

## The Effect of Three Coating Materials on the Candidal Growth, on the Surface and Color of A heat-Cure Acrylic Resin Denture Base

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### ABSTRACT

**Aims:** To evaluate the effect of three coating materials: Acrylic resin coating "monopoly", olive oil and grape seeds oil on the *Candida albicans* growth, and on color of a heat – cured acrylic resin denture base. **Materials and methods:** Three coating materials on the acrylic denture base surface was determined via *Sensitivity test* in which twenty discs of acrylic resin 6mm diameter and 2mm thickness were prepared and divided into four groups: control (uncoated), monopoly, olive, and grape oil coated groups. These discs were added to Sabouraud Dextrose agar surface and incubated for 24 hours at 37 °C and the diameter of growth inhibition zones were measured. Then the *Turbidity test* was taken place, in which 60 acrylic specimens (1cm x 1cm x 2mm) were prepared and immersed in 60 test tubes that were divided into four groups, each group (15 specimens) was subdivided into three groups of incubation: 7, 20, and 30 days incubation period, each test tube contained 4 ml Brain Heart Infusion Broth, 0.1 ml of 24 hours *Candida albicans*, and its corresponding acrylic specimen, turbidity (yeast growth) was measured by a spectrophotometer at 530 nm wave length. For color change measurement test, 20 acrylic resin specimens (45 x 10 x 2.5 mm) were prepared and distributed into four studied groups. The absorbed light was measured by spectrophotometer and termed optical density at 345 nm. **Results:** All coating materials were fungicidal in pores method. Statistical analysis of sensitivity test showed that *Candida albicans* was susceptible at highest extent to monopoly coated denture base followed by the oils coated groups. In turbidity test, monopoly specimens showed antifungal activity in all periods of incubation, but the effect decreased gradually, olive and grape oil coated specimens exhibited their highest antifungal activity after 30 days incubation. Color change measurements showed that monopoly coated group had the highest optical density, while the olive and grape groups showed a decrease in the optical density when compared to uncoated group. **Conclusions:** Use of coating materials on the acrylic denture base surface was beneficial, especially monopoly in terms of antifungal activity against *Candida albicans* followed by natural oils (olive and grape oils). However, coating materials showed a significant change in the color of acrylic denture base.

**Key words:** Acrylic resin denture base, coating materials, *Candida albicans*, color.

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### INTRODUCTION

The oral mucosa is considered to be a unique environment offering a variety of ecological niches for microbial and fungal colonization <sup>(1)</sup>. The presence of prosthetic device is one of the several reasons for the rise in fungal infection <sup>(2)</sup>. Microorganisms and the oral deposits that adhere to a dental

appliance bring about several undesirable effects; therefore, unclean dentures represent both an esthetic and a health concern for the person who use them <sup>(3)</sup>. The fitting surface of the acrylic resin denture base is the main reservoir of Candida Species, and the growth of these organisms in large number is one

of the most important source of infection through direct cytotoxicity<sup>(4,5)</sup>.

*Candida albicans* plays a major role in the pathogenesis of denture stomatitis, oral candidiasis and inflammatory hyperplasia of the palate<sup>(6-8)</sup>. Several different ways were effective in disinfection of inoculated dentures: The use of cleansers, ultrasonic treatment, exposure to oxygen through air drying, incorporation of antifungal agents<sup>(9)</sup>. The use of coating agents with TiO<sub>2</sub> photocatalyst which is effective for maintenance of tissue conditioners when the dentures are removed during the sleep<sup>(10)</sup>. Addition of anionic charge on the denture base acrylic resins has been shown to inhibit *Candida albicans*<sup>(11)</sup>. The interference with bacterial adherence through daily application of a silicone polymer to the surface of the denture is a promising development in denture cleaning<sup>(3)</sup>, and the removal of the denture during night can be considered as an efficient procedure in reduction of Candida count, because continuous denture wear predispose Candida infection<sup>(12)</sup>.

The use of coating agents may bring beneficial effects in reducing Candida accumulation in order to maintain denture base for oral hygiene and odor free appliances. Coating materials could be natural or prepared, the antimicrobial activity of plant oils and extracts has formed the basis of many applications like pharmaceuticals alternative medicine and natural therapies<sup>(13)</sup>. Olive oil and grape seeds oil are examples of natural oils, additionally they are stable and safe enough to be used in foods. These advantages have shown much attention in concerning the antifungal effect when they are used to coat the acrylic resin denture base as these oils have been used in the formulation of products intended for oral hygiene, like a tooth paste, a mouth wash, a spray or a chewing gum, to decrease plaque accumulation; thereby achieving a reduction in the occurrence of dental diseases and halitosis<sup>(14)</sup>.

An example of a coating material that could be prepared is an acrylic resin coating material, termed "monopoly". This coating was used as a glaze on the acrylic resin obt-

urator to achieve a smooth and a polished surface<sup>(15)</sup>, as the smooth surface is less susceptible to Candida growth than the rough one; also this coating material has been used to seal the surface of temporary soft lining materials to preserve the integrity and viscoelastic properties of these materials, by preventing movement of substances to or from the soft liner; thereby enhancing the longevity of these materials<sup>(16-18)</sup>.

The use of coating materials whether natural or artificial may affect the color property of acrylic resin denture base, but should match the color of soft tissue surrounding the denture; the color property is already affected by the particle size of the acrylic resin and the porosities present on the surface<sup>(19,20)</sup>.

This study aims at evaluating the effect of three coating materials on the growth of *Candida albicans* on the surface and color of a heat-cure acrylic resin denture base.

## MATERIALS AND METHODS

### ▪ Denture Base Specimens Preparation:

A single brand heat-cured acrylic resin denture base (Major base 2/ Major Prodotti Dentari, SPA, Italy) was used and prepared in dimensions according to the test required, whatever the test used; the curing of the heat-cured denture base specimens was achieved according to the manufacture's instructions in a hot water bath at 70°C for 30 minutes, then at 100°C for other 30 minutes.

### ▪ Microorganism Growth Conditions:

*Candida albicans* was obtained from the central laboratory in the College of Science, to ensure the purity, the *Candida albicans* was cultivated on Sabouraud Dextrose agar and germ tube test as well as microscopical examinations were performed. A loop-full of *Candida albicans* was transferred to 4 ml of BHIB and incubated at 37°C for 24 hours, then transferred to haemocytometer slide to get 1x10<sup>8</sup> cell/ml.

### ▪ Coating Materials and Antifungal Activity:

Two food plant oils: Olive oil (*Oleum olivae*), Turkey product; and grape seed oil (*Vitis vinifera*), Syria product; also one prepared acrylic resin coating material termed:

monopoly were used. Monopoly was prepared by mixing one part of heat-cured clear methyl methacrylate powder with ten parts of auto polymerizing clear methyl methacrylate liquid together in a glass beaker at 130°F water bath that stirred for 8–10 minutes, until the mixture started to thicken<sup>(21)</sup>.

Determination of susceptibility of *Candida albicans* isolates to coating materials was achieved by pores method<sup>(22)</sup>. Sabouraud Dextrose agar was prepared in order to enhance the growth of *Candida albicans*, pores on the surface of agar were made by a sterile Pasteur pipette, then 0.1 ml of *Candida* suspension was transferred by a micropipette and the inoculums was streaked by L-shape spreader. After that 0.1 ml of each coating material was transferred to each pore and the plates were incubated at 37°C for 24 hours, then the diameters of inhibition zones were measured<sup>(22)</sup>.

▪ **Sensitivity Test (Disc Diffusion Method):**

To determine the antifungal effect of coating materials on the surface of acrylic denture base, 20 discs of acrylic resin (6 mm in diameter and 2 mm thickness) were prepared and divided into 4 groups: Control group (the discs were left uncoated), olive oil, grape oil, and monopoly coated groups, each group had 5 specimens. For the olive and grape oils specimens, they were immersed into the corresponding containers for 72 hours, then they were dried; while monopoly coating material was applied with a smooth brush to the whole surface of specimen and allowed to dry 4–5 minutes under 60 Watt-lamp, this was repeated until three coats have been added<sup>(21)</sup>, and the acrylic resin discs were ready to undergo sensitivity test. *Candida albicans* broth was streaked on Sabouraud Dextrose agar by L-shape spreader; after 15 minutes, discs of acrylic resin were added to the surface of agar by a sterile forceps using 5 replicate plates and the plates were incubated at 37°C for 24 hours, then the diameters of growth inhibition zones were measured<sup>(23)</sup>.

▪ **Turbidity Test:**

The effect of three incubation periods on the antifungal activity of the coating ma-

terials that coated the acrylic resin surface, was performed using microdilution method. Sixty acrylic resin specimens which measure (1 cm x 1 cm x 2 mm) were prepared and turbidity test was carried out using series of 60 test tubes. These tubes were divided into three groups according to the period of incubation (7, 20, and 30 days) and incubated at 37°C. Each period of incubation had twenty test tubes which were subdivided into four groups according to the acrylic resin specimens (uncoated, olive oil, grape oil and monopoly coated specimens), with five replicates for each group. Each test tube contains 4 ml of sterile BHIB, 0.1 ml of 24 hr. *Candida albicans* and the corresponding acrylic resin specimen. The turbidity (yeast growth) in test tube was measured using a spectrophotometer at 530 nm wave length<sup>(24)</sup>. Positive control, negative control and treatment tubes were also needed. Positive control: Represents a turbidity caused by *Candida albicans*, while the negative control tube: Represented the turbidity related to the coating itself. So to determine the antifungal effect of each coating material, we must exclude the turbidity caused by the coating materials (negative control).

▪ **Color Change Examination:**

Twenty acrylic resin specimens (45 x 10 x 2.5 mm) were prepared and divided into four groups (control, olive oil, grape seed oil and monopoly coated groups) and each group had 5 specimens. Application of coating materials was achieved as mentioned before and the color measurement was performed using an Ultra-violet visible spectrophotometer CECIL 2000<sup>(25)</sup>. A single clear acrylic denture base sample was prepared with the same dimensions of the tested samples to make the spectrophotometer scale = zero, in order to exclude the absorbance of light by the colored acrylic denture base component.

The surface of the acrylic resin specimen from which the data was collected, was identified by a numerical value. The absorbed light is measured and termed optical density, the wave length at which maximum absorption of light occurred is termed  $\lambda$  max, for the Major base heat-cured acrylic resin the  $\lambda$  max was 345 nm<sup>(26)</sup>.

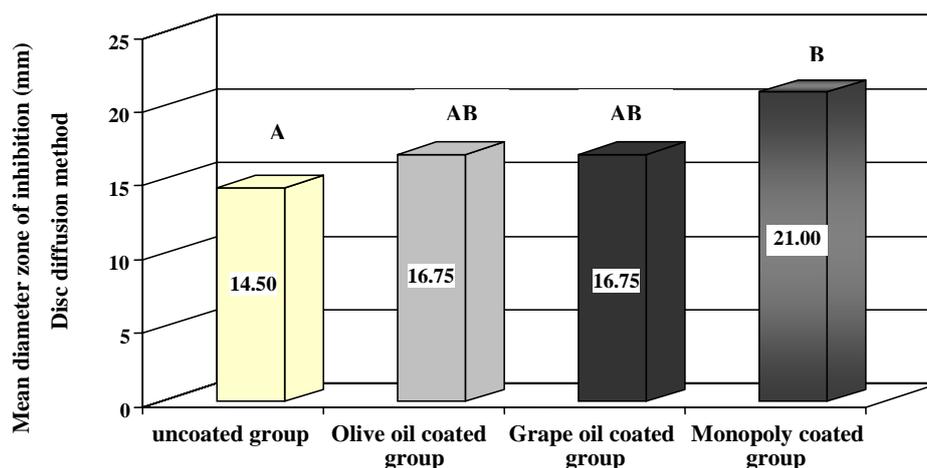
## RESULTS

To find out whether the effect of the coating materials that were used in the study is fungicidal or not, *Pores Method* was carried out, and showed that *Candida albicans* was susceptible to all coating materials, especially to the monopoly coating material, and in less extent to olive oil and grape seed oil, as appeared in Table (1).

The effect of coating material on the acrylic denture base was determined by *Disc Diffusion method*. Duncan's Multiple Range Test at Figure (1) showed that there was differences in fungicidal effect between study groups, as following: Monopoly, olive, grape oils coated groups and uncoated group, respectively in descending order; but this difference was not significant ( $P > 0.05$ ), Table (2).

**Table (1):** Susceptibility of *Candida albicans* to coating materials (Pores method)

Coating materials	Mean diameter zone of inhibition (mm)
Monopoly	26
Olive oil	20
Grape oil	18



Note: Different letters represent the significant difference between the studied groups.

**Figure (1):** Duncan's Multiple Range Test for the susceptibility of *Candida albicans* to the denture base groups.

**Table (2):** One – way Analysis of Variance for susceptibility of *Candida albicans* to the denture base groups

	Sum of squares	df	Mean square	F value	Significances
<b>Between groups</b>	88.5	3	29.5	2.798	0.085
<b>Within groups</b>	126.5	12	10.542		
<b>Total</b>	215	15			

df: degree of freedom.

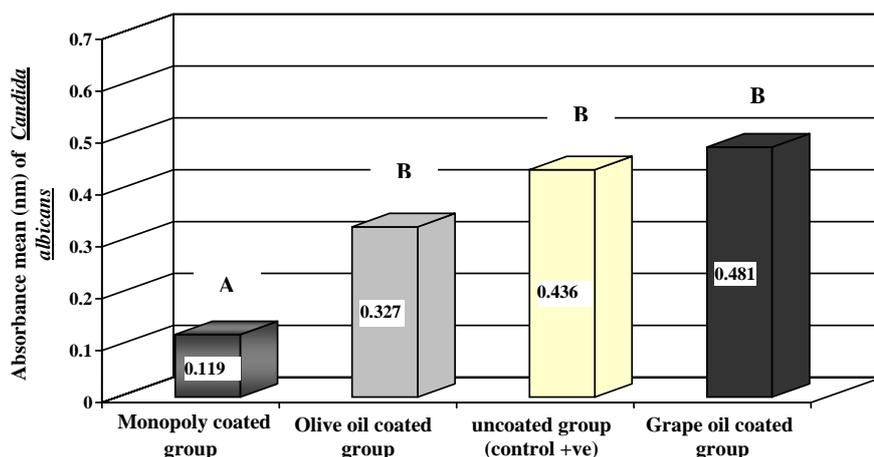
The efficacy of coating material on the acrylic resin denture base surface against *Candida albicans* through Turbidity Test after three periods of incubation, was analyzed by One-way ANOVA and Duncan's Multiple Range Test, Table (3) and Figures (2– 4). The results showed that the differences were significant ( $P \leq 0.05$ ), the antifungal effect of the monopoly coated group was the highest followed by the olive

oil coated, while the antifungal effect of the grape oil was lower than the uncoated group after 7 days of incubation. After 20 and 30 days incubation, the monopoly coated group gradually decreased in its antifungal effect, olive and grape oils coated groups showed their greatest antifungal effect after 30 days incubation, lastly the uncoated group of denture base showed the least effect among the studied groups.

**Table (3):** One – way Analysis of Variance for the antifungal effect of the denture base groups against *Candida albicans* during the periods of incubation

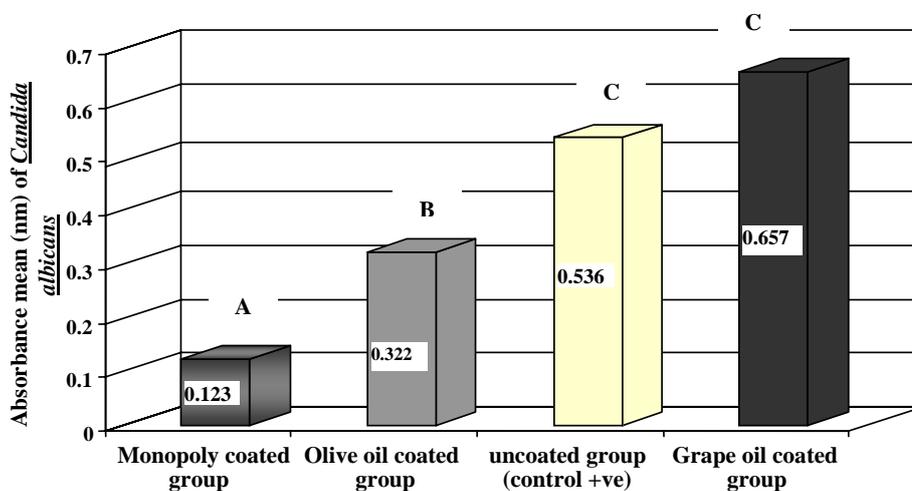
Period		Sum of squares	df	Mean square	F value	Significances
After 1 week	Between groups	0.39	3	0.13	5.884	0.007
	Within groups	0.354	16	0.022		
	Total	0.744	19			
After 20 days	Between groups	0.833	3	0.278	12.714	0.0
	Within groups	0.35	16	0.022		
	Total	1.183	19			
After 30 days	Between groups	0.545	3	0.182	8.315	0.001
	Within groups	0.35	16	0.022		
	Total	0.895	19			

df: degree of freedom.



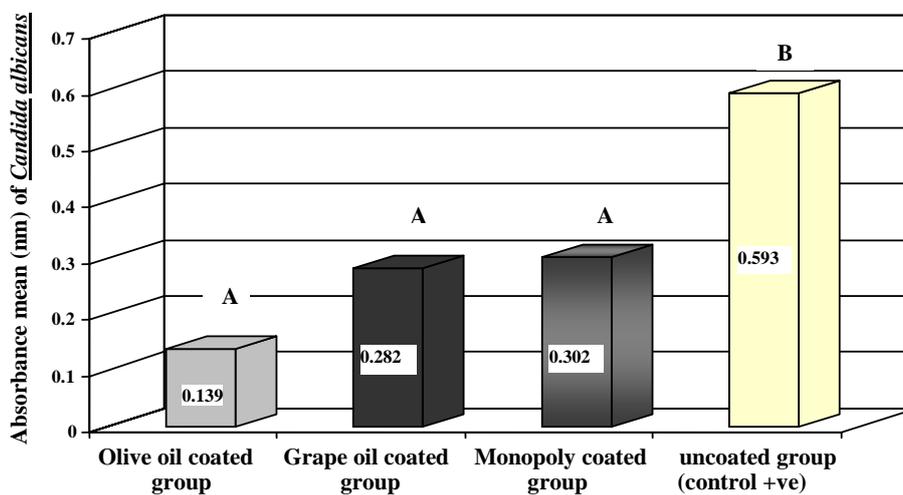
Note: Different letters represent the significant difference between the studied groups

**Figure (2):** Duncan's Multiple Range Test for the antifungal effect of the denture base groups against *Candida albicans* after 7 days incubation period.



Note: Different letters represent the significant difference between the studied groups.

**Figure (3):** Duncan's multiple range test for the antifungal effect of the denture base groups against *Candida albicans* after 20 days incubation period.



Note: Different letters represent the significant difference between the studied groups.

**Figure (4):** Duncan's Multiple Range Test for the antifungal effect of the denture base groups against *Candida albicans* after 30 days incubation period

Table (4) and Figure (5) gave an idea that there was a significant difference in the optical density of the denture base groups, monopoly coated group showed the highest increase in the optical density (2.99 nm), and olive oil and grape oil coated groups showed a reduction in the optical density (1.74, 1.85 nm, respectively) when compared to the uncoated denture base group (control group).

**DISCUSSION**

The fungal organisms that are most commonly associated with denture plaque are of the genus *Candida*. These yeast are present in the saliva of a majority of denture wearers and display an affinity of adherence to methacrylate resin<sup>(3)</sup>.

The control of denture hygiene is important for the prevention and treatment of denture stomatitis<sup>(27)</sup>. There have been sev-

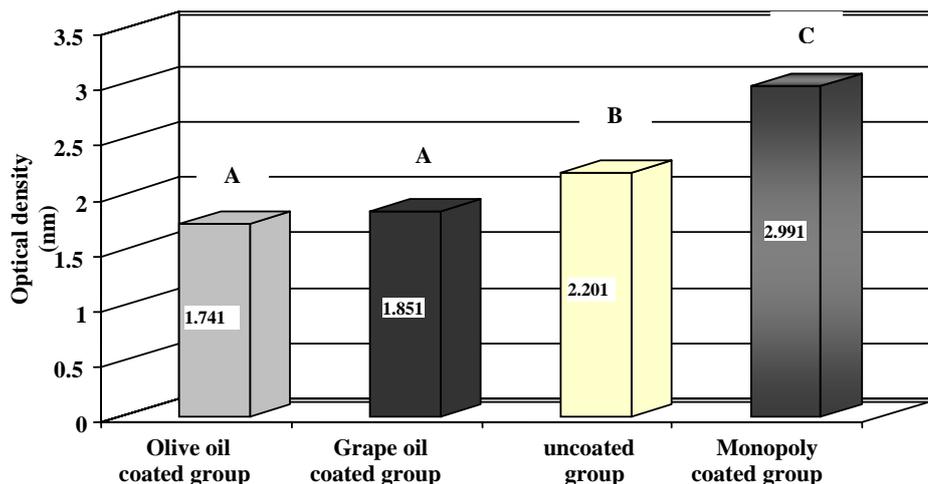
eral therapeutic measures to eradicate *Candida albicans* on the denture surface, the use of coating materials was undertaken in this study.

Coating the acrylic denture base with monopoly coating material exhibited a significant inhibitory effect against the *Candida albicans* among other coating materials, this effect agreed with Aslan and Avci<sup>(28)</sup> who reported a significant reduction in the number of *E. Coli* colonies when monopoly was painted on the unpolished surface of an open bulb of buccal obturator. Because roughness of the material facilitate the *Candida albicans* proliferation, the monopoly was used as a glaze on the temporary soft liner and acrylic resin surface to produce a smoother surface when it is impossible to be polish<sup>(15, 29)</sup>.

**Table (4):** One – way Analysis of Variance for the optical density of the acrylic resin denture base groups

	Sum of squares	df	Mean square	F value	Significances
<b>Between groups</b>	4.787	3	1.596	24.744	0.000
<b>Within groups</b>	1.032	16	0.064		
<b>Total</b>	5.819	19			

df: degree of freedom.



Note: Different letters represent the significant difference between the studied groups.

**Figure (5):** Duncan's Multiple Range Test for the optical density of the acrylic resin denture base groups

The antifungal activity of monopoly coated denture base decreased with time of incubation gradually, this is because monopoly is a temporary coating and lose its effect within 1–2 months as investigated by Dominguez and others that reported: The monopoly prevents loss of plasticizers and absorption of water from a temporary soft liner over 1 month. Furthermore, monopoly prevents leaching of toxic and allergic components of acrylic resin denture base<sup>(16)</sup>.

Historically, many medical researchers employ plant oils for most bacterial, viral, parasitic and fungal infection<sup>(30, 31)</sup>. In this study, olive and grape seeds oils coated denture base exhibited different antifungal activities against *Candida albicans*, but the highest effect of both oils was recorded after 30 days incubation, these findings were supported by Markin and Pretty's colleagues<sup>(14, 32)</sup>. Olive and grape oils are habitually used in treating oral ulcers, gingivitis, periodontitis, pockets, abscess, reducing dentin demineralization, herpes and in the preparation of a product for oral hygiene and dentifrice<sup>(31, 33)</sup>.

The activity of olive oil is due to a presence of active ingredient (Oleuropein) which is thought to give olive tree its disease resistance. The primary ingredient in the Oleuropein, which inhibit the fungi, bacteria and parasites' growth is elenolic acid<sup>(34)</sup>.

The therapeutic value of grapes is said to be due to high magnesium content. Grape oil is anti inflammatory, analgesic, a mouth wash for ulcer and can support the body through illness, because the nutrient content of grapes is close to that of blood plasma<sup>(35)</sup>. Supplementation with grape seeds extract substantially increases the level of antioxidants in the body, which destroy free-radicals damaging compounds in the body that tamper DNA and cause cell death<sup>(36)</sup>. Grape seed extract is rich of Vit. E, flavonoids, linoleic acid and proanthocyanidins, which are synergistic with Vit. C and help it to enter the cells; thus protect the cell from oxidative damage<sup>(37)</sup>.

The power of olive and grape oils include an ability to contain the infection, by inactivating the microorganisms and directly

penetrate the infected cells to stop the infection<sup>(36)</sup>.

In terms of color change measurement, monopoly coating material caused a significant increase in the optical density, this may be due to the fact that monopoly is an acrylic resin material and when applied to a denture base, an additional layer of acrylic resin preserve the colored substances. Olive and grape seeds oils' coatings, showed the least optical density, the discoloration has been attributed to penetration of the colored substances through absorption and oxidation of the polymer matrix or oxidation of unreacted double bonds of unpolymerized MMA monomer, because the degree of polymerization would never be 100% completely<sup>(38–40)</sup>.

## CONCLUSIONS

The use of coating materials was an effective mean for controlling fungal growth. The highest antifungal activity exhibited by monopoly coated denture base, but its effect reduced with the time of incubation gradually; on the other hand, coating with monopoly caused a significant increase in the optical density of the denture base. Olive oil and grape seeds oil coated denture base showed their highest antifungal activity after 30 days incubation. Also a notification was recorded, that these oils caused a significant decrease in the denture base optical density.

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