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Application of yeasts as biocontrol agents for controlling foliar diseases on sugar beet plants

El-Sayed H.E. Ziedan[1*] and Eman S.H. Farrag[2]

[1]Department of Plant Pathology, National Research Centre, Dokki, Cairo, Egypt
[2]Agricultural Botany, Department of Plant Pathology, Faculty of Agriculture, Qena, South Valley University, Egypt

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Survey of foliar diseases on sugar beet plants during 2008-2009 in Kafr El-Sheikh Governorate, Egypt, indicated that sugar beet plants are attacked by some foliar diseases *i.e* Cercospora leaf spot caused by *Cercospora beticola*, powdery mildew caused by *Erysiphe betae* and rust caused by *Uromyces betae*. Cercospora leaf spot disease was more epidemic followed by powdery mildew, meanwhile rust disease recorded at lowest levels. Five applications by yeasts *i.e., Saccharomyces cerevisiae, Pichia albicans, Candida sake* and commercial biocontrol agent Rhizo-N (*Bacillius subtilis*) as well as fungicide (Topsin-M 70%) significantly reduced powdery mildew disease incidence on sugar beet than untreated plants. Topsin M-70% was the best treatment, it completely suppressed powdery mildew followed by *S. cerevisiae, C. sake* and *P. albicans*, meanwhile Rhizo-N was the highly effective treatment in this respect. Also, all treatments were significantly reduced Cercospora leaf spot incidence on sugar beet than untreated plants. Topsin M-70% was the best and significant treatment followed by *C. sake, S. cerevisiae* and *P. albicans*. Scanning electron microscopy (SEM) showed that *S. cerevisiae* highly colonized on leaf surface of sugar beet and adhere itself around mycelium of *Erysiphe betae*. Sucrose in sugar beet roots was recoded as highly percentage on sugar beet treated by *P. albicans* followed by Rhizo-N and Topsin M-70% treatments. Topsin M-70% was the best and significantly treatment increased of root weight yields followed by *S. cerevisiae* then *P. albicans* and *C. sake*. On the other hand, Rhizo-N treatment was the lowest treatment increased yields of sugar beet.

Key words: *Bacillius subtilis,* yeasts*, Saccharomyces cerevisiae, Pichia albicans, Candida sake*, sugar beet, *Cercospora* leaf spot and powdery mildew

Introduction

In Egypt, sugar beet (*Beta vulgaris* L.) is an important crop for its high content of sucrose after sugarcane. Under field conditions, several pathogenic fungi attack growing sugar beet plants causing serious diseases *i.e.,* Cercospora

* Corresponding author: El-Sayed H. E. Ziedan; e-mail: ziedanehe@yahoo.com

leaf spot (*Cercospora beticola* Sacc.), Powdery mildew (*Erysiphe betae* Vanha Weltzien) and rust (*Uromyces betae* Press) (El-Kholi *et al*., 1994 and El-Kholi, 2000). Cercospora leaf spot and powdery mildew is the most serious and destructive foliar diseases in many sugar beet growing regions of the world. Cercospora leaf spot cause a reduction in gross sugar yield up to 42% (Shane and Teng, 1992). In Germany, the reduction of root yield and sugar content reached to 30 and 50%, respectively (Wolf *et al*., 1995 and Wolf and Verret, 2002). In addition, powdery mildew causes losses of up to 30% (Ruppel, 1995).

Application of fungicides is still the effective method to control these diseases. The wide spread use of the chemical fungicides has become a subject of research concern due to their harmful effect on non-target organisms as well as their possible carcinogenicity. Some reports, which have done, on field isolates of *C. beticola* and *E. betae* indicated that some of these isolates were fungicide resistant's (Weiland and Koch, 2004 and Fernández-Aparicio *et al.*, 2009). Biological control is becoming an increasingly important alternative to chemicals in crop protection. Yeasts were selected for its highly antagonistic activity against fungal pathogens such as *P. anomala*, which improved feed hygiene of cereal, grains by reduction of moulds and Enterobacteriaceae (Olstrope and Passooth, 2011). *Candida oleophila* reduced the development of gray mould rot caused by *Botrytis cinerea* on apple (Mercier and Wilson, 1995). *C. sake* was used to control *Penicillium expansum* on apple and pears, (Nunes *et al.,* 2001). Furthermore, El-Tarabily (2004) reported the yeasts would be suppressed some soil-borne plant pathogens on sugar beet. A yeast-like fungus *Sporothrix flocculosa* (syn. *Pseudozyma flocculosa*) has been tested for control of powdery mildew in greenhouse-grown cucumbers with promising results. It has been formulated as a wettable powder (Sporodex®) for use against powdery mildew on greenhouse crops (Paulitz and Bélanger, 2001). The yeast isolates of *Sporidiobolus* sp. (H 10), *Cryptococcus* sp. (H 15) and *Metschnikowia* sp (H 25) suppressed *Venturia inaequalis*, the causal agent of apple scab (Fiss *et al*., 2003).

The objective of this work was to evaluate of some yeast species to control foliar diseases on sugar beet, compare with commercial biocontrol agent and fungicide.

Materials and methods

Survey of foliar fungal diseases on sugar beet

During annual disease surveys on 2008-2009 at El-Abassia village, Kafr El-Sheikh Governorate, Egypt, foliar diseases were recorded according to external symptoms. Cercospora leaf spot, powdery mildew and rust diseases

were the main symptoms. Fungal pathogens were identified according to morphological characterizes as established by Ellis (1997).

Biocontrol agents

Two isolates of yeasts *P. albicans* and *C. sake* were isolated from the surface of orange fruits following protocol described by Fiss *et al.* (2003) for the isolation of epiphytic fungi. The isolates were grown at 27 °C on a complex medium (10 gm malt extract, 4 gm yeast extract, 4 gm glucose, and 15 gm agar per liter) and transferred every 6 weeks. The first identification was based on the key of Van der Walt and Yarrow (1984). Additional morphological, physiological and biochemical characters of the isolated yeasts were obtained by the methods currently used in yeast taxonomy (Yarrow, 1998). The yeast of *S. cerevisiae* obtained from Arab company for medicines and medicinal plant (Meba Co., Egypt). Rhizo-N (*Bacillus subtilis*) was provided from El-Nasr Company for fertilizers and biocides in El-Sadat city, Egypt.

Inocula preparetion of yeasts and E. betae

Pichia albicans and *C. sake* were grown at 24°C for 48 h on nutrient yeast broth (20.0 g glucose; 20.0 g peptone and 10.0 g yeast extract) with shaking. Cultures were centrifuged and then pellets were resuspended in sterile distilled water and centrifuged again. The resulting pellets were dispersed in sterile distilled water, and the concentration of the yeasts was adjusted to 10^8 colony forming units (cfu) ml^{-1} using a hemacytometer. 100 g of *S. servisiae* as fresh preparations from dry commercial formula was well dissolved in sterile distilled water and the suspension was diluted to 10 liters using sterile distilled water before application.

Erysiphe betae, causing powdery mildew of sugar beet was preserved on vital sugar beet plants in the greenhouse. For inoculation, heavily infested plants were used as an inoculum source of *E. betae*. Before these plants were transferred into an inoculation chamber, old conidia-spores were removed from the leaves by agitating. Young, virulent conidia were formed within 24 h and were used for inoculation. Healthy plants were placed under the infested plants in a chamber where a ventilator ran for 5 seconds in order to distribute *E. betae* conidia evenly on the leaf surfaces. Plants were left overnight and were subsequently transferred to a greenhouse at 20-23 ºC (14/10 hours day/night) and 60±10% relative humidity (RH), separated from the other plants in order to avoid unintentional infections of healthy plants.

Fungicide

Topsin M-70 % w.p (Thiophanate) Diethyl 4-4 (O-phenylene) bis 3-thiollophanate, manufacture by Nippa Soda, Japan.

Field trail

Experiment was carried out at El-Abbassia village, El-Ryiad, Kafr El-Sheikh Governorate, Egypt, during growing seasons, *i.e*. 2009/2010 and 2010/2011. This location was chosen because it has a long history of heavy infection by foliar diseases (El-Sayed, 2000; Hashem and Farrag, 2005 and Gado, 2007). Seeds of sugar beet cv. Pleno were sown in plots (1/100 feddan) in a completely randomized design with three replicates of each treatment. Three plots were left for control. Treatment application was started three months after seed cultivation (the first sign of disease has appeared). Plants were sprayed 5 times during season from January to April during two seasons with 21 days intervals. The treatments were applied as follows:- *Saccharomyces cerevisiae* (100 g /10 L of water), *Pichia albicans* (L /10L of water, *Candida sake* (L /10L of water), Topsin- M-70% (50 g /10 L of water), Rhizo - N (50 g /10 L of water) and untreated control.

Assessment of foliar diseases

The percentage of infected leaves per plant was calculated 12 days after each treatment. Disease severity of Cercospora leaf spot and powdery mildew were calculated 15 and 30 days after spraying.

Cercospora leaf spot disease severity

Disease severity was determined according the scale of Shane and Teng (1983). Scale ranged from 0-10 categories where: 0= no visual infection (1) 1-5 spots/leaf (0.1% severity), (2) 6-12 spots (0.35 % severity); (3) 13-25 spots/leaf (0.75% severity); (4) 26-50 spots/leaf (1.5% severity); (5) 51-75 spots/leaf (2.5 % severity); (6) At higher disease incidences, the average affected area per leaf was estimated from standard area diagrams, and categories 6 through 10 represented 3, 6, 12, 25, and 50% disease severity, respectively.

Powdery mildew severity

Disease readings result for reaction to powdery mildew was made according to scale of 0-9, where 0= no evidence of mildew and 9= 100% of visible mature leaf area covered with mildew (Lewellen, and Schrandt, 2001).

Also, efficiency percentage was calculated as follows:-

$$\text{Efficiency (\%)} = \frac{\text{Disease severity in control} - \text{disease severity in treatment}}{\text{Disease severity in control}} \times 100$$

Antagonism between *S. cerevisiae* and *E. betae*

Yeast inoculum of *S. cerevisiae* was sprayed 24 h prior to inoculation with conidia of *E. betae* as mentioned above. Antagonistic action on the plant cuticle was conducted by scanning electron microscopy (SEM), 1; 4 and 6 days after treatments according to Robinson *et al*. (1984). Samples of sugar beet leaves were fixed in 2.5% glutaraldehyde for 24 h at 48ºC then post-fixed in 1% osmium tetroxide for one hour at room temperature (Harley and Fergusen, 1990). The specimens were then dehydrated with absolute ethanol, critical point dried and finally sputter coated with gold. Samples were investigated using Scanning Electron Microscope model 950 (Zeiss, Germany) in electron microscopy unit, National Research Centre, Dokki, Egypt.

Yield and sucrose percentage

Total weight of sugar beet roots in replicates for each treatment was determined at the season end. Also, five replicate samples, each of ten roots (8-10 kg) for five sprays were randomly collected for determination percentage of sucrose using standard polarimetric method according to Schneider *et al*. (2002). Assessments, including all processing sample such as washing the roots, cutting, milling operations and extract sugar syrup were conducted by laboratory on sugar factory at Delta Sugar Company, El-Hamool city of Kafr El-Sheikh Governorate, Egypt.

Data analysis

The data were subjected to analysis using Duncan's multiple range tests and compared the mean values of disease severity and root yield at the 1% level of significance.

Results

Foliar diseases incidence on sugar beet

Survey of sugar beet plants in Kafr El-Sheikh Governorate indicated that the plants are attacked by foliar diseases *i.e*., Cercospora leaf spot caused by *C. beticola*, powdery mildew caused by *E. betae* and rust disease caused by *U. betae* (Fig 1). Cercospora leaf spot and powdery mildew diseases were shown more epidemic than and rust.

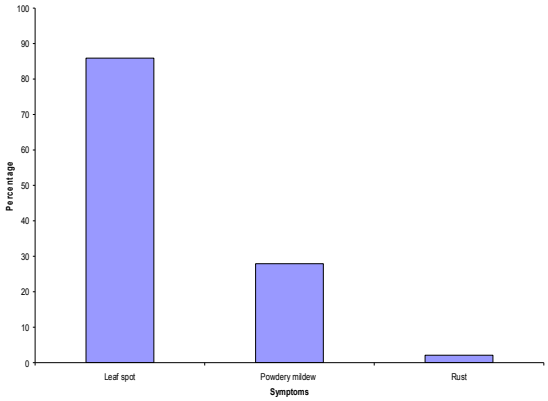

Fig 1. Foliar diseases incidence in sugar beet plants as showed in natural field infestation.

Effect of biological treatments on foliar diseases incidence on sugar beet plants

It is indicated that all treatments, fungicide (Topsin), bacterial (Rhizo-N) and yeasts (*S. cerevisiae, P. albicans* and *C. sake*) applied as spray treatments were significantly suppressed powdery mildew incidence on sugar beet plants compared with untreated ones as shown in Table 1 and Figure 2. Meanwhile, partial reductions were showed against cercospora leaf spot. Topsin was the best treatment; it completely eliminated infection by powdery mildew on sprayed plants, also suppressed Cercospora leaf spot incidence to

Table 1. Efficacy of biological treatments on powdery mildew and cercospora leaf spot incidence on sugar beet

Treatments	Powdery mildew			Cercospora leaf spot		
	Infection (%)	Disease severity (%)	Efficiency (%)	Infection (%)	Disease severity (%)	Efficiency (%)
Control	27.9[a]	67.10[a]	0.0	85.7[a]	39. 22[a]	0.0
Topsin	00.0[d]	00.0[c]	100	14.3[d]	3.33[e]	91.61
S. cerevisiae	6.33[c]	16.00[b]	76.15	78.6[ab]	29.0b[c]	26.06
P. albicans	4.80[c]	21.67[b]	67.70	72.2[b]	30.68[b]	21.77
C. sake	4.50[c]	17.00[b]	74.66	76.4[ab]	18.5[d]	52.83
Rhizo-N	20.0[b]	14.0[b]	79.13	78.9[ab]	17.70[d]	54.87
LSD 0.05	2.32	8.02		3.95	11.65	

Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

Fig 2. Effect of *S. cerevisiae* treatment on foliar disease incidence on sugar beet plants under field conditions (right) compared with untreated ones (left)

The lowest level was followed by yeasts and Rhizo-N treatments. There were considerable differences among the values when yeasts that were used against cercospora leaf spot as illustrated in Table 1. On the other hand, no difference was found between the values in case of powdery mildew. Final determination of disease severity clearly indicated that the sprays by either yeasts gave the best results in management of the powdery mildew disease. The efficiency of tested yeasts indicated clear activity of yeasts against powdery mildew disease compared with Cercospora leaf spot disease. *S. cerevisiae, P. albicans* and *C. sake* gave 76.15, 67.70 and 74.66 % against powdery mildew disease, while gave 26.06, 21.77 and 52.83% against Cercospora leaf spot disease, respectively (Table 1).

Antagonistic of S. cerevisiae against powdery mildew pathogen

The antagonistic action of *S. cerevisiae* on *E. betae* was observed in Figure 3 by scanning electron microscopy. *S. cerevisiae* was highly colonized on sugar beet leaf surfaces and also aggregation of yeast cells on hyphae of *E. betae* (left). Completely malformation and lyses of *E. betae* hyphae with highly yeast colonization was found (right).

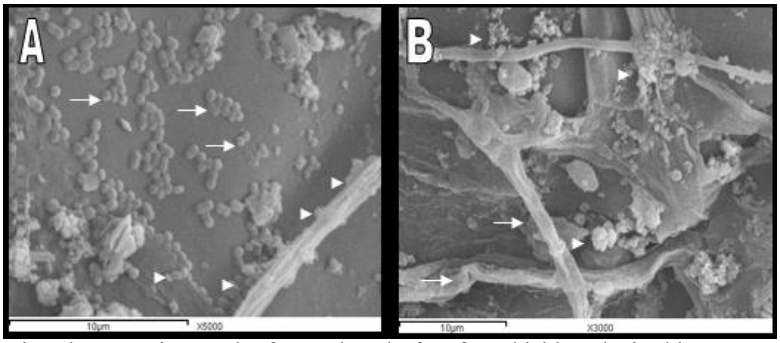

Fig 3. Scanning electron micrograph of sugar beet leaf surfaces highly colonized by antagonistic yeast, *S. cerevisiae* (arrows) and also aggregation of yeast cells on hyphae of *E. betae*, (head arrows), when samples taken 3 days after treatment (A). Malformation of fungal hyphae (arrows) with height colonization on hyphae, (head arrows), when samples taken 6 days after treatment (B)

Effect of biological treatments on sugar beet root yields

It is indicated that there were no great differences among the values concerning root weight per plant (Table 2). Result showed that all treatments led to considerable increase in root weight per feddan of the treated plants comparing to none treated control. *S. cerevisiae, P. albicans* and *C. sake* increased root weight (per feddan) from 19.4 to 29.4, 27.8 and 25.8, respectively. It also increased sucrose content from 18.7 to 19.52, 20.40 and 19.52, respectively.

Table 2. Effect of biological treatments on sugar beet root yield and sucrose percentage

	root weight		
Treatments	**plant (kg)**	**feddan (ton)**	**Sucrose %**
Control	0.69	19.4 c	18.7
Topsin	1.14	31.8 a	20.13
S. cerevisiae	1.05	29.4 a	19.52
Pichia albicans	0.99	27.8 a	20.40
Candida sake	0.81	25.8 ab	19.52
Rhizo-N	0.77	21.6 bc	20.44
LSD 0 .05	NS	6.0	-

Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

Discussion

On sugar beet, Cercospora leaf spot disease caused by *Cercospora beticola*, was more epidemic foliar diseases on sugar beet followed by powdery mildew caused by *Erysiphe betae* and rust caused by *Uromyces betae* (El-Kholi *et al*., 1994; El-Kholi, 2000; El-Sayed, 2000; Hashem and Farrag, 2005 and Gado, 2007). Biological control of different plant diseases was focused using bacteria or filamentous fungi (Whipps, 2001). So, application of yeast as biocontrol agent acts as a new trend against different pathogens. It became in the last few decade a positive alternative to chemical fertilizers safely used for human, animal and environment (Attyia and Youssry, 2001). Yeast as a natural stimulator is richness in protein 47%, carbohydrates 33%, nucleic acid 8%, lipids 4%, and different minerals 8% such as Na, Fe, Mg, K, P, S, Zn, Mn, Cu, Si, Cr, Ni, Va and Li in addition to thiamin, riboflavin, pyridoxine, hormones and other growth regulating substances, biotin, B12 and folic acid (Nagodawithana, 1991).

Applications of yeasts *i.e., S. cerevisiae, P. albicans* and *C. sake* significantly decreased foliar diseases incidence on sugar beet than untreated plants. Topsin M-70% was the best treatment, it completely suppressed powdery mildew and significantly reduced Cercospora leaf spot incidence on sugar beet followed by *C. sake* and *P. albicans*. Meanwhile Rhizo-N was the lowest effective treatment. In this respect, the highly antagonistic activity against fungal pathogens by *Pichia anomala* reduced moulds and Enterobacteriaceae (Olstrope and Passooth, 2011). *Candida oleophila* reduced gray mould rot caused by *Botrytis cineria* on apple (Mercier and Wilson, 1995). *C. sake* was used to control *Penecillium expansum* on apple and pears, (Nunes *et al.,* 2001). commercial formula of yeast-like fungus *Sporothrix flocculosa* (Sporodex®) wettable powder used for control powdery mildew on greenhouse crops (Paulitz and Bélanger, 2001).

Futhermore, *S. cerevisiae* used as biocontrol agent against of soil-borne fungal plant pathogens causing root-rot disease by *Fusarim solani* and *Rhizoctonia solani* of sugar beet as well as plant growth promoters were recent investigated by El-Tarabily and Sivasithamparam (2006), El-Tarabily (2004) and Shalaby and El-Nady (2008). Topsin M-70% was the best and significantly treatment increased of root weight yields followed by *S. cerevisiae, P. albicans* and *C. sake* increased root weight (per feddan) from 19.4 to 29.4, 27.8 and 25.8, respectively. It also increased sucrose content from 18.7 to 19.52, 20.40 and 19.52, respectively. Furthermore, highly sucrose percentage occurred on sugar beet treated by *P. albicans* followed by Rhizo-N. These results agreed with results of Shalaby and El-Nady (2008).

The mechanism of yeast as biocontrol agent involves in nutrient competition (Droby and Chalutz, 1993, El-Ghaouth *et al.,* 1998), site exclusion (Droby and Chalutz, 1993 and Wisniewski *et al.,* 1991), direct parasitism, and perhaps induced resistance (Wisniewski, 1991; El-Ghaouth *et al.,* 1998). The antagonistic action of *S. cerevisiae* on *E. betae* was observed by scanning electron microscopy. *S. cerevisiae* was highly colonized on sugar beet leaf surfaces and also aggregation of yeast cells on hyphae of *E. betae*. Malformation and lyses of fungal hyphae with hight colonization on hyphae and conidia was observed. This research indicates that *S. cerevisiae, P. albicans* and *C. sake* are considered a new promising plant growth promoting and biocontrol agents for controlling plant diseases on different crops.

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