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BIOLOGICAL CONTROL OF *BLACK MOLD* OF ONION CAUSED BY *Aspergillus niger* BY USING *Saccharomyces cervisiae*

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ABSTRACT

Aspergillus niger (Tieghem) attacked onion plants causing black mold. The tested bioagent *Saccharomyces cervisiae* at three concentrations 0.25%, 0.5% and 1%) were evaluated as bioagent on the mycelial growth, incidence and disease severity of black mold. The bioagent *Saccharomyces cervisiae* was more effective on inhibiting mycelial growth, disease severity and disease incidence at high concentration 1% than 0.25% and 0.5%.

Keywords*: Aspergillus niger - Saccharomyces cerevisiae - Biological* control - Onion - Black mold

INTRODUCTION

Onion (*Allium cepa. L.*) is one of the most economical field crops in Egypt. Black mold caused by (Aspergillus niger) is one of the most important onion storage diseases. Mousa *et al*., (1973), Yoo, *et al.,* (1989), Chalutz and Wilson (1990), Tanaka, (1991); Hayden *et al*., (1994); Köycü and Özer (1997); Chand Goyal and Spotts (1997); Droby *et al*., (1998); Özer *et al*., (1999), and Eman, Farrag (2005).

Several studies have documented onion storage losses (Tucker and Drew, 1982 and Suzuki and Cutcliffe 1989).

Mousa *et al*., (1973) reported up to 80% of bulb stored in sudan were infected by black mold. In Japan, black mold caused more than 60% storage losses during summer (Tanaka 1991).

Therefore disease managements are important for reducing storage losses in Egypt. The modern trends are directed to study the effect of the yeast *(Saccharomyas cerevisiae*) against some pathogens causes plant diseases Chalutz and Wilson 1990; Teixido (1998) Johnes Prusky (2002); Hammouda *et al*., (2005) Gianluca *et al*., (2006) Fatten Mansour and Soltan (2006).

The aim of the present work was to study the effect of yeast in controlling black mold disease of onion

MATERIALS AND METHODS

1- Isolation and identification of the causal pathogen:

Samples of onion black mold disease were collected from Assiut Governorate. Isolation procedures was carried out using infected onion bulbs Giza 6 cultivar. Soaking the infected plant parts in 1% sodium hypochlorite solution for 10 minutes followed by complete washing in sterile distilled water for surface disinfestation of infected sample before plating on PDA medium at 25°C for 5-7 days. Pure culture of the developing fungi was obtained by single spore isolation. Primary identification was carried out according

Moubasher (1993) Hoog *et al.,* 2000 then was confirmed by Assiut University Mycological Center (AUMC).

2- Pathogenicity test:

Apparantly healthy onion bulbs, free from obvious infection by *Aspergillus niger* Giza 6 cultivar, were washed thoroughly with tap water, surface sterilized by dipping in 1% sodiumn hypochlorite solution for 2-3 minutes. Then rewashed with sterile distilled water and left for arial at room temperature. Inoculum of *Aspergills niger* was prepared by growing the two tested isolates on 100ml Potato Dextrose liqued in 250ml. concical flasks, then incubated at 25°C. After 6 days, incubated culture filtrates were decanted and the growing mycelium of each tested isolate was washed using sterile distilled water, suspended in 100ml of distilled water and blended in warning blender for two minutes. Healthy onion bulbs were sprayed with the mycelial suspension 4×10^5 propagulus/ml. Fungal suspension was replaced by distilled water and the bulbs were treated in the same manner as a control treatment. Twenty five of bulbs were put in 30 × 40cm. Jute sacks for each treatment replicated four times. Sacks were kept in rows at room temperature about 32°C for three months. A piece of wet cotton with sterilized distilled water was put in each bag to maintain suitable relative humidity around the bulbs. The percentage of infection were recorded after 3 months and disease index of bulbs were recorded after the end of experiment by using scale of o to 4 was used by AICRIP (1968).

Source of *Saccharomyces cervisiae*

Extract of commericall yeasts (*Saccharomyces cervisiae*).

In *vitro* effect of *Saccharomyces cerevisiae* on linear growth of *Aspergillus niger*:

Quarter or half or one gram of *Saccharomyces cerevisiae* was dissolved in 100ml of PDA broth medium to give final concentration of 0.25, 0.5 and 1% respectively. Flask (250ml) contained 90ml of sterilized PDA medium were melted, cooled to about 52°C and 10ml of each yeast concentration were separately added to them, mixed and poured in sterilized Petri dishes (20ml/plate). Inoculation was done with fungal discs, (5mm) in diameter obtained from *Aspergillus niger* 7 days old culture. Three replicates were used for each tested concentration. Another group of PDA plates free from *Saccharomyces cerevisiae* inoculated with the fungus as control. All plates were incubated at (25°C) for 7days. Linear growth was recorded until the full growth of comtrol plates. The obtained data were statistically analyzed according to Snedeor and Cochracn (1967).

Effect of *Saccharomyces cerevisiae* on the disease incidence of black mold during storage:

This experiment was carried out in 2005 and 2006 growing seasons. Two isolates of *Aspergillus niger* were used. Healthy onion bulbs were sprayed with the mycelial suspension (4×10^5) propagulus/ml and soaked in different concentrations of *Saccharomyces cerevisiae* 0.25% or 0.5% and 0.1% respectively for 5 minutes. The previously treated bulbs were kept in 30 × 40cm. Jute sacks each containing 25 bulbs and a piece of wetted cotton to

maintain suitable humidity around bulbs. Then kept at room temp. (about 32°). Four replicates were used for each treatment. Healthy onion bulbs were sprayed with the fungal suspension only used as control. After 90 days from inoculation, percent of infection and disease index of bulbs were recorded after the end of experiment by using scale of 0 to 4 which was used by A.I.C.R.I.P. (1968).

Statistical analysis:

The collected data were statistically analyzed using two factors Completely Randomized Block Design. Treatments were compared at 0.05 and 0.01 level of probability L.S.D. (Fisher 1984).

RESULTS AND DISCUSSION

Data presented in Table (1) indicated that all isolates of *Aspergillus niger* causes black mould disease of onion during storge. Data also show that isolate (No.1) was the highly pathogenic than isolate (No. 2).

These results were in agreement with the finding of Mousa *et al*., 1973; Yoo *et al*., (1989); Tanaka (1991) Haydan *et al*., (1994); Köycü and özer (1997); Özer *et al*., (1999) and Eman Frrag 2005.

Table (1): Pathogencity test of *Aspergillus niger* causal pathogen of black mold of onion:

Isolates	Disease incidence %	Disease severity %
I_1	59	15.5
I_2	57	14.5
Control	0	0
L.S.D. 1%	5.243	2.141
5%	3.461	1.413

Data in Table (2) indicate that all concentrations of *Saccharomyces cerevisiae* inhibited linear growth of *Aspergillus niger* in *vitro* when compared with the control. The most reduction was obtained when using the concentration 1% followed by 0.5% and 0.25%.

Theses result are in agreement with finally Richard, Jones and Prusky 2002 and Gianluca et al., (2006).

Table (2): Effect of *Saccharomyces cerevisiae* on the linear growth of *Aspergillus niger* in *vitro*:

Saccharomyces cervisiae concentration	Linear growth (mm)		Mean
	$Isolate_1$	$Isolate_2$	
0.25%	55	52	53.5
0.5%	46.3	45	45.65
0.1%	31	30	30.5
Control	89	89	89
L.S.D.	1%	5%	
I	0.484	0.354	
C	0.689	0.500	
IXC	0.975	0.707	

I Isolates C. concentration of *Saccharomyces cerevisiae*
IXC isolates interaction X concentration of saccharomyces *cerevisiae*

Data presented in Table (3) indicated that the tested *Saceharomyces cerevisiae* reduced the effect of the two testing isolates developing of onion black mold disease after 90 days of storage when compared with the control. The concentration 1% was the most effective in controlling disease incidence and severity through the two tested growing seasons followed by concentration 0.5% and 0.25% respectively this results agree with Jones and Prusky 2002 they found that *Saccharomyces cerevisiae* inhibited the growth of germinated *Colletotrichum coccodes* spores and inhibited the decay development caused by *Colletotrichum coccodes* in tomato fruits. Fatten, Mansour and Soltan 2006 they mentioned that *Saccharomyces cerevisiae*, *Candida teunis*, *Bacillus subtilis* and the bioagent *Biozeid* at high concentration were the most effective treatment for controling fruit rots caused by *Botrytis cinerea* and *Alternaria alternatia* naturally infected persimmon fruit with efficacy exceeded 80% and Gianluca *et al*., (2006) found that Antagonistic yeasts showing a killer activity against *Aspergillus carbonarius* and *Aspergillus niger* on grape.

The use of microorganisms particularly yeasts occurring naturally on the surface of fruits or vegetables, usually has been preferred for the control of post harvest disease Jeffries and Jeger 1990 Chalutz and Wilson 1990; Droby *et al*., 1991. Janisiewicz 1991; Smilanick *et al*., 1993 and Janisiewicz *et al*., 1994.

Table (3): Effect of *Saccharomyces cerevisiae* on the disease incidence and disease severity of black mold during storage 2005 and 2006 growing seasons:

Isolate	Concentrations	2005 season		2006 season	
		Disease incidence %	Disease severity %	Disease incidence %	Disease severity %
I_1	0.25%	26	6.5	20	5
I_2		24	4	19	4.75
Mean		25	5.25	19.5	4.87
I_1	5.0%	19	4.75	18	4.5
I_2		17	4.25	16	4
Mean		18	4.5	17	4.25
I_1	1%	17	5.25	15	2.75
I_2		13	3.25	11	3.66
Mean		15	4.25	13	3.205
I_1	Control	60	15	56	14
I_2		56	14	52	13
Mean		58	14.5	54	13.5
L.S.D.		I 3.230	I 1.044	I 1.850	II 0.654
1%		C 4.567	C 1.477	C 2.616	C 0.654
		IXC 6.459	IXC 2.088	IXC 3.700	IXC 0.926
		I 2.383	I 0.771	I 1.365	I 0.342
5%		C 3.370	C 1.090	C 1.931	C 0.483
		IXC 4.766	IXC 1.541	IXC 2.730	IXC 0.463

I Isolates C. concentration of *Saccharomyces cerevisiae*
IXC isolates interaction X concentration of *Saccharomyces cervisial*.

Yeasts are suitable as bio-control agents of postharvest diseases because they rapidly (i) colonize and survive on fruit surfaces for long periods of time under different conditiones (ii) use available nutrients to proliferate

rapidly, limiting nutrient availability to the pathogen (iii) are generally unaffected by fungicides used commercially: In the end we must use bioagent to save our environment from pollution caused by fungicides.

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المقاومة البيولوجية للعفن الأسود فى البصل المتسبب عن اسبرجلس نيجر باستخدام السكارواميسس سيرفسيا

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****معهد بحوث أمراض نبات مركز البحوث الزراعية – الجيزة – جمهورية مصر العربية**

يصاب البصل بمرض العفن الأسود ويهدف هذا البحث إلى اختبار الخميرة (سكارواميسس سيرفسيا) بثلاث تركيزات (٠,٢٥% و ٠,٥% و ١%) فى مقاومة الفطر المسبب للمرض . فى الدراسة المعملية لوحظ أن التركيز العالى من الخميرة ١% كان أكثر فاعلية فى مقاومة العفن الأسود فى البصل بالمقارنة بالتركيز ٠,٥% و٢٥%. ونفس النتائج المتحصل عليها فى المعمل أعطت نتائج مماثلة لها فى المخزن تحت ظروف العدوى الصناعية.