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Control of Sour Rot Disease of Lime Fruits Using Saprophytic Isolates of Yeast

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Sour rot disease incited by *Geotrichum candidum* causes serious damage to lime fruits during handling, transportation, marketing and exportation processes. Saprophytic yeast isolates were isolated from the surface tissues of various fruits and vegetables with high frequency in addition to two identified yeast isolates, *i.e. Saccharomyces cerevisiae* and *Candida tennis* were individually assayed against the growth and spore germination of *Geotrichum candidum in vitro*. The tested yeast isolates were able to inhibit pathogen linear growth and decrease the percentages of spore germination as well. Yeast isolates *C. tennis*, *S. cerevisiae*, *Candida* sp.T_1 and *Saccharomyces* sp.P_1 inhibit the fungal growth by 51.1, 44.1, 38.7 and 35.5 %, respectively. While yeast isolates *Candida* sp.O_{12} and *Cryptococcus* sp.L_2 gave a moderate effect on the fungal growth. The identified yeast isolate *S. cerevisiae* significantly inhibit the percentage of spore germination of *G. candidum* by 82.2% followed by the isolate *Saccharomyces* sp.P_1 and *Cryptococcus* sp.L_2 which reduce spore germination by 76.8 and 74.1 %, respectively.

Under storage conditions, non-disinfested lime fruits treated with different yeast isolates gave the best reduction in disease incidence and rotted tissue as compared with disinfested fruits. Yeast isolates *Cryptococcus* sp.L_1 and *Candida* sp.T_1 gave the highest reduction in disease incidence (3.3% infection) followed by fruits treated with *S. cerevisiae*, *Candida* sp.O_{12} and *C. tennis* (8.3%, 16.6% and 22.3%, respectively). The obtained results concerning the rotted tissues showed the same trend. The obtained results might lead to the conclusion that the natural existence of epiphytic yeasts on the fruits surface could provide an expecting protection against *G. candidum*. Therefore, the usage of such yeast isolates could be suggested as cheap, easily applied and safe bio-treatment for controlling sour rot disease of lime fruits and such postharvest diseases.

Keywords: Bioagents, *Geotrichum candidum*, lime, sour rot and yeast.

Lime fruits (*Citrus aurantium* L.) is considered as an important exportation fruits in Egypt. Postharvest decay of lime fruits caused by *Geotrichum candidum* (sour rot); *Penicillium digitatum* (Green mould) and *P. italicum* (blue mould) are the most important factors affecting harvested lime fruits during handling, transportation, exportation and storage (Morris, 1982; Brown and Eckert, 1988 and Morsy and Abdel-Kader, 1994). Several investigators found that number of fungicides successfully control postharvest decay pathogens of citrus fruits (Mahmoud, 1978;

Eckert and Brown, 1986). However, chemical control programs face many problems such as chemical fungicides imposes, selective pressure upon the pathogens population and having residual harmful effect to the human health and environment (Eckert and Ogawa, 1988). Moreover, some fungal isolates developed significant resistance to used fungicides (Spotts and Cervantes, 1986). Therefore, alternative approaches are needed for controlling postharvest diseases of citrus and other fruits. Biological control using either natural products or antagonistic microorganisms proved to be successful for controlling various plant pathogens in many countries (Papavizas and Lumsden, 1980). It still not costly and easy in application, however it can serve as the best control measure under restricted conditions. In addition, its application is safe, un-hazardous for human and avoids environmental pollution (Sivan and Chet, 1989).

The objective of the present study was designed to evaluate the efficacy of some saprophytic yeast isolates for controlling lime sour rot incidence during storage.

Materials and Methods

Isolation, purification and identification of saprophytic yeasts:

Potential antagonists were isolated from the surface of various fresh apparently healthy fruits collected from three principle fruits and vegetables markets at Cairo and Giza governorates. Mandarin, tomato, green pepper, orange and lime fruits were placed individually into a 500ml beaker containing 200 ml sterilized distilled water. Each beaker was placed on a rotary shaker at 100 r.p.m. for 10 minutes. A volume of 0.1 ml of suspended water was then spread on a nutrient yeast extract dextrose agar (NYDA) plates and incubated at 25°C for 24 hr. Developed several yeast colonies were selected and purified by streaking NYDA plates in order to obtain single cell colony. Single cell colonies were picked up and maintained onto another slants and kept in a refrigerator for further studies (Roberts, 1990 and Mehrotra *et al.*, 1996). The purified yeast isolates were kindly identified as genus at the Chemistry of Natural and Microbial Products Dept., National Research Centre, Egypt. Identification was carried out with the guide procedures described by Kreger-Van Rij (1984); Odds (1988) and Barnett *et al.* (2000).

Pathogen isolation and identification:

Five g of rotten lime tissue were suspended into 50 ml sterilized water. A loopful of the suspension was streaked onto PDA plates containing 1.6 µg/ml penicillin for avoiding bacterial contamination and incubated at 25°C for five days. Appeared colonies were picked up and re-inoculated onto PDA plates for obtaining pure fungal colonies, then identified according to Barnett and Hunter (1972).

Preparation of yeast cells and fungal spore suspension:

Different isolated yeast isolates in addition to *S. cerevisiae* and *Candida tennis* (kindly obtained from the Microbiology Dept. . NRC. Egypt) were grown at 25°C for 48 hr in shaked NYD broth cultures. Yeast cells were pelleted by centrifugation at 3000 r.p.m. for 20 min, re-suspended in sterile distilled water. and centrifuged again. The resulting pellets were dispersed in sterilized distilled water and the

concentration of the yeast cells was adjusted to 10^8 cfu/ml using a haemocytometer slide. Conidia of *G. candidum* were obtained from 7day-old PDA cultures and were also adjusted to a concentration of 10^6 conidia/ml.

Antagonistic effect of yeast isolates on G. candidum in vitro:

The interaction between yeast isolates and *G. candidum* was evaluated as fungal growth inhibition and growth clear zone as well. Yeast isolates which recorded a frequent occurrence as 25% and more were used in the present test. Dual culture technique after Ferreira *et al.* (1991) was followed. Yeast isolates (48-h-old) were streaked individually on one side of 9 cm Petri dishes containing PDA medium, while 5 mm disks of *G. candidum* were placed on the opposite side of yeast inoculated plates. Both tested microorganisms were placed 2 cm from the plate edges. A set of only fungal inoculated plates were used for check treatment. All plates were incubated at 25±2°C until full fungal growth in check plates. Percentage of fungal growth reduction was calculated in yeast treatments relative to the fungal growth in the check treatment after 7 days. Clear growth zone lay between tested yeast isolates and fungal growth was assessed visually at the end of the test and indicated as high, moderate and low degrees of antagonistic effect.

The antagonistic effect of yeast isolates on spore germination of *G. candidum* was evaluated in potato dextrose broth (PDB) following the method described by (Droby *et al.*, 1997). A volume of 0.1 ml of 10^8 cfu/ml active yeast cell suspensions was added to a 10 ml glass tube containing 5 ml PDB. At the same time, aliquots (0.1 ml) of spore suspensions (10^6 spores/ml) of *G. candidum* was added to each tube. The same volume of *G. candidum* spores suspension and 0.1 ml sterilized distilled water were added to another set of test tubes containing 5 ml PDB and reserved as control treatment. All test tubes were incubated for 20 h at 25±2°C in a rotary shaker (50 r.p.m), after that 100 fungal spores per replicate were observed microscopically to determine germination percentage. All yeast isolates were represented in three replicates, and experiments were performed three times.

Percentages of non-germinated spores referred to those germinated in the control treatment were estimated to calculate the percentages of germination inhibition due to the effect of yeast isolates.

Effect of different yeast isolates on sour rot disease incidence in vivo:

Selected lime fruits apparently healthy were divided into two groups: the first, was surface-disinfested by immersing into 0.5% sodium hypochlorite solution for 5 min and then washed with sterilized distilled water, then left for air drying at room temperature (18-20°C), while the second, were left without disinfestations. Three marked wounds were made up for all infested and non-infested fruits at its equator using sterilized head of an inoculated needle (Wilson and Chalutz, 1989). After wounding, 50µl of an aqueous yeast suspension (10^8 cfu/ml) were immediately pipetted into each wound site. The treated fruits with yeast suspension were left to air dry for 2 hr at ambient temperature (24-26°C), then artificially inoculated by spraying with *G. candidum* (10^6/ml) spore suspension and left again for air drying. Control treatments included infested and non-infested fruits inoculated only with *G. candidum* spore suspension at the same concentration. All lime fruits were

placed into carton boxes (20 fruits per each) in relevant to each particular treatment and stored in a cold room at 20±2°C for two weeks. Three boxes as replicates were used for each particular treatment as well as control.

Diseases assessment:

At the end of storage period average percentages of infected fruits with sour rot disease were recorded. Moreover, estimation of disease development was recorded as percentages of rotted tissue which was calculated by referring the weight of rotted part in relative to the whole infected fruit weight.

Statistical analysis:

Tukey test for multiple comparisons among means was employed by Neler *et al.* (1985).

Results and Discussion

Isolation, purification and identification of saprophytic yeasts:

The numbers of potential saprophyte yeast isolates taken from the surface of fresh healthy fruits of mandarin, tomato, green pepper, orange and lime are represented with their genus and codes referred to the host and source of collected samples. Results presented in Table (1) indicate that the saprophytic yeast isolates namely: *Saccharomyces* sp.M_2, *Saccharomyces* sp. P_1; *Candida* sp. O_{12} *Candida* sp. T_1; recorded the most dominant isolates which represented high frequency, being 85.3, 84.4, 68.5 and 66.6%, respectively. Meanwhile, isolates of *Cryptococcus* sp. L_2; L_3 and L_1 showed moderate frequency, being 41.2, 30.2 and 28.6%, respectively. The rest of yeast isolates represented a low frequency, which did not exceed 18.4%.

Table 1. Frequency occurrence of naturally saprophytic yeast isolates from different fruits surface

Tested fruit	Yeast isolate	Frequency (%)
Lime	*Cryptococcus* sp. L_1	28.6
	Cryptococcus sp. L_2	41.2
	Cryptococcus sp. L_3	30.2
Mandarin	*Saccharomyces* sp. M_1	14.7
	Saccharomyces sp. M_2	85.3
Pepper	*Saccharomyces* sp. P_1	84.4
	Candida sp P_2	10.0
	Candida sp P_3	5.6
Tomato	*Candida* sp. T_1	66.6
	Candida sp. T_2	18.4
	Candida sp. T_3	12.0
Orange	*Saccharomyces* sp. O_{11}	8.5
	Candida sp. O_{12}	68.5
	Candida sp. O_{13}	12.0
	Candida sp. O_{14}	11.0

The high frequently isolated yeasts were chosen for further studies under *in vitro* and *in vivo* conditions. Natural occurrence of different yeasts on the surface of agricultural products was previously recorded by Spotts *et al.* (1998); He Dan *et al.* (2003) and Sugar and Spotts (1999).

Pathogen isolation and identification:

The morphologically and microscopically examined characters for purified fungal cultures revealed that the isolated fungus from rotten lime tissues is *Geotrichum candidum*. The present finding is confirmed by Morsy and Abd El-Kader (1994) who recorded that the first occurrence of lime sour rot in Egypt caused by *Geotrichum candidum*.

Antagonistic effect of yeast isolates on G. candidum in vitro:

Presented data in Table (2) indicate that, all the nine yeast isolates tested were able to reduce mycelium growth of *G. candidum*. Isolates of *C. tennis*, *S. cerevisiae*, *Candida* sp.T_1 and *Saccharomyces* sp.P_1 showed a high inhibitory effect on the fungal growth, being 51.1, 44.1, 38.7 and 35.5%, respectively. A moderate inhibitory effect was observed in case of isolates *Cryptococcus* sp.L_1, *Candida* sp. O_{12} and *Cryptococcus* sp. L_2, being 31.5, 27.7 and 20.5%, respectively.

The visual clear zone of fungal growth indicated as (-) or (+);.(++) and (+++) depends on the width of zone of inhibition. Data also show that the ascending order of the width of clear zone of growth inhibition followed the same order of recorded efficacy of yeast isolates as fungal linear growth inhibition. This observation could be attributed to the produced metabolism by different yeast isolates and which diffused into growth medium to various extents relatively to each diverse isolate. In this regard, several investigators recorded the efficacy of yeast as an inhibitor agent against pathogenic fungi (Mehrotra *et al.*, 1996; He Dan *et al.*, 2003; Guozheng *et al.*, 2004 and Ozgur *et al.*, 2005).

Table 2. Effect of different yeast isolates on mycelium growth of *G. candidum* under *in vitro* conditions

Yeast isolate	Linear fungal** growth (mm)	Growth reduction (%)	Visual antagonistic effect (zone of inhibition)
Cryptococcus sp. L_1	61.6 b	31.5	++
Cryptococcus sp. L_2	71.5 a	20.5	+
Cryptococcus sp. L_3	79.2 a	12.0	+
Candida sp. O_{12}	65.0 b	27.7	++
Candida sp. T_1	55.1 c	38.7	+++
Saccharomyces sp. P_1	58.0 c	35.5	+++
Saccharomyces sp. M_2	85.1 a	5.4	-
Saccharomyces cerevisiae	50.3 d	44.1	+++
Candida tennis	44.0 d	51.1	+++
Control	90.0 a	-----	-----

Figures with the same letter are not significantly different (P= 0.05).

* (+++) High antagonistic action: (++) Moderate.(+) Low and (-) No antagonism.

** Incubation period was extended to 7 days at 25±2°C

On the other hand, it is interesting to note that although *Saccharomyces* sp.M_2 recorded high frequency as 85.3 (Table1), it caused low inhibition to the fungal growth not exceed 5.4% and no antagonistic effect as clear zone of growth inhibition was observed as well. Therefore, this isolate was neglected from the further work.

Presented data in Table (3) indicate that, all the yeast isolates tested varied in their inhibition on spore germination of the pathogen. *Saccharomyces cerevisiae* has significantly inhibited spore germination of *G. candidum* by 82.2% followed by *Saccharomyces* sp.P_1 and *Cryptococcus* sp.L_2, being 76.8 and 74.1%, respectively. It is interesting to note that *Cryptococcus* sp.L_3 showed the lowest effect either on growth or spore germination of *G. candidum* when compared with the other yeast isolates tested. Moreover, although *C. tennis* occupied the top order of inhibition 51.1% to mycelial growth (Table 2), it backward to have a moderate effect (57.2%) on spore germination. These results could be attributed to the varied bio products produced by yeast isolates which affect fungal growth or its spore germination and might not be the same. The obtained results in the present study are in agreement with those reported by Burkhead *et al.* (1995). Also, Brown (2000) found that yeast isolates *Candida* spp. were among microorganisms isolated from plant tissues, and were successfully found to be fungal antagonist. El-Gamal and Abd-Alla (2002) reported that yeast isolates of *S. cerevisiae* and *C. tennis* were highly inhibitive to fungal growth and sclerotia formation of *Sclerotinia sclerotiorum* the causal agent of white rot disease of bean green pods.

Table 3. Effect of different yeast isolates on the percentages of spore germination of *G. candidum*, 20 h after incubation at 25±2°C

Yeast isolate	Spore germination (%)	Reduction (%)
Cryptococcus sp. L_1	35.7 e	63.3
Cryptococcus sp. L_2	25.2 f	74.1
Cryptococcus sp. L_3	75.6 b	22.3
Candida sp. O_{12}	55.3 c	43.2
Candida sp. T_1	33.1 e	66.0
Saccharomyces sp. P_1	22.6 f	76.8
Saccharomyces cerevisiae	17.3 g	82.2
Candida tennis	41.6 d	57.2
Control	97.3 a	-----

Figures with the same letter are not significantly different (P= 0.05).

Moreover, antagonists which have been found in micro-ecosystems on the surfaces of fruits and vegetables were developed as biological control agents for controlling postharvest diseases of fruits and vegetables (Janisiewicz, 1991; Wilson *et al.*, 1993 and Cheah *et al.*, 1996). On the other hand, several antagonistic yeasts showing high protection to various fruits against postharvest pathogens have been also reported (Janisiewicz, 1988; Roberts, 1990; Pusey *et al*, 1993; Guozheng *et al.*, 2004; Ozgur *et al.*, 2005 and Hongyin and Xiaodong, 2007).

Effect of different yeast isolates on sour rot disease incidence in vivo:

Presented data in Table (4) indicate that the non-disinfested lime fruits treated with different yeast isolates showed better reduction in disease incidence when compared with the disinfested fruits. *Cryptococcus* sp.L_1 and *Candida* sp.T_1 showed the highest suppressive effect on disease incidence estimated as 3.3%. Meanwhile, *S. cerevisiae*, *Candida* sp. O_{12} and *Cryptococcus* sp. L_2 were able to reduce sour rot disease by 8.3, 16.6 and 18.3%, respectively. On the other hand, similar trend in a lower extent of sour rot incidence was observed with disinfested lime fruits inoculated with yeast isolates. *Cryptococcus* sp.L_2, *Cryptococcus* sp.L_1 and *S. cerevisiae* recorded significant reduction in percentages of disease incidence, being 11.7, 13.3 and 18.3%, respectively. Many workers have also successfully used different yeast species for controlling postharvest diseases during storage (Chang-Goyal and spots, 1996 and Benbow and Suger, 1999). They reported that preharvest application with natural saprophyte yeasts were able to control postharvest pear diseases.

Table 4. Effect of different yeast isolates on sour rot disease incidence after two weeks of storage at 20±2°C

Yeast isolate	Disease incidence (%)	
	Non-disinfected fruits	Disinfected fruits
Cryptococcus sp. L_1	3.3 f	13.3 f
Cryptococcus sp. L_2	18.3 d	28.3 e
Cryptococcus sp. L_3	25.0 c	31.7 d
Candida sp. O_{12}	16.6 d	26.7 d
Candida sp. T_1	3.3 f	11.7 g
Saccharomyces sp. P_1	31.7 b	41.7 b
Saccharomyces cerevisiae	8.3 e	18.3 f
Candida tennis	23.3 c	33.3 c
Control	86.6 a	91.6 a

Figures with the same letter are not significantly different (P= 0.05).

The efficacy of the tested yeast isolates on the development of disease infection represented as rotted tissue of lime fruits followed the same observed order of their effect on disease incidence. Data shown in Table (5) indicate that yeast isolates *Cryptococcus* sp. L_1, *Cryptococcus* sp. L_2, *Candida* sp. T_1 and *Saccharomyces* sp. P_1 showed significant reduction in the percentages of non-disinfested rotted fruit tissues calculated as 14.4, 18.3, 16.7 and 18.6%, respectively. Meanwhile, *Cryptococcus* sp. L_1 and *Saccharomyces* sp. P_1 occupied the first order in minimizing rot disease development of disinfested lime fruits. In this regard, Vero *et al.* (2002) reported that two yeast antagonists, *Cryptococcus laurentii* (strain 317) and *Candida ciferrii* (strain 283) isolated from the surface of healthy apples, suppressed blue mould of apple caused by *Penicillium expansum*. Both antagonists reduced the incidence of blue mould by 80% at 25°C. Furthermore, Jones and Prusky (2002) investigated the possibility of expressing a DNA sequence in *Saccharomyces cerevisiae* to allow the

Table 5. Effect of different yeast isolates on the percentages of rotted fruit tissues after two weeks storage

Yeast isolate	Fruit rotted tissues (%)	
	Non-disinfected fruits	Disinfected fruits
Cryptococcus sp. L.1000	14.4 f	16.7 f
Cryptococcus sp. L.2000	18.3 f	58.3 b
Cryptococcus sp. L.3000	41.6 b	49.8 c
Candida sp. O.1002	20.3 e	25.1 d
Candida sp. T.1000	16.7 f	41.7 c
Saccharomyces sp. P.001	18.6 f	18.3 f
Saccharomyces cerevisiae	25.5 d	33.0 e
Candida tennis	33.6 c	31.2 e
Control	91.6 a	100.0 a

Figures with the same letter are not significantly different (P= 0.05).

production of an antifungal peptide. Yeast was found to inhibit the growth of germinated *Colletotrichum coccodes* spores and inhibited decay development caused by the pathogen in tomato fruits. The lack of activity toward nontarget organisms by the peptide and the use of *S. cerevisiae* as a delivery system suggest that this method could provide a safe alternative for postharvest disease control.

It is clear from presented data in Tables (4 & 5) that the non-disinfected lime fruits treated with different antagonistic yeast isolates resulted in a higher reduction in disease incidence and also reduced disease development as percentage of rotted tissues compared with disinfested lime fruits. These results might be attributed to the increasing the number of microflora persistence on the fruit's surface and/or synergistic effect between them which may probably occur. Similar conclusions regarding these results are reported.

The success of antagonists (non-disinfested fruits) than individual one (disinfested fruits) referred to different mode of action of mixture numerous modes of actions have been postulated and demonstrated for antagonist effective in controlling postharvest diseases, including nutrient competition, antibiotic production. enzymes that act on fungal cell wall components such as chitinases and ß-1,3 glucanase and induced host resistance (Droby and Chalutz, 1994 and Burkhead *et al.*, 1995). Also, Janisiewicz (1996) reported that mixtures of microbial antagonists have been used successfully to increase the level of biological control above that achieved with individual isolates of the mixture. The mechanisms by which yeast exert their biocontrol activity have not been fully elucidated. Biological activity of antagonistic yeasts may involve nutrient competition (Droby *et al.*, 1989), site exclusion (Droby and Chalutz, 1993), direct parasitism, and perhaps induced resistance (Wisniewski *et al.*, 1988; Wisniewski *et al.*, 1991 and Wilson and El-Ghaouth, 1993). Furthermore, several antagonistic yeasts have been isolated and shown to protect a variety of fruit against postharvest pathogens (Janisiewicz, 1988; Roberts, 1990; Pusey *et al.*, 1993; Wilson and Wisniewski, 1994; Chang-Goyal and

Spotts, 1996 and Ortu, *et al.*, 2003). Although the use of antagonistic yeasts to control postharvest diseases have been demonstrated with several commodities, their commercialization will be depend on whether they are capable of effectively controlling decay of fruit from different locations with variable inoculum loads, types of infection, and levels of mechanical injury. In addition, microbial biocontrol agents will be expected to display curative activity comparable to that observed with synthetic fungicides. Currently available antagonistic microorganisms do not appear to be able to control previously established infections and are most effective when applied prior to infection by the pathogen (Wilson and El-Ghaouth, 1993 and Wilson and Wisniewski, 1994). The obtained results of the present study lead to suggestion that epiphytic yeast naturally occurring on the surface of fruits and vegetables tissue could be applied as safe bio-treatment for controlling sour rot disease of lime fruits.

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مكافحة مرض العفن المر فى ثمار الليمون باستخدام بعض عزلات من الخمائر المترممة

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تعتبر ثمار الليمون من أحد أهم صادرات الفاكهة فى مصر ، ويتسبب مرض العفن المر والذى يسببه الفطر "*Geotrichum candidum*" فى خسائر ملموسة للثمار المصابة فى جميع مراحل ما بعد الحصاد والتى تشمل التداول والنقل والتسويق وكذلك التخزين والتصدير.

تم عزل وتعريف وتقدير نسبة تكرار تواجد العديد من عزلات الخمائر المترممة بصورة طبيعية على أسطح العديد من ثمار الخضر والفاكهه.

تم اجراء الأختبارات المعملية وكذلك اختبار التخزين واستخدم فى ذلك عزلات الخمائر التى أظهرت نسبة تواجد عالية وأضيف اليهم عزلتان سبق استخدامهما بنجاح كمضادات للفطريات فى دراسات سابقة وهما "*Saccharomyces cerevisiae*" و "*Candida tennis*".

وجد أن جميع عزلات الخمائر المختبرة لها القدرة على تثبيط النمو الميسليومى وكذلك خفض النسبة المئوية لانبات جراثيم الفطر "*G. candidum*" تحت ظروف المعمل. وأثبتت النتائج أن عزلات الخمائر "*C. tennis*" ، "*S. cerevisiae*" ، "*Candida* sp.T_1" و "*Saccharomyces* sp. P_1" كان لهم القدرة على تثبيط النمو الميسليومى للفطر ، بينما كان لكل من العزلتان "*Candida* sp.O_{12}" ، "*Cryptococcus* sp. L_2" تأثير متوسط على نمو الفطر. كما وجد أن العزلة "*S. cerevisiae*" قد أعطت تأثيرا معنويا فى تثبيط انبات جراثيم الفطر قدر بحوالى ٨٢,٣% وتلى ذلك العزلات "*Saccharomyces* sp. P_1" ، "*Cryptococcus* sp. L_2" بنسبة ٧٦,٨ ، ٧٤,١% على التوالى.

تحت ظروف العدوى الصناعية بالفطر الممرض خلال التخزين وجد أن ثمار الليمون غير المعقمة سطحيا والمعاملة بعزلات الخمائر المختبرة أظهرت قدرة افضل على خفض نسبة حدوث المرض وأيضا نسبة الجزء المتعفن من نسيج الثمار المصابة وذلك مقارنة بالثمار المعقمة سطحيا.

أشارت النتائج أن عزلة الخميرة المعزولة من ثمار الليمون "*Cryptococcus* sp. L_1" وكذلك الخميرة المعزولة من ثمار الطماطم "*Candida* sp.T_1" أظهرتا قدرة عالية على خفض نسبة حدوث المرض تحت ظروف التخزين حيث كانت نسبة اصابة الثمار بنسبة ٣,٣% ، وتلى ذلك "*S. cerevisiae*" والخميرة المعزولة من البرتقال "*Candida* sp.O_{12}" ، ثم الخميرة "*C. tennis*" بواقع ٨,٣ ، ١٦,٦ ، ٢٢,٣% على التوالى ، وقد أظهرت هذه العزلات تاثيرها على النسبة المئوية للنسيج المتحلل للثمار المصابة بصورة مشابهه.

تشير نتائج الدراسة الى أن التواجد الطبيعى للخمائر المترممة على أسطح ثمار الخضر والفاكهه تمثل دورا هاما فى حماية هذه الثمار من الاصابة بمسببات الأعفان المختلفة ، كما يمكن استغلال هذه الخمائر فى طرق المكافحة البيولوجية حيث أنها تتميز بكونها سهلة التطبيق ورخيصة الثمن كما أنها تعتبر طريقة أمنة وفعالة ضد الأمراض التى تصيب الثمار بعد الحصاد وأثناء التخزين.