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INHIBITION EFFECT OF LASER, GAMMA RAYS, AND SOME NATURAL PLANT EXTRACTS ON FUNGAL DETERIORATIN ON STUCCO MONUMENTS

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Abstract:
Architectural and museum stucco belonging to several civilizations are suffering from microbiological deterioration aspects. Several types of laser, gamma rays and natural plants extract were performed to assess their suitability and sustainability for inhibition of fungal deterioration in stucco models. The Fourth Harmonic of Nd: Yag266 (power density= 71 mW/cm2) is the most potent type in inhibition of fungi in a least time for exposure (20: 30 minutes). Irradiated A. parasiticus stucco mockup confirmed that results. The use of ND-YAG Laser 266 nm (Power density= 7.03× 108W/cm2) was effective in eliminating fungi from stucco models without damaging its surface and preserving the patina layer. The time required to clean 1 cm2 of flat parts of the stucco mockup is 8: 10 min and for 1 cm2 of sunken parts whose depth is 3 mm is 15: 20 min. Gamma ray was successful in elimination of the total tested fungi at the rays level of 750 Kr, without any effects on stucco models. The inhibitory mixture of seed extract of neem and thyme (1:1) was effective in inhibition of fungal infection. The result of the monthly follow-up for a period of six months indicated that the fungal growth appears at the end of the fifth month with a heavy growth within the sunken places and represented by A. niger, A. flauvs and A. flavipes, in a ratio of 34:19:1.

1. Introduction

There are many types of architectural and museum stucco belonged to several civilizations with different support and components [1-6]. Mechanical, chemical, physical, and biological deterioration factors have a severe impact on stucco and need intervention and treatment to rescue the stucco monuments [7-9]. Identification of microbiological deterioration of monuments and its treatments with safe, innovative, and effective conservation with retention of historical and archaeological values is a very important goal [10-12]. Laser is effective and well-accepted method in many of conservation processes generally but disappointingly, thermal effect, dissolution or removal of surface pats, and yellowing of the restored materials due to its complex photo-thermal, photochemical and mechanical interactions [13-17]. It was also in many cases successfully applied removing black crusts, fungi, bacteria, lichens, birds, animal wastes [18-22]. It stated that the rays with a 405 nm laser had a significant bactericidal and fungicidal effect on some pathogenic bacteria and fungi. [15,19,23]. The success of using laser in cleaning depends on choosing a suitable device and type of laser [24] Nd-YAG laser 532 nm is efficient, but its reaction differed in the elimination of fungi [25,26] (Sanjeevan, Klemm & Klemm 2007; Pauili et al., 2008). Conversely, the wavelengh of 1064 nm is more efficient in eliminating fungi and their spots, but performs yellowing [27,28]. In addition, the Nd: YAG laser was more successful in cleaning sub-aerial biofilm (SAB) developed on granite surfaces more than Er: YAG laser [29]. Several other types of laser such as UV Laser excimer laser Krf, third harmonic laser, He-Ne Laser, Ruby Laser, Nd-Glass Laser, nitrogen Laser, Nd: YVO4 Laser and UV & IR were used successfully for conservation and restoration of different types of archeological materials. [30-37]. Gamma rays (Caesium-137) were firstly used in imaging the archeological objects. It was used in restriction

of the microbial growth as pesticides and fungicides [38,39]. Fumigation of archival resources by gamma radiation was considered in libraries and museums antiques science 1960_s [40-42]. Gamma radiations is efficient in removing of micro-organisms from the archeological objects [43,44]. It is stated that gamma rays (CO-60) doses from 14.5 to 25 k Gy were eliminated several fungal species, with minimum lethal effect at 16 kGy [45]. Furthermore, the most efficient and safe dose for removing fungi without any damaging of archeological materials, ranges from 500gy to 10kgy. Conversely, the higher doses of 10 KGY [46] and 12 KGY [47] may led to impairment of the mechanical properties of the materials and the death of fungi. In general, efficient conservation processes must apply regularly [48,49]. Natural plant extracts have been used for inhibition of fungal growth with no side effects on humans and animals [50,51]. *Azerdiracta indica* L (Neem) is one of the powerful fungicides that inhibited fungi like *A. niger, A. flavus* and *Alternaria alternata* [52-54]. It is also stated that the neem oil component; neem-76, was the most potent in inhibition of bacteria and fungi [55]. *Thymus vulgaris* L (Thyme) is powerful fungicide and it has been reported to be effective in elimination of *A. ochraceus* and *dermatophytes* [56-59]. The purpose of this study was to investigate the efficiency of using laser, gamma rays and natural extracts for fungal det-erioration inhibition of designed stucco models, executed according to the results of the previous examination study to some architectural and museum stucco monuments.

2. Materials and Methods

2.1. *Stucco models preparation and development of selected fungal colonies*

Stucco models with selected fungi were prepared [60]. Fungi which were isolated, purified and identified from Gamali Yusuf, Tatar El-Higaziya mosques, Bayt El-Kredlea Museum and Zeinab Khatoon house. There were *Aspergillus niger*, *A. flavipes*, *A. flavus*, *A. ustus*, *A. fumigutas*, *A. parasiticus*, *A. clavatus*, *Penicillium chrysogenum*, *Cladosporium Cladosporoides*, *Fusarium* sp., *Alternaria alternata* [60,61].

2.2. *Inhibition methods*

2.2.1. Laser rays

The experiments were carried out with He Ne laser 632 nm, Nd: Yag laser 532 nm with lower wavelength, a Diode laser 405 nm, LED 405 nm and Nd: Yag 266 nm., with a CW Diode laser 405 nm (power density= 254 mW/cm^2); CW Helium neon laser 632 (power density= 4 mW/cm^2), the second harmonic wavelength of Nd: Yag laser 532 nm pulsed (power density= 38.5 mW/cm^2), LED laser 405 nm (power density= 95 mW/cm^2) and the fourth harmonic wavelength of Nd: Yag laser 266 nm pulsed (power density= 71 mW/cm^2).

2.2.1.1. *Application rays*

a) Preliminary test: Tested fungi in broth culture and on models were exposed to different rays' periods of 5, 10, 15, 20, 30, 45 and 60 minutes. Fungal inoculum was picked up using sterile needle, and placed directly into sterile Czapek's-Dox agar plates; 3 replicates for each treatment, incubated at 28 °C for a week. Control experiments were carried out.

b) Confirmation test using A. parasiticus: A. parasiticus was chosen owing to its infrequent response to the different laser rays. From stored previously treated tubes inoculum was transported to laser rays, pre-stucco mockup, and left for a period of two weeks. Swabs were taken and cultivated on Czapek's Dox agar plates to confirm the existence of the same fungus. The stucco models were left in incubator for a month. Control non-infected models were also carried out.

c) Cleaning with Nd-YAG Laser 266 nm device with special lens: Nd-YAG Laser 266 nm (Surelite continuum, Modile: SLT-10, pulsed, power out: 20 mw, wavelength: 266 nm) with special lens (focus= 9 cm Model: SLT-10) was applied to clean three infected stucco models. Three distances were chosen as follow: first (work distance: 10 cm, power density: 1.4×109 W/cm^2), second (work distance: 11 cm; Power density: 1.05×109 W/cm^2) and third (work distance: 11.5 cm; power density: 7.03×108 W/cm^2).

2.2.2. Gamma rays

Different gamma rays levels were applied with Dose rate: (3.056 K. Gy/h), Activity 9199 carries, speed 1000 Kr/ 3h.23 mins. (90 Kr, 100 Kr, 250 Kr, 500 Kr, and 750 Kr) were used at different times. The duration of exposure to gamma rays extends until the time at which fungal growth was stopped. All experiments were prepared in three replicates in addition to control one at Gamma unit, Atomic Energy Center, Cairo, Egypt.

2.2.3. Natural plants extract

2.2.3.1. *Extraction method*

Five grams of the dry plant sample (*Azerdiracta indica* and *Thymus vulgaris*) are placed in 250 solvent, and the extraction is carried out in a Soxhlet device for 2 to 3 hours. The extract is then filtered under vacuum using an air vacuum device, and near dryness, each extract is supplemented with exactly 10 ml of the same solvent, and these are the concentrates of the extracts. Thus, each 1 ml of concentrated extract contains 50 mg of dry matter.

2.2.3.2. *Spore germination test*

A modified method of Mandels and Darby [62] was used, where 120 test tubes containing 10 ml Czapek's-Dox broth, tested extract (1:1) and fungus spore were incubated at 28°C in three replicates for each fungus. The no. of germinated spores was counted using Hemocytometer after consecutive incubation periods of 6, 12, and 24 hours. Ten isolated species were treated.

2.2.3.3. *Application of inhibitory mixture*

A mixture of *Az. indica* and *Th. vulgaris* (1:1 v/v) seeds extracts were applied using sterile sprayers on models.

a) Inhibition of fungi before cleaning: The inhibitory mixture sprayed on the surface of the stucco models every two days for three times. Using a sterile needle, a fungal inoculum was picked up, placed in Czapek's Dox broth tubes, and counting the germinating spore using the hemocytometer for consecutive times of 12, 24 and 48 h. Mechanical cleaning occurs by using scalpel to reduce

the density of the fungus in a circular manner from bottom to top direction, leaving a thin layer to avoid surface scratching. After that scalpel with cotton piece (wetted with distilled water) on its tip, clean the left layer in a circular manner. After that, patina layer appears except at sunken parts that are in dark in color. regular swabbing and culturing on Czapek's-Dox agar occurs.

b) Testing the inhibitory mixture for maintaining stucco against fungal infection: After mechanical and chemical cleaning, the inhibitory mixture was applied three times for six days with two days interval and placed in natural air. A monthly follow-up of one, three and six months was performed. Paper swabs from each stage were transplanted into Czapek`s-Dox Agar plates and incubated at 28 °C for a week.

3. Results

3.1. *Laser rays*

The results of exposure tested fungi to different laser rays periods (5 to 60 min) were illustrated in fig. (1). The data revealed that not all laser types are effective in complete ablation of fungal growth, at low and safe exposer time but it clearly induces various types and degrees of damages to the fungal growth. It is illustrated that the Fourth Harmonic of Nd: Yag266 nm laser (power density= 71 mW/cm^2 laser), was the most potent fungal inhibitor. The data revealed also that *A. ustus*, *A. parasiticus*, *A. fumigatus* and *P. chrysogenum* are 4HNdYag266 low tolerant **(**completely inhibited after 10 min of rays). Moreover, the growth of *A. flavus* and *A. flavipes* are moderate tolerant (inhibited after 20 minutes) and so for *Cl. Cladosporoides*, *A. niger* and *Al. alternata*, but their growth decreased after 15-minute exposure to about 76%, 88.1% and 89% respectively. The use of Diode laser 405 nm (power density= 254 mW/cm^2) is less effective and it had no effect on most of the fungi (*A. ustus*, *A. fumigatus*, *Cl. Cladosporoides*, *Alterna alternata*, *P. chrysogenum*, *Fusarium* sp.) so they are laser philic. But it reduced the growth of *A. niger* by 62%, *A. flavus* by 88.2% and *A. flavipes* by 77.3% after an hour, as the growth decreases gradually from 15 min up to 1 h. exposure so they are moderate laser tolerant. Conversely, *A. parasiticus* was higher laser tolerant, as its growth starts to reduce after 45 min rays. It is noted that before 15 min exposure, all fungi have the same growth rate as the non-treated one. The response to He Ne laser 632 nm (power density= 4 mW/cm^2) was variable. *Cl. Cladosporioid*es, is a high HN laser tolerant, where its growth eliminated after 1hr exposure. Similarly, *A. ustus A. parasiticus*, G3 and A. flavus are a high HN laser tolerant, but their degree of growth inhibition after 1 h exposure is variable. By the way, Conversely, *Al. alternata*, *P. chrysogenum*, Fusarium and A. niger are HN laser-philic. The Second Harmonic of Nd: Yag laser 532 nm (power density= 38.5 mW/cm^2) had different reactions. The growth of *A. flavus* and *A. ustus*, began to decrease after 30 minutes of exposure, thus they all are moderate 2HNdY laser tolerant. Moreover, *A. fumigatus*, *P. chrysogenum*, *Cl. Cladosporoides* are not affected by all treatments and so they are 2HNdY laser-philic. For LED 405 nm (power density= 95 mW/cm^2), almost all tested fungi (*A. flavus*, *A. flavipes*, *A. fumigatus*, *A. parasiticus*, *A. ustus*, *P. chrysogenum*, *Cl. Cladosporoides* and *Fusarium* sp.) are LED 405 laser-philic, where they are continued to grow within all periods of exposure without inhibition with respect to the control one. Conversely, it has an inhibitory effect on *A. niger* (25%) and *Al. alternata* (86.2%) after 45 minutes of exposure. Generally, based on the foregoing experiments, the Fourth Harmonic of Nd: Yag266 nm (power density= 71 mW/cm^2) was the effective mean for inhibition of the tested fungi at the least time for exposure (20 & 30 minutes) comparing to other tested laser types. But for the 2nd Harmonic of Nd:Yag laser 532 nm inhibited *A. niger* and *Al. alternata* growth after1 hour and 45 min of exposure respectively. It had no effect on *A. fumigatus*, *P. chrysogenum*, and *Cl. Cladosporoides*, and reduced growth of the rest of the fungi. Correspondingly [63], stated that laser rays was affected the abundance of fungi and Nd:YAG laser 3rd and 4th harmonic are effective in the abolition of *Verrucaria nigrescens* from marble substrates, due to the high optical absorption property of melanin pigments of fungal partner, with consequent disruptive photothermal and photomechanical effects [64,65].

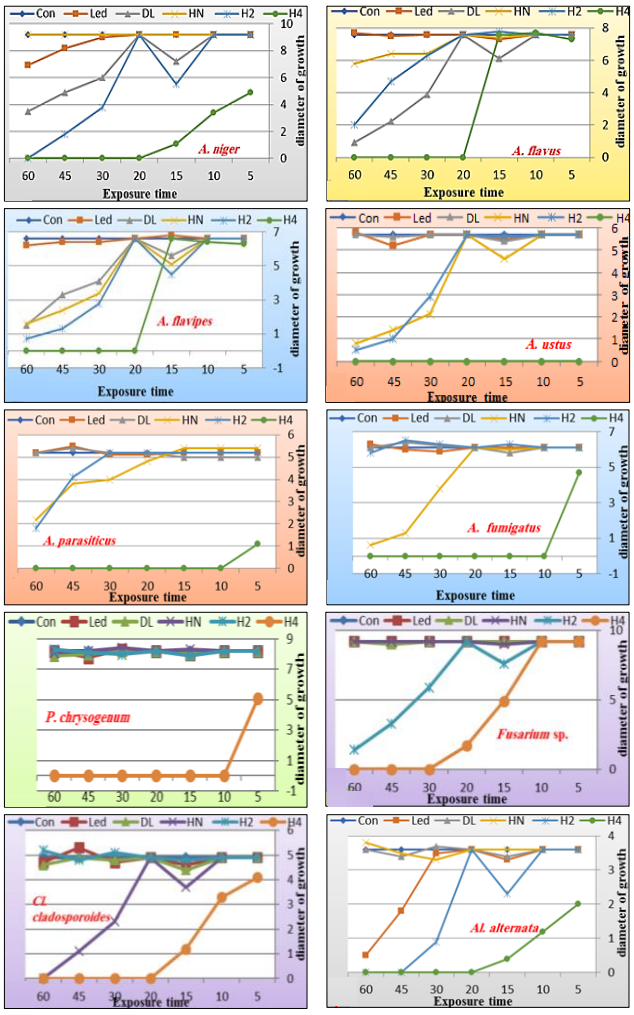

Figure (1) exposure time (mins) of tested fungi to different laser rays with time ranging from 5 minutes to an hour. Con; control, led; led 405 nm, DL; Diode laser 405 nm, HN; He Ne laser 632 nm, H2; the 2nd Harmonic of Nd:Yag Laser 532 nm; H4; The 4th Harmonic of Nd:Yag laser 266 nm.

Figure (2) illustrated the result of confirmatory experiment using several stucco models infected with *A. parasiticus* and exposed to tested laser types. It is observed that, the medium intensity greenish brown growth of *A. parasiticus* covering the entire surface of the stucco mockup, fig. (2-a) become less dense and in light greenish brown color, fig. (2-b) up on exposure to He & Ne laser 632 nm for an hour. This confirms the result of testing this type of radiation on the fungus, which led to reducing its growth only by 58%. Conversely, fig. (2-c) treated with Diode laser 405 nm beams for an hour, the growth become condensed with clear greenish brown color compared to control one. This confirms the result of testing this type of radiation on the fungus, which led to an increase in the growth of the fungus by 21.5%. Thus, the use of 4th Harmonic of Nd:Yag 266 nm laser for ten min was completely inhibited the growth of the fungus and this confirms the above results, fig. (2-d).

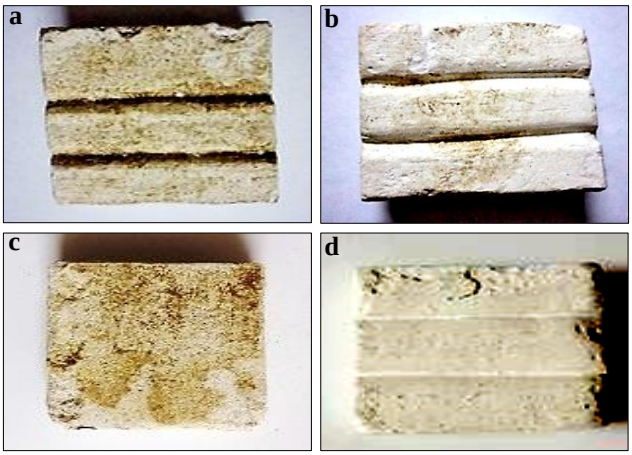

Figure (2) stucco (5×5 cm) models infected with *A. parasiticus* without exposure to any type of radiation; **a.** (control), **b.** a 1-hour (60 min.) exposure to He & Ne laser 632 nm, **c.** diode laser 405 nm, **d.** 4th harmonic of Nd:Yag 266. nm for ten minutes.

Nd-YAG Laser 266 nm device is the most common type of laser involved in the field of restoration and used in maintenance of several archeological objects [24,66,67-69] and its effect differed relying on the wavelength used [23,25-28]. The results obtained, fig. (3-a) indicate that the ideal conditions for laser ablations of the bio-deteriorated layers are highly dependent on the treated species and the length of the rays path. The first test led to a successful cleaning of the fungus layer, but the patina layer was removed. Thus, this result is not suitable for the archeological materials, because it is missing the features of the originality of the stucco, fig. (3-b). In the second test, the fungus layer was successfully cleaned, but a portion of the patina layer was also removed, it was better than the previous one, but it also did not preserve the patina layer, fig. (3-c). In the third test, there is a success in removing the fungus layer and preserving the patina layer of the stucco mockup, fig. (3-d). By completing the cleaning process, the time required to clean 1.0 cm^2 of flat parts was from 8:10 min, where 1.0 cm of cavernous parts that are 3.0 mm deep of the same model take from 15 to 20 min. SEM micrograph of stucco model infected with fungi after exposure to the YAG Laser 266 nm for an hour show that the fungal hyphae and spore appeared morphologically unaffected, fig. (3-d). These fungi were not grown when sub-cultured again

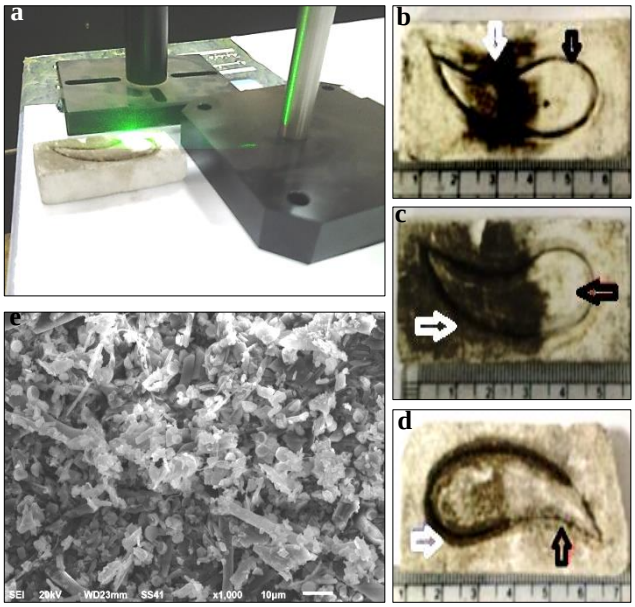

Figure (3) cleaning infected stucco mockup (2.5×5 cm) by ND -YAG laser 266 nm device with special lens; **a.** diameter of the laser beams after attaching a lens for the ND-YAG laser 266 nm., **b.** infected area before and after cleaning at distance of 10 cm, **c.** infected area before and after cleaning at distance of 11 cm, **d.** infected area before and after cleaning at distance of 11.5 cm, **e.** SEM of an infected sample with G3 fungi after exposure to YAG Laser 266 nm for an hour, show fungal hyphae extended between the dense stucco fibers covered with mucilage.

3.2. *Gamma rays*

Al. alternata was adversely affected by all radiation levels and so it was the most sensitive fungus, fig. (4). The rays level of 100 kr led to a reduction in the growth of *Cl. Cladosporoides*, *Fusarium* sp., A. *ustus* and *A. parasiticus* comparable to control.

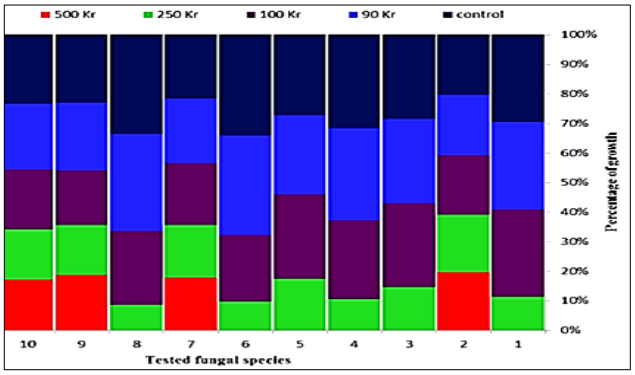

Figure (4) percentage of radial fungal growth after exposure to variant levels of gamma rays. Note; growth was 0 at 750 kr so not represented graphically. **1**; *A. niger*, **2**; *A. flavipes*, **3**; *A. flavus*, **4**; *A. ustus*, **5**; *A. fumigatus*, **6**; *A. parasiticus*, **7**; *P. canescens*, **8**; *Clad cladosporoides*, **9**; *Fusarium* sp., **10**; *Al. alternate*.

The rays' level of 250 kr was effective in reducing the growth of all tested fungi, fig. (5-a). Increasing dose level to 500 kr is fatal for *A. niger*, *A. flavus*, *A. ustus*, *A. fumigatus*, *A. parasiticus* and *Cl. Cladosporoides*. The remaining radio tolerant fungi (*P. chrysogenum*, *Fusarium* sp., *Al. alternata*, G3 and G4 fungi) were completely inhibited at level of 750 kr, fig. (5-b). Likewise, [48] stated that fungal growth was gradually decreased over 250 Gy, Gamma rays until reach the lethal level at 2000 Gy. SEM of stucco models infected with *A. niger*, *A. flavus*, *Cl cladosporoides*, *Al. alternata* and G4 fungi after exposure to doses of 500 kr and 750 kr gamma rays indicated that the fungal hyphae and spore appeared morphologically unaffected, fig. (5-c & d), and failed to germinate when sub-cultured on suitable substrate, without affecting stucco structure. According to microscopic examination this method can be used to inhibit fungal damage of the transported archeological materials.

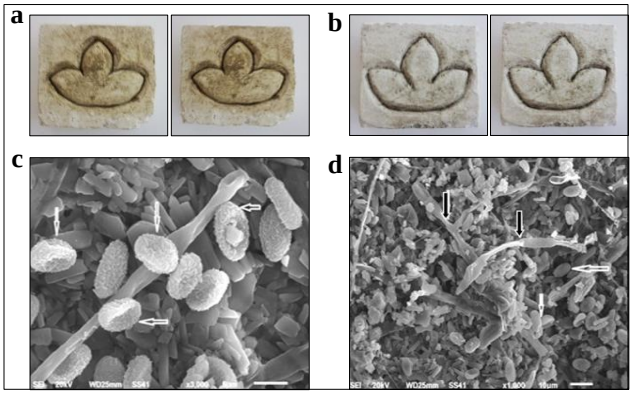

Figure (5) examination of stucco models infected with *A. flavus* and *Alternaria alternate*, **a.** original mockup, **b.** mockup after irradiated by gamma ray of 750 kr., **c.** & **d.** SEM micrograph of irradiated sample showing the fungal hyphae and spore, aerial hyphae and spores stretching on the surface of the stucco. Hyphae spread between the stucco crystals; d, spores mixed within stucco.

Results of Natural plants extract usage were illustrated in tab. (1) that the percentage of conidial germination was variable. It is observed that *Melia azederachta* extract was completely inhibit the gemination of *A. ustus* and *A. flavus* spores and to certain degree the conidia of *A. fumigatus* and *Fusarium* sp. and does not have any effect on the other tested fungi. Alike, *Azerdiracta indica* L (Neem) seed extract inhibits the spore germination of some species (*A. flavus*, *P. chrysogenum*, *Al. alternata*, *Cl. cladosporoides*, *Fusarium* sp.), reduced it in others (*A. ustus*, *A. niger*, *A. flavipes* and *A. fumigatus*) and had no effect on the rest of the tested fungi. Similarly, it is stated that neem is effective fungicides [52-54], that inhibited fungi like *A. niger*, *A. flavus* and *Al. alternata*. Moreover, *Th. vulgaris* seed extract, inhibit the growth of *A. fumigatus*, and *Fusarium* sp., reduced the gemination of *A. ustus*, *A. niger*, *A. flavus*, and *Cl. Cladosporoides* spores and affect the rest of the fungi to certain levels. It is reported to be effective against *Aspergillus* spp., *Penicillium* spp. and several other mitosporic fungi from different habitats [56,57]. Also, *Aspergillus* exhibited maximum enzyme activity [10]. In fact, the effectiveness of the extracts on fungi in consortium was variable. *Az. indica* seed extract was more effective followed by *Th. vulgaris* seed extract. Thus *Az. indica* and *Th. vulgaris* were the most potent and in order to get more effective inhibition a mixture of them (1:1) was made and an appropriate aliquot was prepared for the application in further experiments. Results of many studies [12,58] reported that thyme oil was the most efficient in killing fungi isolated from archaeological objects in Saqqara Excavation, Egypt.

Table (1) percentage of germinating conidia of fungi treated with extract of *Azadirachta indica* and *Thymus vulgaris* after 6, 12 and 24 hr compared to untreated control.

Essential oil	*Azadirachta indica*			*Thymus vulgaris*		
Fungal species	**6**	**12**	**24**	**6**	**12**	**24**
A. niger	-	+++	++	-	++	++
A. flavus	-	-	-	-	+	++
A. flavipes	-	-	-	-	-	+
A. fumigatus	-	-	-	-	-	-
A. ustus	-	-	-	+++	+	+
A. parasiticus	++	++	++	++	++	++
Penicillium chrysogenums	-	-	-	-	++	+
Alternaria alternata	-	-	-	-	-	++
Cl. cladosporoides	-	-	-	-	-	+++
Fusarium sp.	-	-	-	-	-	-
G1	-	-	-	+	+	+
G2	-	-	-	+	+	+

Percentage of germination (0 = -, 1-25 = +, 25>50% = ++, 50>75 %= +++, 75>100 % (++++)

4. Discussion

The application of different laser rays conditions lead to different degrees of ablation that are highly dependent on the treated fungal species, where the substrate is stucco in all samples. Many researches are also stated that, the effectiveness of laser in ablation depends on the laser-material interaction, which in turn, is related to wavelength, fluency and optical properties of the sample, such as absorption of light and heat diffusion [26,30,31,70]. It was confirmed that suitable device and type of laser is important for success in cleaning. Depending on the species involved, different complex interactions are observable [71,72]. These variations are probably due to the high concentration of endogenous or exogenous absorbing compounds. Therefore, according to the laser type and the rays period tested fungi were classified as low tolerant (inhibition after 5-10 min rays), moderate tolerant (inhibition after 15-30 min rays), high tolerant (45-60 min rays) and laser-philic fungi (no inhibition). The use of ND-YAG Laser 266 nm (Power density= 7.03×108W/cm^2) was effective in cleaning the fungi from the surface of the stucco models without damaging the stucco surface and preserving the patina layer that expresses the originality and date of the stucco carvings and the time required to clean 1.0 cm^2 of the flat parts is 8 to10 min and 1.0 cm^2 of the cavernous parts that are deep of 3.0 mm is 15 to 20 min. Gama rays can inhibit microbes in the archeological materials [43,73]. This radiation type was used to inhibit fungal growth, starting with a low level of 90 Kr, then 100 Kr, 250 Kr, 500 Kr and 750 Kr at variable rays periods. It was observed that the level of rays 90 Kr was not effective in inhibiting tested fungi or fungal consortia and conversely all fungi were prohibited by 750 kr rays. A mixture of neem (*Az. Indica)* and thyme (*Th. vulgaris)* seed extract (1v:1v),

can be used as a conservation material periodically every six months; to keep the stucco surfaces from fungal infection. Consequently, this inhibitory mixture will be used in the disinfection of Dakhla (decorative solid stucco window with stucco veils) of the Tatar Hijaz Mosque. The use of natural extract in the control of fungal growth on archaeological objects was effective and free of toxicity [74-76]. The inhibitory mixture of neem and thyme 1:1 has no damage to the stucco material. It must be used periodically, before the end of every five months. Therefore, it is recommended to use natural materials extensively in the conservation of antiquities as eco-friendly and green materials. Reaching a safety level for the restorer and the monument, by inhibition of the fungus growth is the main target. To clarify the effectiveness of the treatment processes before starting cleaning operations, the model was repeatedly checked to find germinating conidia. Thus, repeated samples of conidia (each 12, 24 and 48 h) were cultured in a liquid nutrient tube and no. of geminating one, counted using a hemocytometer. Repeating the average number of conidia indicated that fungi did not grow again, and the cleaning process could be started directly. The use of essential oils is esthetically acceptable for disinfection with negligible toxicity to humans and the environment, ensuring a good quality of life for employees [59]. Consequently, the result of six month follows up of germinating spores was observed in the sunken areas in black color of *A. niger* (predominant) and *A. flavus* and in in dark greenish brown color of *A. niger* and *A. flavipes*. It is reported that *Aspergillus niger* was the most resistance species [58]. Also, *Aspergillus* species tolerate to high temperature. This indicates that the effect of the inhibitor has begun to weaken and allow the fungi to grow on the surfaces of the models

5. Conclusion

The fungi were successfully removed from the stucco models' surfaces using an ND-YAG Laser 266 nm (Power density= 7.03x108W/cm^2), all while maintaining the patina layer that indicates the originality and date of the stucco carvings and reducing the cleaning time. Eight to ten minutes for 1.0 cm^2 of the flat areas and fifteen to twenty minutes for 1.0 cm^2 of the deep 3.0 mm cavernous parts. Gamma rays have the ability to inhibit microorganisms found in archaeological objects. However, fungus or fungal consortia studied were not harmed by gamma rays at 90 Kr, while all fungi were forbidden by 750 Kr rays. The methods of inhibition of tested fungi, had given different results for each method. The success of the fourth harmonic of Nd:Yag Laser and gamma rays in inhibition of all fungi, they can be used with only transported or museum stucco. A portable laser device needed for field in situ treatment. Natural plants extract (neem: thyme 1:1) is effective to be applied in various fields of conservation in a period of six months at maximum.

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