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Efficacy of Different Vinegars as Antifungal Agents Against *Aspergillus-niger.*

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ABSTRACT: *Natural sources have caught the scientist's eye for the development of new active substances because of the side effects of chemically synthetic preservatives and their ultimate hazardous effects on human health. In the present study, three types of vinegars; white distilled vinegar, grape vinegar and apple cider have been used to test the antifungal activity against Aspergillus niger. For the isolation of the fungal strain, lemon was used. Microscopy was performed for identification of fungus followed by application of vinegars through agar well diffusion assay technique. All kinds of vinegar showed a significant size of zone of the inhibition against fungi. Among all these vinegars, apple cider showed the best effect against A. niger forming zone of inhibition of 0.7±0.05 mm. While, grape vinegar and white distilled vinegar forms a zone of inhibition of 0.3±0.05 mm and 0.45±0.05 mm respectively. The results showed that vinegars can be used as an alternative to synthetic antifungal agents in order to overcome the drawbacks because they have active components i.e. phenolics and flavonoids etc. which have antifungal and anti-oxidant properties.*

Key words: *Aspergillus niger, preservatives, synthetic vinegars, lemon.*

INTRODUCTION

A large number of preservation techniques are being developed to preserve the food and to satisfy the consumer with regard to nutritional and other aspects of food. In order to destroy the vegetative microorganisms, foods are thermally processed by giving a temperature of about 60 to 100°C for just a few seconds. Due to the transfer of heat, energy is transferred to food. This energy may induce unwanted reactions, leading to undesirable organoleptic and nutritional effects (Vega-Mercado et al.,1997). To ensure food safety and fulfill the demands of nutrition and quality has resulted in increased interest in preservation techniques. These preservation techniques were used to inactivate the microorganisms and their enzymes in food. Quality of food may include

flavor, color, odor, texture and other nutritional value. The increasing demand for the quality products may attribute towards the use of natural preservatives rather than chemically synthesized preservatives. These natural preservatives are derived from plants, animals and micro flora (Hugas et al., 1995). Antimicrobials enhance the shelf life of food by inhibiting or reducing the bacterial growth rate and the viability rate. Antimicrobials including spices and herbs may add to improve or change the taste of food (Beuchat andGolden, 1989).

The *Aspergilla, Aspergillus* section *nigri* (David et al., 1985) includes species with uniseriate or biseriate conidial heads, vesicles are spherical to pyriform, smooth-walled stipes are present and conidia of *A. niger* are black or near black-colored. This group of fungi contains about 26 species. The most common species is *A. niger.* It is worldwide in its distribution and can be isolated from all continents and from every environmental condition. Other species of *Aspergillus* that have been related to human and animal infection include *A. acidus, A. aculeatus, A. brasiliensis* and *A. tubingensis.* The most common species of *Aspergillus* is *A. niger* and it is worldwide in its distribution. It is found in almost every environmental condition (Geiseret al., 2007).

The conidia of *A. niger* are white to yellow in color at first which becoming black in color later on. This species is commonly found in *Aspergillomas* and is the most frequently encountered agent of *Otomycosis.* It is also a common laboratory contaminant. Colonies are white or yellow and are covered by the thick or dark layer of black conidial heads. The conidial heads are large in size and the diameter is about 3 mm by 15 to 20 µm (Geiseret al., 2007). Infections of *A. niger*are severe enough to cause mortality and morbidity. This species is reported as a source of pneumonia (Person et al.,2010).

Different natural products including plant extracts, herbs, spices, oils, vinegars, garlic extracts and certain foods contain such substances that possess the antimicrobial activity (Shethet al., 1988). Among the different natural products, vinegars are also contributing to sanitizing properties. Vinegar is also known as acetic acid and plays a major role as fungicide. Vinegar is an acidic liquid. It is made by the process of fermentation from alcoholic beverages. The most widely used alcoholic beverages are wine (Nascimento et al., 2003). Vinegars have the antioxidant activity, antitumor, anti-glycemic and antihypertensive effects (Kanaokaet al., 2007). For example, in white distilled vinegar, certain nutrients have been added, which shows the antimicrobial activity. This vinegar is made from pure alcohol and is diluted with water. White distilled vinegar is the most commonly used vinegar in industries (Young,1974).

Wine vinegar is also extensively used as acidifying and food preservative agent and it is mainly produced in Mediterranean countries (Tesfaye et al., 2002).

Among the vinegars, apple cider has an important element; Maleic acid with bactericidal and fungicidal activity. Maleic acid has been reported for the treatment of denture stomatitis (Boffo et al., 2006).Apple cider vinegar contains polyphenolic compounds and these compounds have healthy effects on human health (Budak et al., 2011).

Although vinegar has been traditionally used as a food flavoring and preservative, recent investigations demonstrate the potent bioactive effects of vinegars which may benefit human health. Agar well diffusion method is the most widely

used method for evaluating the antimicrobial activity of plants or microbial extracts.

MATERIALS AND METHODS

Isolation and culturing of fungus:

For isolation, potato dextrose agar (containing 3.9g PDA in 100ml) was prepared and the medium was sterilized. A small piece of rotten lemon was taken and was inoculated in the center of the Petri plate. Then, the plate was incubated at 37°C for 72 hours.

Identification:

Microscopy was done in order to identify the isolated fungus. For this purpose, the fungal smear was made and stained with the help of crystal violet stain. Then, the strain was observed under the microscope.

Agar-well Diffusion Method:

The antimicrobials present in the natural antifungal (vinegars) are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition are mostly circular in shape as there is a confluent lawn of growth. The diameter of the zone of inhibition is measured in millimeters. PDA plates were prepared and the wells were made in the center of each plate. Using a sterile swab, all the plates were swabbed. 100 µl of each of the vinegar i.e. apple cider, white distilled vinegar and grape vinegar, was pipetted out in each of the respective wells. The vinegars were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The plates were then incubated at 37°C for 72 hours. After 72 hours, there appeared a clear zone of inhibition. The diameter of zone of inhibition was measured in millimeters with the help of the Vernier caliper and diameter of each zone of inhibition was measured from three different directions. The mean values of zone of inhibition are mentioned in results.

RESULTS

In the present study, fungal species was isolated and identified based on their colony morphological characteristics, color charts and microscopic examination from Lahore Garrison University, Laboratory. The isolated fungal species was *A. niger*.

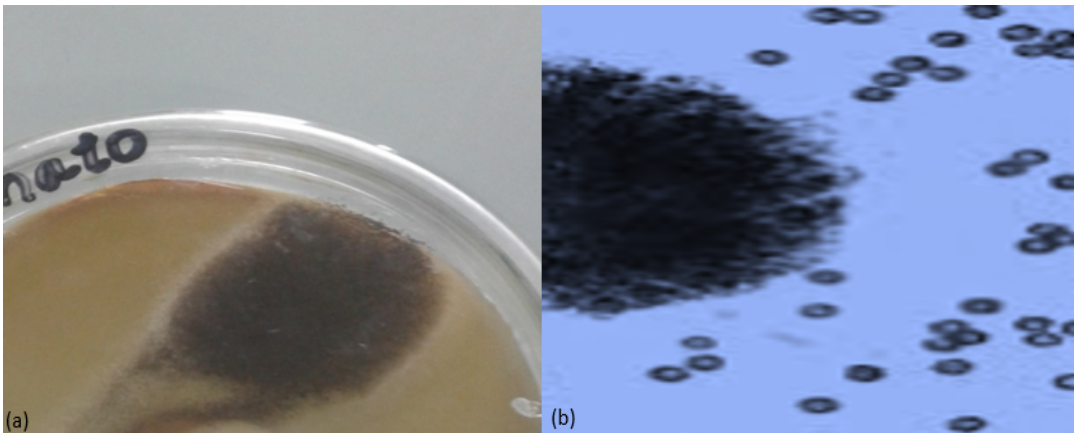

Fig. 1: (a) Morphological examination of *A. niger* (b) Microscopic examination of *A. niger*

Table 1: Microscopic identification of *Aspergillus niger*

Fungus	Stipes color	Texture of conidia	Diameter of Conidia (µm)	Shape of conidia	Conidia surface
Aspergillus niger	Slightly brown	Wrinkled	3-5	Globular	Very rough, irregular

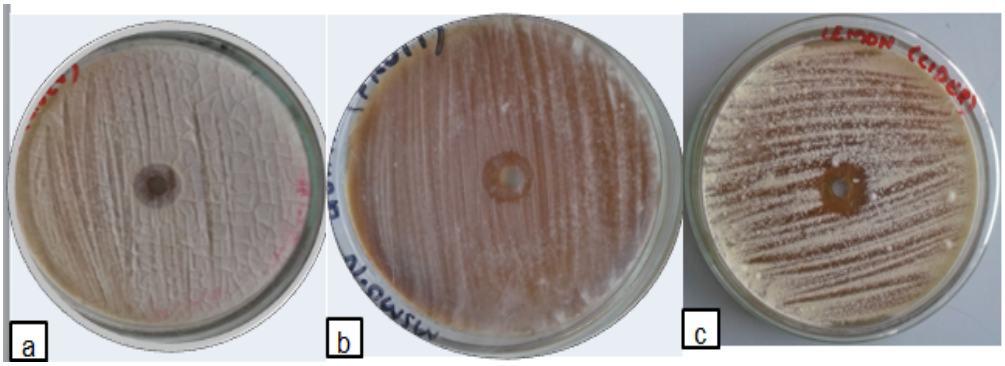

Fig. 2: (a) White vinegar showed the activity against *A. niger* by forming zone of inhibition of 0.4±0.05 mm after 72 hours. (b) Grape vinegar showed activity against *A. niger* by forming zone of inhibition of 0.2±0.05 mm after 72 hours. (c) Apple cider vinegar showed antifungal activity against *A. niger* by forming zone of inhibition of 0.7±0.05 mm after 72 hours.

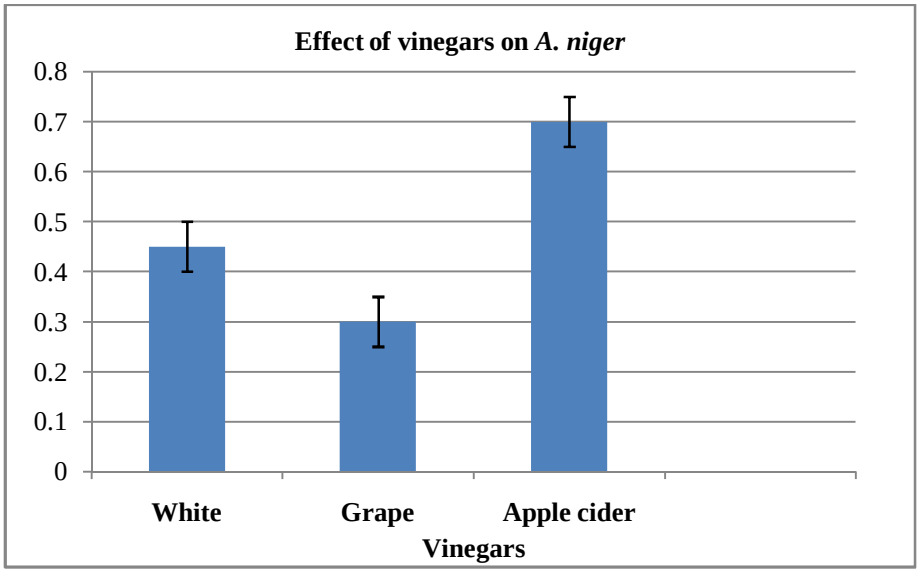

Fig. 3: showing the zones of inhibition formed by vinegars against *A. niger*. Apple cider has the best effect against fungal species showing zone of inhibition of 0.7±0.05 mm.

DISCUSSION

Vinegars have been used as antifungals from ancient times to preserve food from fungal and bacterial pathogens. The key component in vinegar is acetic acid which has antifungal activity. The use of vinegar to cure infections is reported since the time of Hippocrates. A combination of honey and vinegar was also used to treat cough in ancient times. This study is also conducted to evaluate antifungal activity of different types of vinegars. Vinegars are used against fungus in various applications which have been demonstrated to prevent the germination of conidia of vegetable decaying fungi. Vinegar has shown antifungal properties against conidia of fungi growing on fruits. Hence it is a known antimicrobial agent and there is evidence to suggest that is has some antifungal properties (Sholberg et al., 2000).

In the present study, *A. niger* has been isolated from the vegetable source i.e. rotten lemon. Three types of vinegars named distilled white, grape and apple cider have been utilized against the growth of fungus to test their antifungal potential. Vinegars showed significant zone of inhibition of different diameters against fungal species indicating their effectiveness.

They have antioxidant (Buonocore et al., 2010), antidiabetic (Salbe et al., 2009) and antitumor effects. Previous studies have shown that vinegars could be used to reduce or completely inhibit the microbial growth on fruits and vegetables (Wu et al., 2000). In the present study, *A. niger* was more sensitive towards the apple cider with the zone of inhibition of 0.7±0.05 mm. The rest of the two also show the significant zones with the mean values of 0.45±0.05 mm and 0.3±0.05 mm.

The best effect of Apple cider is due to the presence of phenolic compounds like Gallic acid, catechin, epicatechin, chlorogenic acid, caffeic acid, and p-coumaric acid which contain antioxidant and antimicrobial properties (Budak et al., 2011). In the previous study, the antifungal activity of apple cider vinegar was tested at different concentrations. Apple cider vinegar exhibited fungistatic activity when tested at MICx4 (Arneborg et al., 2000;Guldfeldt et al., 1998). White vinegar has the ability to reduce the blue mold decay in apples. White vinegar is much more effective than balsamic vinegar (Peter et al., 2000).

CONCLUSION

Although, traditionally vinegars have been used as a food flavoring and preservative agents, but recent studies have shown that the active components present in vinegars are beneficial for human health as well e.g. maleic acid, phenolics and flavonoids are antifungals as well as antioxidants. In the present study, three different types of vinegars are used against fungi namely *A. niger*. The resultant antifungal activity of vinegars showed that they could be used as an alternative to synthetic antifungal agents. Phenolic compounds in vinegar can scavenge superoxide anion and free radicals in vivo resulting in a potent antioxidant activity.

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