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Effect of Fresh Garlic and Ginger on the Shelf-Life of Gelatin Waste Used for Improvement of Plant Growth

[1]Eman M. Hegazy and [2]Nabil H. El-Sayed Khamis

[1]Food Toxicology and Contaminates Department, National Research Center, Dokki, Giza, Egypt
[2]Chemistry of Tanning Department, National Research Center, Dokki, Giza, Egypt

Abstract: In this study, gelatin waste was hydrolyzed to get amino acids and was supplemented with garlic and ginger to increase shelf-life then used for improving plant growth. Elements and amino acids in the hydrolysate were determined. Fresh crushed garlic as well as ginger powder at concentrations of (12.5, 17.5, 20.0, 22.5, 25.0 and 50.0 g/l) were used as antifungal agent during storage of the amino acids solution for 455 days at room temperature (25 °C) and refrigerator temp. (8 °C). Determination of the antifungal activity of the extract from both fresh garlic and ginger against *Aspergillus flavus* NRRL 3357 growth were studied. Afterwards, hydrolyzed gelatin was used as fertilizer during cultivation of cucumber in a greenhouse. Results showed that, garlic extract inhibited growth of *A. flavus* NRRL 3357 in agar gel diffusion method to a zone of 15 mm.Also, all concentrations of fresh crushed garlic prevented the fungal growth during storage under different conditions for 455 days and proved to be better antifungal than the ginger powder. Complete inhibition was observed to the growth of *A. flavus* and aflatoxins production in the liquid amino acid. Finally, using the amino acid hydrolysate of gelatin waste as a fertilizer for cultivation of cucumber caused an increase in the number of leaves and fruits.

Key word: Garlic · Ginger · Gelatin Waste · Amino Acids · Elements · Antifungal · Aflatoxins · *Aspergillus flavus* · Cucumber

INTRODUCTION

Gelatin, an animal protein derived from collagen has many industrial applications, mainly in food and pharmaceutical products. Gelatin is a mixture of peptides produced by partial hydrolysis of collagen extracted from the skin, boiled crushed bones, connective tissue, organs and intestines of animals of domesticated cattle, chicken, pigs and horses. Gelatin is classed as a food in its own right and not subject to the food additives legislation in Europe. The worldwide production of gelatin amount to about 300.000 tons per year (roughly 600 million lb).Although gelatin contains 98-99% protein per dry weight, it has less nutritional value than many other complete protein sources. Gelatin is unusually high in the non-essential amino acids glycine and proline (produced by the human body). While lacking certain essential amino acids (not produced by human body) [1].

Mycotoxins are secondary metabolites produced by various molds. *Aspergillus, Penicillium* and *fusarium* are the most common genera. Fungal contamination of plants can occur in the field, during plant growth, at transport and storage under certain environmental conditions. Aflatoxins (B1, B2, G1 and G2) have toxic carcinogenic the teratogenicity and mutagenic activity. Secondary metabolites are produced by fungi *Aspergillus flavus*. These toxins were first isolated form Brazilian peanut meal in 1961 [2,3].

Allium sativum, commonly known as garlic, is a species in the onion genus Allium. Its close relatives include the onion, shallot, leek, chive and rahkyo with a history of human use of over 7,000 years. It was first isolated and studied in the laboratory by Chester Cavallito and John Hays Bailey in 1944. Allium is one of most commonly used spices that enhance flavor in food. It has a wide spectrum of actions which include antibacterial,

Corresponding Author: Nabil H. El-Sayed Khamis, Chemistry of Tanning Department, National Research Center, Dokki, Giza, Egypt.

antifungal and antioxidative as well as its beneficial effects on the cardiovascular and immune system of human [4]. Over the past 20 years, there has been much interest in the investigation of natural products as sources of new antibacterial and antifungal agents [5].

Ginger (*Zingiber officinale*) has been used widely as a food spice and herbal medicine. In particular, its gingerol-related components have been reported to possess antimicrobial and antifungal properties, as well as several pharmaceutical properties [6]. Dehydrozingerone (DZ) which is a constituent of ginger is a potential antifungal agent and can find application as an additive or adjuvant in food and pharmaceutical industries after appropriate toxicological studies [7].

Substitution of synthetic preservatives with natural antimicrobial compounds is preferable for food safety and control of fungal contamination and mycotoxin production [8].

Cucumber (*Cucumis sativus*) is a widely cultivated plant in the gourd family Cucurbitaceae. The cucumber originated in Nepal, where a great many varieties have been observed, from *Cucumis hystrix*. It has been cultivated for at least 3,000 years and was probably introduced to other parts of Europe by the Greeks or Romans. Records of cucumber cultivation appear in France in the 9th century, England in the 14th century and in North America by the mid-16th century [9].

This study aims to characterize some chemical composition of hydrolyzed gelatin waste and possibility of preservation using fresh garlic and ginger. The application of hydrolyzed gelatin waste used as fertilizer during cucumber cultivation was investigated.

MATERIAL AND METHODS

Gelatin Waste: The waste products from national industry for gelatin was hydrolyzed with a mixture of phosphoric and citric acids at pH 1.5 for 5 hours at 100°C. The hydrolysate was neutralized with potassium hydroxide (KOH), filtered then analyzed.

Elements Analysis: Atomic Absorption Spectrometer was used for the quantitative determination of the elements (K, Mn, Fe, Zn, Mg and P).

Standards: Standard solutions of the metals K, Mn, Fe, Zn, Mg and P were provided as solution 1000 ppm from Merck (Merck, Darmstadt, Germany).

Amino Acids Analysis: Liquid chromatography (3000 amino acids analyzer-appendorf-Germany) was used for amino acid analysis. The analyzer was operated at flow rate 0.2ml/min. The reaction temperature was 123°C. The pressure of buffer was in the range of 0-50 bar. The pressure of reagent was in the range of 0-150 bar.

Antifungal Agents: Fresh garlic and ginger were obtained from the Agricultural Research Center, Ministry of Agriculture, Egypt.

Preparation of Antifungal Agent: Bulb of garlic was crushed and ginger was ground in electric grinder. Six concentrations and control (12.5, 17.5, 20.0, 22.5, 25.0, 50.0 and 0.0 g/l) of both garlic as well as ginger were added to the gelatin waste in closed vial. The fourteen vials where stored in refrigerator at 8°C and another fourteen vials where stored at room temperature (25°C). The vials were daily observed for fungal contamination.

Aspergillus flavus: *Aspergillus flavus* NRRL 3357 was obtained from standard Association of Australia 80 Astum St., orth Sydney, NSW.

Inhibitory Test for Garlic and Ginger Extracted: Fresh garlic as well as ginger were ground in electric grinder and filtered through gauze to get the extract. 0.5 ml from 48 hour old cultures of *A. flavus* NRRL 3357 was each poured into separate twelve sterile Petri dishes. 20 ml of sterile media of potato Dextrose Agar (PDA, Oxoid) was poured into each dish. After solidification of PDA agar wells were dug in the plates with the aid of sterilized cork borer (6 mm diameter). 50 ul of each extract was put in the wells with properly labeling then the Petri dishes were incubated at 25°C for 7 days. The diameter of the clear zone of inhibition around the wells (Zone of inhibition) was measured to the nearest millimeter using a transparent ruler [10].

Cucumber Cultivation: Cucumber (*Cucumis sativus*) Class hybrid option RS 27 was cultivated in green house (Middle East Agricultural Production Company in Tnipol city, Egypt). The Cucumber plant was divided into two equal parts. After one month of cucumber cultivation the seedling of the first part was sprayed with the gelatin waste using a machine gun, at concentration of 5 cm/ Littre, water the other part was not sprayed and used as control. After spraying with gelatin waste the percentage of dwarf plants, lengths and number of both securities, as well as fruits were counted.

RESULTS AND DISCUSSION

Elements Content: Elements content were measured in the gelatin waste by Atomic Absorption Spectrometer. Results of analysis are presented in Table 1. The data from Table (1) showed that Potassium (K) recorded the highest concentration at a level of 1026.7 mg / L. On the other hand the lowest concentration was found for both Zinc and Magnesium at 0.481 and 0.466mg/L respectively.

Elements Content: The results are shown in Table (2) was reported. A relatively high percentage of amino acids reached to > 5% and found Four essential amino acids (Threonine, Valine, Isoleucine and Leucine) It can be seen from results that the main amino acids were Proline (PRO) which recorded the highest value at concentration of 0.482g/100 ml sample which resistant to adverse weather condition like drought and salinity, followed by Glycine (GlY) at 0.410g /100ml sample of gelatin waste. The lowest value recorded (0.014 g / 100 ml) was for Tyrosine (TYR). Low values were also reported for both Serine (SER) and leucine (LEU). Both Cysteine (CYS), Methionine (MET) and Tryptophan (TRY) were not recorded at any value.

In is respect, the amino acids composition of gelatin as reported by Stevens [11] minor constituents, Glycine 21%, Proline 12%, Hydroxyproline 12%, Glutamic acid 10%, Alanine 9%, Arginine 8%, Aspartic acid 6%, lysine 4%, Serine 4%, Leucine 3%, Valine 2% Phenylalanine 2%, Threonine 2%, Isoleucine 1% Hydroxylysine 1%, Methienine and Histidine 1% and Tyrosine < 0.05%.

Table 1: Element content in gelatin waste

Element	Concentration* (mg/L)
Potassium (K)	1026.7
Magnesium (Mg)	26.800
Iron (Fe)	1.208
Zinc (Zn)	0.481
Magnesium (Mn)	0.466
Phosphorous (P)	4.000

* Concentration come from 1.5 kg waste

Table 2: Amino acids present in the gelatin waste

Amino Acids	g/100ml (sample)	Amino Acids	g/100ml(sample)
Aspartic (ASP)	0.201	Isoleucine (ILE)	0.049
Threonine (THR)	0.065	Leucine (LEU)	0.101
Serine (SER)	0.101	Tyrosine (TYR)	0.014
Glutamic (GLU)	0.338	Phenylalanine (PHE)	0.059
Proline (PRO)	0.482	Hisitidine (HIS)	0.068
Glycine (GLY)	0.410	Lysine (LYS)	0.151
Alanine (ALA)	0.283	Arginine (ARG)	0.251
Valine (VAL)	0.075	Cysteine (CYS)	0.000
Methionine (MET)	0.000	Tryptophan (TRY)	0.000

Table 3: Effect of different concentrations of crushed garlic on fungal growth in gelatin waste during storage at room and refrigerator temperatures

	Storage period (days)		
Concentration of crushed Garlic (g/L)	4	42	455
0.0 (Control)	*Pencillium spp.**	*A.niger***	-
12.5	N	N	N*
			N**
17.5	N	N	N*
			N**
20.5	N	N	N*
			N**
22.5	N	N	N*
			N**
25.0	N	N	N*
			N**
50.0	N	N	N*
			N**

*Storage at room temp. (25°) C** Storage at Refrigerator temp. (8°C)
N: NO fungal growth through storage period for both room and refrigerator temp.

These values vary, especially the minor Constituents, depending on the source of the raw material and processing technique.

Antifungal and antimycotoxic effects of adding crushed garlic and ginger powder were evaluated in the hydrolyzed gelatin stored at room refrigerator temperatures for 455 days (Tables 3) and at refrigerator temperatures for 365 days (Table 4). Obtained results show that, crushed garlic proved to possess higher antifungal activity than the ginger powder. *Penicillium* spp was found in the untreated gelatin (control) after four days storing at room temperature. On the other hand, *Aspergillus niger* was found after 52 days storing in refrigerator.

Results in Table (3) show that, all concentrations of crushed garlic realized antifungal activity during the storage period of the hydrolyzed gelatin. In this respect, Groppo *et al.* [12] proved that Allicin is the main active compound present in crushed garlic cloves which is responsible for its antibacterial activity. Also, garlic was proved to have antifungal, antiprotozoal and antiviral properties [13, 14] proved the antifungal activity of six fractions derived from garlic in a vitro system. They found that Ajoene had the strongest activity in these fractions. They added that the growth of both *Aspergillus niger* and *Candida albicans* was inhibited by Ajoene at 20 ug /ml. They also showed that, Ajoene had stronger antifungal activity than Allicin. Although the mechanism is not clear Ajoene may damage the cell walls of the fungi.

Table 4: Effect of different concentrations of ginger powder on fungal growth in gelatin waste during storage at room and refrigerator temperatures

Concentration of Ginger powder (g/L)	Storage period (days) 4	5	6	42	61	365
0.0 (Control)	*Pencillium spp.**	N	-	*A.niger***	-	-
12.5	N	N	N	*A. niger**	N	N**
17.5	N	*P. spp**.	N	*A.niger***	-	-
20.0	N	N	N	N	*A. niger**	N**
22.5	N	N	N	*N*	*P. spp**.	*A.niger***
25.0	N	N	*P. spp.**	N	N	N**
50.0	N	N	*P. spp.**	N	N	N**

* Storage at room temp. (25°C)** Storage at refrigerator temp. (8°C)

N: NO fungal growth through storage period for both room and refrigerator temp.

Fig. 1: Zone of inhibition around the wells by Garlic extract against *A.flavus* NRRL 3357 growth

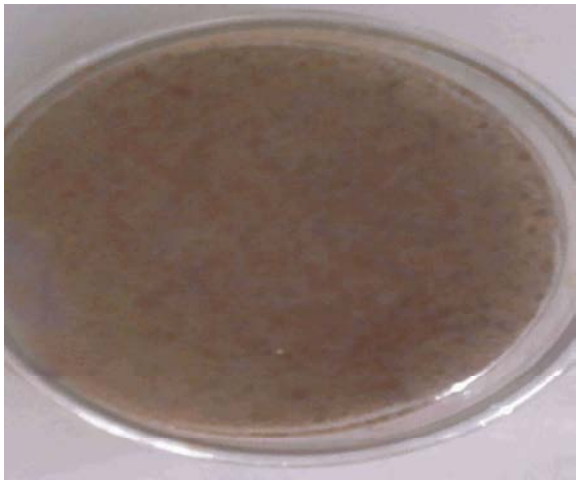

Fig. 2: *A.flavus* NRRL 3357 growth with out any extract (control)

The antimicrobial activity of garlic is believed to be due to the effect of Allicin, the main ingredient in garlic, generated by the Phosphopyridoxal Enzyme Allinase [15].

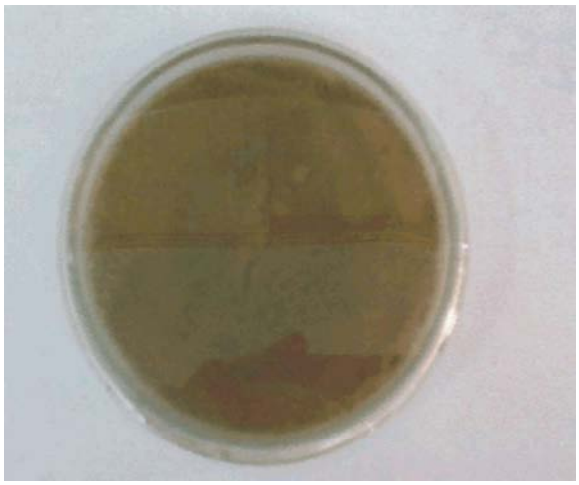

Fig. 3: No zone around the wells by ginger extract against *A.flavus* NRRL 3357 growth

Fungal growth in treated gelatin with ginger powder was investigated during storage at room and refrigerator temperatures. Results in Table (4) showed that ginger powder was active as antifungal agent at refrigerator temp. (8°C) with low concentrations (12.5 and 20.0 g / L) and the high concentrations (25.0 and 50.0 g / L). In this respect, Touba, Zakaria and Tehereh [16] found that the crude hot water extract of ginger at three concentrations (10, 20 and 30%) showed the best anti-fungal activity against *Phoma exigua; Fusarium nygamai* and *Rhizoctonia solani*

Inhibitory Action of Garlic and Ginger Extracts: The antifungal activity of the garlic and ginger extracts against *A.flavus* NRRL 3357 growth (*A.flavus* only) was assessed by the wells method[10] as showed in (Figs. 1-3).

The results revealed that the garlic extract showed antifungal activity toward A. *flavus* NRRL 3357 growth, the recorded inhibition zone was 15 mm (Figure 1).

Figure 2 showed the growth of *A.flavus* without extract (control) as shown in Figures 2 and 3 no zone was observed. Touba, Zakaria and Tehereh [16]proved that, the cold water extract of garlic exhibited good anti-fungi activity against *Phoma exigua, Fusarium mygamai* and *Rhizoctonia* than crude extract of six spices like cardamom, chili, coriander, onion, ginger and galangals at three concentrations (10, 20 and 30 of the crude extract) in-vitro. Our results are not comparable with the previous investigations of Ali *et al. and* Senhaja Faid, Ichraq [17,18] they reported that ginger essential oils exhibited an inhibitory effect against a wide range of pathogenic bacteria and fungi. Maybe that, they used ginger essential oils which proved to be stronger as antifungal agent than ginger powder used in our study.

Cucumber Cultivation: The hydrolyzed gelatin waste was used for plant nutrition through cucumber cultivation. The results revealed that hydrolyzed gelatin waste has high nutrition value on cucumber plant. The results showed that, after 15 days of spraying the cucumber plant, the percentage of dwarf plants length was 5%, also increased the number of securities witch reached 13.7%. More-over, increased the number of fruits by 37.7 % through five days after spraying.

CONCLUSION

The hydrolyzed gelatin (from gelatin industry waste) contain essential elements and amino acids that can be used for plant nutrition.Low concentrations of crushed garlic was used as antifungal agent at room and refrigerator temperatures and the extract was stored for 445 days. This treatment could protect hydrolyzed gelatin from any fungal growth. The hydrolyzed gelatin improved growth of cucumber plant, which signify its use for plant nutrition.

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