Antifungal Effect of Aloe Vera Gel on Penicillium Citrinum in Culture Media and UF Cheese

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***Abstract*—Inhibitory effect of natural ingredients such as essential oils and plant extracts against fungal activity was evaluated on several researches. *Aloe vera* gel contains a blend of carbohydrates (polysaccharides), glycoprotein (enzymes) as well as a variety of nutrients, vitamins and minerals and has antimicrobial, anti-fungal and anti-oxidant properties. In this study, the effect of *Aloe vera* gel at concentrations of 0.5%, 1%, 2%, 5%, 10% and 15% on inhibition of *Penicillium citrinum* (PTCC 5304) growth in culture media and UF cheese was investigated. The maximum percentage of mold growth inhibition on UF cheese at 15% concentration was 37.3%.**

***Index Terms*—*aloe vera* gel, *penicillium citrinum*, antifungal, UF cheese**

I. Introduction

Cheese is one of the most important milk products, which has a specific value in human nutrition. One third of the milk in the world is used to produce cheese [1]. Ultrafiltrated white cheese is one of the most consumed types of cheese in Iran [2] and is produced by ultrafiltration of pasteurized (72°C, 15 seconds) cow's milk with five times higher concentration and addition of mesophilic lactic starter bacteria and rennet [3]. Other characteristics of this type of cheese are: at least 34% (w/w) dry matter, 2 to 4 percent salt and pH 4.6-4.8. UF cheese according to this recipe after a short period of ripening at 27 ℃ for 1 day and maintenance at 8 ℃ for 1 to 2 weeks can be supplied to the market [4].

The presence and growth of fungi on food can reduce the quantity and quality of food [5], [6]. The growth of fungi can also cause risks to human health because some species of fungi are able to produce mycotoxins [7], [8]. Mycotoxins are compounds that originate from different species of secondary metabolites of fungi and they can contaminate foods and cereals with adverse effects on animals and humans [9], [10]. Penicillium species are one of the main causes of cheese pollution [11]. Penicillium species isolated from cheese are identified by producing some types of mycotoxins [12]. *Citrinin* is a secondary toxic metabolite that was isolated from *Penicillium citrinum* for the first time. *Citrinin* has a nephrotoxic property [5]-[13]. Treatments such as the use of chemical preservatives: sorbates, propionate and natamycin have been applied as mold inhibitors in cheese [14]. Because of public awareness of carcinogenicitic and teratogenicitic side effects of chemical preservatives used in foods, the demand for healthy foods with fresh ingredients, or at least less processed foods have risen. This has caused a lot of research for the purpose of substitution of chemical preservatives by adding natural compounds to prevent fungal growth and toxin production [15]-[17]. *Aloe vera* is a perennial herb belonging to the family of Liliaceous [18]. All species of Aloe have gel including different polysaccharides, but commercial use of this gel is limited to species of *Aloe arborenseans, Aloe ferox* and *Aloe vera* which the latteris more widespread than others in the world. *Aloe vera* is used as a source of functional ingredient in food industry. *Aloe vera* gel contains 75 nutrients and 200 active compounds, 20 minerals and 12 vitamins [19]. It also contains a variety of enzymes, sugars, lignin, anthraquinone, saponins, amino acids and salicylic acid [20]. The results showed that *A. vera* gel can control the growth of *Fusarium oxysporum* [21]. Several studies have shown that the hydroalcoholic extract of fresh leaves have inhibitory effect on growth of *Botrytis gladiolorum*, *Fusarium oxysporum*, *Heterosporium pruneti* and *Penicillium gladioli* [22]. Jasso *et al.* (2005) investigated antifungal activity of Aloe vera pulp on the development of mycelium of *Rhizopus solani*, *Fusarium oxysporum* and *Colletotrichum coccodes* and found positive results. [23] Yoltana and Golan (1955) experimented on the antifungal activity of natural aloe gel beyond the four phytopathogenic fungi including *Penicillium digitatum*, *Penicillium expansum*, *Botrytis cinerea* and *Alternaria alternata*. The results showed that

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the natural gel suppressed the growth of the fungus *Alternaria alternate* and *Penicillium digitatum*. [24] Coopoosamy and Magwa (2007) proved that *Aloe vera* has an antifungal effect on *Aspergillus glaucus*, *Candida tropicalis*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. [25] Cock (2008) showed the inhibitory effect of gel on *Aspergillus niger* [26]. According to Arunkumar and Muthuselvam (2009), the maximum antifungal activity of *Aloe vera* was witnessed in acetone extract on *Aspergillus niger* and *Aspergillus flavus* [27]. Navarro *et al*. (2011) examined the effects of *Aloe vera* gel alone and with thymol on reduction of nectarine decay due to *Botrytis cinerea* and *Rhizopus stolonifer* [28]. The results showed that *Aloe vera* gel alone and with thymol can be used as anatural antifungal agentinstead of synthetic fungicides. The purpose of this study was to evaluate the antifungal effect of *Aloe vera* gel on *Penicillium citrinum* in laboratory conditions and in UF cheese.

II. Methodology

A. Aloe Vera Gel

4-Years *Aloe vera* plants from Golazin agro-industrial complex in Shahriar, Tehran, Iran were used in order to extract fresh gel. Each time, roughly 0.5 to 1 hour before the experiment, leaves were picked and kept in laboratory sterile conditions.

B. Preparation of Conidial Suspension

The mildew fungus used in this study was *Penicillium citrinum* (PTCC 5304) obtained from fungal collection center of Iranian Research Organization for Science and Technology (IROST). It was activated by culturing on potato dextrose agar (PDA, Merck, Germany) slope for 10 days at 26 ℃. Then, conidia were harvested by adding 10ml 0.85% saline solution to cultureand gently scraping the mycelia with a sterile inoculating loop to free spores. Afterwards, it was filtered through sterile glass wool to remove mycelial fragments. The number of spores was determined by a haemocytometer and adjusted by 0.85% saline solution to final concentration of 10^6 spores per ml.

C. Antifungal Effect of Aloe Vera Gel in PDA Medium

Antifungal effect of *Aloe vera* gel on cheese spoilage fungi (*Penicillium citrinum*) was examined by mixing the gel with PDA medium. The aloe gel extracted from the leaves under sterile condition and transferred to the mixer (the Bellagio brand) and mixed at maximum speed for 6 min. Concentrations of 2%, 5%, 10%, 20% and 30% of extracted gel was added to the flask containing sterile PDA medium and mixed thoroughly. Then, they were distributed in the Petri dishes. PDA plates without gel as control were taken along the treated samples. After solidification of the medium, Whatman No. 1 filter papers with 5mm diameter were placed in the center of each Petri dish. 5 µl of spore suspension was inoculated on it and then incubated for 10 days at 26 ℃. The experiment was repeated three times for each treatment. To obtain the percentage of mold growth inhibiting by *Aloe vera* gel, the following equation (equation 1) was used:

$$Inhibitionofgrowth(\%) = \frac{Dc-Ds}{Dc} \times 100 \qquad (1)$$

Dc = mean diameter of colonies in the control sample.
Ds = mean diameter of colonies in treated samples

D. Antifungal Effect of Aloe Vera Gel in Cheese:

UF Feta cheese was prepared by adding different concentrations of *Aloe vera* gel (0.5, 1, 2, 5, 10 and 15%) in Pakara factory Sanandaj, Iran. After maturation time, it was aseptically removed from the package and placed on sterile piece of aluminum foil, then cut in to pieces proportional to the diameter of the plates. For each concentration, 25 to 30 plates were prepared. Then, 3 µl of spore suspension (10^6 spores per ml) was inoculated in the center of cheese in each plate. Plates were incubated for 10 days at 26 ℃.

III. Results

A. The Effect of Different Concentrations of Aloe Vera Gel on Penicillium Citrinum in PDA Medium

Table I illustrates the effect of different concentrations of *Aloe vera* gel on the mean diameter and percent of growth inhibition of *Penicillium citrinum* in PDA medium.

TABLE I. Percent of Growth Inhibition of *Penicillium Citrinum* by *Aloe Vera* Gel in PDA Medium

Concentration of aloe Vera gel (%)	Colony diameter (cm)	Percentage of inhibition
control	4.1[a]	0
2	3.76[ab]	8.2926
5	3.63[b]	11.4634
10	3.517[b]	14.2195
20	2.93[c]	28.5365
30	2.636[c]	35.7073

In each column means with different letters are significant at 5% level according to LSD.

On the tenth day of measurement, treatment with 2% did not show a significant difference from treatments of 5% and 10%. Treatments 20 and 30% had a similar effect. *Penicillium citrinum* colony growth on control treatment was more than other treatments. In treatments of 20% and 30%, the lowest growth was observed. The percentage of inhibition at the highest concentration of gel (30%) was 35.7%.

B. The Effect of Different Concentrations of Aloe Vera Gel on Penicillium Citrinum in UF Cheese

The percentage of fungal growth inhibition was determined according to (1). The results are presented in Table II.

The comparison of the mean diameter of *Penicillium citrinum* with LSD test at tenth day of measurement (Table II) showed that treatment with 1% were not statistically different from 0.5% and 2% treatments.

Control treatments, 5%, 10% and 15% showed significant difference. The growth of *Penicillium citrinum* colony on UF cheese was more in the control group and less than others in 15% treatment. The percentage of inhibition at the highest concentration of gel (15%) was 37.3%. The study also showed that 100% inhibition of growth could not be achieved.

TABLE II. PERCENT OF GROWTH INHIBITION OF *PENICILLIUM CITRINUM* BY *ALOE VERA* GEL IN UF CHEESE

Aloe vera Concentration (%)	Colony Diameter (cm)	Percentage of Inhibition
Control	3.48[a]	0
0.5	3.25[b]	6.7528
1	3.16[bc]	9.339
2	3.04[c]	12.7873
5	2.85[d]	18.2471
10	2.38[e]	31.6091
15	2.18[f]	37.3563

In each column means with different letters are significant at 5% level according to LSD.

IV. DISCUSSION

The effects of plant antifungal compounds have been investigated for many years and antifungal effects of these compounds have been demonstrated in many studies. The results of this study showed the effect of *Aloe vera* gel on reduction of *Penicillium citrinum* growth. The extent of this effect depends on the concentration of the gel so that higher concentrations have a greater impact on reducing mold growth. Navarro *et al.* (2011), concluded that *Aloe vera* gel as a coating on two varieties of nectarines, could be a natural antifungal compounds against some fungi and a suitable substitute for synthetic fungicides.

Castillo *et al.* (2010) examined the different concentrations of *Aloe vera* gel to inhibit the growth of fungi responsible for the decay of fruits including *Botrytis cinerea* and *Penicillium digitatum*. For both fungi, mycelium growth inhibition was observed with increasing the concentration of *Aloe vera* gel. [29]

V. CONCLUSION

The inhibitory effect of *Aloe vera* gel on the growth of *Penicillium citrinum* in PDA medium and UF cheese was investigated. With increase in the concentration of *Aloe vera* gel, the mold growth was significantly reduced. The percentages of inhibition at the highest concentration of gel in PDA medium and in UF cheese were 35.7% and 37.3%, respectively.

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