

**Chemical control of fungi infesting easel
oil paintings at the University of Santo
Tomas, Museum of Arts and Sciences**

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Full Length Research

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Two hundred paintings of Filipino masters, all accessioned at the UST Museum of Arts and Sciences, were surveyed to determine their state of preservation. Forty-three (21.5%) oil paintings were found to be in different states of deterioration with mold attack as the most common cause. Fungal infestations were most evident on pigments, wood support and paper backing. The infested paintings, based on the records at the museum, are between 50 and 100 years old. Infesting molds were isolated by swab method and purified through a series of transfer in plates of Malt extract agar (MEA) with pH 3.5 and incubation temperature of 28^oC. Forty-eight isolates in six genera as follows were obtained and identified based on cultural and morphological characteristics in MEA: *Aspergillus* and *Penicillium* (Deuteromycota); *Rhizopus*, *Mucor* and *Cunninghamella* (Zygomycota); and *Chaetomium* (Ascomycota). *Aspergillus* is the most prevalent with 77% occurrence in all the paintings sampled. It is followed by *Penicillium*, 13%, and then *Chaetomium* sp., 4%. The lesser isolates wherein each has an occurrence rate of 2% are *Mucor*, *Cunninghamella*, and *Rhizopus*.

Key words: Fungi, infesting, oil painting, and museum of arts and sciences.

INTRODUCTION

Paintings are fragile creations susceptible also to different kinds of deterioration. One of the most common causes of deterioration is microbial attack, particularly by molds. These microorganisms often attack and infest paintings when the environmental conditions to which the art objects are exposed are conducive for their growth and development. Researchers have shown, for example, that molds rapidly developed on the paintings when relative humidity was high (around 70%) and temperature increases (Dhawan et al., 1991; Pavlogeogatos, 2003). Moreover, the organic nature of the materials composing a painting may serve as nutrients for most molds (Agrawal et al., 1989), just as they do on painted parts of ordinary homes.

Biodeterioration of paintings due to infestation by fungi is a common problem in most tropical countries like the Philippines where rainy season is longer, temperature is higher (25-31^oC), and most of the time the air is very humid (Tse et al., 2008).

Architect Clarissa Avendaño (pers. comm., 2002), Assistant Director of the Museum of the University of

Santo Tomas, admits that mold infestation is a problem in the museum especially with their stocked paintings. There is no air conditioner in the storage room due to budget limitations. Louvers have been installed on the door of the room so that air may circulate and partly prevent fluctuation of temperature and humidity inside. Still, high humidity in the storage room during the rainy season subjects the collections to fungal growth.

The UST Museum has in its holdings art objects of immeasurable value to Philippine culture and history. Of the collections, the paintings are among those most susceptible to deterioration caused by fungi. This is the first study of its kind focusing on the valuable paintings in the museum.

It hopes to provide (a) much-needed knowledge on biodeterioration of paintings in our country, (b) baseline information to restorers and conservators regarding specific guidelines on the conservation of the kinds of paintings under study, and (c) background on the fungicides against specific fungi that attack local paintings.

The study focuses on the problem of fungal infestation of easel oil paintings at the UST Museum. It aims to:

- (1) Isolate and identify the molds responsible for the deterioration of aforementioned paintings at the Museum;
- (2) Determine the degree by which painting components favor the growth of the isolated fungi;
- (3) Evaluate selected fungicides for their inhibitory or eradivative effects on the isolated fungi; and
- (4) Test the solubility of pigments of a mock painting in selected fungicides.

MATERIALS AND METHODS

Survey of the paintings

The paintings used for the study came from the Museum of the University of Santo Tomas. Surveying included careful visual inspection as well as taking photographs of oil paintings observed to be infested with molds. Other conservation problems of the paintings were also noted.

Isolation of molds

For isolation of molds, a modified method of Dhawan (1983) was adopted. Sterilized cotton buds were rubbed gently on the affected surface and the spores/mycelia collected were aseptically shaken in flask containing 100 ml sterile water. The resultant suspensions were used to prepare dilutions up to 10^{-6} from each dilution 1.0 ml was transferred into a sterile petridish. Afterwards each plate was aseptically poured with 9.0 ml sterilized Malt extract agar medium (Malt extract, 20 g; agar, 20 g; distilled water, 1 L; pH 3.5 with 1 N HCl) pre-cooled at 45-47°C. Each plate was rotated to distribute the agar and the inoculum evenly. The petri dishes were then incubated at room temperature for 3-5 days. Fungal colonies that developed were transferred to MEA slants to obtain pure cultures suitable for identification.

Identification of molds

Fungal isolates were identified based on cultural and morphological characteristics by observing pure culture growth, and performing wet mount and slide culture technique. Fungal structures, including asexual and sexual structures, were examined under the stereomicroscope and light compound microscope. Mounting fluids used for identification processes were either water or lactophenol without dyes. Fungal genera were identified using literature in Fungal Taxonomy and Mycology (Ainsworth, 1973; Alexander, 1977; Barnett and Hunter, 1972;; Ellis, 1976; Garry and Robert, 1979 ; Bryce, 1971; Raper and Fennel, 1977 ; Raper and Thom, 1968; Subramanian, 1971; Sutton, 1980)

Experiments on the fungi's use of painting materials

In this procedure, sterilized and triplicate plates of agar with a specific concentration of painting materials (12 pigments, canvas, linseed oil, varnish) were prepared. Then each plate was inoculated with a selected

fungal isolate from each genera. A control plate with zero amount of painting materials was also prepared. The inoculated plates were then incubated for 7 days at 30°C. After a week, diameters of fungal growths were carefully measured and recorded.

Evaluation of the selected fungicides

Isolated pure cultures of the molds were used as test organisms to evaluate the inhibitory or biocidal effect of five selected fungicides: Preventol R-80, Umonium-38, Boric acid, Clotrimazole, and Dithane M-45.

Disk diffusion assay

This was carried out on a solid medium suitable for growing the test organisms. Malt extract agar seeded with appropriate fungal spore suspensions were poured into petri dishes. Whatman paper discs (Becton-Dickinson) 6-mm. in diameter soaked with different concentrations (1-10%) of fungicides were transferred carefully on the solidified agar plates. The plates were incubated at room temperature. The diameter of the clear zones around the discs were measured after three days incubation.

Minimum Inhibitory and Lethal concentrations

These were done for fungicides with zones of inhibition in Disk Diffusion Assay. In this method, a series of broth tubes (Mueller-Hinton broth) containing fungicide concentrations in the range 0.1% to 12% (at 0.1% interval) is prepared and inoculated with fixed cell density of the test organisms. The lowest concentration of the fungicide resulting in no growth (no turbidity) after 3 days of incubation was the Minimum Inhibitory Concentration (MIC). The Minimum Lethal Concentration (MLC) was ascertained if the tubes showing no growth were subcultured into fresh medium lacking fungicide. The lowest fungicide concentration from which the microorganism does not recover or grows when transferred to fresh medium is the MLC (Prescott et al., 1999).

Experiments to determine reaction of fungicides with painting pigments

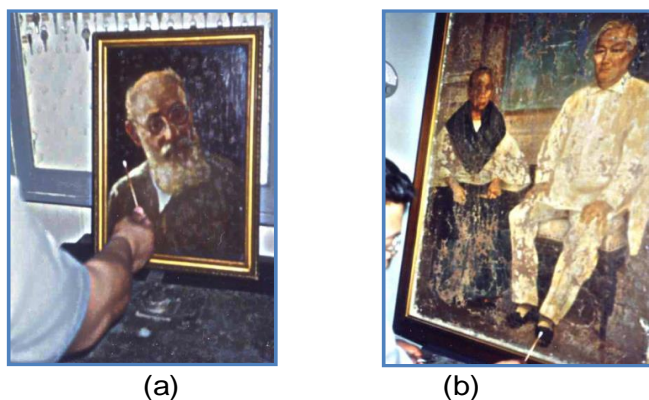
In this method, a series of concentrations (0.2%, 0.39%, 0.78%, 1-10%, 12.5%, 25%, 50%, 100%) of the different fungicides were prepared. Then 50 µL from each concentration of the test fungicides were dropped on the selected surfaces of two oil paintings:

1) a mock- painting - this was prepared by brushing a ready-made canvas (8" x 10") with black, white, green, blue, orange, yellow, violet and red pigments which were mixed with linseed oil as binder. Turpentine was also used to thin out the pigments. Then the painting was allowed to dry up to two weeks

2) On an old oil painting on canvas (dated 1975, unvarnished) from a personal collection. Then fungicide

Table 1: Types of deterioration caused by fungi on oil paintings

Painting No.	Type of deterioration	CFU/gm	Fungi isolated
1-19, 21, 23-26 30-33,35-41,43	Surface damage	1.0×10^6	Aspergillus(29), Penicillium(6), Chaetomium(2), Rhizopus(1), Mucor(1), Cunninghamella(2),
20, 22, 27-28, 34, 42	Discoloration	2.0×10^5	Aspergillus(6)
7,29	Structural damage	1.0×10^3	Aspergillus(2)

**Figure 1:** Two of the oil paintings under study showing signs of fungal deterioration.

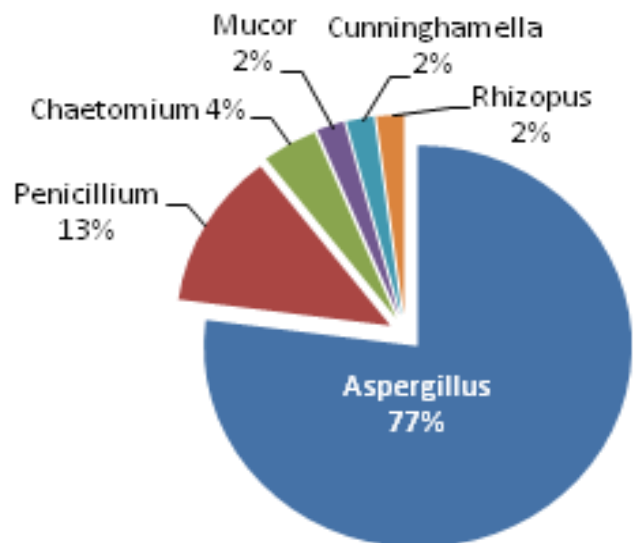
- a) Molds colonies on the surface appears as white patches
 b) This painting exhibits flaking of pigments in almost all areas as a result of molds' enzymatic digestion of the pigments.

drops from each painting was allowed to stand for at least three minutes. At the end of the period, the droplets were pipette-off and examined under light microscope to look for the presence of pigment crystals, precipitates, and other signs of solubility. Reaction of fungicides was either positive or negative in comparison with the control wherein only plain water was used in lieu of the fungicides. Likewise, the surface of the paintings was also examined by digital macro-photography to look for signs of color change or discoloration.

RESULTS AND DISCUSSIONS

Of around 200 paintings that were visually surveyed under normal and raking light, forty-three easel oil paintings were found to exhibit signs of molds infestation. Most evident of these deteriorations were observed in various parts of the paintings such as pigments, wood support and paper backing.

Further inspection found that neither airconditioner nor dehumidifier were present in the stock room. With the use of thermo-hygrometer instrument, the room's temperature was found to be very high at 31°C while the relative humidity was around 72%. This condition was far beyond the standard temperature and RH requirement for a museum and/or storage facility for cultural objects which is between $20\text{-}25^{\circ}\text{C}$ and 45-65% relative humidity, respectively (Ascione et al., 2008; Van Schijndel et al.,

**Figure 2:** Percentage distribution of fungi isolated from easel oil paintings

2008).

A Study conducted by Florian (1996) had found that when paintings are stored in an environment of 75-85% RH and $20\text{-}30^{\circ}\text{C}$ temperature, conidia and ascospores of foxing-causing fungi germinate and make their colonies of about 2-5 mm diameter around the microdusts.

Standard microbiological cultivation methods had produced forty-eight fungal isolates from the deteriorating oil paintings. Characterization through nutritional and morphological methods as well as by referring to standard reference works such as Ainsworth (1973), the following fungal genera (figure 2) were identified as follows: *Aspergillus*, *Penicillium*, *Mucor*, *Cunninghamella*, *Chaetomium*, and *Rhizopus*.

Percentage distribution of the isolates showed *Aspergillus sp.*, with 77% rate of occurrence as the most abundant and prevalent genus. Next was *Penicillium sp.*, with 13%, and then followed by *Chaetomium sp.*, 4%. The lesser isolates wherein each had occurrence rate of 2% were *Mucor*, *Cunninghamella*, and *Rhizopus*.

Surface damage (table 1) had been found as the most common type of fungal deteriorations observed among paintings surveyed. It comprised over 81% of the total number of deterioration types observed. Next was discoloration, 14% and then structural damage (figure 1),

Table 2: Average growth diameters of fungal isolates in different painting materials

Painting material	Average growth diameter in mm.*	% Inhibition**
Varnish	0	100
Canvas	55	-10
Linseed oil	66	-32
Ivory black	36	28
Burnt umber	19	62
Yellow ochre	27	46
Viridian hue	28	44
Sap green	41	18
Ultramarine	35	30
Phthalo blue	33	34
Medium yellow	26	48
Brilliant red	29	42
Crimson	26	48
Lemon yellow	39	22
Chinese white	25	50

*based on control (ave. 50 mm.)

**data were interpreted applying Neumann-Keuls mean values according to the formula below (Kharbade et al., 2008):

$$\% \text{ Inhibition} = 100 - \frac{\text{Ave. growth in painting material} (100)}{\text{Ave. growth in control (w/o painting material)}}$$

5%. Surface damage causes obstruction of the image by growing colonies; discoloration is manifested by pigment production which reacts with the pigments of the painting causing color change; while structural damage is due to enzymatic digestion of painting materials. The visible manifestation is thinning and softening of the pigments and canvas according to Strzelczyk and Alicja, 1981. Although it is something to be expected, but microbiological analyses had confirmed that majority of paintings with *surface damage* had also the greatest population and variety of fungal organisms (table 1).

Further microbiological analysis of the isolates had revealed that majority of the fungi which attacked the pigments belong to *Aspergillus*. *Penicillium* was found to occur mostly at the wooden support. *Chaetomium*, on the other hand, had been isolated from wooden support as well as from the pigments. Other fungi that occurred in less numbers were *Mucor*, *Cunninghamella* and *Rhizopus*. They had been found dispersed in different areas of the paintings.

Experiments in order to test the degree by which fungi use painting materials to support their growth (table 2) had shown some surprising results: *varnish* was the only painting material that totally inhibited the growth of the 6 fungal isolates representing different fungal genera such as *Aspergillus*, *Penicillium*, *Chaetomium*, *Cunninghamella*, *Mucor* and *Rhizopus*. The reason for this inhibition is due to its chemical property. Varnish which is usually composed of a resin called dammar forms a very thick protective film when varnish gradually dries out. This

makes the painting very impenetrable to fungi. *Canvas* was 10% higher in growth diameters compared with the control in supporting the growth of fungi. Its susceptibility to fungal infestation can be traced to its cellulose fiber component which is a complex polysaccharide but which can be enzymatically simplified into sugars and then serves as a source of energy for most fungi. But canvas is actually second only to Linseed oil in terms of degree of susceptibility. Linseed oil, with 32% higher growth diameter compared with the control appeared as the most susceptible of all painting materials to fungal attack. The mechanism behind it is that linseed oil being an organic compound which composed generally of unsaturated fatty acids is more easily assimilated as energy source by most fungi through enzymatic reactions (Agrawal et al., 1989). On the other hand, *pigments* in general showed various degrees of inhibition in the growth of fungi. On top of the list was Burnt umber, with 62% growth inhibition compared with the control. It is followed by Chinese white, with 50% inhibition compared with the control. The following is the overall arrangement of pigments according to decreasing capacity to inhibit the growth of fungal growths: Burnt umber > Chinese white > Crimson, Medium yellow > Yellow Ochre > Viridian hue > Brilliant red > Phthalo blue > Ultramarine > Ivory black > Lemon yellow > Sap green. This arrangement shows Sap green in the last position and therefore it means that it is most susceptible pigment to majority of fungi causing painting deterioration. This finding corroborates a similar study conducted by Winters et al. (1976). Statistically, however, using Anova 1 at .05% significance level, there was no significant difference among growth zones of different genera of fungal isolates in different painting materials

Furthermore, Petushkova and Lyalikova (1986) explained that fungi attack pigments by producing organic acids which dissolve the pigments. In the case of Sap green, the main reason behind is that it is made from a natural extract of ripe *Rhamnus* sp. The presence of low molecular weight sugars in the extract makes it easily degraded by enzymatic reaction in majority of the fungi. In contrast, Burnt umber's ability to resist the growth of most fungi is due to its chemical property wherein as Manganese dioxide it can react with fungi as enzyme inhibitor, thereby affecting the normal metabolic function of fungus cells which then lead to death. Likewise, Chinese white being chemically known as Zinc oxide (ZnO) and therefore contains a heavy metal, inhibits the growth of fungi by binding with the protein molecules of the cell particularly at sulfhydryl groups thereby causing denaturation.

Evaluation of the selected fungicides against the fungal isolates by Disk-diffusion Assay (table 3) had revealed that there had been in general a large zones of inhibition in both Preventol R-80 and Umonium-38 in all concentrations (from 1%-10%) against *Aspergillus* isolates. These large zones indicate "susceptibility" of the fungal isolate in both

Table 3: Average MIC and MLC in Mueller-Hinton broth of fungicides against fungal isolates^a

Fungal isolates	Fungicides and Concentration(%)				
	Preventol R-80	Umonium-38	Clotrimazole	Boric acid	Dithane M-45
Aspergillus	1.5 ^b	2.5 ^b	3.2 ^b	9.5 ^c	4.0 ^c
Penicillium	2.0 ^b	2.7 ^b	3.7 ^b	9.6 ^c	4.3 ^c
Chaetomium	2.3 ^b	3.1 ^b	4.1 ^b	9.8 ^c	4.7 ^c
Cunninghamella	2.2 ^b	3.1 ^b	3.4 ^b	9.3 ^c	4.5 ^c
Mucor	2.0 ^b	2.6 ^b	3.3 ^b	10.0 ^c	4.4 ^c
Rhizopus	1.8 ^b	2.6 ^b	3.3 ^b	9.7 ^c	4.1 ^c

^asince $p < .05$, then there are significant differences among MIC/MLC of different fungicides in fungal isolates

^a Based on 48 isolates

^b Minimum lethal concentration

^c Minimum inhibitory concentration

Preventol and Umonium. In contrast, the smaller zones of inhibition for both Clotrimazole and Boric acid, indicate high “resistance” of the isolates in the fungicides. Dithane M-45, on the other hand gave only “intermediate” susceptibility as a result of their fair values in zones of inhibition.

As indicated by very low zones of inhibition, susceptibility of *Penicillium* isolates were generally “highly resistant”. Similarly, both *Chaetomium sp.* and *Cunninghamella* also showed “high resistance” in all of the fungicides. A significant result showed that *Cunninghamella sp.* was only susceptible in Preventol R-80; while it exhibited resistance in the rest of the other fungicides. *Mucor* was resistant in all the five fungicides. *Rhizopus*, however, gave mixed reactions with 5 fungicides. It was susceptible with Preventol R-80, while it gave intermediate susceptibilities with Umonium, Clotrimazole and Boric acid. Finally, even in Dithane M-45, *Rhizopus* had also exhibited resistance.

In order to quantitatively determine the exact concentration in which fungicide would cause inhibition or lethality, further test was conducted through Mueller-Hinton broth method. More specifically, this method would determine the Minimum Inhibitory Concentration(MIC) or the Minimum Fungicidal Concentration (MFC). MIC is the lowest concentration of the fungicide that has no growth or turbidity with the test fungal isolates. On the other hand, MFC is obtained if the tubes showing no growth are sub-cultured into fresh Mueller-Hinton broth lacking fungicide; then the lowest fungicide concentration from which the fungal isolate does not recover and grows when transferred to fresh medium is the MFC (Prescott et al.,1999). Summarizing the significant findings (table 3), it was found out that those with FMC included Preventol R-80, Umonium-38, and Clotrimazole, respectively. On the other hand, those with MIC were the Boric acid and Dithane M-45.

Further analysis of the experiment results revealed that the fungicides could be arranged into the following according to increasing effectivity in eradicating and / or inhibiting the 6 genera of fungal isolates: Preventol R-80 > Umonium-38 > Clotrimazole > Dithane M-45 > Boric

acid. As the arrangement shows, Preventol R-80 with 2.1% mean MFC was the most effective of all the fungicides since it had the lowest concentration needed to eradicate all fungal isolates. Next to Preventol R-80 in effectivity were Umonium-38, with 2.8% mean MFC, then followed by Clotrimazole, with 3.6% mean MFC, and Dithane M-45, with 4.4% mean MIC. On the other hand, Boric acid, having 9.7% average MIC was the least effective of all test fungicides since it required the highest concentration in order to cause inhibition.

The fungicidal effects of Preventol, Umonium and Clotrimazole can be explained through their mechanism of action. Preventol R-80 (Benzalkonium chloride) inhibits growth by acting as surface active agents on cell membrane of the microorganism, thereby causing cell leakage (Ascaso et al., 2002). Likewise, Umonium-38 since it also contains benzalkonium chloride (0.32%), stops the growth of fungi by acting as surfactants which emulsifies lipids causing leaks in cell membranes. Furthermore, its two alcohol components namely, alcohol tridecyl (< 15%) and 2-propanol ceteth (< 15%) function to increase the penetrating power of the fungicide as well as to act on proteins by denaturation. According to Umonium product information brochure (2001) released by the Manufacturer (Huckert’s International) , studies conducted at Catholic University of Rome, Italy in 1998 and in Laboraco, Brussels Belgium, 1998 had proven the fungicidal efficacy of Umonium at 2.50% within 10 minutes and at 0.5% within 20 minutes for *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404). On the other hand, Dithane M-45 was found to be a weak fungicide even though its mechanism of action is also to inhibit growth by destroying sulfhydryl enzyme system of the fungi. It would take very high concentrations before Dithane M-45 would be able to penetrate the thick sporangiospores of the fungi. But compared with Dithane M-45, Boric acid showed the least in inhibiting the growth of fungi because chemically it is also a weak acid and therefore in order to cause toxicity in fungi would have to require also very high concentrations. But as in any acid, Boric acid inhibits growth by denaturing protein components of the fungi

particularly the enzyme system responsible for ATP production.

For experiments determining solubility of painting pigments with selected fungicides, the following were the significant findings: a) all the five fungicides namely, Preventol R-80, Umonium-38, Clotrimazole, Boric acid, and Dithane M-45 proved stable with the pigments of the two oil paintings up to 100% concentration for 3 minutes, and up to 24 hours extended exposure time. In other words, there were no observed chemical reactions as manifested by color change, dissolution, or precipitation when the painting pigments were exposed to drops of fungicide; b) however, Clotrimazole, Dithane and Boric acid had left a residue upon evaporation of their water solvent. Because of this, they may not be recommended for treatment of infested paintings since their residues might affect the aesthetic appearance of the painting. Both Preventol and Umonium provided no residues and therefore may be recommended for treating fungi infested paintings. But between the two fungicides, Preventol was much better to use for painting conservation because it was observed that even after 24 hours exposure the surface of the paintings still appeared cleaner and had increased luster or brilliance. It was also observed that the surface of the painting treated with Preventol R-80 still appeared slightly moist even after 24 hours exposure. This may be attributed to the hygroscopic property of the quarternary salt compound, which in a way could prove an advantage for the treated painting because it would mean extended time for its fungicidal activity. A similar study conducted by Koestler et al., (1991) had also confirmed the stability of quarternary salt fungicide with majority of the pigments used.

CONCLUSIONS

The present study provides step-by-step procedures in the conservation of paintings attacked by molds. The approach explored by the researcher is a chemical control. In the case of paintings which have been already attacked by molds, the best way to stop further the destruction or deterioration is to chemically treat them and at the same time have them quarantined to avoid further spread of the infection to other paintings or art works. But side by side with the treatment, it is also important that the environment should also be normalized. In other words, exerting effort in maintaining the environment with right temperature (between 20°C-25°C) and right humidity (between 45%-65%). Actually, this is just part of the so called Integrated Pest Management (IPM) wherein all aspects of the museum like facilities, environment, personnel, activities, and so on. are geared towards reducing if not totally eradicating common pest problems in the museum like the molds and the insects.

Furthermore, the researcher has found that the best fungicide to be used to treat these infested paintings is

Preventol R-80 at a concentration of at least 2.1%. Umonium-38 is next in efficacy and was proven to exhibit eradicated property at a minimum fungicidal concentration of 2.8%. Both of these fungicides were found to be stable with mock-paintings up to 24 hours exposure time.

Other significant findings in the study are as follows: it was found out that different painting materials have various degrees of susceptibility with fungal pests. Varnish, is the least susceptible, while linseed oil is the most easily attacked by molds. Canvas is next to linseed oil in susceptibility, while in general pigments inhibit the growth of most fungi.

The above findings have some important implications to the artists and people involved in art conservation and restoration. With regards to varnish, application of it on paintings particularly those that will be stocked for a long time can be part of the recommendation. For linseed oil, since it is normally used when painting is being made by the artist, mixing it with a certain amount of the best fungicide can protect the painting from future attack. In the case of pigments, the artist should avoid as much as possible using sap green or for an alternative instead. Using more of the pigments that have inhibitory property with fungi like Chinese white could avert possible fungal attack in the future.

Knowing the types of fungal damage in fungal infested paintings and isolating and identifying the culprit genera can guide conservators in determining the kind of fungicides to be used as well as how much of the fungicides should be applied. But to know which fungicide is the best in eradicating culprit fungi is not enough. Before it is even used, the fungicide should be able to pass first the solubility test with pigments or varnish of the painting. Because even though a fungicide may be excellent in eradicating fungi but if it is going to destroy the paintings, then it cannot also be recommended for conservation. Moreover, in performing solubility test one should not use the actual painting under study but should prepare or look for possible mock-paintings that can be used. This is in order to avoid possible problem of destroying the actual painting in case the fungicide reacts destructively with the painting materials.

Other things to consider in choosing the best fungicide are its availability, cost and toxicity. Of course it would be better if the fungicide is locally available, cheaper and less harmful for the restorer who is applying the fungicide including the people who might be exposed to the treated paintings like museum staff and visitors (Agrawal et al., 1989; Dawson, 1982; Agarossi et al., 1988; Caneva et al., 1991). A fungicide must also have the ability to remain effective for a long time. Although this is impossible to achieve because there are a lot of underlying factors that may reduce the efficacy of the fungicides as time passes by. Some of these factors however, include rate of evaporation, rate of absorption by painting materials,

effects of pollutants, and capacity of the organisms to neutralize the toxic effects of the fungicide. In fact, the present study has confirmed the ability of several fungal isolates to even use fungicide as a source of energy. Therefore, finding for a fungicide that would last long enough will always be a continuing pursuit and a challenge to every researcher.

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