Conference Proceeding of
1st International Conference "Food and Agriculture: New approaches"-2013, Cairo, Egypt.
Original Research Article

Evaluation the Efficacy of Baker Yeast (*Saccaromyces cerevisia*e) and Chitosan to Controlling *Penicillium digitatum* Sacc. that Cause Green Mold Decay of Kumquat Fruits.

ABSTRACT

The commercial backing yeast of *Saccharomyces cereivisae [Meyen ex E.C.Hansen]* and/or chitosan was evaluated for their in vitro activity against the fungal growth of *P.digitatium* the causal agents of kumquats fruit decay. Baker yeast *S.cerevisiae* at 2% resulting a highly and significantly reduction of *P.digitatum* linear growth by 32.4% if compared with control treatment. All chitosan concentrations were tested result a significant reduction of *P. digitatium* linear growth, chitosan at 2% resulting highly reduction of pathogen growth by 78.3% followed by 71.5% at 1% concentration. Chitosan at 2% was mixed with backer yeast (B.Y) at 2% resulting significant and highly reduction of *P.digitatium* linear growth by 82.5% followed by chitosan 1% mixed by baker yeast (B.Y) 2% by 77.5% reduction of pathogen linear growth if compared with control treatment. Under application trials, kumquat fruits were coated with chitosan ½% decreased the green mold incidence by 83.6% while, fruits were coated with chitosan at 2% and 1% resulting a highly reduction of green mold disease incidence by 80.3% and 78.4%, respectively. Kumquat fruits were coated with baker yeast (*S.cerevisiae*) at 2% concentration reducing the green mold disease incidence by 79.5% and the same concentration was reducing the percentage of disease severity by 72.3% if compared with un- coated fruits. In combination treatments, kumquat fruits were coated with chitosan at 2% combined with baker yeast (B.Y) at 2% resulting a highly and , significant reduction of green mold incidence and disease severity by 75.1% and 90.0% , respectively. The combination of baker yeast (B.Y) at 2% and chitosan at 2% could be a promising a safe and cheep method for the control of green mold disease of kumquat fruits.

Keywords: *Baker yeast – Chitosan – Kumquat fruits – Green mold disease.*

1. INTRODUCTION

The kumquat (genus *Fortunella)* is subject to losses from postharvest decay during shipment. Due to its popularity with some ethnic groups it commands a high price on the market and is usually shipped in small packages. Kumquat production in Egypt is a small volume operation, amounting to only about 10,000 bushels before the 1984 & 1985 freezes greatly reduced the amount of fruit available. A true citrus, the kumquat fruit is small in size, typically 3/4 to 11/4 inches diameter [1]. Depending upon variety, the fruit will be round to elongated in shape [2,1,3]. The fruit are used for decoration in gift packs and for use in various jams and preserves (Templeton.HFS 845 and Templeton.HFS846). They are also eaten fresh, peel included [2,4,1]. The problem of stimulation of endogenous defense mechanisms in kumquat has a special economic importance because export of this exotic fruit is limited by its high susceptibility to decay mainly that of *Penicillium digitatum* Sacc. (Fig.1.),[5]. The application of fungicides for decay control in kumquat, as proposed by Hall, seems undesirable because the peel of this fruit is consumed along with the pulp. [6]

Fig.1. Kumquats fruits infected with *Penecillium digitatium* the causal agent of green mold disease.

As is known, synthetic fungicide treatment has long been the main method for controlling postharvest diseases [7]. However, there is increasing international concern over the indiscriminate use of synthetic fungicides on crops because of the possible harmful effects on human health[8,9] .and the emergence of pathogen resistance to fungicides[10,11] .Therefore, new alternatives for controlling postharvest diseases which have good efficacy, low residues, and little or no toxicity to non-target organisms are in urgent demand [12]. The use of microbial antagonists to control postharvest diseases of fruits and vegetables has shown during the last 30 years to be one of the most promising alternatives to fungicides [9, 13,14]. Some bacteria, actinomycetes and yeasts showed effectiveness against postharvest diseases of fruit and vegetables [15, 16, 17] . Among these microbial antagonists, yeasts that naturally occur on fruits and vegetables have attracted the attention of several researchers as potential antagonists of postharvest diseases due to the fast colonization on fruit surfaces [9,18]. Chitosan is a linear polysaccharide consisting of β-(1→4)-linked 2-amino-2- deoxy-D-glucose residues, originating from deacetylated derivative of chitin, which is the second most abundant polysaccharide in nature after cellulose. It was non-toxic, biodegradable, biofunctional, and biocompatible. Chitosan has strong antimicrobial and antifungal activities that could effectively control fruit decay [18]. It could easily form coating on fruit and vegetable, and the respiration rate of fruit and vegetable was reduced by adjusting the permeability of carbon dioxide and oxygen.Combining antagonistic yeasts with chitosan will make it possible to exploit the antifungal and eliciting properties of chitosan and the biological activity of the antagonists [21]. The purpose of the present research was to test the activity of commercial backing yeast (*S.cerevisiae*) applied alone and or in combination with chitosan on *Penicillium digitatum* growth under vitro conditions and green mold incidence and disease severity in kumquat fruits.

2. MATERIALS AND METHODS

Fruits

Mature fruit of 'Nagami' kumquat was obtained directly from orchards (Kalubia) or packing houses from (Cairo , Egypt) , before the application of any postharvest treatment. Fruit samples of uniform size and appearance from one orchard were subjected randomly to various treatments.

Pathogen

Penicillium digitatum (Pers.:Fr.) Sacc. was isolated from naturally infected kumquat fruits after storage of several weeks. This isolate was the most aggressive one in our collection and produced the largest lesions on inoculated fruit. This fungus was purified and maintained on potato–dextrose agar (PDA) and stored at 4°C, with periodic transfers through kumquat fruit to maintain its aggressiveness. It was identified as *Penicillium digitatium* (Pers.:Fr.) Sacc.)**.** Conidia suspension was prepared from 7-day-old cultures on potato dextrose agar (PDA) plate and adjusted to 10^6 conidia ml^{-1}. The number of conidia was determined with a haemocytometer slid.

Effect of different baker yeast (Saccharomyces *sereivisae*) concentrations on linear growth of *Penicillium digitatium* under vitro conditions

The inhibitory effects of Baker yeast (B.Y) (*S. sereivisae*) suspension on mycelia growth of *P. digitatium* was tested *in vitro* using the agar dilution technique. An aqueous solution of B.Y(commercial formulation) was prepared in sterile distilled water and was added aseptically to autoclaved and cooled PDA medium at 50℃ to achieve final concentrations of 1/4 ,1/2 , 1.0 and 2.0% . The amended medium was dispensed (15ml/plate) aseptically into 9-cm-diameter Petri plates. Un-amended plates served as control. Hyphal plugs (5 mm diameter) were cut from the periphery of actively growing colonies (7 to 10 day-old) and transferred aseptically, mycelium down, to three replicate Petri plates containing PDA medium supplemented with chemicals. The plates were sealed with parafilm and incubated at 20-22℃. Fungus linear growth was measured daily until the growth in the control reached the edge of the Petri plates.[38] The antifungal activity was expressed in terms of percentage of reduction of mycelium growth calculated according to the following formula:

Reduction (%) = [(Diameter in control– Diameter in treatment) / Diameter in control] × 100.

Effect of different chitosan concentrations on linear growth of *Penicillium digitatium* under vitro conditions

Chitosan was purchased from Sigma-Aldrich.

Different concentrations of chitosan solution prepared by the method described by El-Gaouth[22,24]. Chitosan solution was added to conical flasks containing melted PDA medium to obtain final concentrations of 1/4 ,1/2, 1.0 and 2.0% and mixed gently and then dispensed in sterilized Petri plates (10 cm diameter).Plates were individually inoculated at the center with equal disks (10-mmdiameter) of the same physiological age of each *P.digitatiu*m**,** Three plates were used per treatment and sealed with parafilm and then incubated at 22-25℃. Fungus line ar growth was measured daily until the growth in the control reached the edge of the Petri plates. The antifungal activity was expressed in terms of percentage of reduction of mycelium growth calculated according to the previous formula.

Effect of different combinations of chitosan concentrations and backer yeast (B.Y) 2% on linear growth of *P.digitatium* under vitro conditions.

Different concentrations of chitosan solution *i.e*. 1/4 ,1/2, 1.0 and 2.0% were prepared and then add to B.Y 2% individually , to obtained four combinations as follow :

1- Chitosan 1/4 % + B.Y 2%.

2- Chitosan 1/2 % + B.Y 2%.
3- Chitosan 1 % + B.Y 2%.
4- Chitosan 2 % + B.Y 2%.

All these treatments were dispensed in sterilized Petri plates (10 cm diameter).Plates were individually inoculated at the center with equal disks (10-mmdiameter) of the same physiological age of each *P.digitatiu*m**,** The plates were sealed with par film and then incubated at 22-25℃. Fungus linear growth was measured daily until the growth in the control reached the edge of the Petri plates. The antifungal activity was expressed in terms of percentage of reduction of mycelium growth calculated according to the previous formula.

Effect of kumquat fruits coating with different concentrations of chitosan on green mold incidence and disease severity after 30 days .

Different concentrations of chitosan were tested to study their effect on green mold incidence of kumquat fruits. Fresh kumquat fruits apparently free from physical damage and diseases were artificially wounded using sterilized scalpel. Inoculation of wounded fruits about 3 wounds 3mm deep and 3mm wide was carried out by spraying fruits with spore suspension (10^6 spores/ml) of *P.digitatiu*m then air dried at room temperature, 23-25℃. Inoculated fruits were dipped in chitosan solutions at concentrations of 1/4 ,1/2, 1.0 and 2.0% for 2 min, then air dried. All treated or un-treated (control) kumquat fruits were placed into sterilized foam trays at the rate of 20 fruits /tray. Each particular concentration as well as control treatment was represented by one carton box. All foam trays were stored at 20±2C° for 30 days. Percentage of infected fruits as (disease incidence) and disease severity as (rotted parts of fruits) were recorded.[39]

Effect of fruits coating with different concentrations of backer yeast (B.Y) on green mold incidence and disease severity after 30 days .

Four backer yeast B.Y (*S. sereivisae*) concentrations were tested to study their effect on green mold incidence of kumquat fruits. Fresh kumquat fruits apparently free from physical damage and diseases were artificially wounded using sterilized scalpel. Inoculation of wounded fruits was carried out by spraying fruits with spore suspension (10^6 spores/ml) of *P.digitatiu*m then air dried at room temperature, 23-25C°. Inoculated fruits were dipped in baker yea st B.Y solutions containing 0.01% Tween 80 at concentrations of 1/4 ,1/2, 1.0 and 2.0% for 2 min, then air dried. All treated or un-treated (control) kumquat fruits were placed into sterilized foam trays at the rate of 20 fruits /tray. Each particular concentration as well as control treatment was represented by one tray. All foam trays were stored at 20±2C° for 30 days. Percentage of infected fruits a s (disease incidence) and disease severity as (rotted parts of fruits) were recorded.

Effect of fruits coating with different concentrations of chitosan combination with Backer yeast (B.Y) 2% on green mold incidence and disease severity after 30 days .

Different combinations of chitosan concentrations and backer yeast (B.Y) 2% were prepared as follow : Chitosan 1/4 % + B.Y 2%,Chitosan 1/2 % + B.Y 2% ,Chitosan 1 % + B.Y 2% and Chitosan 2 % + B.Y 2% were tested to study their effect on green mold incidence of kumquat fruits. Fresh kumquat fruits apparently free from physical damage and diseases were artificially wounded using sterilized scalpel. Inoculation of wounded fruits was carried out by spraying fruits with spore suspension (10^6 spores/ml) of *P.digitatiu*m then air dried at room temperature, 23-25℃. Inoc ulated fruits were dipped in different combinations of chitosan concentrations and backer yeast (B.Y) 2% chitosan solutions for 2 min, and then air dried. All treated or un-treated (control) kumquat fruits were placed into sterilized foam trays at the rate of 20 fruits /tray. Each particular concentration as well as control treatment was represented by one carton box. All foam trays were stored at 20±2°C for 30 days. Percentage of infected fruits as (disease incidence) and disease severity as (rotted parts of fruits) were recorded.[39].

Statistical Analysis

Tukey test for multiple comparisons among means was employed [23].

3. RESULTS AND DISCUSSION

3.1.Effect of different concentrations of chitosan on linear growth of *Penicillium digitatium* under vitro conditions

Results presented in Table .1. showed that , all chitosan concentrations were used resulting a significant reduction of *P. digitatium* linear growth, but chitosan at 2% resulting highly reduction of pathogen growth by 78.3% followed by 71.5% at 1% concentration, while ,other tested concentrations showed a moderate effect to reducing pathogen growth. Abd-Alla and Wafaa [24], studied the effect of various concentrations of chitosan solution on the mycelium growth and spore germination of *Colletotrichum gloeosporioides* (Penz.) the causal agent of anthracnose disease of mango fruits was studied under vitro conditions. Chitosan solution at 0.6mg/l obtained significantly reduction of *C. gloeosporioides* growth and inhibited spore germination, while, chitosan solution at 0.8mg/l resulted a complete reduction and inhibition of fungal mycelium growth and spore germination.[24]. In vitro evaluations, it was demonstrated that the combination of chitosan at 10 mg ml_1 and thyme essential oil at 300 mgml_1 had a fungicidal effect on *Rhizopus stolonifer* (Ehrenb.) Vuill**.**, inhibiting mycelia growth, spore germination and sporulation of this fungus.[25].

Table .1. Effect of different concentrations of chitosan on linear growth of *P.digitatium* under vitro conditions.

Treatment	Linear growth (mm)	% Reduction
Chitosan ¼ %	50.2 b	44.2
Chitosan ½ %	33.3 c	63.0
Chitosan 1%	25.6 d	71.5
Chitosan 2%	19.5 e	78.3
Control	90.0 a	00.0

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

3.2.Effect of different Baker yeast concentrations on linear growth of *Penicillium digitatium* under vitro conditions

Results presented in Table .2. Showed that, baker yeast (B.Y) *S. cerevisieae* at 2% resulting a highly and significantly reduction of *P.digitatium* linear growth by 32.4% if compared with other (B.Y) tested concentrations and with control treatment. Other backer yeast concentrations were used showed a slightly effect against the pathogen linear growth. Petersson and Schnurer, [26], reported that, the yeast *Pichia anomala* inhibits the growth of *Penicillium roqueforti* and *Aspergillus candidus* on agar. In this investigation, antagonistic activity on agar against 17 mold species was determined. The abilities of *Pichia anomala*, *Pichia guilliermondii*, and *Saccharomyces cerevisiae* to inhibit the growth of the mold *Penicillium roqueforti* in nonsterile high-moisture wheat were compared by adding 103 *Penicillium roqueforti* spores and different amounts of yeast cells per gram of wheat. [27,28],reported that , yeast isolates *Saccharomyces cerevisea* and *Candida tennis* were a highly significantly inhibitive to fungal growth and sclerotia formation for *Sclerotinia sclerotiorum* the causal agent of white rot disease of bean green pods. [28], tested the yeast, *Saccharomyces cerevisiae*, *Candida tenuis* and the commercial backing yeast of *Saccharomyces cerevisiae* mixture (CBY) and/or peppermint, melon and rose essential oils were evaluated for their in vitro activity against the fungal growth of *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternate* the causal agents of tomato fruit decay, and they found that , *S.*

cerevisiae mixture (CBY) proved itself to have the highest inhibitory effect on the growth of the pathogenic tested fungi followed by the two other yeast isolates *S. cerevisiae* and *C. tenuis*.

Table .2. Effect of different concentrations of baker yeast (B.Y) solution *S.serveiseae* on linear growth of *P.digitatium* under vitro conditions.

Treatment	Linear growth (mm)	% Reduction
B.Y ¼ %	90.0 a	00.0
B.Y ½ %	88.1 a	2.1
B.Y 1 %	80.6 b	10.4
B.Y 2 %	60.8 c	32.4
Control	90.0 a	00.0

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

3.3.Effect of different combinations of chitosan concentrations and backer yeast (B.Y) 2% on linear growth of *P.digitatium* under vitro conditions.

Results presented in Table.3.3 Showed that, chitosan at 2% was mixed with backer yeast (B.Y) at 2% resulting significant and highly reduction of *P.digitatium* linear growth by 82.5% followed by chitosan 1% mixed by baker yeast (B.Y) 2% by 77.5% reduction of pathogen linear growth if compared with control treatment. Other tested combinations result a moderate effect for the pathogen linear growth reduction. On postharvest control chitosan application was applied in combination with biocontrol agents, such as *Candida satoiana*or and *Cryptococcus laurentii*, microorganisms that show an antagonistic activity toward postharvest pathogens [21,29,30,31,32,33]. Chitosans and *Pichia guillermondii* were evaluated on the growth of *Penicillium digitatum*. a low and high degree of acetylation (DA)chitosan was selected for use against moulds combined with yeasts. Biopolymer and yeasts presented an additive effect, since chitosan were effective to delay spore germination, whereas yeast decreased apical fungal growth.[34].

Table. 3.3 Effect of different combinations of chitosan concentrations and backer yeast (B.Y) 2% on linear growth of *P.digitatium* under vitro conditions.

Treatment	Linear growth (mm)	% Reduction
Chitosan ¼% + B.Y.2%	48.9 b	45.6
Chitosan ½ % + B.Y.2%	40.3 b	55.2
Chitosan 1 % + B.Y.2%	20.2 c	77.5
Chitosan2 % + B.Y.2%	15.7 d	82.5
Control	90.0 a	00.0

The same letter are not significantly different (p=0.05)

3.4.Effect of kumquat fruits coating with different concentrations of chitosan on green mold incidence and disease severity after 30 days .

Results presented in Table3.4. Showed that, kumquat fruits were coated with chitosan ½% decreased the green mold incidence by 83.6% while, fruits were coated with chitosan at 2% and 1% resulting a highly reduction of green mold disease incidence by 80.3% and 78.4%, respectively. On the other hand, the same trend was shown when determined the green mold severity, kumquat fruits were coated with chitosan at 2% and chitosan at ¼ % reducing the disease severity by 92.0% and 90.3%, respectively. Several mechanisms were proposed for the antimicrobial activity by chitosan. Chitosan interacts with the membrane of the cell to alter cell permeability. The other mechanism involves the binding of chitosan with DNA to inhibit RNA synthesis [35]. Kevin *et al.*, 2009,[36],reported that, coating fruits with chitosan solutions can reduce respiration rate and ethylene production and internal O2 increased internal CO2; concentrations and therefore the fruit are firmer with less decayed. [24], reported that, coating mango fruits with 0.2 and 0.4% (w/v) chitosan solution obtained a highly protective effect against anthracnose

disease incidence of mango fruits, by 98.1% and 95.4% after 30 days of storage, respectively. At the same treatments were reducing the percentage of fruit rotted tissues by 89.3 and 95.0%, respectively.[24,24].

Table 3.4. Effect of fruits coating with different concentrations of chitosan on green mold incidence and disease severity after 30 days .

Treatment	% of green mold incidence	% Disease severity
Chitosan ¼ %	58.8 b	10.3
Chitosan ½ %	14.2 c	8.5
Chitosan 1%	18.7 c	10.5
Chitosan 2%	16.6 c	7.0
Control	86.5 a	88.5

The same letter are not significantly different (p=0.05)

3.5.Effect of fruits coating with different concentrations of backer yeast (B.Y) on green mold incidence and disease severity after 30 days of storage at 5C.

Results presented in Table3.5. Showed that, kumquat fruits were coated with baker yeast (*S.cerevisiae*) at 2% concentration reducing the green mold disease incidence by 79.5% and the same concentration was reducing the percentage of disease severity by 72.3% if compared with un- coated fruits and others (B.Y) concentrations. While, fruits coated with (B.Y) 1% gave a highly reduction of green mold incidence and disease severity by 35.7% and 62.3%, respectively. These results were agreement with [37], they found that, 'Choke Anan' and 'Nam Doc Mai' mangoes were wounded and treated with one of two yeast antagonists (*Candida* sp. isolate ns 5 and ns 9) for 12 h before soaking with chitosan (0.25% and 0.5%) and followed by inoculation with the anthracnose pathogen *Colletotrichum gloeosporioides*. Treated fruits were stored at 25℃ for 7 days. The results reveale d that anthracnose lesions decreased on fruit in whose wounds antagonistic yeasts had been allowed to colonize before inoculation with the pathogen.

Table 3.5. Effect of fruits coating with different concentrations of backer yeast (B.Y) on green mold incidence and disease severity after 30 days .

Treatment	% of green mold incidence	% Disease severity
B.Y ¼ %	85.3 a	51.6
B.Y ½ %	81.0 a	55.0
B.Y 1 %	55.6 b	33.3
B.Y 2 %	17.7 c	24.5
Control	86.5 a	88.5

The same letter are not significantly different (p=0.05)

3.6.Effect of fruits coating with different concentrations of chitosan combination with Backer yeast (B.Y) 2% on green mold incidence and disease severity after 30 days.

Results presented in Table3.6. Showed that, kumquat fruits were coated with chitosan at 2% combined with baker yeast (B.Y) at 2% resulting a highly and, significant reduction of green mold incidence and disease severity by 75.1% and 90.0%, respectively, followed by fruits were coated with chitosan at 1% combined with (B.Y) at 2% resulting a moderate effect to reducing the disease incidence by 58.7% and gave a highly effect to reducing the disease severity by 88.7% if compared with other treatments and or un-coated fruits. Combining antagonistic yeasts with chitosan will make it possible to exploit the antifungal and eliciting properties of chitosan and the biological activity of the antagonists [21,37]. reported that, The combination of antagonistic yeast with chitosan was more effective on the reduction of anthracnose

incidence than yeast or chitosan alone. *Candida* sp. ns 9 in combination with 0.5% chitosan was the most effective in controlling anthracnose fruit rot in 'Choke Anan' and 'Nam Doc Mai' mangoes in which the average percentages of disease incidences were 6.7% and 13.3%, respectively, compared with 93.3% and 100% infection in the control fruits. Fresh lime fruits were artificially wounded using sterilized scalpel and inoculated with spore suspension (106 spores/ml) of *G. candidum* then treated with citral and /or chitosan. Results indicate that the most effective treatments are combined treatments between citral at 4.0 or 5.0 ml / l and chitosan at 6.0 or 8.0 g / l which reduced the disease incidence and rotted part tissue more than 89.5 and 93.5% respectively.[38].

Table 3.6. Effect of fruits coating with different concentrations of chitosan combination with Backer yeast (B.Y) 2% on green mold incidence and disease severity after 30 days .

Treatment	% of green mold incidence	% Disease severity
Chitosan ¼% + B.Y.2%	53.3 b	35.8
Chitosan ½ % + B.Y.2%	51.5 b	22.1
Chitosan 1 % + B.Y.2%	35.8 c	10.0
Chitosan2 % + B.Y.2%	21.5 d	8.8
Control	86.5 a	88.5

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

4. CONCLUSION

Combining antagonistic yeasts with chitosan can be expected to provide better control of green mold of kumquat fruit than the use of biocontrol agent alone. Future research will explore the possibility of bio-control enhancement using mixtures of antagonists or some additives and try to formulate them into commercial products, and it could be suggested that combined treatments between chitosan and yeast might be used commercially as easily , safely , and applicable method for controlling post harvest diseases.[37].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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