EFFECT OF ALLIUM SATIVUM ON THE GROWTH OF SOME SPECIES OF FUNGI.

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KEY WORDS: garlic, antifungal effect, fungi, *Paracoccidioides brasiliensis, Cryptococcus neoformans, Candida albicans.*

***ABSTRACT*:**

This study included studying some properties of the Extract Concentrated Fresh of Garlic (ECFA), (AlliumSativum), and evaluated the activity of (ECFA) on the inhibition of the growth of some fungi species: (*Histoplasma capsulatum, Coccidioides immitis, Exophiala dermatitidis, Paracoccidioides brasiliensis, Cryptococcus neoformans, Candida albicans* and different Species of *Aspergillus)* .

Also some physical condition which enhances the activity of (ECFA)as antifungal ,were studied like (dilution, incubation temperature, protein Concentration, half-life and its treatment with activated charcoal).

The result shows that the (ECFA) has inhibition action on growth of fungi species which included in this study.

تاثير خلاصة الثوم على نمو بعض انواع الفطريات

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الكلمات المفتاحية:-

الثوم ، تاثيرمضاد للفطريات ، الفطريات ، باراكوتشيوادس براسيلينزيس ، المكورات الخيطية ، المبيضات البيض.

الخلاصة:

الدراسة توضح بعض خصائص المادة المستخلصة من الثوم الطازج (ECFA) وفحص تاثيرها على نمو بعض بعض الانواع الفطرية مثل:(هيستوبلازما كابسولاتم ، كوسيديويديس اللدودة اكسفوليا ديرماتايتس، نظيرة الكروانية البرازيلية، الأدعومية المستخفية، المبيضات البيض وأنواع مختلفة من الرشاشيات) .

كما دراسة بعض الظروف الفيزيائية مثل (التخفيف، ودرجة حرارة الحاضنة و تركيز البروتين وعمر النصف والمعاملة مع الفحم المنشط.).

اظهرت النتائج ان جميع الانواع الفطرية التي تضمنتها هذه الدراسة كانت عرضة للتاثر بمادة (ECFA **)** من حيث النمو.

INTRODUCTION

The harmful effects of most of the antifungal agents mainly of the polyenes and The poor enzymatic balance that regulates the azole derivatives, allylamines and Thiocarbamates, as well as the narrow range of action will stimulate the development of new lines of research in this area (1). This research act on the level of ergosterol synthesis-Fungal membrane without interference with similar mammalian mechanisms, or the Research with new compounds that exert their action at different levels of plasmatic membrane. Also, the research that based on the Polysaccharide synthesis of the cell wall of fungi. Many of them are Limited to an experimental use, since it is not yet known if they can have side effects in humans, also they represent a line of research Promising in the field of

antifungal therapy (2).other lines of research on antifungal antibiotics have focused on the use of some fungal toxins (3), which originated in Central Asia.

Belongs to the family of *Liliáceas*. This plant grows in almost of all the world, and in all temperate and warm climates (4). It is often has the following properties: antiseptic, antibacterial and hypocholesterolemic effect. its also effective to decrease platelet aggregation, antimycotic,and Expectorant. (5), (6).

Allium sativum mainly in the bulb, an odorless sulphurous substance called Alliin which by the action of Allinase available in the garlic itself, that convert the alliin of garlic to allicin and sulphides of allyl, vinyl and propyl (0.6%) which provide the characteristic odor of garlic(19).

In addition, it contains vitamin A, B1, B2, C, a nicotinic acid amine, choline, hormones, alicyclic I and II, sulfocianic acid, iodine and traces of uranium. This complex Composition makes the bulb have different effects on the organism (6). The effects of Antimicrobials and antimycotics are attributed to the action of allicin which has Demonstrated to be active in vitro, against *Candida albicans*, some species of *Trichomonas, Staphylococcus aureus, Escherichia coli, Salmonella typhi, S. paratyphi, Shigella dysenteriae, Vibrio cholerae*, Herpes simplex virus, Influenza virus ,Some fungi, mainly dermatophytes and yeasts pathogenic to man (7).

There are studies on a substance derived from A. sativum, ajoeno and its Action in vitro against dermatophytes, in which it succeeded in inhibiting growth at the Minimum Inhibitory Concentration (MIC) of 60 μg / mL and Minimum Concentration Fungicide (CMF) of 75 μg / mL. The in vivo cream effect was also tested 0.4% applied once per day for a period of five days, with a low percentage of healing; However, an excellent clinical response was obtained in patients with Pityriasis versicolor achieving a cure of 87.5% (8). Others report the antifungal action on *C. albicans* and *Aspergillus niger* (9) and the inhibition of the growth of other yeasts like Saccharomyces Cerevisiae at concentrations below 20 μg / mL (7).

In recent years the achievements of the pharmaceutical industry have been very important In antimicrobial production, although the costs are very high (8). when the action of A. sativum become satisfactory, so, it will be consider a useful product, since it possesses therapeutic properties, it is also cultivable at the long of the country, it grows in any type of land and it is very economic.

By all the above, this study is tried to demonstrate the in vitro antifungal effect of A. sativum *C. albicans*, dermatophytes,with different environmental conditions in order to obtain a competitive product with the Antifungal agents that are used in the market and easy reached for most patients. Therefore, the objective of this study was to demonstrate some Characteristics of the ECFA, and to analyze its effect on the growth of some Species , and then to obtain a competitive product with the Antifungals that are used in market in the future,.

Characteristics of ECFA

For the study of some characteristics of ECFA, such as dilutions, concentration ECFA, half-life, and temperature, yeast *C.Albicans*, can be tested because of its grow fast. 50 μL aliquots of the ECFA were taken,Which were added to Petri dishes containing 1 x 106 CFU / mL of *C.Albicans* , and incubated at 28 ° C for 96 h, comparing the growth with respect to a control without ECFA (all inhibition experiments described Were carried out with the same protocol), whereas for the study of The antifungal properties were analyzed the effect of ECFA on the following strains of fungi Yeasts: *C. albicans, C. tropicalis, C. Krusei, C. lamtia, C. parapsilosis, Cryptococcus neoformans,* and *Exophiala dermatitidis*.

The systemic: *Histoplasma capsulatum, Paracoccidioides brasiliensis* and *Coccidioides immitis*. The contaminants: *A. flavus, A. terreus, A. clavatus, A. Ochraceus, A niger, Mucor rouxii, Paecilomyces sp., Malassezia furfur and Trichotecium Sp*. The dematiaceous Alternaria alternata and the causes of chromoblastomycosis and Sporotrichosis as: *Cladophialophora carrionii and Sporothrix schenckii* and some *Dermatophytes*.

Determination of the antifungal effect of ECFA on the growth of different species of fungi:

A batch of the different species of fungi to be analyzed was taken, resuspending In 1 mL of 0.85% sterile saline, and a 10 µL aliquot was taken, Applying it in a Neubauer chamber to determine the number of cells.

Subsequently, from each fungus, 1 x 106 cells / mL of ECFA were taken and then planted in Petri dishes containing Sabouraud Dextrose Agar (SDA), and for each fungus species under study, 50 µL of the ECFA was added, spreading it throughout the box with a wand Sterile glass in the form of a triangle, and incubated at 28^{O}C, observing their growth At different times (4 days to 6 weeks), comparing growth with a groups of the fungus to be analyzed without the ECFA. all experiments were performed 3 times and duplicate.

Protein determination of ECFA was determined by the Lowry method (10).

MATERIAL AND METHODS

Prepare Fresh Garlic Concentrated Extract (ECFA) by taking Approximately 15 heads of garlic, raw and previously peeled, ground in A mortar, a suspension of the garlic was obtained; This suspension was filtered on gauze by pressing it , to obtain a larger amount of filtrate, and storing in closed container at 4^{O}C, to save material extract and its activity.

RESULTS

The results obtained from the properties of the antifungal activity of ECFA, half life, temperature, effect of different dilutions, minimum concentration Inhibition, using *C. albicans* as standard, were as follows:

With respect to the effect of different dilutions of ECFA (1:10, 1: 100 and 1: 1000 v / v), any dilution with 0.85% sterile saline solution was found to inhibit Antifungal properties, which does not occur when tested without diluting it (Figure 1). On the other hand, the minimum inhibitory concentration (MIC) of ECFA was between 40 and 50 µL of the ECFA (0.8-1.0 mg / mL protein), while the concentration of 50 µL Used similarly inhibited the growth of different concentrations of *C. Albicans* (1 x 10 6 to 10 x 10 6 yeast / mL), as little or no Growth (Figure 2).

Figure 1. Effect of different dilutions of ECFA on the growth of *C. albicans*. (1 x 106 CFU / mL, SDA 28 ° C, 96 h, 50 µL). 1.- *C. albicans* + ECFA undiluted. 2.- *C. albicans* + ECFA dilution 1:10. 3.- *C. albicans* + ECFA dilution 1: 100. 4.- *C. albicans* + ECFA dilution 1: 1000.

Figure 2. Effect of ECFA on the growth of different concentrations of *C. albicans*.

(SDA ,28 ° C, 96 h, 50 µL). 1.- *C. albicans* 1 x 106 CFU / mL without ECFA. 2.- *C. albicans* 10 x 106 CFU / mL with out ECFA. 3.- *C. albicans* 5 x 106 CFU / mL with ECFA.4.- *C. albicans* 6 x 106 CFU / mL with ECFA. 5.-*C. albicans* 7 x 106 CFU / mL with ECFA. 6.- *C. albicans* 8 x 106 CFU / mL with ECFA.

In relation to the half-life of the antifungal activity of the ECFA, it was found that it is totally lost between 55 and 60 days after its production, at 4ºC in covered containers (although the loss of activity starts at 39 days, with A loss of activity of 10%), while in containers uncovered at both 4ºC and 28 ºC, loses its activity at 24 h incubation, and 60ºC totally loses its activity in 60 minutes, both in capped and uncovered tubes.

On the other hand, when testing the antifungal effect of ECFA on different Yeasts and fungi, it was found that the growth of all Conditions analyzed (Figures 3, 4 and 5).

Figure 3. Effect of ECFA on the growth of *H. capsulatum*. (SDA , 28^{o}C, 21 days, 50 µl) .1-*H. capsulatum* 1 x 106 spores / mL without ECFA. 2.- *H. capsulatum* 1 x 106 spores / mL with ECFA after 20 days. 3.-*H. capsulatum* 1 x 106 spores / mL with ECFA after 40 days.

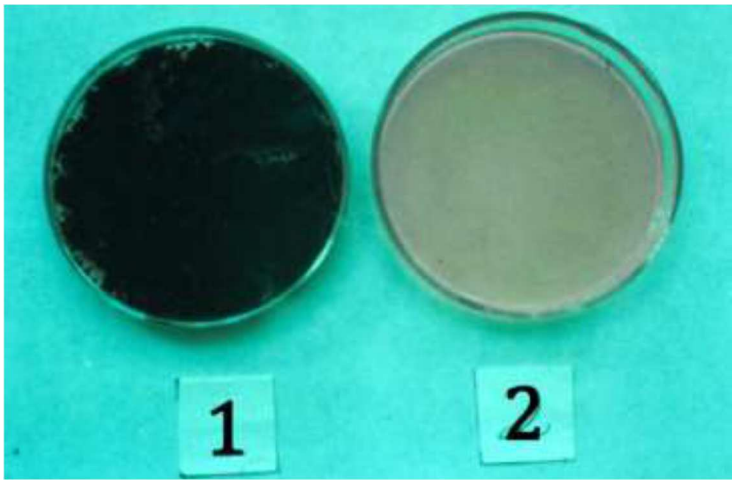

Figure 4. Effect of ECFA on the growth of *E. dermatitidis*. (SDA ,28 ° C, 96 h. 50 µl) . 1-*E.Dermatitidis* 1 x 106 spores / mL without ECFA. 2- *E. dermatitidis* 1 x 106 spores / mL with ECFA.

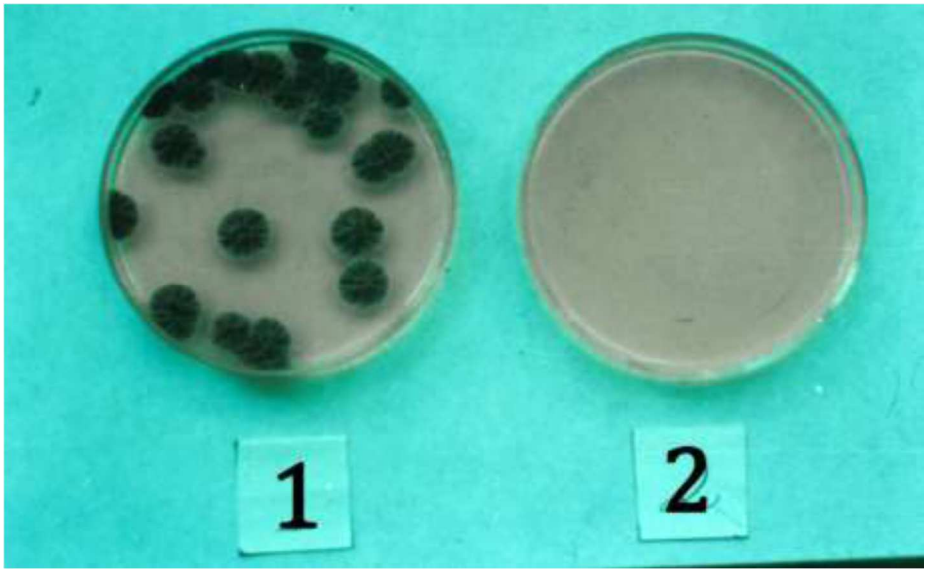

Figure 5. Effect of ECFA on the growth of *C. carrionii*. (SDA ,28 ° C, 96h, 50 µl).

1.-*C.carrionii* 1 x 106 spores / mL without ECFA. 2.- *C. carrionii* 1 x 106 spores / mL with ECFA.

DISCUSSION

Azole derivatives are perhaps the most commonly used antifungal group in the present. Its interference in the synthesis of ergosterol of the fungal membrane Leads to structural deformations and eventual cell death. *P. brasiliensis* Is very sensitive to the azo derivatives. In vitro, the minimum concentrations Inhibitory, fluctuate between 10-10 and 10-8 M in the sequence: Saperconazole> itraconazole> ketoconazole (1). In practice Medical attention, itraconazole (a triazole) and ketoconazole (an imidazole) are Used for the treatment of paracoccidioidomycosis (12).

Amphotericin B deoxycholate is also indicated in the treatment of cases Patients with severe dissemination, with recommendations for follow-up Sulfonamide to prevent relapse (13). already researchers try to find some new agent to treat fungal infections in state of old one as mentioned above.

[(E, Z) -4,5,9-trithiadodeca-1,6,11-triene 9-oxide], derived from allicin, to its Instead a natural garlic product, is another compound currently in Potential antifungal drug (6). Ajoeno is effective in the topical treatment of Tinea pedis, Cruris and corporis, with results similar to terbinafine (14), Inhibits the growth of P. brasiliensis and the dimorphic transition to phase Levaduriform. Its effect is on the blockade of phosphatidyl choline synthesis, with An accumulation of the precursor compound phosphatidyl ethanolamine, which results in an alteration in the cell membrane structure and consequent cell death (15).

Therefore, in this work, we studied some properties of the ECFA, the Information has not been described in the literature (6)For example, the dilution of the ECFA decreases its activity, which is Reported for Staphylococcus aureus and Bacillus subtilis (16), and for C. albicans with lyophilized garlic extract (17) and oil (18), but is different from that reported by (19) for essential oils of oregano, thyme, rosemary and Clove, which at different dilutions do not lose their antifungal effect, also the 5% (v / v) ECFA inhibits the growth of athogenic fungi isolated from roots of rotted cassava (Fusarium oxysporum, F. solani, Botryodiplodia theobromae, Macrophomina phaseolina, Penicillium oxalicum and A. niger) by 53.3% (20).

ECFA of the ECFA was 0.8-1.0 mg / mL protein, while Feldberg and Chang (21), report 0.3 mM for *Salmonella typhimurium* (although this concentration is not lethal) using the essential oils of oregano, thyme, rosemary and clove, in addition to some of its main components as: cloveugenol, carvacrol and thymol.

Against *A. niger*, the IMC repo Both were 60 μg / mL (8), and ajoene concentrations of 2.5 Mg / mL completely inhibited the growth of five clinical isolates of *Histoplasma capsulatum* (22) . report an IMC of 0.0625 and 0.125% (v / v) with the oil of Garlic for Penicillium funiculosum.

With respect to the half-life of ECFA, it loses its activity between 55 and 60 days After obtaining them, at $4^{O}C$ in covered containers. In this regard, That the loss of allicin during the process of obtaining pulp of different Commercial varieties of Allium sativum, was 22% at 180 days of storage, With a large loss of allicin content after processing (23).

In relation to the antifungal properties of ECFA, it is verified that it is a Excellent ntimycotic for the different fungi analyzed between Found: *C. albicans, C. neoformans, E. dermatitidis, T. mentagrophytes, T.Tonsurans, T. rubrum, M. canis, M. gypseum, H. capsulatum, P. brasiliensis, C. M. aureus, A. flavus, A niger, Paecilomyces sp., M. furfur* and *Trichotecium sp., A. alternata, C. carrionii* and *S. schenckii*, which is in agreement with most reports in the literature (3,17).

CONCLUSIONS

ECFA shows a good antifungal effect against a wide variety of fungi species, which makes it possible to be applied in medical therapy and agriculture, as well as being economical, easy to obtain and not causing side effects, although further studies are required for its use for Therapeutic application.

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