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EFFECT OF AQUATIC EXTRACT OF CHILI PEPPER AND HONEY EXTRACTS ON FUNGUS ISOLATED FROM *MUSCA DOMESTICA* AND SOME PATIENTS

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Abstract

This experiment was conducted to separately study the effect of the aquatic extract of honey and chili pepper, as well as their effect when combined on fungus. Three concentrations (6.25, 12.5, 20) µl of the aquatic extract of chili pepper and honey extracts were prepared on the growth of five types of pathological skin fungus isolated from domestic flies Musca domesticat and from infected people, including *Penicillium parasiticus, Aspergillus niger*, *Rhizopus stolanifer*, *A. flavus* and *Microsporium cains*. The results showed that the aquatic extract of chili pepper contains many medicinal compounds: Glycosids, Tannins, Flavones, Resins, Saponins, Capsaicin Alkaloids, and Phenols. Also, the results of the present study showed that the aquatic extracts have a different effect depending on the concentration and type of fungus. The concentration of honey extract did not affect the growth of all fungal species, and the optimal inhibitory concentration of fungi growth was 20 µl. All chili pepper extract concentrations showed significant different effects on the inhibition of the fungal species. Also, concentrations of honey and chili pepper extract showed significant effects when combined compared with when they were used separately.

Key words : Chili pepper, honey extract, fungi, colony diameters.

Introduction

The excessive and indiscriminate use of various antifungals led to the emergence of resistant strains. There is not enough information about the mechanics of its resistance so far, although some varieties of fungi have the ability to resist many antibiotics and with different concentrations. It becomes necessary to find alternatives to fungal antibiotics by using many phyto medical extracts, including honey, chili, and other plant extracts (Mohamed, 1999). The *Capsicum annuum* L. belongs to the solanaceae family, one of most important marketing and export vegetable crops. Pepper is a plant characterized by high nutritional value because it contains vitamins, which are needed for the body, especially in the winter season to resist cold and flu (Hussein, 1981). Capsaicin is a substance extracted from hot pepper varieties, and can be used as a paste to treat bone pain caused by rheumatism. Fluoride is also extracted from chili varieties that protect teeth from decay (AOAD, 1988).

Pepper is used in the manufacture of pickles and hot sauce, and it is used as an appetizer, particularly in tropical regions. The original home of the pepper is South America, and the crop then moved to the warm tropical regions of all continents of the world, such as the East Indies, China, Spain, Greece, and Africa where it is dried and crushed and used as an appetizer (Al-Sarraf and Abbas, 1982). Chili pepper is grown in Iraq as a secondary crop during the months of September to November and matures in April. Chemical analysis of commercial chili powder shows that it consists of 23.8% protein, 1.7% raw fat, 11% moisture, 6.6% raw fiber, 7.5% ash, 0.4% calcium, and 0.6% phosphorus (Trakhtenberg, 2006). Also, it contains sulfur compounds with pilot oil, allicin, vitamins A, E, B1, B2, D, mineral salts and antifungal substances that help lower blood pressure (Wattar, 1986). Chili also contains vitamin C. Chili is one of the most important foods known to prevent cancer (Oluwole, 2001).

Research indicates that hot peppers cause cellular poisoning of cancer cells and destroy them by interacting with and inhibiting enzymes that contain the sulfadil group (SH) in the cancer cells, as these cells contain this compound at concentrations higher than the rest of the tissues (Moon *et al*, 2000). Chili also stimulates lymphocytes to eliminate cancer (Amagase *et al*., 2001). Chili pepper strengthens the immune system, so it is recommended to eat to prevent epidemics (Kyo *et al*., 2001).

A study was conducted to investigate the immune stimulation of chili among people who eat 35 g / day of pepper for three weeks; that level of consumption caused an increase of the effectiveness of their immune cells (Thomson *et al*., 2006). Researchers found that Alliin substance (S-allyl-cystiene Sulfoxide) is derivative of amino acid (Cysteiene) responsible for the release of all active ingredients in chili peppers, while Alicin is produced by chipping the chili peppers, as the allinase enzyme releases compounds responsible for the transfer of Alliin into Alicin (Kojuri *et al*., 2007).

As for honey, it's a thick aromatic substance produced from the nectar of flowers by the bees, which convert it to a thick texture. Honey contains a variety of sugars and vitamins (Palma, 1992). It has the ability to maintain its active ingredients for a relatively long period of time. It has an anti-bacterial and fungal effect because it contains Formic acid and is highly resistant due to the existence of prostaglandin. There is several explanations for the mechanism of honey that already belongs to the high osmosis of honey, or enzymes, or substances that inhibit bacterial growth (Medhi *et al*., 2008). Researchers report that honey has the ability to prevent infections, fungal skin diseases, the incidence of eczema, herpes and psoriasis because it contains vitamin B3 and E (Medhi *et al*., 2008). In medicine, honey is included in the treatment of burns and wounds contaminated with bacteria and fungi, an important drug used in particular alternative medicine (Hanes and Simuth, 1992). As others have noted, honey inhibits the growth of microorganisms due to its lower pH level (Nafadi Alaa, 2004). Other studies have indicated that the degree of inhibition of honey on fungal growth varies by the type and concentration of honey, especially non-heat-treated honey. This has been explained by the fact that heat affects the active components that inhibit the growth of microorganisms in honey (Al-Hindi, 2003). Because honey and chili peppers are reservoirs rich in active ingredients, this experiment studies health impacts resulting from mixing hot pepper extract and honey and their role in inhibiting fungal growth.

Materials and Methods

Collection of specimens

The experimental samples were collected during January to March 2017 from people, who suffer from skin diseases, who were diagnosed clinically by a dermatologist, as well as specimens isolated from domestic flies. A standard insect fishing net and nylon bag were used to collect adult insects of domestic flies from several sites in Diwaniyah city, such as garbage dumps, dirty environmental sites, and palm waste by placing sugar in the bag to attract fly insects. 75 insects were obtained from the family of domestic flies. Insects varied between the infected and non-infected, so some of the symptoms of disease can be observed on the infected insect, including turning the insect on its back so it cannot stand and fly. Then, insects were sterilized with 2% sodium chloroate for 2 minutes and washed with sterilized distilled water twice. After that, the flies were transferred on to clean filter paper and isolated in the laboratory. A sterile swab was used, and samples were taken from damaged skin. The samples were then transferred to the laboratory for the necessary tests.

Culture media

Sabouraud's Dextrose gar (SDA)

SDA was prepared by using methods of Merz and Roberts (1995) by dissolving 40 g of dextrose, 10 g of peptone, 20 g of Agar and 0.05 g of antibiotic (Chloramphenicol). Then, the volume was added to (1000) ml of distilled water. The mixture was placed in a clean glass flask and sterilized with the device under temperature (121^0) and pressure (1) for half an hour after adding antibiotic to the center (after sterilization). This media has been used before to isolate opportunistic fungi (Silva *et al*., 2004).

Potato Dextrose Agar (PDA)

This media was prepared in the laboratory from the following materials: (200) g of potatoes, (20) g of dextrose, and (20) g of agar. Also, culture media prepared by the company was used by dissolving (39) g in one liter of distilled water and then sterilizing the medium under the ambient temperature at (121° C) and pressure (1) atmosphere for half an hour. In both paragraphs (2.1) and (2.2) above, the culture media were planted in dishes and prepared for the purpose of planting and re-sampling.

Cultivation of fungal isolates

After the fungal swabs were obtained from the laboratory, they were grown on the dish with SDA media with three replicates per swab. Then, the dishes were incubated in the incubator at a temperature of (37° C)

Table 1 : The active compounds of the chili pepper extract.

Appearance	Active compounds in chili pepper extract
+	Tannins
+	Alkaloids
+	Glycosides
+	Phenols
+	Flavones
+	Resins
+	Saponins

for 3-7 days and the fungi were visible in the dishes as following :

1- *Penicillium parasiticus*

2- *Aspergillus niger*

3- *A.flavus*

4- *Rhizopus stolanfer*

5- *Microsporium cains*

These were diagnosed through phenotypic and microstructure traits.

Collect plant sample

Plant samples (chili pepper) were brought from grocery markets in the Al-Qadisiyah city, taken to the laboratory, cleaned from dust and put in bags for usage.

Prepare aquatic extract

50 g of chili pepper was taken and crushed by electric mixer, then dried in the electric oven. After that, it was put in a flask, the distilled water added to the beaker and this completed the size to 1000 ml for three hours. The aquatic extract of the chili pepper was then filtered using filter paper until a thick liquid was obtained, and then concentrations (6.25, 12.5, 20 µl) were prepared.

Preparation of honey

The honey was brought from a beekeeper with 100% concentrated and prepared concentrations (6.25, 12.5, 20 µl) of honey. In a special method, a mixture of honey and pepper extract was prepared and the same concentration used above was studied to evaluate their effect on the fungi selected in the experiment.

Chemical detection of active compounds of chili pepper

Detection of tannins

Detect the tannins by mixing 10 g of pepper powder with 50 ml of distilled water to make solution and heat the solution until boiling, then the solution was filtered. After that, the solution was divided into two groups. The first group was added to it (1%) of lead acetate. The appearance of gelatine deposits indicates the existence of tannins, while the second group was added to it (1%) of ferric chloride to indicate the presence of tannins with the appearance of bluish green colour (Shihata, 1951).

Detection of alkaloids

The method of Harborne (1984) was used and was as follows: Boil (10%) g of chili pepper with (50) ml of distilled water cidified by (4%) of hydrochloric acid. The solution was cooled and then filtered with filter paper and took about 0.5 mL of leachate in the watch glass with each of the following reagents:

- Dragangrove Reagent and its result, the appearance of the deposit (orange) Mayer's reagent and its result, the appearance of the deposit (white)-
- Wagner's reagent and its result the emergence of a deposit (brown).

Detection of glycosides

Use the method of Shaikhly *et al*. (1993) and was as following:

Place 1 mL of pepper extract in a test tube and add 2 ml of Benedict's Reagent as previously indicated, Transfer the solutions to a boiling water bath and leave for 5 minutes. The positive examination (presence of the glycosides) was demonstrated by the appearance of red color. To confirm the result, two equal amounts of Fehling's Reagent were combined with the aquatic extract of the pepper powder and left in the water bath for 10 minutes. The appearance of a red deposit indicated of the positive test.

Detection of phenols

The method of Harbone (1979) was used and was as following:

By adding 3 ml of pepper extract to 2 ml of ferric chloride solution, the appearance of bluish green indicates the presence of phenols.

Detection of flavones

The method of Jaffer *et al*. (1983) was used and was as following: solution was prepared as follows:

- By dissolve (10) g of pepper powder or its extract in (5) ml of ethyl alcohol at a concentration (95%), then solution was filtered and prepared.
- By adding 10 mg of ethyl alcohol at a concentration of 50% to 10 mL of potassium hydroxide at a concentration of 50%. When mixing equal amounts of both solvents, the appearance of yellow color is evidence of the presence of flavone.

Detection of resins

The method according to Shihata (1951) was used and was as following: adding 50 ml of ethyl alcohol at a concentration of 95 to 5 g of pepper powder its extract. After that, the solution was left in a water bath to boil for 2 minutes. Then, 100 ml of distilled water was added to acidify with the hydrochloric acid. The presence of the resin materials is indicated by the appearance of a crumb.

Detection of Saponins

There are two ways to detect the Saponins according to Shihata (1951).

1. By shaking the aquatic solution of the powder pepper strongly in the test tube, the appearance of dense foam for several minutes is evidence of the presence of Saponins.
2. By adding 5 ml of mercuric chloride to 1.5 ml of aquatic extract of the pepper, the emergence of a white deposit indicates the positive result.

Results and Discussion

In table 2, the results showed that the colony diameters of fungus *A.niger* was affected with concentrations 12.5 and 20 µl of chili extract, which were 0,1.2 cm. There was no significant difference among honey concentrations on colony diameters of the fungus *A. niger* and this is consistent with Mustafa (1995). There was a clear inhibition in the diameters of colonies of the fungus *A. niger* (1.2, 0, 0) cm when mixing the extract of chili pepper with honey extract using the same concentration. The extract of chili pepper has an inhibitory effect on many fungal pathologies such as skin fungi and fungi that cause ear infections such as *Aspergillus* sp. and *Penicillium* (Abdul Hussain, 2001).

The results of table 3 showed that there were significant differences in the diameters of the colonies with concentration (20 µl) of the chili pepper extract, which were (5.5, 4.3, 1.4), respectively. As for the honey extract, the concentration (20 µl) had a significant effect on the diameters of the colonies of the fungus *A. flavus*, which were (7.1, 3.2, 2.2) respectively. Also, when mixing the extract of chili pepper and honey with same concentrations, the concentration (20 µl) has significantly inhibited on the diameters of the colonies of the fungus *A. flavus*, which were 5.2, 3.1, 0, respectively.

With regard to table 4, the extract of chili peppers with all its concentrations showed significant differences compared with the control treatment. The colony diameters of the fungus *P. parasiticus* were 6, 0 and 0, respectively. Honey treatment was significantly different

Table 2 : Effect of different concentrations of aquatic extract of chili pepper and honey extracts on colony diameters of the fungus *A. niger*.

Colony diameters	Concentrations	Extracts type
9	0	Control
5.3	6.25	Chili pepper extract
1.2	12.5	
0	20	
4.2	6.25	Honey
6.3	12.5	
3.1	20	
1.2	6.25	Honey + chili pepper extract
0	12.5	
0	20	
0.37		LSD 0.05

Table 3 : Effect of different concentrations of aquatic extracts of chili pepper and honey extracts on colony diameter of the fungus *A. flavus*.

Colony diameters	Concentrations	Extracts type
9	0	Control
5.5	6.25	Chili pepper extract
4.3	12.5	
*1.4	20	
7.1	6.25	Honey
3.2	12.5	
*2.2	20	
5.2	6.25	Honey + chili pepper extract
3.1	12.5	
*0	20	
0.08		LSD 0.05

compared to the control treatment in terms of inhibition of fungi *P. parasiticus*, which was the rate of colony diameters (5.2, 1.3, 0), respectively. The fungal colonies were inhibited by (100%), the rate of colony diameters was (0, 0, 0) respectively when mixing honey and chili pepper extracts.

P. parasiticus fungus has been significantly affected by honey and chili extracts despite the resistance mechanism that is represented by enzymes and this is consistent with what was found (Abdul Hussain, 2001).

From table 5 results showed that there was a clear

Table 4 : Effect of different concentrations of aquatic extracts of chili pepper and honey extracts on colony diameter of the fungus, *Penicillium parasiticus*.

Colony diameters	Concentrations	Extracts type
7	0	Control
6	6.25	Chili pepper extract
*0	12.5	
*0	20	
5.2	6.25	Honey
*1.3	12.5	
*0	20	
*0	6.25	Honey + chili pepper extract
*0	12.5	
*0	20	
0.34		LSD 0.05

Table 5 : Effect of different concentrations of aquatic extracts of chili pepper and honey extracts on colony diameter of the fungus, *Rhizopus stolanifer*.

Colony diameters	Concentrations	Extracts type
9	0	Control
8	6.25	Chili pepper extract
6.5	12.5	
5.1	20	
9	6.25	Honey
9	12.5	
7.2	20	
7.3	6.25	Honey + chili pepper extract
5.4	12.5	
*2.1	20	
0.27		LSD 0.05

resistance by *R. stolanifer* when the aquatic extracts were used in all its concentrations. The colony diameters were (8, 6.5, 5.1 cm) respectively when using chili pepper extract. In the case of the use of honey extract, there was no inhibitory growth of the colonies of the fungi, as the diameters of colonies (9, 9, 7.2) cm, respectively, compared to the control treatment, had a rate of colonies of 9 cm. In the case of mixing the extracts, it was observed that there was a clear effect and a significant difference in the use of concentration (20) µl, and the rate of colonies was (2.1) cm compared to the treatment of control, consistent with (Al-Rejo and Maha Akram, 2004). The

Table 6 : Effect of different concentrations of aquatic extracts of chili pepper and honey extracts on colony diameter of the fungus *Microsporium cains*.

Colony diameters	Concentrations	Extracts type
9	0	Control
4.2	6.25	Chili pepper extract
2.2	12.5	
3.1	20	
1.6	6.25	Honey
1.3	12.5	
1.2	20	
1.5	6.25	Honey + chili pepper extract
0	12.5	
0	20	
0.06		LSD 0.05

fungus, when exposed to inappropriate conditions such as temperature or the use of active substances or drugs fungus, has a mechanism of resistance, which produces enzymes to help analyze these materials, and this is consistent with the findings of Pia and Platt (1995).

The results of table 6 showed that the significant differences in the diameters of the colonies (4.2, 2.2 and 3.1 cm), respectively in the use of the chili pepper. As for the honey extract, all its concentrations had a significant effect on colony diameters, which were 1.6.1.3, 1.2 cm, respectively. Also, when mixing the chili pepper extract and the honey under the same concentrations, the concentration (20, 12.5 µl) significantly inhibited the growth of colonies of fungi - *Microsporium cains*, with a rate of colonies of colonies of (1.5, 0, 0) cm, respectively. Where the fungus *Microsporium cains* was inhibited by the extract that used in the research, it showed that the enzymatic property did not enable resistance to that change in the media.

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