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YEAST APPLICATION AS A BIOFERTILIZER AND BIOCONTROL AGENT FOR ONION NECK ROT DISEASE IN RELATION TO BULB PRODUCTIVITY AND QUALITY

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Abstract: Using yeast as a biofertilizer influenced incidence of neck rot disease and onion bulb yield and quality.

Dipping onion transplants cv. Shandaweel 1 before transplanting and foliar spray, after 45 days from transplanting, with active dry yeast were significantly influenced the exportable, total and culls onion bulb yield as well as incidence of neck rot disease. Yeast applications at concentration 0.1% gave the highest exportable and total bulb yield in 1998/1999 and 1999/2000 seasons, followed by yeast application either at concentration 0.05% or Ronilan fungicide treatment. Untreated treatment (control) gave the lowest value of exportable and total bulb yield, while it gave the highest value of culls yield in both seasons of study.

Yeast application at 0.1% reduced the incidence of both neck rot and other rot diseases in storage, while; yeast application at 0.05% and Ronilan treatment gave the same effect under natural and artificial bulb infection with *Botrytis allii*.

Introduction

Winter onion (***Allium cepa*** L.) is one of the most important vegetable crops in Egypt. Egyptian onion is famous all over the world for its superior quality and early appearance in European markets. During the last few years the cultivated area with onion in Egypt profoundly decreased because of serious damage of the crop yield due to infection by several diseases either during the growing season or storage as well (Hussien, 1983; Abd Elrazik *et al.*,1988 a & b; Hassan, 1996 and Monnet and Thibault, 2000). Neck rot disease caused by *Botrytis allii* Munn. is one of the most serious diseases, which attack onion plants, grown for bulb or seed production. It causes serious quantitative and qualitative losses in onion grown for exportation and during storage (Hussein, 1983; Abd Elrazik *et al.*,1988 a & b; Hassan, 1996 and Schwartz and Mohan, 1996).

The yeast (***Saccharomyces cerevisae***) is considered a new promising biofertilizer for crops. It became in the last few decade a positive alternative to chemical fertilizers safely used for human, animal and environment Omran (2000). Leifert *et al.* (1990), Ahmed *et al.* (1997), Hegab *et al.* (1997) and Mansour (1998) showed that yeast application resulted in improvement in leaf area; vegetative growth and crop yield of different crops.

Fokkema and Lorbeer (1974), Fokkema (1978), Fokkema *et al.* (1979), Blakeman and Fokkema (1982), Gullino *et al.* (1991), Chamberlain *et al.* (1999) and Tylkowska and Szopinska (2000) reported that some microorganisms may act, as bicontrol agent of certain plant diseases, simply by making the plant grow better.

The main objective of the present investigation was to study the effect of yeast application on incidence of neck rot disease in field-grown onion and during storage, as well as its beneficial effect as a biofertilizer on exportable, culls and total bulb yield of winter onion crop.

Materials and Methods

Source of *B. allii* isolates:

Naturally diseased onion bulbs of Shandaweel 1 cultivar showing neck rot symptoms were collected from different localities of Assiut and Sohag Governorates. Infected parts of onion bulbs were cut into small pieces, surface sterilized by dipping in 2% sodium hypochlorite solution for 3 minutes, washed thoroughly with sterile distilled water, then plated on Potato Dextrose Agar medium (PDA). Inoculated plates were incubated at 25°C for 7 days. Fungal hyphal tips taken from the growing colonies were transferred to PDA slant, incubated at 25°C for 7 days and then kept in refrigerator at 5°C as stock cultures for further studies. Isolated fungi were identified using the morphological characteristics of mycelia and spores described by Owen *et al.* (1950) and Barnett (1960).

Pathogenicity tests:

Five different isolates of the fungus were tested for their pathogenicity on Shandaweel 1 onion cultivar bulbs free from obvious infection were washed thoroughly with tap water, surface sterilized by dipping in 2.0% Sodium hypochlorite solution for 2 – 3 minutes, rewashed with sterile distilled water and left for drying at room temperature. Bulbs were divided into two groups. The first group was wounded and inoculated with the suspensions of the tested fungal isolates ($1x10^4$ C.F.U. / ml) using a sterilized atomizer. The second group was inculcated with fungal suspension using the flowing procedure. A hole was made near the neck of each bulb by using a cork borer (1 cm diam. and 1 cm

depth) then, 0.5 ml of the fungal suspension of each isolate (1 x 10^4 C.F.U / ml) was poured into the hole, and then covered with paraffin wax. Fungal suspension was replaced by distilled water and the bulbs were treated in the same manner as control treatment. Twenty-five bulbs were put in 30 x 40 cm jute sacks for each treatment. Sacks were kept in rows at room temperature (27 ± 5 °C) for three mouths. The percentages of diseased bulbs and/ or percentage of rotted parts were calculated as a ratio between the weights of the infected parts to the total weight of each bulb after 3 months from inoculation.

Effect of yeast application on exportable, total and culls onion bulb yield:

The experimental work was carried out during 1998 / 1999 and 1999/2000 seasons at Experimental Farm of Faculty of Agriculture, Al-Azhar University, Assiut. The soil type was silty loam with pH 7.4. The normal methods of onion cultivation were used. All experiments were carried out using completely randomized block design and the plot size was 3 x 3.5 meter (1/400 feddan) with four replicates.

Transplanting took place on November 16th in both seasons. Shandaweel 1 onion cultivar transplants (60 days old) were dipped for 5 minutes in suspension of active dry yeast at 0.05 %, 0.1% concentration and Ronilan fungicide (3-[3-5 dichlorophenyl] [5-ethenil-5-methil-2, 4-oxazolidi-nedione) at concentration 20 g/l. A treatment without yeast or fungicide (control) was also included in this test. This gives a total of four treatments. Plants were sprayed with the above-mentioned treatments after forty-five days from transplanting,. Triton B 1956 was admixed to each treatment at rate of 0.05% as an adhesive agent.

Harvesting took place at maturity i.e. when 75% of plants tops were down. Plants were left to cure for three weeks after which the tops and roots were removed. After curing, bulbs were divided into exportable and cull bulbs and the following data were recorded: 1 – Exportable yield (ton/ feddan), 2 – Total yield (ton/ feddan) and 3 – Yield of culls (sound, bolters, doubles, off color and damaged). The exportable bulbs were put in jute sacks and stored for three months at room temperature, then percentages of neck rot and other rots were recorded.

Effect of yeast application on incidence of neck rot in storage:

The effect of yeast application on incidence of neck rot disease in storage was studied under artificial inoculation with the highly pathogenic fungal isolate (No. 5). Onion bulbs were taken from each of previous treatment after curing process. The above-mentioned procedure in pathogenicity tests was

used in this study. After three months storage period at room temperature (27 ± 5 °C). percentages of neck rot and rotted parts were recorded.

All data were subjected to statistical analysis using the normal F test and the means were compared using Duncan's Multiple Range Test (Gomez and Gomez, 1984).

Results and Discussion

Isolation and identification of the causal pathogen:

Five *B. allii* isolates were isolated from diseased onion plants collected from different localities of Assiut and Sohag Governorates of A.R.E. showed sever neck rot symptoms. Isolates were identified to be *Botrytis allii* Munn. according to their morphological characteristics of mycelia and spores as described by Owen *et al.*, (1950) and Barnett (1960).

Pathogenicity tests:

Table 1indicate that the tested isolates were able to infect onion bulbs and caused neck rot. Results also revealed that the fungal isolates were varied in their pathogenicity. Isolate No.5 showed the highest percentage of infection and amount of rotting, followed by isolate No.1 and 3, whereas isolates No. 2 and 4 were the least pathogenic ones. Ellerbroch and Lorbeer (1977), Abd Elrazik *et al.* (1988 a, b) and Hassan, (1996) mentioned that *B. allii* is able to infect onion bulbs during storage causing complete death for the parenchymatous cells of the infected tissues.

Table 1: Pathogenicity tests of *B. allii* isolates during storage on Shandaweel 1 onion cultivar.

Isolate No.	Neck rot infection %	Rotting parts %
1	* 22.00 b	7.35 c
2	12.75 d	6.75 c
3	16.25 c	12.00 a
4	15.00 cd	9.88 b
5	25.25 a	13.63 a
Control	0.0 e	0.0 d

- Means in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at 5% level.

2) Effect of yeast application on exportable onion yield:

Dipping onion transplants before transplanting and foliar spray, after 45 days from transplanting, with active dry yeast was significantly influenced the exportable yield (Table 2). Yeast applications at concentration 0.1% gave the highest exportable yield in the tested season 1998/ 1999 and 1999/ 2000 (14.4 and 13.84 ton / fed., respectively), followed by yeast application at concentration 0.05% and Ronilan.

Untreated treatment (control) gave the lowest value of exportable bulb yield (8.20 and 8.88 ton / fed. in 1998/ 1999 and 1999/ 2000 season, respectively).

These results are in agreement with those obtained by Elad (1986) and Fokkema (1992).

Table 2: Effect of yeasts application on onion exportable yield (ton / fed.) in 1998 / 1999 and 1999 / 2000 seasons

Treatments	1998 / 1999 season	1999 / 2000 season
Yeast 0.05 %	* 11.34 b	11.08 b
Yeast 0.1 %	14.40 a	13.84 a
Ronilan (20 g/l)	10.92 b	12.76 b
Untreated (control)	8.20 c	8.88 b

* Means in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at 5% level.

3) Effect of yeast application on total bulb yield:

Dipping onion transplants before transplanting and foliar spray with active dry yeast was significantly influenced the total bulb yield (Table 3). Yeast application at concentration 0.1% gave the highest total bulb yield in both seasons (16.7 and 16.32 ton / fed.), followed by yeast application at 0.05% (15.00 and 14.76) and Ronilan treatment (14.72 and 15.76 ton /fed.) in the first and second seasons respectively. Control treatment gave the lowest value of total bulb yield (8.20 and 10.88 ton / fed.) in both seasons.

Table 3: Effect of yeasts application on onion total yield (ton / fed.) in 1998/ 1999 and 1999/ 2000 seasons

Treatments	1998 / 1999 season	1999 / 2000 season
Yeast 0.05 %	*15.00 b	14.76 b
Yeast 0.1%	16.70 a	16.32 a
Ronilan (20 g/l)	14.72 b	15.76 a
Untreated (control)	13.28 c	13.72 c

- Means in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at 5% level.

Such results are in agreement with those obtained by Elad (1986), Fokkema (1992), Akl *et al* (1997) and Hegab (1997). Omran (2000) who reported that the various positive effect of yeast application on growth, nutritional status of the crops and productivity could be due to: 1- Encouragement the uptake of various elements, 2- Activating the photosynthesis process and both cell division and cell enlargement, and 3- Control the incidence of pests and diseases. They also noted that all of the previous merits greatly affect the vegetative growth and yield.

4) Effect of yeast application on culls bulb yield:

Data presented in Table 4 indicate that dipping onion transplants before transplanting and foliar spray, after 45 days from transplanting, with active dry yeast were significantly influenced the culls bulb yield. Untreated (control) treatment gave the highest record of culls yield (5.08 and 4.84 ton / fed. in the first and second seasons respectively). Yeast applications at concentration 0.1% gave the lowest value of culls bulb yield in both season (2.30 and 2.48 ton / fed.), followed by yeast application at concentration 0.05% (3.66, 2.96) and Ronilan treatment (3.80, 4.00 ton/ fed.).

Such results are on line with those obtained by Fokkema *et al.*, (1979), Elad (1986) and Fokkema, (1992).

Table 4:Effect of yeasts application on culls onion bulb yield (ton / fed.) in 1998 / 1999 and 1999 / 2000 seasons

Treatments	1998 / 1999 season	1999 / 2000 season
Yeast 0.05 %	* 3.66 b	2.96 bc
Yeast 0.1 %	2.30 b	2.48 c
Ronilan (20 g/l)	3.80 b	4.00 ab
Untreated (control)	5.08 a	4.84 a

- Means in the same column followed by the same letter or letters are not significantly different according to Duncan's multiple range test at 5% level.

5) Effect of yeast application on neck rot and other rots under natural storage condition:

Data in Table 5 show that, treatments had a significant effect on neck rot and other rots affecting onion in storage in both seasons of study. Untreated (control) gave the highest percentage of infection for both neck rot and other rots in both seasons. Yeast application of 0.1% gave the lowest percentages of rots. However, yeast application of 0.05% and Ronilan treatment showed approximately a similar effect on incidence of rots on bulbs during storage.

Table 5: Effect of yeasts application on infection percentage of neck rot disease and other rots in storage under natural storage conditions.

Treatments	1998 / 1999 season			1999 / 2000 season		
	Neck rot	Other rots	Total	Neck Rot	Other rots	Total
Yeast 0.05 %	* 0.44 b	7.71 b	8.15 b	0.41 b	11.49 c	11.90 b
Yeast 0.1 %	0.65 b	4.10 c	4.75 c	0.55 b	6.92 d	7.47 c
Ronilan	0.54 b	8.67 b	9.21 b	0.46 b	13.58 b	14.04 b
Untreated (control)	1.34 a	11.05 a	12.39 a	1.68 a	15.26 a	16.94 a

* Means in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at 5% level.

6) Effect of yeast application on incidence of neck rot in storage under artificial bulb inoculation:

Results presented in Table 6 indicate that yeast application significantly reduced incidence of neck rot disease in storage under artificial inoculation with the pathogen.

Yeast application 0.1 gave the highest reduction of neck rot infection percentage, followed by yeast application 0.05% and Ronilan treatment. Such results are in agreement with those reported by Fokkema and Lorbeer (1974), Fokkema (1978), Fokkema *et al.* (1979), Blakeman and Fokkema (1982) and Sallam (1998).

In conclusion, using yeast may provide a safety and successful mean for controlling onion neck rot disease as biocontrol methods and thus overcoming the problems of using chemicals in disease control and biofertilization.

Table 6: Effect of yeast application on neck rot incidence in storage under artificial infestation.

Treatments	Neck rot infection %	Rotting parts %
Yeast 0.05 %	* 11.00 b	11.00 b
Yeast 0.1 %	6.00 c	4.00 c
Ronilan	10.00 bc	11.25 b
Untreated (control)	22.00 a	25.25 a

* Means in the same column followed by the same letter or letters are not significantly different according to Duncan's multiple range test at 5% level.

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إضافة الخميرة كسماد حيوي وكعامل للمقاومة الحيوية لمرض عفن الرقبة في البصل وعلاقتها بإنتاجية وجودة محصول الأبصال

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أجريت هذه الدراسة بهدف التعرف على تأثير معاملة شتلات البصل صنف شندويل ١ بالخميرة الجافة النشطة كسماد حيوي بديل للأسمدة الكيماوية وكذلك دراسة تأثيرها على خفض نسبة الإصابة بمرض عفن الرقبة المتسبب عن الفطر بوترايتس ألياي والذي يسبب فقد كبير في المحصول وخفض في جودة الأبصال ويعوق عملية تصديرها للأسواق العالمية.

ولقد بينت الدراسة أن معاملة الشتلات قبل الزراعة بمعلق الخميرة الجافة النشطة بتركيز ٠,١ % بالإضافة إلي رش المجموع الخضري للنباتات بعد ٤٥يوم من الشتل بنفس التركيز من الخميرة أدى إلى زيادة كل من محصول البصل القابل للتصدير وكذلك المحصول الكلي للأبصال كما أدت تلك المعاملة أيضاً إلى خفض محصول الأبصال النقضه وذلك بالمقارنة بالمعاملة بالمبيد الفطري الرونيلان، الموصى به لمعاملة الشتلات. أيضاً، تبين من الدراسة أن معاملة الشتلات بالخميرة الجافة النشطة قبل الزراعة أدى إلى خفض نسبة الإصابة بمرض عفن الرقبة وكذلك خفض نسبة الإصابة بالأعفان الأخرى في المخزن وتحت ظروف التخزين العادية وظروف العدوى الطبيعية وتحت ظروف العدوى الصناعية بالفطر المسبب لمرض عفن الرقبة في البصل.