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WASABI, A PROMISING ALTERNATIVE FOR THE BIODETERIORATION CONTROL OF THE EGYPTIAN ARCHAEOLOGICAL PAPYRI

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ABSTRACT

Wasabi (*Wasabia japonica*) is an edible plant containing different phytochemicals. It is very interesting to exploit its natural antimicrobial impacts in controlling the biodeterioration of organic artifacts such as papyrus. In this study, the authors investigated the *in vitro* antifungal activity of Wasabi towards several fungal species associated with the biodeterioration of archaeological papyri in the Grand Egyptian Museum-Conservation Center. The examined fungi showed antifungal activity with maximum activity against *Penicillium lanosum* III. The minimal inhibitory concentration ranged between 0.2 to 1.6% while the minimum fungicidal concentration increased to 2.5%. *In vivo* treatment studies were applied using thermally aged biodeteriorated papyrus samples. The microbiological test revealed that Wasabi can completely eradicate *Aspergillus flavus* and *P. lanosum* after three days of exposure. Finally, Fourier Transform Infrared Spectroscopy and Colorimeter were used to assess the treatment after short and long terms. These findings suggest that Wasabi can be useful sources of fungicidal preparations for the disinfection of biodeteriorated papyrus artifacts. Our research introduces the practical use of Wasabi for the first time in the conservation field, especially after confirming its long-term safety.

KEYWORDS: Wasabi, antifungal activity, biodeterioration, FTIR, colorimeter, archaeological papyrus.

1. INTRODUCTION

Wasabi belongs to the family Brassicaceae, its rhizome is a very popular pungent spice which is used in the Japanese cuisine. It arises naturally in Japan in mountain river valleys. Today, it also arises in some areas in New Zealand, North America, Korea, and China as it needs shady, cool, and humid atmosphere to grow. Previous studies show that Wasabi possesses several physiological roles, such as; appetite enhancement (Kojima, 1988), antimicrobial activity (Isshiki and Tokuoka, 1993), anticancer (Fuke et al., 1997; Yano et al., 2000; Nomura et al., 2005) and antiplatelet roles (Morimitsu et al., 2000). It is thought that Wasabi has a strong herbal medicinal impact, participating in the safety of eating raw fish and other foods (Yano et al., 2006). It differs from other Brassicaceae species in that it contains a higher concentration of isothiocyanate (ITCs).

ITCs are degradation products from glucosinolates (GLSs); secondary metabolites which are formed of amino acid, glucose, nitrogen and sulfur (Du and Halkier, 1998). GLSs are stored in the cell vacuole and come into contact with the myrosinase enzyme which is located in the cell wall or the cytoplasm during the damage of tissue (Magrath et al., 1994). Then, they are hydrolyzed into a number of products, where the ITC is quantitatively dominant. The GLSs degradation products possess biological activities that include fungicidal, herbicidal and nematocidal properties, which are beneficial effects on human health (Bonnesen et al., 1999; Lazzeri et al., 2004; Keum et al., 2005). Allyl isothiocyanate (AITC) reportedly possesses antimicrobial activity towards different varieties of microorganisms (Lin et al., 2000a, b; Masuda et al., 2001; Mari et al., 2002; Dufour et al., 2012; Lu et al., 2016).

The molds are considered as one of the most popular daily plagues that threaten archival collections and libraries that contain paper-based documents (Michaelsen et al., 2010). The fungi function under considerably wider climate limits, including normal museum conditions. Hence, they are considered as the most dangerous organisms for museum artifacts and archaeological collections. And since papyrus is an organic material, it is susceptible to microbiological degradation. Appropriate conservation treatments is required to deal with fungal problems include chemicals (Abdel-Kareem, 2010; Stupar et al., 2014), essential oils (Abad et al., 2007; Rakotonirainy et al., 2007; Othman et al., 2014) and physical methods (Michaelsen et al., 2013; Elamin et al., 2019).

Now there is an ample evidence for the antimicrobial properties of Wasabi, but reports of suppression of fungi are still limited and no information is acquired regarding its introduction in the conservation application or its safety towards the artifacts. Thus, it seems reasonable to explore the possibility of introducing the Wasabi for eradication of mold-infected papyri. Consequently, the aim of this study is to develop a new fungicide as an alternative for the synthetic chemical ones in the conservation application.

2. MATERIALS AND METHODS

2.1. *Tested Fungi and Preparation of Wasabi Extract*

Ten fungal species were chosen for this study (7 days-old fungal culture). These species were isolated from different bio-deteriorated archaeological papyri. These papyri belonged to the Egyptian Museum in Tahrir and El-Minya storages, before being transferred recently to the organic storage room of the Grand Egyptian Museum Conservation Center (GEM-CC).

A Wasabi powder (S&B Foods, Japan) was used for this study. The Wasabi extract was freshly prepared by adding distilled pure water to the powder until it turned into a dumpling-like state (60% w/v).

2.2. *Preparation of Papyrus Experimental Materials*

Papyrus samples of 30×15 mm underwent two steps of accelerated aging processes; the thermal aging process, followed by the induction of a microbial infection using Petri Dish Test Chamber to simulate papyrus artifacts. Since heating the samples for 72 hour at 120°C is regarded as an equivalent to about twenty-five-years of aging under normal conditions (Feller, 1994), the samples were kept in the oven at 120°C for 4 months, which can be simulated for 1000 years of aging.

Regarding induction of a microbial infection, Each Petri dish test chamber (150 in diameter×25 mm in height) contained four layers of blotting paper that was saturated with a 30 ml sterile distilled water and was covered with a polyethylene mesh spacer to elevate the samples. Thermally aged papyrus samples were then sprayed with a 1ml of individual mold inoculum. Each chamber was then sealed in polyethylene bag and incubated at 28±2°C for four months or until the appearance of any deterioration signs.

2.3. *In Vitro Antifungal Activity of Wasabi, Determination of its Minimum Inhibitory Concentration (MIC) and its Minimum Fungicidal Concentration (MFC)*

The systematic evaluation of the antifungal activity of the Wasabi extract (2%) was carried out using the inverted Petri dish technique (Kienholz, 1959;

Pandey et al., 1982). The antifungal activity is reported as the inhibition ratio (IR) and calculated according to the equation set by Ikeura and Kobayashi (2015).

IR (%) = [diameter of inhibition circle (mm)/diameter of plate (100 mm)] × 100

Also the degree of sporulation of each fungus was used to evaluate the antifungal activity qualitatively.

Concerning MIC, Wasabi extract in concentration (0.2-3.0%) was used to determine the MIC value for each of the tested fungus using the inverted Petri dish technique. The MFC was regarded as the lowest concentration that did not show any fungal growth after removing the antimicrobial agent. It was determined by taking the plates that exhibited no fungal growth in the MIC experiment and replace the cover lid that contained Wasabi by other sterilized one under septic condition. The plates with the new cover were then incubated for 2-4 weeks.

2.4. *In Vivo Treatment*

It Started with vacuum cleaning (Guild and MacDonald, 2004) where the contaminated specimens were set in a fume hood surrounding with silica gel and kept overnight for drying. Vacuum cleaning was carried out with a vacuum cleaner provided with a HEPA filter. Then, vapour exposure treatment was followed the vacuum cleaning as each contaminated specimen was put in a closed glass Petri dish (12 cm in diameter). Three different concentrations of Wasabi extract were used based on the concentration that was recommended by the MFC experiment (one concentration before and one after). The Wasabi was then placed on an aluminum foil paper next to the specimen. The mold growth on the Specimens was examined at 0-72 hours. The percentage of the fungal reduction (R) was determined according to the following equation:

$$R(\%) = \frac{B - A}{B} * 100$$

Where A and B represent the number of colony forming unit after and before treatment, respectively

2.5. *Evaluation of Treatment Process*

It was evaluated using colorimetric and Fourier Transform Infrared Spectroscopy (FTIR) Analyses. The colors of all the examined samples were determined by spectrophotometry (CM-700d, Konica-Minolta). The CIEL*a*b* color coordinates, L (lightness), a (red/green axis), and b (yellow/blue axis), for the samples were detected to compare the color changes before and after treatment using Wasabi. The color changes were calculated and expressed as ΔL, Δa and Δb, and a calculation of the total color change (ΔE*ab) was done according to Normal 43/93 (1994) using the equation of Abdel-Kareem (2005).

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

The ΔE results were interpreted according to the scale (Drzewinska, 2002):

- ΔE <1.00 the difference unnoticeable
- 1.00<ΔE <2.00 very small difference, noticeable to an experienced observer only
- 2.00<ΔE <3.50 medium difference, noticeable to an experienced observer only
- 3.50<ΔE <5.00 significant difference
- ΔE >5.00 very prominent difference

The chemical structural change in the treated surface of the specimens was estimated by using FTIR, model Shimadzu IR Prestige 21. The FTIR light source is used in the middle range infrared (4000-400 cm^{-1}).

Regarding the evaluation of the long-term effect of Wasabi, the treated papyrus samples were divided into two groups. The first was subjected to accelerating thermal aging that simulated a 100 year of aging to detect the changes that may happen. The other group was preserved in control environment for one year. The changes happened to the first group were evaluated using colorimetric and FTIR analyses, while the microbiological analysis for the second group was made to determine the possibility of fungal recovery after one year.

3. RESULTS AND DISCUSSION

3.1. *In Vitro Antifungal Activity of Wasabi, Its MIC and MFC*

All of the examined fungi had antifungal activity as shown in Table 1. Based on the degree of sporulation, Wasabi was found to possess the ability of decreasing the sporulation of all the tested fungi in which *Penicillium purpurogenum* showed the weakest sporulation. The maximum value of the inhibition ratio was recorded by *P. lanosum* III (100%) followed by *Aspergillus niger*. The AITC is well known for its strong antimicrobial activity (Dufour et al., 2012; Lu et al., 2016). This activity towards the cheese-related fungi as *A. flavus*, *Penicillium commune*, *P. notatum* and *Geotrichum candidum* was also investigated by Winther and Nielsen (2006); Tunc et al. (2007). The antimicrobial mode of action of ITCs is a result of binding to sulfhydryl groups present in the active sites of enzymes responsible for the growth and survival of microbes. The reduction in the cellular levels of important thiol groups consequently happened, leading to the formation of oxygen and other free-radicals (Jacob and Anwar, 2008; Aires et al., 2009). Moreover, the ITC could cause damage to cell membrane which leads to cellular metabolites leakage (Lin et al., 2000b).

Results of MIC and MFC showed that Wasabi could be fungistatic or fungicidal depending on the

concentration of Wasabi. However, *A. flavus* showed the same MIC and MFC (1.6%). Our result was in agreement with that obtained by Irkin and Esmer (2015) who reported that AITC could be fungicidal and fungistatic depending on the concentration of AITC and the number of fungus spores. Also we found that the MFC differs with different species where *Penicillium* sp. and *Ulocladium* sp. needed higher concentration for complete eradication (2.5%) while *P. purpurogenum*, *P. simplicisimum* and *A. ochraceous* needed lower one. The MIC of Wasabi towards *Staphylococcus aureus* or *Escherichia coli* was 1%. However, 4% of the Wasabi showed higher bactericidal activity towards *S. aureus* than *E. coli* (Lu et al., 2016).

Table 1. In vitro antifungal activity of Wasabi, Its MIC and MFC

No.	Tested fungi	IR[a] (%)	DS[b]	MIC	MFC
1	*Penicillium purpurogenum*	0	+1	0.4	1
2	*P. simplicisimum*	0	+3	0.8	1
3	*P. lanosum* I	5	+1	0.4	2
4	*P. lanosum* II	12	+3	0.8	1.5
5	*P. lanosum* III	100	0	0.2	0.8
6	*Penicillium* sp.	0	+4	1.5	2.5
7	*Aspergillus flavus*	0	+4	1.5	1.5
8	*A. niger*	20	+3	0.4	2
9	*A. ochraceous*	0	+2	0.4	1
10	*Ulocladium* sp.	8	+3	1.5	2.5

[a]IR (%): Inhibition ratio = [diameter of inhibition circle (mm)/diameter of plate (100 mm)] × 100
[b] DS: Degree of sporulation: + 5 maximum sporulation, 0 No sporulation

It is important to mention that the Wasabi extract should be prepared just before treatment, as it starts to lose its activity once it becomes moist. The addition of water activates the hydrolysis reaction of GLSs that are present in the Wasabi using myrosinase enzyme. AITC is one of GLSs hydrolysis products which is responsible for the antifungal activity and is evaporated on adding water.

3.2. *In Vivo Treatment (Dose and Duration Assessment for Treatment of Biodeteriorated Papyrus Samples)*

The ability of Wasabi to decontaminate papyrus samples was determined *in vivo* against thermally aged, biodeteriorated papyrus samples. The dried papyrus samples (Fig. 1) were first subjected to vacuum cleaning to reduce the number of mold spores (Guild and MacDonald, 2004) and consequently reduce the required dose for treatment.

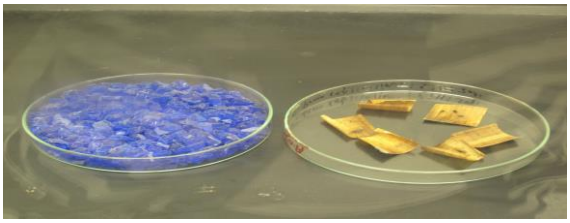

Figure 1. Drying of biodeteriorated papyrus samples using silica gel.

Papyrus samples were then treated using the vapor exposure method to investigate the effectiveness of Wasabi. After 72 hour of application, the fungal growth was completely eradicated by the three tested concentrations for each fungal species (Fig. 2 A and B). It was clear that the increase in the duration of application was preferable than the increase in concentration. Goncalves et al. (2009) reported that the AITC sachets were effective in reducing the mold and yeast counts after 7 days in cottage cheese. Ethyl isothiocyanate (EITC), AITC and their combination decreased the fungal infection by more than 85% after 3-4 days of apple incubation (Wu et al., 2011). Moreover, the treatment of *Penicillium* sp. and *A. flavus* infected papyrus samples with thyme or lemon grass oils needed a long period, reaching two weeks as mentioned by Othman et al. (2014).

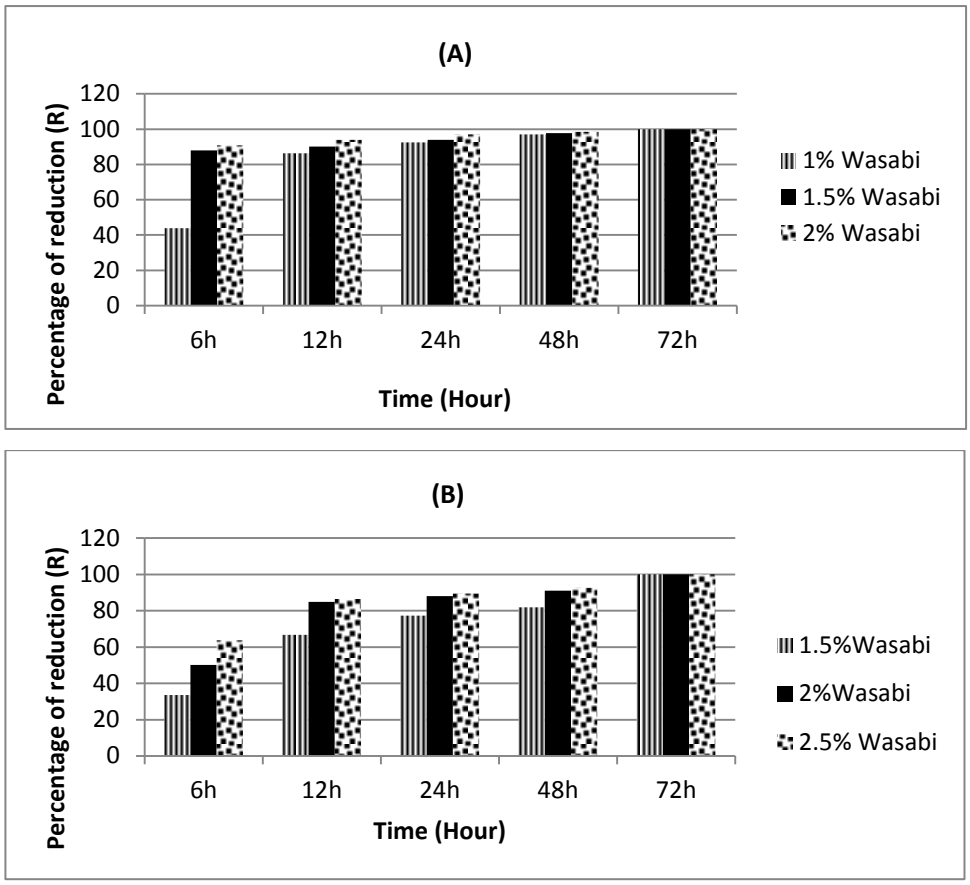

Figure2. Dose and Duration assessment for treatment of infected papyrus samples from A. flavus (A) and P. lanosum (B).

3.2.1. Evaluation of Treatment Process after Short and Long -Term Preservation

3.2.1.1. Colorimetry

Wasabi treatment caused invisible changes in the optical properties in papyrus samples either infected by *A. flavus* or *P. lanosum* (ΔE <1.00), however, after long-term treatment a very small differences were observed only for an experienced observer (1.00<ΔE <2.00) as shown in Table 2. This indicates that Wasabi can keep the color of papyrus significantly, unchanged for at least 100 years. The same experiment was done by Othman et al. (2014) and it was found that a moderate difference in the ΔE*ab values was detected for *A. flavus* and *Penicillium* sp. infected papyrus samples after being treated with thyme and lemon grass oils.

Table 2. Color changes of A. flavus and P. lanosum infected papyrus samples after short long term treatment

Color change	*A. flavus* (1%)				*P. lanosum* (1.5%)			
	ΔL	Δa	Δb	ΔE	ΔL	Δa	Δb	ΔE
Short term	0.75	0.06	0.48	0.89	0.82	0.07	0.44	0.93
Long term	1.4	0.45	1.25	1.93	1.38	0.40	1.25	1.90

3.2.1.2. FTIR analysis

The chemical modification induced in papyrus samples after Wasabi treatment was investigated by FTIR spectroscopy (Fig. 3). Because of the cellulosic inherent property in papyrus, the characteristic peaks for papyrus were detected as follows; those at 3433cm^{-1} and 2918 cm^{-1} could be attributed to O-H and C-H stretching, respectively, while at 1427 cm^{-1} was corresponded to C-H bending (lignin and cellulose). The peaks at 1375, 1157 cm^{-1} attributed to characteristic bands of carbohydrate were observed. By comparing the spectral bands of infected papyrus sample with those after short and long terms treatment we found that there is no significant structural changes happened to papyrus samples even after long terms indicating that Wasabi could keep papyrus unchanged for at least 100 years.

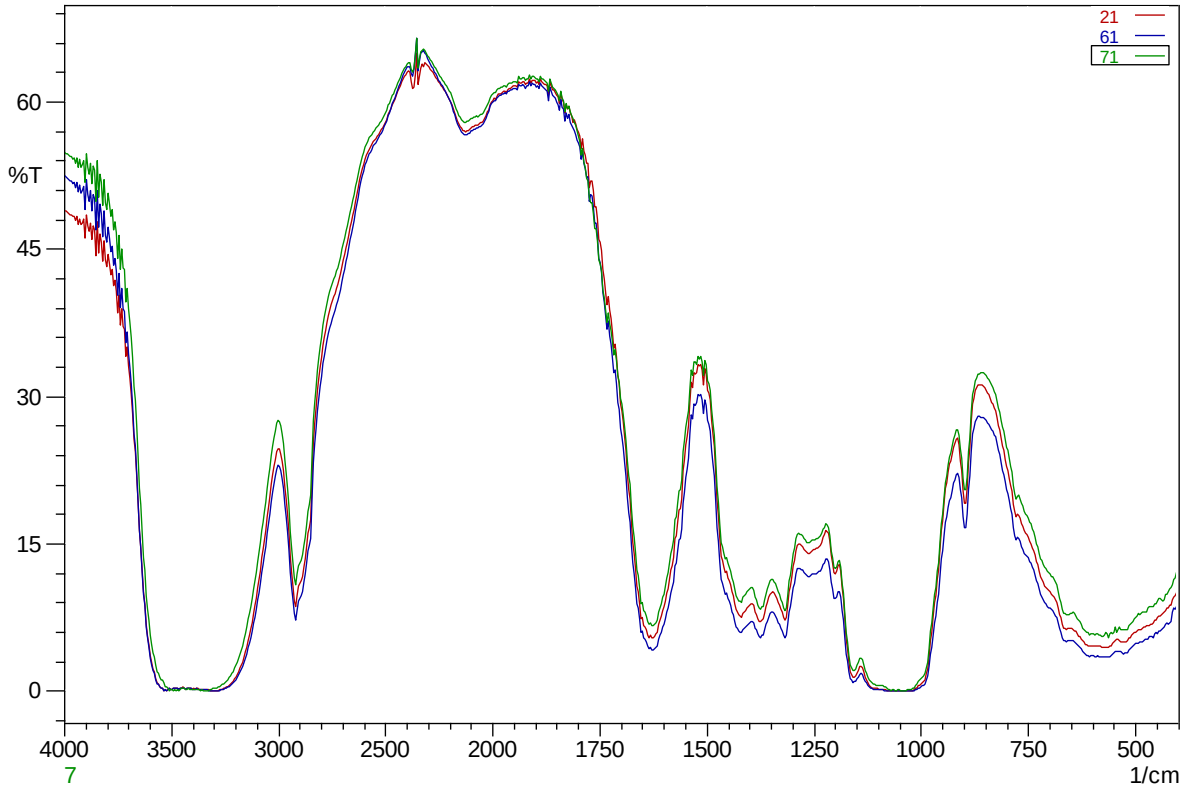

Figure 3. FTIR Spectra (red color) control infected papyrus, (blue) after short term Wasabi treatment (green) after long term Wasabi treatment.

3.2.1.3. Microbiological Test

The percentage of the recovery of fungi after one year from treatment was found to be zero. This confirms the ability of wasabi to keep papyrus healthy for at least 100 year provided that the temperature and RH were controlled.

4. CONCLUSIONS

The results of this search could help in explore a novel natural product; Wasabi, and ultimately contribute to the conservation field, which will provide benefits to human, artifacts, environment and the whole society. The results of this study may form the basis of further investigations to cover the safety toward painted papyrus as well as other artifact materials. To our knowledge, this is the first report on the introduction of Wasabi as fungicidal material in conservation filed.

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