed back, and tightly sealed with plastic film aseptically. Control bulbs were inoculated with sterile PDA agar plugs. Each fungus was replicated three times in a total of 45 bulbs/ tested isolates. To maintain high humidity, the inoculated bulbs were placed separately in individual plastic trays, each of which has five rows, and each row has three bulbs with wet filter paper and is covered with cling film. Incubation was carried out at 27±1 ºC under laboratory conditions, constantly observed and checked weekly.

Disease severity was determined 4 weeks after bulb inoculation (**Mohamed *et al.*, 2017)**. Measurement of percentage of disease severity was conducted according to **Gálvez and Palmero (2021)** as the infested bulbs were rated visually according to the following scale with five categories. (1) no visual infection (bulbs are completely healthy); (2) a few it rotten covering as less than 10% of the bulb area; (3) moderately rotten covering up to 25% of the bulb area; (4) heavily rotten covering until it reaches 50% of the bulb area and (5) rotten covering up to 75% of the bulb area (dead by the completely decayed bulb).

$$\text{Percentage of disease severity} = \frac{\sum(nR)}{5\,N} \times 100$$

Whereas: n = Number of infected bulbs, R= Category number, N = Total number of examined bulbs, and 5 = The highest category number of infections. The disease symptoms of artificially infected bulbs were also reisolated and compared with the original isolates at the end of the test of each fungus.

III. Molecular identification of *Fusarium oxysporum* fungal isolate

Molecular identification of the recovered *F. oxysporum* fungal isolate that showed the highest disease severity of bulb rot in the pathogenicity test. The isolate was grown in sterile petri plates containing autoclaved potato dextrose agar (PDA) medium and incubated for 7 days at 28°C **(Pitt and Hocking, 2009)**. The cultures were sent to the Molecular Biology Research Unit, Assiut University for DNA extraction using a Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea. Samples of fungal DNA were sent to SolGent Company, Daejeon, South Korea for polymerase chain reaction (PCR) and 18S sequencing. PCR was performed using ITS1 (forward) and ITS4 (reverse) primers which were incorporated in the reaction mixture. Primers have the following composition: ITS1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). The purified PCR products (amplicons) were sequenced with the same primers with the incorporation of ddNTPs in the reaction mixture (**White *et al.*, 1990)**. The obtained sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05.

IV. Greenhouse experiment

Evaluation of the efficacy of some bio-fertilizers and silicates compared to Topsin-M® fungicide against *F. oxysporum* under greenhouse conditions

The above-mentioned bio-fertilizers and silicates were tested for their efficacy to control *F. oxysporum* f. sp. *gladioli* (as the isolated *F. oxysporum* in the survey was identified in the molecular analysis as *F. oxysporum* AUMC15124**).** This was conducted under greenhouse conditions using two ostensibly healthy *Lilium* cultivar *i.e.,* '*L. corleone*' and '*L. litouwen*'. Inocula of the tested fungus were prepared by growing them at 27±1 ºC in pure cultures (PDA). After 7 days, one mycelial disc 5 mm in diameter was taken from the margins of colony growth in plates and transferred onto pure sterilized sorghum sand medium (75 and 25 g) from the sorghum and fine washed sand, and 50 ml tap water, respectively in 200 ml glass bottles (**Muhanna 2020**) for 20 days at 27±1 ºC (**Mohamed *et al.*, 2017**). Plastic pots (30-cm-diameter) were sterilized with sodium hypochlorite (5%), then inverted, and left to dry for a day before being used for planting. The used soil was made up of a mix of peat moss, loam, and sand (at 1:1:1 g/g) Loam and sand were sterilized over a sequenced three-day period by Autoclaving at 121°C for 30 minutes. Each soil in a plastic pot (9 kg) was pre-infested with *F. oxysporum* tested isolate at a rate of 3% (w/w) one week before planting. *Lilium* bulbs were dipped in each treatment as shown in **Table 1** for 20 min immediately before planting. Then treatments were applied again 4 weeks after sowing with irrigation water. Each treatment contained 45 replicate pots (1 bulb/pot).

The disease incidence (percentage of infection), DI %, was recorded 12 weeks after planting according to **Raju and Naik (2007)** using the following formula:

$$DI\,\% = \frac{\text{Number of infected bulbs}}{\text{Total number of bulbs}} \times 100$$

Also, the efficacy of the tested treatments (TE %) was calculated relative to the untreated control according to **Farag *et al.* (2018)** according to the following formula:

$$TE\,\% = \frac{DI\% \text{ in control} - DI\% \text{ in treated}}{DI\,\% \text{ in control}} \times 100$$

V. Field experiment

Evaluation of calcium silicate and Phosphorine® in comparison to Topsin-M® to control bulbs rot of lily under field conditions

Based on the results of the greenhouse experiment, calcium silicate and phosphorine® in comparison to Topsin-M® 70% fungicide was selected to be tested for their efficacy on the highly susceptible *'L. litouwen'* cultivar of lilium. The field trial was conducted in a field known for having intensive infection with bulb rot of lily. The cultivar was planted highly susceptible to infection of diseases. The plot (sandy loam soil with good drainage) was divided into three sections (three replications) for each treatment, each of which has five rows, and each row has 12 bulbs, with a total of 60 bulbs of spacing between them (widths x lengths cm) (20 x 20), with a total area of (100 x 250) per replications. The sections were separated by a one-meter free space. Prior to planting, all necessary horticultural precautions were taken. Bulbs were planted at a depth of 6-8 cm, with the pointy part of it facing upwards. It was watered as needed throughout the entire trial.

Lilium bulbs were dipped in solutions each treatment of Phosphorine® (5g/l), calcium silicate (5g/l), and Topsin-M® 70% wp fungicide (3g/l) for 20 min immediately before planting. Then treatments were applied again 4 weeks after sowing with irrigation water.

Each separately was carried out in the early morning (**Khalaj 2010)**. At the end of the test (twelve weeks after planting), plant growth parameters, *i.e.*, the number of flowers per plant, plant height (cm), and bulbs weight (g) were determined. The percentage of disease incidence (DI %) was calculated according to **Raju and Naik (2007)**. Also, the efficacy of the tested treatments (TE %) on bulbs and plants were calculated relative to the untreated control according to **Farag *et al.* (2018),** as above mentioned.

Statistical analysis

A randomized complete block design was used with three replications for each treatment. The collected data were statistically analyzed with the Statistix programmer. And means comparisons were performed at the 5% level using the least significant difference (LSD) according to **Snedecor and Cochran (1989)**.

RESULTS

I. Frequency of fungi associated with *Lilium* spp. bulb rot

Data in **Table 2.** showed that six fungal species fungi have been isolated and identified from the collected samples. However, most of the mean frequency (%) isolated fungi from greenhouses and fields were *Fusarium oxysporum* isolates and constituted 44.77% of the total isolates recovered (370) from both greenhouse and field collected samples. This was followed by *F. verticillioides* (16.80%), *F. proliferatum* (15.46%), and then *Pythium splendens, F. semitectum,* and *Rhizoctonia solani* were isolated at lower frequencies less than 10.65% of the total isolates recovered.

Table 2: Frequency (%) of the isolated fungi from infected *Lilium* bulb rot collected from different greenhouses and fields in Egypt

Isolated fungi	Greenhouse		Field		Mean of Frequency (%)
	No. of isolates	Frequency (%)	No. of isolates	Frequency (%)	
Fusarium oxysporum	77	49.36	86	40.20	44.77
F. proliferatum*	22	14.10	36	16.80	15.46
F. semitectum	10	06.41	17	07.94	07.18
F. verticillioides*	24	15.38	39	18.20	16.80
Pythium splendens	15	09.62	25	11.70	10.65
Rhizoctonia solani	08	05.13	11	05.14	05.13
Total	156	100.00	214	100.00	100.00

* *F. proliferatum* (Matsoshima, Nirenberg= *F. moniliforme* in previous publication)
**F. verticillioides* (Saccardo, Nirenberg = *F. moniliforme* in previous publications)

II. Pathogenicity tests

Data of the pathogenicity test on *'L. corleone'* and *'L. litouwen'* cultivars, shown in **Table 3.,** showed the ability of all fungi to cause bulb rot, but to different degrees. *F. oxysporum* showed the highest disease severity with mean over the two tested cvs. being 93.33%. This was followed by *F. verticillioides*, and *F. proliferatum*, with much lower disease severity values while *Rhizoctonia solani* showed the lowest value being 29.83% over the two tested cvs.

Table 3: Pathogenicity (and disease severity %) of isolated fungal species from natural infection of *Lilium* spp. four weeks post inoculation on two tested cvs., *L. corleone* and *L. litouwen*

Isolated fungi	Disease severity %		
	'L. corleone'	***'L. litouwen'***	**Mean**
Fusarium oxysporum	91.67a	95.00a	93.33
F. proliferatum	57.00b	65.67b	61.33
F. semitectum	36.00d	40.33d	38.17
F. verticillioides	59.33b	66.33b	62.83
Pythium splendens	44.33c	56.00c	50.17
Rhizoctonia solani	29.67e	30.00e	29.83
Control (untreated)	00.00	00.00f	-
Mean	45.43	50.48	-
LSD at 5%	2.65	1.88	

*At 0.05 of probability, values followed by a different letter (s) differ significantly and the values represent the average of 45 bulbs/ treatment

III. Molecular identification of *Fusarium oxysporum* recovered in the survey

F. oxysporum isolate was further identified by molecular methods as it represents the most dominant isolated fungus which caused the highest disease severity in the pathogenicity test. The good sequence quality was aligned to get the nearest relative with the highest similarity by the sample TF-5 *F. oxysporum* AUMC15124 (GB: MZ715094) (540 letters) *i.e.*,

[TAGGTGAACCTGCGGAGGGATCATTACC
GAGTTTACAACTCCCAAACCCCTGTGA
ACATACCACTTGTTGCCTCGG
CGGATCAGCCCGCTCCCGGTAAAACGGG
ACGGCCCGCCAGAGGACCCCTAAACTC
TGTTTCTATATGTAACTTCTG
AGTAAAACCATAAATAAATCAAAACTTT
CAACAACGGATCTCTTGGTTCTGGCAT
CGATGAAGAACGCAGCAAAA
TGCGATAAGTAATGTGAATTGCAGAAT
TCAGTGAATCATCGAATCTTTGAACGC
ACATTGCGCCCGCCAGTATTCT
GGCGGGCATGCCTGTTCGAGCGTCATTTC
AACCCTCAAGCACAGCTTGGTGTTGGG
ACTCGCGTTAATTCGCGTTC
CCCAAATTGATTGGCGGTCACGTCGAGCT
TCCATAGCGTAGTAGTAAAACCCTCGT
TACTGGTAATCGTCGCGGCC
ACGCCGTTAAACCCCAACTTCTGAATGTT
GACCTCGGATCAGGTAGGAATACCCGC
TGAACTTAAGCATATCAATA
AGCGGAGGA]

The tested strain showed 100% identity and 100% coverage with several strains of *F. oxysporum* f. sp. *gladioli* accessed from the GenBank as seen in **Figure 1**.

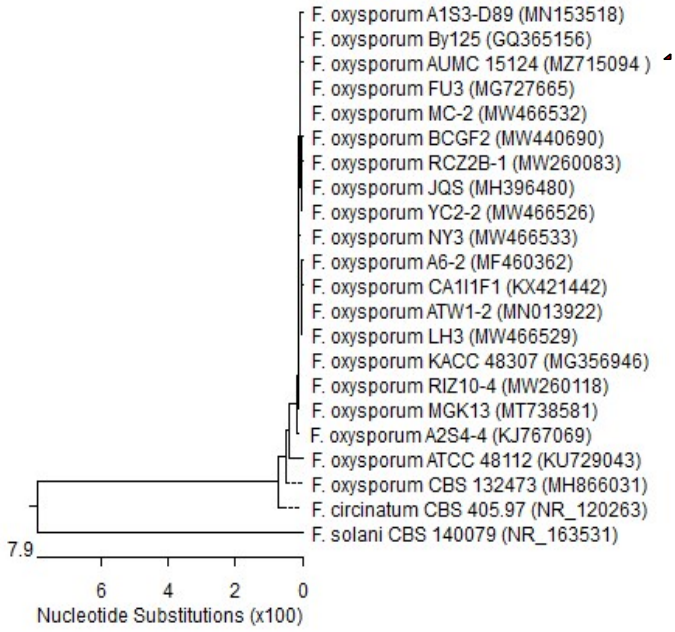

Fig. 1: Phylogenetic tree of the *Fusarium oxysporum* AUMC15124 isolate

IV. Efficacy of some bio-fertilizers and silicates compared to Topsin-M fungicide against the native isolate *F. oxysporum* f. sp. *gladioli* under greenhouse conditions

All the treatments shown in **Table 1.** were tested against the aggressive pathogenic isolate of *F. oxysporum* f. sp. *Gladioli* AUMC15124 and were evaluated under greenhouse conditions.

Table 4. showed that all the tested treatments significantly reduced the percentage of disease incidence (DI %) in bulbs, compared to the untreated control. Calcium silicate was the most effective and significantly decreased mean (DI %) to 12.5% over the two tested cvs. which was not significantly different from Topsin-M® fungicide which showed 11.11%, his was followed by Phosphorine® as showed 23.34%. The other treatments, however, showed a much lower effect compared to the untreated control 84.44%.

Table 4: Effect of the different treatments on the percentage of disease incidence (DI %) in bulbs after 12 weeks post-planting of two cultivars (*L. corleone* and *L. litouwen*) in potting soil infested with *Fusarium oxysporum* AUMC15124** under greenhouse condition

Treatments & conc.	Disease incidence %		
	'L. corleone'	*'L. litouwen'*	Mean
Bio-fertilizers			
Biogen®(5g/l)	54.44b*	56.67b	55.56
Phosphorine®(5g/l)	21.11e	25.56e	23.34
Rhizobactrien®(5g/l)	33.89d	35.56d	34.73
Silicate (sources of silicon)			
Aluminum silicate(5g/l)	38.33cd	40.00c	39.17
Calcium silicate (5g/l)	11.11f	13.89f	12.50
Magnesium silicate (5g/l)	38.89c	40.56c	39.73
Fungicides			
Topsin-M®(3g/l)	10.00f	12.22f	11.11
Control (untreated)	79.44a	89.44a	84.44
Mean	35.90	39.24	-
LSD at 5%	4.54	3.95	

** *F. oxysporum* = *F. oxysporum* f. sp. *gladioli* (according to the conducted molecular analysis), *At 0.05 of probability, values followed by a different letter (s) differ significantly and the values represent the average of 45 bulbs and or plants/treatment.

Meanwhile, it is evident in **Fig. 2** that there were no significant differences between the efficiency of calcium silicate and Topsin-M® on both tested cvs. against *Fusarium oxysporum* f. sp. *gladioli* when it was used to infect soil under greenhouse conditions. Topsin-M® gave the

highest treatment effectiveness (87.34 and 86.28%) followed by calcium silicate (85.96 and 84.52 %), while Phosphorine® was of a lower effective (73.53 and 71.34%) than either calcium silicate or Topsin-M®, while Biogen® was the least effective (40.09, 45.65 %, respectively).

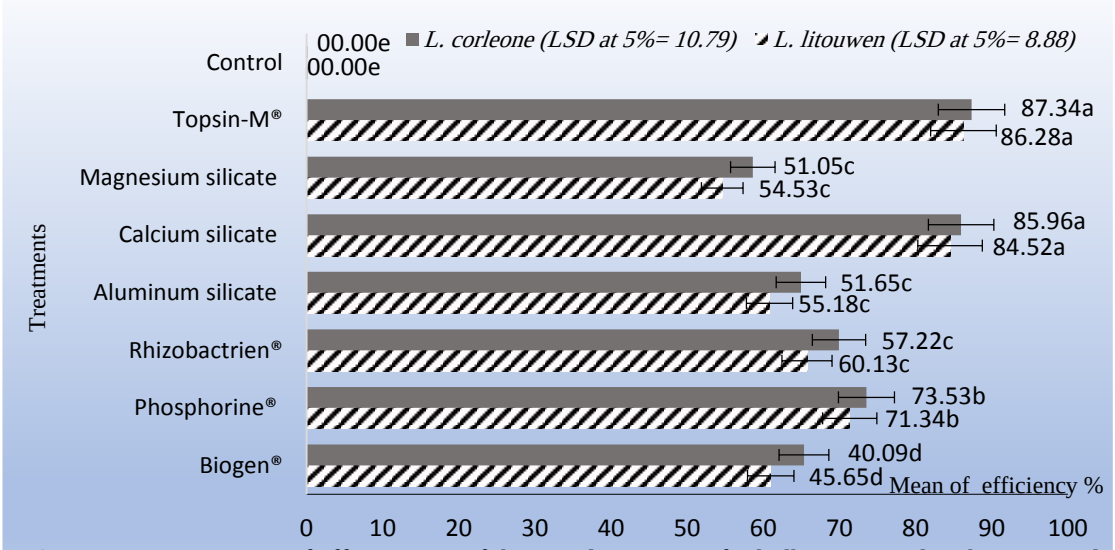

Fig. 2: Mean percentage of effectiveness of the tested treatments for bulbs compared to the untreated control at the end of the experiment after 12 weeks planting *'L. corleone'* and *'L. litouwen'* in soil infested with *F.* oxysporum f. sp. *gladioli* AUMC15124 under greenhouse conditions

V. Evaluation of calcium silicate and Phosphorine® in comparison to Topsin-M® to control bulbs rot under field conditions

This trial is conducted in a field naturally heavily infested with bulb rot of lily to investigate the efficacy of calcium, Phosphorous® silicate, and Topsin-M® on *'Lilium litouwen'* under natural field infection conditions

Data in **Table 5** revealed that the tested treatments significantly reduced the incidence of the bulb rot disease on *Lilium litouwen* cultivar under natural field infection conditions **(Table 5 & Fig. 3).**

As shown in **Table 5,** Topsin-M® as a fungicide was effective and showed a significant decrease in the bulb rot. The percentage of disease incidence in bulbs reached 15.56, followed by calcium silicate with 18.89, then Phosphorine® with 20.0, compared to 50.00 % for the untreated control.

Concerning plant growth parameters, (plant height [cm], number of flowers [No.], and bulbs weight [g]), it was noticed that these parameters were significantly increased with calcium silicate treatment and the average of these values was 427.67 cm, 40.00, and 848.33 g, followed by Phosphorine® as bio-fertilizers (420.00 cm, 39.33, and 675.67 g). This was followed by the application of Topsin-M® as fungicide 306.00 cm, 25.67, and 501.33g compared to the untreated plants with 193.33 cm, 19.33, and 268.33 g, respectively.

Table 5: Effect of calcium silicate, Phosphorine® and Topsin-M® on bulb rot disease incidence (%) and growth parameters of lily (cv. *'Lilium litouwen')* under field conditions

Treatments & Conc.	Bulb rot disease incidence %	Plant growth		
		Height (cm)	No. of flowers	Bulbs weight (g)
Bio-fertilizers				
Phosphorine® (5g/l)	20.00b*	420.00a	39.33a	675.67b
Silicate (sources of silicon)				
Calcium silicate (5g/l)	18.89b	427.67a	40.00a	848.33a
Fungicides				
Topsin-M® (3g/l)	15.56c	306.00b	25.67b	501.33c
Control (untreated)	50.00a	193.33c	19.33c	268.33d
LSD at 5%	2.94	24.60	1.97	45.70

Bulbs were dipped in each treatment for 20 min immediately before planting, then treatments were applied again 4 weeks after sowing with irrigation water. *Values followed by a different letter (s) differ significantly at 0.05 of probability, the values represent the average of 180 bulbs and or plants/treatment

Fig. 3 shows that the fungicide Topsin-M® gave the highest significant percentage of efficiency in controlling bulb rot that infected *Lilium litouwen*, with an average efficiency

percentage on both plants and bulbs (73.83 and 68.83), followed by calcium silicate (68.16 and 62.19) and Phosphorine® (64.75 and 60.01%, respectively), compared with untreated control.

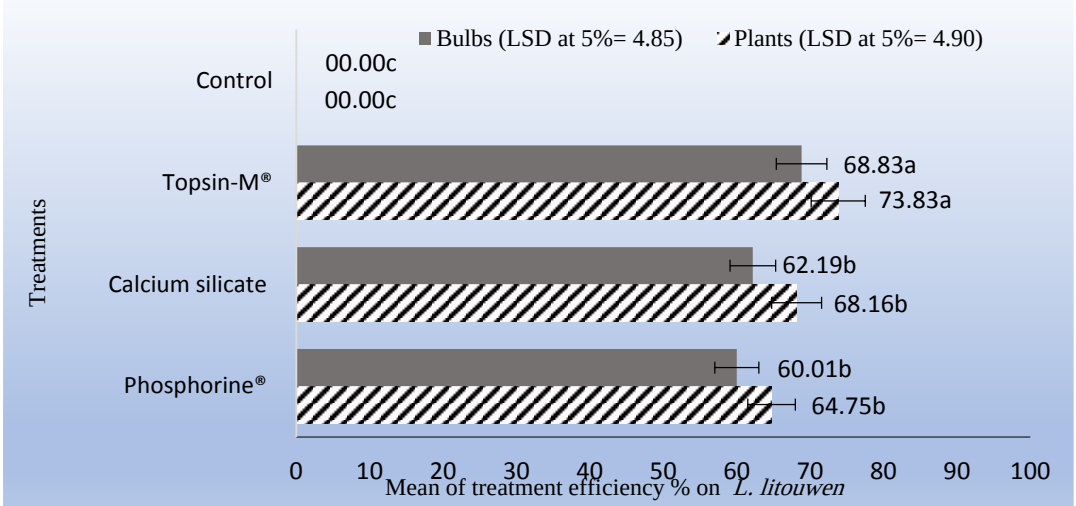

Fig. 3: Efficiency percentages of calcium silicate, Phosphorine® and Topsin-M® on basal rot of *Lilium litouwen* cultivar under natural field infection conditions

DISCUSSION

The *Lilium* spp. is one of the most beautiful and popular flowers especially in the vase and the garden and it has become one of the most popular and important flower bulbs and cut flowers all over Egypt and worldwide. lily bulb rot, caused by soil-borne diseases, is considered to be an important fungal disease in Egypt **Mohamed *et al.* (2017)**. In order to overcome the disease, some biofertilizers and silicate compounds were tested in the present study as effective, safe and environmentally friendly compounds compared to fungicides to reduce the spread of bulb rot in *Lilium* spp.

Six fungal species, *i.e*., *Fusarium oxysporum, F. proliferatum, F. semitectum, F. verticillioides, Pythium splendens* and *Rhizoctonia solani,* were isolated from naturally infected bulbs of lily plants showed typical symptoms of bulb rot collected from different greenhouses and fields in Egypt, where *F. oxysporum* was the most prevalent and constituted 44.77% of the total isolates made (370 isolates). These results are consistent with those obtained by **Lebiush-Mordechai *et al.* (2014), Bakhshaie *et al.* (2016)** and **Lakshman *et al.* (2017).** Meanwhile, significant differences for pathogenicity were found between the recovered fungal species where *F. oxysporum* showed the highest mean percentages of disease severity being 93.33% over the two tested cvs. (*L. corleone* and *L. litouwen)*, followed by *F. verticillioides*, then *F. proliferatum*, while *Rhizoctonia solani* exhibited the lowest rates of disease severity. Meanwhile, the molecular study for the recovered *F. oxysporum* strain showed 100% identity and 100% coverage with strains of *F. oxysporum* f. sp. *gladioli* accessed from the GenBank. These findings are in harmony with **Zhou *et al.* (2018)** and **Hua *et al.* (2019) and Guiying-Shi *et al.* (2020)**.

Under greenhouse conditions, the efficacy of three silicate compounds (calcium silicate, aluminum silicate, magnesium silicate), biofertilizers (Biogen®, Phosphorine®, Rhizobacterine®), in comparison with the fungicide Topsin-M® against *F. oxysporum* f. sp. *gladioli* were evaluated over two tested cvs. (*L. corleone* and *L. litouwen)*. Data revealed that all the tested treatments significantly reduced the disease incidence in bulbs, compared to the untreated control. Calcium silicate was the most effective and significantly decreased the mean percentage of disease incidence (DI %) to 12.50% over the two tested cvs. which was not significantly different from Topsin-M® fungicide which showed 11.11%, this was followed with Phosphorine® as showed 23.34%. The other treatments, however, showed much lower effect. These results are in harmony with those obtained by **Sakr (2016), and Khalifa *et al.* (2017**).

Meanwhile, under field conditions in a field naturally heavily infested with bulb rot of lily, the efficacy of the most effective treatments in the laboratory test, (*i.e*., calcium silicate, Phosphorous®, and Topsin-M®) were evaluated on the susceptible cv. *'Lilium litouwen'*. All tested

treatments significantly reduced the incidence of the bulb rot disease on the tested cultivar and a trend was obtained similar to the greenhouse test but at a bit lower effect as disease incidences were 15.56%, 18.89, and 20.0%, for Topsin-M®, calcium silicate, and Phosphorine®, respectively, compared to 50.00% for the untreated control. This was accompanied by an enhancement in plant growth parameters (plant height, number of flowers, and bulbs weight) with the highest effect was recorded with calcium silicate. These results are in harmony with several investigators **(Fortunato *et al.*, 2012, Khalifa *et al*., 2017 and Mohamed *et al*., 2017).**

There are numerous explanations of the silicone role in suppressing bulbs rot *i.e.*, silicon's emerging role as a biologically active element capable of improving the plant and bulbs' natural defense system (**Detmann *et al.*, 2012).** Sources of silicon availability lead to increase mesophyll conductance, which improves plant photosynthesis and enhances root exudates, which play an important role in the plant's and bulbs' resistance to soilborne fungal pathogens **(Kablan *et al.*, 2012, Jayawardana *et al*., 2014** and **Elsharkawy *et al*., 2015)**. Moreover, silicon is preferentially deposited in plant epidermal tissues, where it improves tissue rigidity and increases resistance to biotic and abiotic stressors, resulting in increased plant growth and yield (**Meharg and Meharg, 2015**).

Too, silicone may interfere with cationic co-factors of the enzymes which influence plant and bulbs resistance and suppress bulb rot **(Sakr, 2016 and Jacoby *et al*., 2017).** Also, silicates play a vital role in activating disease resistance by improving plant growth and the physiological characteristics of the plant cell and the accumulation of silicon beneath the cuticle significantly fortify the cell wall against pathogen attack **(Sakr, 2016, and Khalifa *et al*., 2017**).

On the other hand, **Bektas and Kusek (2020)** and **Eid *et al.* (2021)** stated that biofertilizers improved plant nutrition by increasing nutrient availability in soils because beneficial bacteria and fungi successfully colonize the rhizosphere (**Riaz *et al*., 2020** and **Fasusi *et al*., 2021)**. Biofertilizers are regarded as an important component of organic farming because it aids in the long-term preservation of soil fertility and sustainability by fixing atmospheric di-nitrogen, mobilizing fixed macro and micronutrients, and converting insoluble phosphorus in the soil into plant-available forms (**Hua *et al.*, 2019, Nosheen *et al*., 2021** and **Pirttilä *et al*., 2021)**.

Consequently, it can be concluded that treatments with calcium silicate, and Phosphorine® biofertilizer can be used successfully in ornamental plant production to help to control bulb rot of Lilies, or even reduce the excessive use of fungicides, and stimulate their plant growth.

REFERENCES

Abdel-Rahman, T.F.M., S. A. El-Morsy and A. E. A. Halawa. 2020. Occurrence of stem and leaf Spots on Lucky bamboo (*Dracaena sanderiana* hort. ex. Mast.) plants in vase and its cure with safe means. J. Plant Protec. Pathol., Mansoura Univ., 11 (12):705 – 713.

Bakhshaie, M., S. Khosravi, P. Azadi, H. Bagheri, and J. M. van-Tuyl. 2016. Review biotechnological advances in *Lilium*. Plant Cell Rep., (35): 1799–1826.

Bektas, I. and M. Kusek. 2020. Biological control of onion basal rot disease using phosphate solubilising rhizobacteria. Biocontrol Sci. Technol., 31, 190–205.

Detmann, K. C., W. L. Araújo, S. C. Martins, L. M. Sanglard, J. V. Reis and E. Detmann. 2012. Silicon nutrition increases grain yield, which, in turn, exerts a feed-forward stimulation of photosynthetic rates via enhanced mesophyll conductance and alters primary metabolism in rice. New Phytol. 196, 752–762. doi: 10.1111/j.1469-8137.2012.04299.x.

Dhingra, O. D. and J. B. Sinclair. 1995. Basic Plant Pathology .2nd ed. Lewis Publ., CRC Press, U S A,434 pp.

Domsch, K. H., W. Gams and T. H. Anderson. 1980. Compendium of soil fungi. vol. 1, Academic Press, London. 887 pp.

Eid, A. M., A. Fouda, M. A. Abdel-Rahman, S. S. Salem, A. Elsaied, R. Oelmüller, M. Hijri, A. Bhowmik, A. Elkelish and S. E.-D. Hassan. 2021. Harnessing bacterial endophytes for promotion of plant growth and biotechnological applications: An Overview. Plants, 10 (5): 935-969. https://doi.org/ 10.3390/plants10050935.

Elsharkawy, M. M., T. Hase, M. Shimizu and M. Hyakumachi. 2015. Suppressive effects of a polymer sodium silicate solution on powdery mildew and root rot diseases of miniature rose. African J. Biotechnol., 14 (42): 2917-2927.

Farag, M. F., E. W. R Ghebrial and H. S. Zawam. 2018. Efficacy of some medicinal and aromatic plants combined with different biocides against *Fusarium oxysporum* f. sp. lycopersici-Meloidogyne incognita disease complex in tomato. Egypt. J. Phytopathol., 46 (2): 85-106.

Fasusi O. A., Cristina Cruz and O. O. Babalola. 2021. Agricultural sustainability: microbial biofertilizers in rhizosphere management. Agriculture, (11): 163. https://doi.org/10.3390/agriculture11020163.

Fortunato, A. A., F. A. Rodrigues, P. J. C. Baroni, B. G. C. Soares, D. M. A. Rodriguez and O. L. Pereira. 2012. Silicon suppresses *Fusarium wilt* development in banana plants. J. Phytopathol., 160: 674-679.

Gálvez, L. and D. Palmero. 2021. Incidence and etiology of postharvest fungal diseases associated with bulb rot in garlic (*Alllium sativum*) in Spain. Foods 10, 1063. https:// doi.org/10.3390/foods10051063.

Guiying-Shi, H. Sun, A. Calderón-Urrea, X. Jia, H. Yang and S. Guoli. 2020. Soil fungal diversity loss and appearance of specific fungal pathogenic communities associated with the consecutive replant problem (CRP) in Lily. Front. Microbiol., 15 (11):1649.

Hua, C., Z. Xie, Z. Wu, Y. Zhang, Z. Guo, Y. Qiu, L. Wang and Y. Wang. 2019. The Physiological and biochemical effects of phthalic acids and the changes of rhizosphere fungi diversity under continuous cropping of Lanzhou Lily (*Lilium davidii* var. *unicolor*). Hort Sci., 54 (2): 253–261.

Jacoby, R., M. Peukert, A. Succurro, A. Koprivova and S. Kopriva. 2017. The role of soil microorganisms in plant mineral nutrition—current knowledge and future directions. Front. Plant Sci., 8:1617. doi: 10.3389/fpls.2017.01617.

Jayawardana, H. A. R. K., H. L. D. Weerahewa and M. D. J. S. Saparamadu. 2014. Effect of root or foliar application of soluble silicon on plant growth, fruit quality and anthracnose development of capsicum. Trop. Agric. Res., 26 (1): 74-81.

Kablan, L., A. Lagauche, B. Delvaux and A. Legrève. 2012. Silicon reduces black sigatoka development in banana. Plant Dis., 96: 273–278.

Khalaj, M. A. 2010. Study on the possibility of replacement of phosphorus bio-fertilizers for common chemical phosphorus fertilizer (triple super phosphate) in Tuberose. National Ornamental Plant Research Station. Mahallat, Iran.

Khalifa, M. M. A., N. A. Mahmoud and N. M. Abou-Zeid. 2013. Performance of some biofungicides on the most onion economic diseases compared to recommended fungicide in Egypt. I-White rot disease control and economical feasibility. Egypt. J. Appl. Sci., 28 (1): 40-65.

Khalifa, M. M. A., N. A. H. Fetyan, M. S. Abdel Magid and N. I. El-Sheery. 2017. Effectiveness of potassium silicate in suppression white rot disease and enhancement physiological resistance of onion plants, and its role on the soil microbial community. Middle East J. Agric. Res., 6 (2): 376-394.

Klich, M. A. 2002. "Identification of common *Aspergillus* species". Centraalbureau voor schimmelcultures, 116: 70-90

Lakshman, D. P. Vieira, R. Pandey, J. Slovin, and K. Kamo. 2017. Symptom development in response to combined infection of *in vitro*-grown *Lilium longiflorum* with *Pratylenchus penetrans* and soil borne fungi collected from diseased roots of field-grown lilies. Plant Dis., 101 (6): 882-889.

Lebiush-Mordechai, S., O. Erlich, M. Maymon, S. Freeman, T. Ben-David, T. Ofek, E. Palevsky and L. Tsror. 2014. Bulb and root rot in lily (*Lilium longiflorum*) and onion (*Allium cepa*) in Israel. J. Phytopathol., 162 (7-8): 466-471.

Leslie, J. F. A. and B. A. Summerell. 2006. The fusarium laboratory manual. Ames, Iowa, Blackwell, pp. 388.

Meharg, C. A. and A. Meharg. 2015. Silicon, the silver bullet for mitigating biotic and abiotic stress, and improving grain quality, in rice?. Environ. Exp. Bot., 120: 8–17. doi: 10.1016/j.envexpbot.2015.07.001.

Mohamed, T. N., N. A. Reyad and A. A. Halawa. 2017. Management of root and bulb rot affecting some flower bulb production in Egypt. Egypt. J. Biol. Pest Control, 27 (2): 271-278.

Muhanna, N. A. S. 2020. Distinguished positive reactions of *Macrophomina phaseolina* (Tassi) Goid host plants, laboratory Media, and potting soil. Egypt. J. Phytopathol., (48) 107-121.

Nelson, P. E., Toussoum, T. A. and W. F. O. Morasas. 1983. *Fusarium* species, an illustrated manual for identification. The Pennsylvania State University Park, Pennsylvania, Penn St Univ Press, 193pp.

Nirenberg, H. and K. O'Donnell. 1998. New *Fusarium*s pecies and combinations within the *Gibberella fujikuroi*s pecies complex Mycologia, 90: 434-458.

Nosheen, S., I. Ajmal and Y. Song. 2021. Microbes as biofertilizers, a potential approach for sustainable crop production. Sustainability, 13:1868-1898.

Pirttilä, A. M., H. M. P. Tabas, N. Baruah and J. J. Koskimäki. 2021. Biofertilizers and biocontrol agents for agriculture: How to identify and develop new potent microbial strains and traits. Microorganisms, 9 (4): 817-834.

Pitt, J. I. and A. D. Hocking. 2009. Fungi and food spoilage. Springer Nature Switzerland AG. Part of Springer Nature, 524 pp.

Raju, K., and M. K. Naik. 2007. Survey and assessment for the postharvest diseases of onion in North-Eastern Karnataka. Karnataka J. Agric. Sci., 20 (1): 164-165.

Riaz, U., S. M. Mehdi, S. Iqbal, H. I. Khalid, A. A. Qadir and W. Anum. 2020. "Bio-fertilizers: eco-friendly approach for plant and soil environment," *In*: Bioremediation and Biotechnology: Sustainable Approaches to Pollution Degradation, K. R. Hakeem, R. A. Bhat and H. Qadri (eds) (Cham: Springer), 188–214. doi: 10.1007/978-3-030-35691-0.

Riker, A. J. and R. S. Riker. 1936. Introduction to Research on plant diseases, St. louis, chicago, New York and Indianapolis, John's Swift Co., 117p.

Sakr, N. 2016. The role of silicon (Si) in increasing plant resistance against fungal diseases. Hellenic Plant Protection J., 9: 1-15.

Sirin, U. 2011. Determining the effects of *Trichoderma harzianum* and some mycorrhizal fungi on plant growth and against *Rhizoctonia solani* Kühn in *Lilium* under *in vivo* conditions. Afr. J. Biotechnol., 10 (67): 15142-15150.

Snedecor, G.W. and W. Cochran. 1989. Statistical Methods, Eighth Edition, Iowa State University Press, USA. 491 pp.

Tsegaye, Z., F. Assefa, G. Tefera, T. Alemu, B. Gizaw and E. Abatenh. 2018. Concept, principle and application of biological control and their role in sustainable plant diseases management strategies. Int. J. Res. Studies Biosci. (IJRSB), 6 (4): 18-34.

White, T. J., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: PCR Protocols: A Guide to Methods and Applications (ed. M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White), pp. 315-322. Academic Press: San Diego, U.S.A.

Zhou, L., Y. Wang, Z. Xie, Y. Zhang, S. S. Malhi, Z. Guo, Y. Qiu, and L. Wang. 2018. Effects of lily/maize intercropping on rhizosphere microbial community and yield of *Lilium davidii* var. unicolor Correction(s) for this article. J. Basic Microbiol., 18 (58): 892–901.

الملخص العربى

فاعلية بعض المخصبات ومصادر السيليكون في مكافحه عفن أبصال الليليم *Lilium* spp. بمصر

تغريد فكرى محمد عبد الرحمن

قسم بحوث أمراض نباتات الزينة والطبية والعطرية – معهد بحوث امراض النباتات – مركز البحوث الزراعية – جيزه – مصر

تم عزل وتحديد ستة أنواع فطرية من نباتات الليليم المصابة بعفن الابصال. وقد تم جمعها من مختلف الصوب الزراعيه والحقول بمصر. وهم *Fusarium oxysporum* ، *F. proliferatum* ، *F. semitectum* ، *F. verticillioides* ، *Pythium splendens* و*Rhizoctonia solani*. أظهرت النتائج أن *F. oxysporum* حقق أعلى نسبة لحدوث المرض بلغت (44.77٪) من مجموع العزلات والتى بلغت (370). وفى ذات الوقت ، ظهرت فروق معنويه فى اختبار القدره المرضية وأعطى *F. oxysporum* أعلى نسبه مئويه من شده المرض التى بلغت 93.33٪ على صنفى *L.* (*L. corleone* و *L. litouwen*)، بينما أظهر *Rhizoctonia solani* أدنى معدلات الشده المرضية وبلغت 29.83%. ولقد أظهرت الدراسة الجزيئية لسلالة (*F. oxysporum* AUMC15124) هوية 100% وتغطيه 100% بسلالات (*F. oxysporum* f. sp. *gladioli*) التى تم الوصول إليها من بنك الجينات.

وتحت ظروف الصوبة الزراعية، تم تقييم فعاليه ثلاثة من الأسمدة الحيوية (البيوجين®، الفوسفورين® والريزوباكترين®) وثلاثة مركبات من السيليكات (سيليكات الألومنيوم و سيليكات الكالسيوم و سيليكات المغنيسيوم) مقارنة بمبيد فطري "توبسين–إم®" ضد *F. oxysporum* AUMC15124 و تم اختبارهم على صنفي (*L. corleone* و *L. litouwen*). وأظهرت النتائج أن جميع المعاملات المختبرة قللت بشكل كبير من نسب حدوث العفن القاعدى بالابصال ، مقارنة بالنباتات غير المعاملة. وكانت المعامله بسيليكات الكالسيوم هى الاكثر فاعليه ، ولم تكن مختلفة معنويا بشكل كبيرعن "توبسين–إم®".

وفي الوقت نفسه ، أكدت التجربة الحقلية هذه النتائج واتجاهًا مشابهًا لإختبار الصوبة الزراعية ولكن بتأثير أقل قليلاً. وقد ترافق ذلك مع زيادة في معاملات النمو لصنف *L. litouwen*' (طول النبات ، عدد الأزهار ، ووزن البصيلات) مع تسجيل "توبسين–إم®" أعلى نسبة معنوية من الكفاءة في مكافحة عفن الأبصال التى بلغت 68.83 ، يليه سيليكات الكالسيوم 62.19 ، مقارنة بالكنترول.