How to Cite:
AL-Tamimi, M. J. N., & AL-Zobaidy, B. M. H. (2022). Inhibitory efficacy of clove extract against some aflatoxin-producing fungi. *International Journal of Health Sciences*, *6*(S5), 11205–11212. https://doi.org/10.53730/ijhs.v6nS5.10983

Inhibitory efficacy of clove extract against some aflatoxin-producing fungi

Muna J.N.AL-Tamimi
Department Of Biology, College Of Education for Pur Sciences, University Of Karbala, Iraq.

Ban M.H.AL-Zobaidy
Department Of Biology, College Of Education for Pur Sciences, University Of Karbala, Iraq.

Abstract---This study was conducted in the Department of Life Sciences / College of Education for Pure Sciences / University of Karbala, where the effects of the alcoholic and aqueous extract of cloves on 21 aflatoxin-producing fungi isolated from foodstuffs were studied. 2.5 If compared to the comparison treatment and treatments in which concentrations of water and alcoholic extracts of cloves were used, if the alcoholic extract of cloves showed a high effectiveness in inhibiting the tested fungi, it was inhibitory for all fungi producing aflatoxin at a concentration of 2.5 mg/ml. As for the aqueous extract only, the results of the statistical analysis at the probability level of 2.5 mg / ml, there were clear significant differences between the tested fungi and high significant differences between The comparison treatment and the concentrations used for the extract Watery carnation The results of the study also showed that the alcoholic extract is more effective in inhibiting the growth of fungi than the aqueous extract.

Keywords---extract of cloves, aflatoxin, growth of fungi.

Introduction

Aflatoxins are one of the most important and dangerous types of mycotoxins, as they are either fatal or carcinogenic to the liver, produced by *Aspergillus flavus*, *Aspergillus* species (Balina *et al*, 2018). History has recorded many accidents caused by aflatoxin toxins, which claimed the lives of thousands of people in different countries and at different times (Patel *et al*, 2015. Al-Saeedi, 2002). Aflatoxin (AF) was called by this name because its origin is from the fungus A.flavus. The discovery of mycotoxins is the most important event in mycology, during which the so-called (Golden Age) appeared, the era of the emergence and

discovery of many mycotoxins produced by the rest of the fungi such as *Penicillium* and *Fusarium* which The toxins produce Ochratoxin and Pauline (Angele *et al*, 2018. Nagash, 2010). for the fungus AS. Flavus has different colors, it appears in green, blue, white and others. We can see it on bread, oranges, grapes, barley, cherries and corn, and it may grow on clothes and other moist objects that are not exposed to sunlight (Al-Janabi, 1988; Golan Barkia and Paster, 2008). The health problems of people who eat food contaminated with mycotoxins do not appear quickly. Food habits and the dose consumed will determine when the pathological symptoms of poisoning appear. At that time, the mycotoxin has caused great harm within the human body by targeting the vital internal organs in the body such as the heart, kidneys, liver and lymphatic system. Among the main problems resulting from mycotoxins are cancers, cirrhosis of the liver, weak immune system, kidney failure, heart failure ... and others (Matani, Najm Uday, 2014). Fungicides play a major role in combating fungi for many years, but their frequent use has shown many problems such as environmental pollution in addition to the emergence of resistant strains by fungi. polluting the environment (Khalil *et al.*, 2005). Accordingly, this study aimed to determine the efficiency of clove extracts with different concentrations on the growth of toxic fungi that produce aflatoxin toxin.

Materials and Working Methods

Collecting plants

The cloves were purchased as herbs from herbalists' shops, not ground. The herbs included cloves, and they were diagnosed in the herbarium of the College of Education for Pure Sciences, and then they were ground with an electric grinder and kept in bags until they were used. Preparation of the aqueous extract of cloves Prepare the aqueous extract with a weight of 50 g of dry powder of the clove plant and add to it 1000 ml of distilled water in a glass beaker and leave for a whole day at room temperature, then filter the mixture using several layers of clean gauze to get rid of plankton, then filter the extract using 0.1 type filter papers Whatman No . The extract was dried in clean glass dishes at laboratory temperature for several days until the weight was stabilized. The process was repeated to obtain a sufficient amount of the extract, then collected and preserved in opaque and sterilized bottles that were placed in the refrigerator at -4°C until use (Ahmed *et al*, 1998).

Preparation of alcoholic extract of clove

The alcoholic extract of clove was prepared in the same way as the aqueous extract, but ethyl alcohol with a concentration of 96% was used instead of distilled water (Khanzada *et al.*, 2006). The plant extracts were sterilized with a Milipor Filter (0.22) in diameter.

Method of preparing concentrations of aqueous and alcoholic plant extracts.

The concentrations (2.5, 5, 10, 15, 20) mg/ml were prepared, where the concentrations were calculated according to the law C1V1 = C2V2 as follows

1- 2 g of the extract was dissolved in 100 ml of culture medium to obtain a concentration of 20 mg/ml
2- 1.50 g of the extract was dissolved in 100 ml of culture medium to obtain a concentration of 15 mg / ml
3- 1 g of the extract was dissolved in 100 ml of culture medium to obtain a concentration of 10 mg/ml
4- 0.5 g of the extract was dissolved in 100 ml of culture medium to obtain a concentration of 5 mg/ml
5- 0.25 g of the extract was dissolved in 100 ml of culture medium to obtain a concentration of 2.5 mg/ml

Testing the effect of alcoholic and aqueous extract of clove plant on the growth of aflatoxin-producing fungi isolated from foodstuffs

The PDA culture medium was prepared, sterilized by an osmosis device, cooled to a temperature of 45 °C, and the antibiotic Amoxicillin was added at a rate of 250 mg / liter to inhibit bacterial growth. Then, the dried plant extracts were mixed with the medium according to the method of Sundhakar and his group (2009) to prepare concentrations (2.5, 5, 10, 20, 15) mg/ml culture medium at the rate of three replicates for each concentration, and after solidification in the plates, the growing fungus disc with a diameter of 5 mm was placed on the 7-day-old PDA medium in the center of the plate. A negative control was used that included a plate containing culture medium without any addition of any substance (Al-Janabi, 2004). The dishes were incubated at a temperature of (25 +-2 C) for 7-10 days, then the diameter of the developing fungal colony (average of two perpendicular diameters) was measured. The results were recorded and the percentage inhibition was calculated according to the following equation Inhibition percentage = (average colony diameter in control dishes - average colony diameter in treated dishes colony average in control dishes) x 100% (Uma *et al*, 1992; Wanchaitanawong *et al*, 2005) .

Determination of the minimum inhibitory concentration value of the alcoholic extract of clove

Was prepared by mixing the alcoholic extract with the culture medium in order to obtain concentrations (0,25, 0.5, 1, 1.5, 2) mg / ml and an average of three replicates for each concentration (Sundhakar and his group, 2009). The results were recorded based on the presence of growth (+) and the absence of growth (-) If, after the lowest concentration of the extract, no fungal growth appeared, the lowest inhibitory concentration is the minimum inhibitory concentration (Al-Ghazali and Al-Zawahiri, 2012).

Results and Discussion

Effect of aqueous and alcoholic extracts of cloves on the growth of some aflatoxin-producing fungi isolated from foodstuffs

The results showed that the inhibitory activity of plant extracts (aqueous and alcohol) towards the tested fungi depended on the type of extract and its concentration in addition to the type of isolate.

Results of the effect of aqueous extract on the growth of aflatoxin-producing fungi isolated from food

Table (1) shows the effect of aqueous extract on the growth of 21 aflatoxin-producing mushrooms isolated from food. The diameters of the colonies were measured in mm. The diameters were compared in the case of using concentrations (2.5, 5, 10, 15, 20) mg / ml of culture medium with the diameter of the colony in the case of the control, which included culture medium without any addition. Through the results it was found that the fungus *Penicillium griseofulvum* was the most effective in the aqueous extract of clove, with an average diameter of 7.25 mg. The most resistant fungus was *Penicillium oxalicum*, with an average diameter of 17.75 mg. As for the concentrations, the results showed that the aqueous extract inhibited some aflatoxin-producing fungi by 100% at different concentrations. In the fungi *Aspergillus austwicki*, *penicillium griseofulvum*, *Aspergillus caespitosus*, *As.caespitosus*, the complete inhibition occurred at a concentration of 15 mg/ml and in the fungi *Aspergillus oryzae*, *Aspergillus flavus*, *Trichophyton mentagrophytes*, *Cladosporium cladosporioides*, *Penicillium brevicompatum*, *Gibberlla intermedia*, *Debaryomyces hansenii*, *Aspergillus caespitosus*, *Aspergillus versicolor* The complete inhibition was at a concentration of 20 mg/ml.

Table (1) Effect of different concentrations of aqueous extract on the growth rate of aflatoxin-producing fungi isolated from food

Total	20 MG\ML	15 MG\ML	10 MG\ML	5 MG\ML	Concentration 2.5 Mg\ml	control	Type of fungi	
17.75	0.50	1.00	2.00	4.00	35	46	*Penicillium oxalicum*	1
13.00	0.25	1.25	2.00	8.00	14	52.5	*Penicillium expansum*	2
15.00	0.05	0.75	3.50	10.00	15.75	60	*Aspergillus flavus*	3
10.75	0.25	0.50	1.75	6.00	14	42	*Cladosporium uredinicola*	4
10.13	0.25	0.50	2.50	8.00	22	27.5	*Spergillus sydowii*	5
10.75	0	0.75	3.75	10.00	20	30	*Aspergillus oryzae*	6
8.60	0.25	0.25	1.12	3.00	14	33	*Aspergillus tamarii*	7
24.99	0.37	0.56	5.00	18.00	58	68	*Apergillus nomius*	8
14.02	0.05	0.58	4.00	3.50	16	60	*Altenaria triticina*	9
7.25	0	0	1.50	5.00	12	25	*Penicillium griseofulvum*	10
13.33	0	0	2.00	6.00	12	60	*Aspergillus austwicki*	11
13.75	0	0.50	4.50	11.50	27	39	*Asprgillus flavus*	12
13.21	0	0.25	5.50	12.00	19.25	42.25	*Cladosporium cladosporioides*	13
10.16	0	0.25	3.00	6.50	12	39	*Actinomucor elegans*	14
14.42	0	0.50	2.50	10.50	28	45	*Penicillium brevicompatum*	15

10.88	0	0.25	1.50	6.50	12	45	*Trichophyton mentagrophytes*	16
14.79	0	0.50	1.00	6.00	25	56.25	*Gibberella intermedia*	17
10.79	0	0.50	1.25	4.50	10.5	48	*Deparyomyces hansenii*	18
12.08	0	0	1.00	4.00	7.5	60	*Aspergillus caespitosus*	19
11.13	0	0.25	1.50	4.50	12	48.5	*Aspergillus flavus*	20
13.70	0	0.25	2.00	10.00	18	52	*Aspergillus versicolor*	21
12.88	0.10	0.45	2.52	7.50	19.24	47.12	Mean S.D	
						0	P.V	
						4.24	LSD	

The above results represent the average of three replicates of the control treatment, which included culture medium without any addition, and represented a negative control

Results of the effect of the alcoholic extract of cloves on the growth of aflatoxin-producing fungi isolated from foodstuffs

Table (2) shows the effect of alcoholic extract on the growth of 21 aflatoxin-producing mushrooms isolated from foodstuffs. The diameters of the colonies were measured in mm. The diameters were compared in the case of using concentrations (2.5, 5, 10, 15, 20) mg / ml of culture medium with the diameter of the colony in the case of the control, which included culture medium without any addition. Through the results it was found that the fungus *Penicillium griseofulvum* was the most abundant in the alcoholic extract of cloves, with an average diameter of 4.67 mg. The most resistant fungus was *Aspergillus nomius*, with an average diameter of 11.3 mg. As for the concentrations, the results of the statistical analysis showed that the alcoholic extract inhibited all aflatoxin-producing fungi by 100% at a concentration of 2.5 mg/ml. The results of the statistical analysis showed that there were high significant differences at the probability level of 2.5 if compared to the comparison treatment and the treatments in which the concentrations of the water and alcoholic plant extracts of cloves were used. The alcoholic extract of clove showed a high efficacy in inhibiting the tested fungi, as it was inhibiting all aflatoxin-producing fungi at a concentration of 2.5 mg/ml. As for the aqueous extract only, the results of the statistical analysis at the probability level of 2.5 mg/ml, there were clear significant differences between the tested fungi and high significant differences between the comparison treatment and the concentrations used for the aqueous extract of cloves.

Table (2) Average diameters of fungal colonies in mm using alcoholic extract with different concentrations compared to the control treatment

Total	20 Mg\ ml	15 Mg\ ml	10 Mg\ ml	5 Mg\ ml	2.5 Mg\ml	contro l	Type of fungi	
10.67	0	0	0	0	0	64.00	*Penicillium oxalicum*	1

8.67	0	0	0	0	0	52.00	*Penicillium expansum*	2
10.00	0	0	0	0	0	60.00	*Aspergillus flavus*	3
8.33	0	0	0	0	0	50.00	*Cladosporium ueidincola*	4
5.00	0	0	0	0	0	50.00	*Aspergillus sydowii*	5
5.00	0	0	0	0	0	30.00	*Aspergillus oryzae*	6
5.83	0	0	0	0	0	30.00	*Aspergillus tamarii*	7
11.30	0	0	0	0	0	35.00	*Aspergillus nomius*	8
10.00	0	0	0	0	0	68.00	*Alternaria triticina*	9
4.67	0	0	0	0	0	60.00	*Penicillium griseofulvum*	10
10.00	0	0	0	0	0	28/00	*Aspergillus austwicki*	11
6.00	0	0	0	0	0	60.00	*Aspergillus flavus*	12
7.00	0	0	0	0	0	40.00	Cladosporium *cladosporioides*	13
5.96	0	0	0	0 :	0	42.00	*Actinomucor elegans*	14
8.00	0	0	0	0	0	35.00	*Penicillium brevicompatum*	15
8.00	0	0	0	0	0		*Trichophyton mentagrophytes*	16
10.00	0	0	0	0	0	48.00	*Gibbererlla intermedia*	17
9.33	0	0	0	0	0	48.00	*Debaryomyces hansenii*	18
9.33	0	0	0	0	0	60.00	*Aspergillus flavus*	19
8.00	0	0	0	0	0	56.00	*Aspergillus caespitosus*	20
8.33	0	0	0	0	0	56.00	*Aspergillus versicolor*	21
	0	0	0	0	0	48.61	Maen S.D	
						2.94	LSD	
						0	P.V	

The above results represent the average of three replicates of the control treatment, which included culture medium without any addition, and represented a negative control.

The alcoholic extract of cloves proved to be more efficientin inhibiting the tested fungi than the aqueous extract. The reason for this may be due to the ability of ethyl alcohol to extract and dissolve the active substances in the used parts of the plant, the most important of which are resins, fluorides, flavonoids, tins and soaps, some of which dissolve in polar solvents but not others. Solvents such as alkaloids, which work to disrupt the cell membrane permeability, which results in the cell losing small molecules such as amino acids, simple sugars and ions, which leads to a decrease in the metabolic activities of the cell, including energy metabolism, active transport and the process of synthesis of proteins and acids As for the aqueous extract of cloves, it had a lower efficiency than the alcoholic extract in inhibiting the growth of the tested fungi. These results may explain the fact that water is less able to replace most of the active substances such as phenols, terpenes, flavonoids and others that have an inhibitory effect on the growth of fungi, while organic solvents such as Ethyl alcohol has a high ability to dissolve many active substances found in plants, and this agrees with the researcher (Hassan Alaa Khader et al., 2012).

References

1. Angele NT, Paul FM, Félicité MT (2010). Aflatoxin Contamination in Food and Body Fluids in Relation to Malnutrition and Cancer Status in Cameroon. Int. J. Environ. Res. Public Health, 7, 178-188;
2. Alaa Khader Hassan, Iman Abdel Karim Noris, Al Kuwaiti, and Nahida Mohamed Salch. Evaluation of the efficacy of black pepper and clove extracts. Plant Protection Department, College of Science, Agricultural Engineering, University of Baghdad, Iraq.
3. Al-Ghazali, Linda Ahmed Turki, Al-Tuwaihri, Zuhair Hamid Abboud (2012). Study of the inhibitory action of some plant extracts against some Dermatophytes, Master's thesis, College of Science / University of Karbala / Iraq.
4. Al-Janabi, Ali Abdul-Hussein Sadiq (2004), Treatment of skin diseases caused by dermatophytes of dermatophytes with preparations containing some purine compounds, PhD thesis, College of Science / Al-Mustansiriya University.
5. Al-Janabi, Sondos Jamil, 1998. The effect of some food preservatives on the growth of Aspergillus flavus Link ex Fries and its production of aflatoacin in flour, a master's thesis. The mechanism of education Ibn al-Haytham. Baghdad University.
6. Al-Saidi, Ahmed Abdullah Hamad Al-Dabbash. 2002. Investigation of fungi and aflatoxin B1 and M1 toxins in Some samples of cheese produced in Baghdad. Master Thesis. farming mechanism. Baghdad University
7. Balina A, Kebede A, Tamiru Y, (2018). Review on Afltoxin and itsImpacts on Livestock. Journal of Dairy & Veterinary Sciences. 6(2) .Ahmed, I. ; Mehmood, Z. & Mohammad, f. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J. Enthnopharmacole. , 62: 183 – 193

8. Barkia-Golan, R. and N. Paster.2008. Mould fruits and Vegetables as a source Of mycotoxins the Volcanic enter .Agricultural Research Organization. Bet Dagan 50250.Israel. Khalil , B.F. Dabaneh, and G.H. Anfoka.2005. Antifungal activity of Medical Plants. Jordan environmental, plant pathology, 4,130
9. Khanzada,Sh.A ; Iqbal,Sh.M. and Akram,A.(2006). Invito efficiency of plant leaf extracts against Sclerotium rolfsii Saac . Mycopath . 4(1) : 51-53.
10. Matani, Uday Najm, (2014). Mycotoxins: Theory and General Concept « Shaimaa Press, Baghdad, Iraq, p. 2830.
11. Prilosadoso, B. H., Atmaja, N. R. A. C. D., & Murwanti, S. (2021). Revitalization of folklore and actualization efforts through visualization animation media in early childhood character building. International Journal of Social Sciences and Humanities, 5(1), 1–8. https://doi.org/10.29332/ijssh.v5n1.590
12. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. International Journal of Health Sciences, 5(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938
13. Negash D (2018). A Review of Afltoxin: Occurrence, Prevention, and Gaps in Both Food and Feed Safety. Ethiopian Meat andDairy Industry Development Institute, Ethiopia. J. Applied Microbiol.Res.
14. Murtiastutik, D., Citrashanty, I., Endraswari, P. D., Widyowati, R., Ervianti, E., Sawitri, S., Rahmadewi, R., & Harningtyas, C. D. (2022). Antifungal activity of eugenol and clove leaf essential oil (Syzygium aromaticum L.) against clinical isolates of candida species. International Journal of Health Sciences, 6(S5), 6768–6775. https://doi.org/10.53730/ijhs.v6nS5.11530
15. Patel, S. V., Kumar, A., Bosamia, T. C., Bhalani, H. N. and Singh, P. (2015). Aflatoxins: Causes & Effects. A Monthly Magazine of Agricultural and Biological Sciences. XIII (9) : 140-142
16. Sundhakar, P.; Latha, p. ; Sreenivasulu, Y.; Bhaskar Reddy, B.V.; Hemalatha, T. M. ; Balakrishna, M. and Raja Reddy.(2009) . Inhibition of Aspergillus flavus colonization and aflatoxin (AfB1) in peanut by methleugenol. K. Ind.J. Exp.Biol.,47:63-67 1
17. Wanchaitanawong, P. ; Chaungwanit,P. ; Poovavodom, N. and Nitisinprasert , S. (2005). In vitro antifungal activity of Thai herb and spice extracts against food spoilage fungi . Kasetsart. J. Nat. Sci. 39 :400- 4 67.