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Detection of the Presence of Cyclopiazonic Acid and other alkaloid toxins by fungi contaminating the barley malt beverage found in the city of Mosul And inhibit it using thyme oil

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Abstract
This study included investigating fungal contamination in samples of malt beverage Available in Mosul With a focus on detecting cyclopiazonic acid and fungal alkaloids and testing the effectiveness of thyme oil in reducing this pollution The fungi were isolated and identified microscopically and morphologically. The use of Ehrlich's reagent as a colorimetric method for detecting the production of toxins and alkalis In both the malt beverage samples and the fungi isolated from it An experiment was also conducted to evaluate the inhibitory effect of thyme oil. By comparing the growth diameters of fungal isolates before and after treatment with oil at different concentrations And documenting that photographically The contamination level in the samples reached (77.5%) The Aspergillus was the most dominant in (42.8%) . The detector demonstrated its efficiency, as the results showed a positive response to the detector. The color red appeared in the sample R 12 and the color purple appeared in the sample R19 and the color yellow appeared in the sample R*.The fungal isolate Aspergillus fumigatus also showed a red color reaction ,Penicillium expansum give a purple color. And Cladosporium herbarum reacted yellow color. This confirms its contamination with toxins cyclopiazonic acid and other alkaloids Thyme oil demonstrated very high inhibitory efficiency, achieving a maximum inhibition rate of 100% against fungal growth at the specified concentration 0.5% This confirms the oil's effectiveness in inhibiting fungal growth. This opens up prospects for its use as a natural preservative in local food industries in the city of Mosul.

Keywords: Malt Beverage, contaminating fungi, Mosul City, Aspergillus, Cyclopiazonic acid, Ehrlich's Reagent thyme oil , antifungal activity

Introduction

Barley drinks are beverages derived from fermented and unfermented barley. These drinks are consumed in various forms all over the world. From traditional malt beverages to modern non-alcoholic malt drinks (Bhattarai *et al*., 2023) [1]. Which are marketed for their health benefits (Raj *et al*., 2023) [2]. One of the most important fungi that contaminate barley malt beverages Aspergillus. Spp, Pencillium. Spp, Cladosporium. Spp and Fusarium. Spp (Brettrager *et al*., 2022) [2] .Pollution is often produced as a combination of several fungi. This leads to more than one fungal poison in the drink (Pascari *et al*. 2022) [12]. These fungi are characterized by their ability to produce a range of secondary toxins that may be dangerous on human health (Goda *et al*., 2025) [8]. Among these toxins are the toxins of alkaloids In addition to the fungal poison known as Cyclopiazonic acid It is one of the fungal toxins produced by fungi Aspergillus and Pencillium (Jiang *et al*., 2025; Huang *et al*., 2025) [10].

It represents the contamination of barley drinks with Mycotoxin Public health concern (EFSA, 2023 [7]; Codex Alimentarius/FAO & WHO, 2022) [6]

Matereal and Method

Sample collection

A number of samples of malt beverage were collected from different areas of Mosul and its suburbs. Information on each sample was recorded, including the production and expiry dates, the manufacturing company, the beverage's ingredients, and the collection area. These samples were then stored until use.

Isolation of Fungi

The dilution method was used (Ronald *et al*, 1995, Marth, 1978) [11, 15] for different samples of barley malt found in Mosul. Samples were cultured on both media Potato Dextrose Agar (PDA) Sabouraud Sucrose Agar (SSA) (Hoog &Guarro, 1995; Pitt & Hocking, 1997) [5, 13], with four replicates per sample and a control plate. They were incubated for7 days at 28 C°, and the results were recorded.

Detection of toxins Cyclopiazonic acid and ather alkaloids in barley drink using an Ehrlich detector

In order to test for the presence of toxins Cyclopiazonic acid and other alkaloids In barley drink 0.5 ml of Ehrlich reagent was added In a tube containing 5ml of barley drink and Note the color change resulting from adding the indicator And comparing it to the color of the original sample In another tube, the same amount of drink (stander) and recording information and Repeat this process for all samples (Chang-Yen &Bidasee, 1990) [3].

Detection of toxins Cyclopiazonic acid and ather alkaloids by fungi contaminating the malt beverage using an Ehrlich detector.

Filter paper was useda small piece of fiber filter paper was placed over a growing fungal colony in a Petri dish To determine the fungus's ability to produce this type of toxin This is done by placing drops of the reagent On paper, we observe the color change of the paper filter For different time periods, record the color change, and repeat the same steps for the remaining fungi (Chang_Yen &Bidasee, 1990) [3].

Testing the effect of thyme oil on fungi

Tablets with a diameter of 7 ml were taken from fungal colonies .Pencillium ssp. and Aspergillus fumigatus .At the age of 7 days Isolated from barley malt drink They were planted in the center of Petri dishes containing a medium PDA Mixed with thyme oil In different concentrations (0.1%, 0.5%, 1%, 2%, 3%) three repetitions per concentration With a Petri dish without added oil as a comparison sample All steps were carried out under sterile conditions. All Petri dishes were incubated at 25 C° for7 days. The results were obtained by calculating the average of each pair of perpendicular diameters for each fungal colon (Pitt and Hocking, 1997) [13].

Result and discussion

The presence of cyclopiazonic acid and other alkaloids was tested in 40 samples of malt beverage Collected randomly from the city of Mosul 13 samples were observed to be free of these toxins. As for the rest of the samples 27 It contains these toxins With different qualities and concentrations (table: 1) (figure: 1). 15 samples contained cyclopiazonic acid toxins 11samples were reddish-brown in color, and 3 samples gave off a purple color and One sample gave a pinkish color.This is evidence of the high concentration of poison.While 12 samples of the gived yellow, indicating the presence of alkaloids, as shown in the (figure: 2)

Table 1: The extent of contamination of malt beverages in Mosul with cyclopiazolinic acid and other alkaloids using Ehrlich reagent.

Sample number	Number	Ehrlich test color
R1	1	Pink
R13 ,R19 ,R29	3	Purple
R18,R24 ,R26 ,R21,R28,R30 R31, R32,R33,R34,R12	11	Red /Brown
R4 ,R8,R9,R10,R11,R15,R16 R17,R20,R22,R23,R27	12	Yellow
R2,R3,R5,R6,R7,R14,R25,R35 R36,R37,R38,R39,R40	13	Colorless
	40	The total

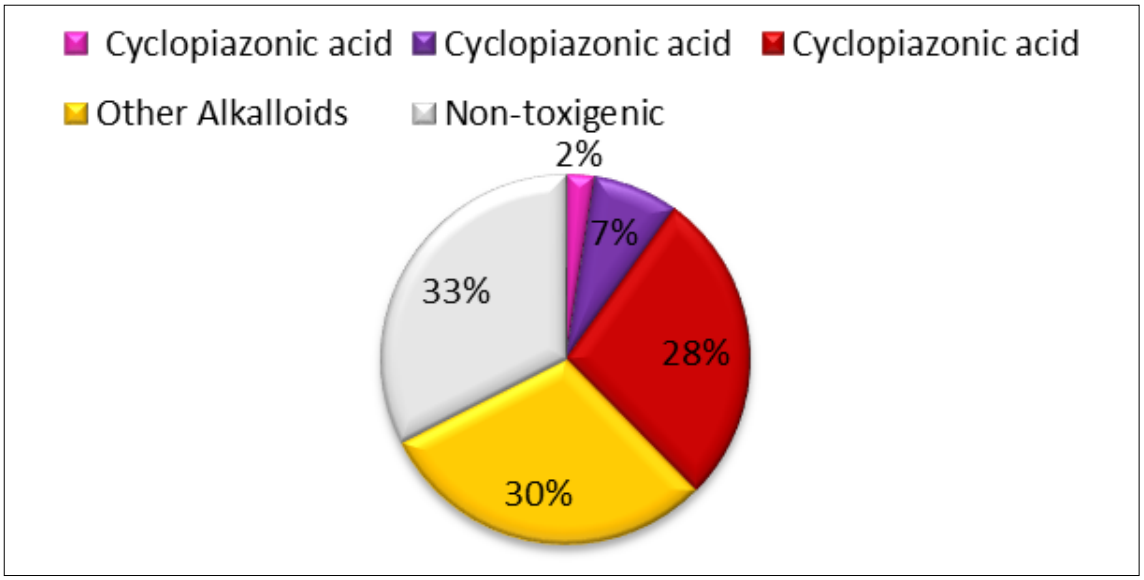
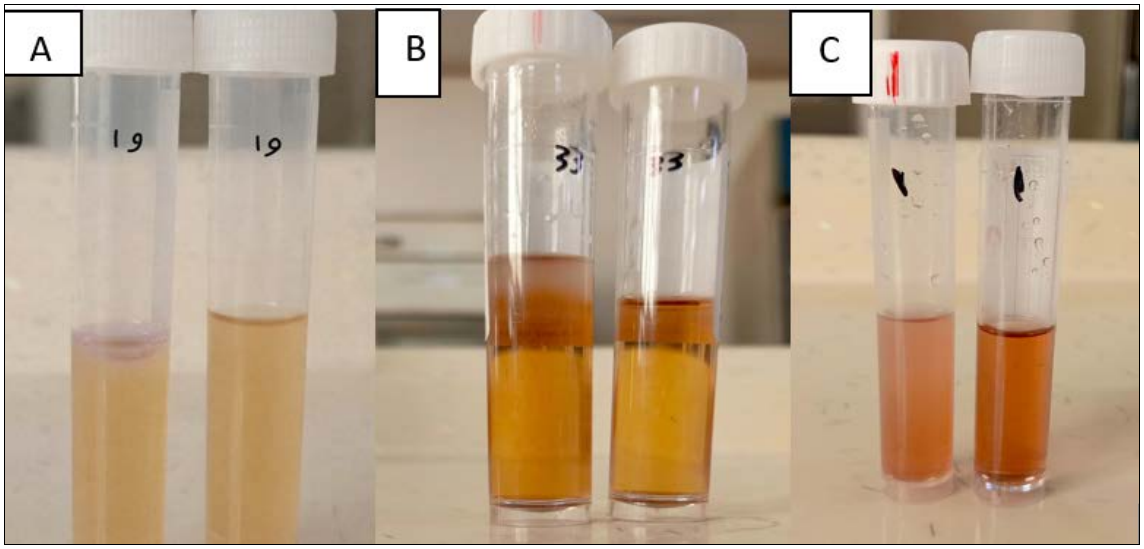


Fig 1: The percentages of contamination of malt beverage samples with cyclopiazonic acid and other alkaloids.



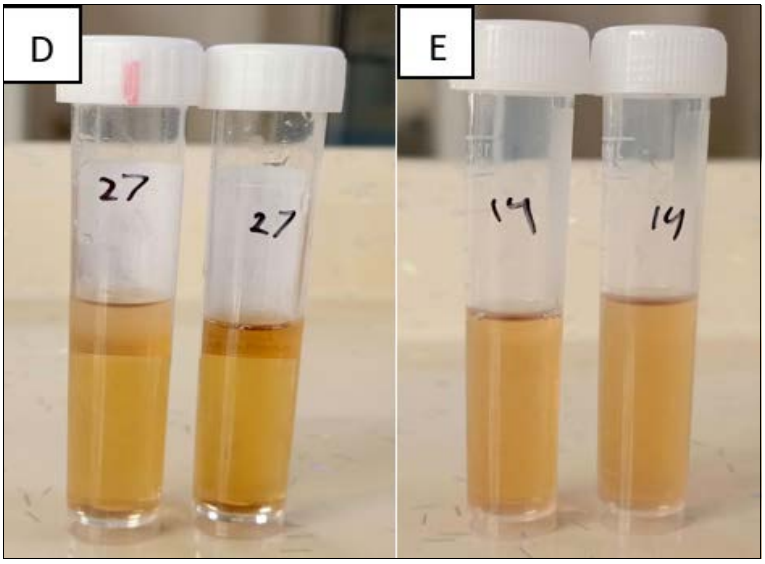


Fig 2: Testing the extent of contamination of malt beverage samples with toxins Cyclopiazonic acid and Other Alkalloids A: Purple color B: reddish-brown in color C: Pink color D: Yellow color E: Colorless

And from ability test of some fungal isolates on producing cyclopiazonic acid and other alkalloids (***Table***: 2) shows 9 fungal isolates that produced toxins Cyclopiazonic acid in different percentages (figur: 3)

Table 2: The extent to which fungal isolates are Capable of Producing toxin cyclopiazolinic acid and other alkaloids using Ehrlich reagent.

Sample number	Name of fungi	Ehrlich reagent test color	Intensity of concentration
R5	*Aspergillus oryzae*	Yellow	+++
R7	*Aspergillus fumigatus*	Yellow	+++
R7	*Aspergillus terreus*	Yellow	+++
R10	*Aspergillus fumigatus*	Yellow	+++
R12	*Aspergillus fumigatus*	Red	+++++
R13	*Pencillium expansum*	Purple	+++++
R13	*Fusarium oxysporum*	Purple	+++++
R14	*Pencillium verrucosum*	Purple	+++++
R15	*Fusarium poae*	Yellow	+++
R18	*Aspergillus fumigatus*	Purple	+++++
R19	*Aspergillus flavus*	Purple	++++
R20	*Rizoctonea solani*	Purple	++++
R22	*Aspergillus flavus*	Yellow	+++
R24	*Astenphylium botryosum*	Yellow	+++
R27	*Cladosporium sphaerospermum*	Yellow	+++
R29	*Cladosporium sphaerospermum*	Purple	++++
R32	*Cladosporium herbarum*	Yellow	+++
R37	*Tricoderma harzianum*	Purple	+++++
R38	*Astenphylium globuliferum*	Yellow	+++

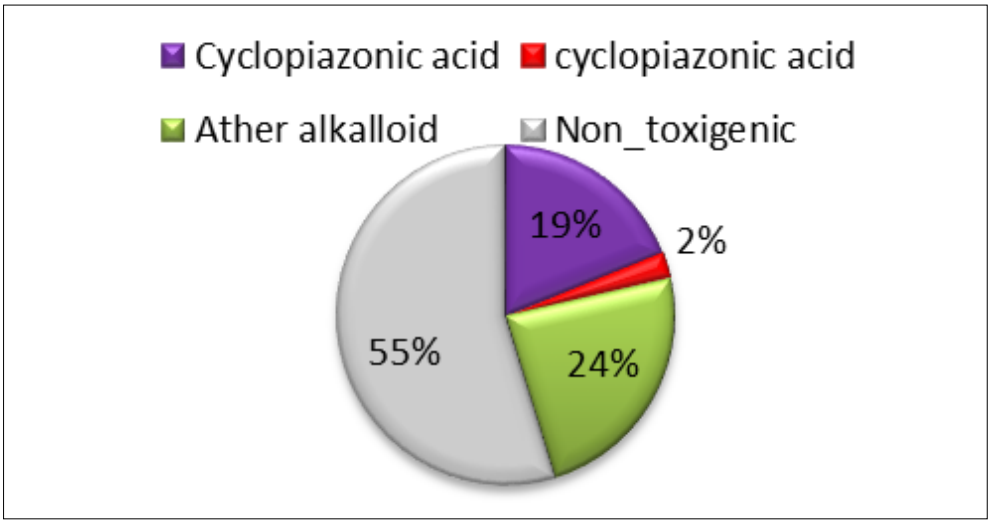


Fig 3: shows the percentages of CPA and other alkaloid production by fungal isolates.

Where 8 isolates gave the purple color (*Pencillium expansum, Tricoderma harzianu, , Fusarium oxysporum, Pencillium verrucosum, Aspergillus fumigates, Aspergillus flavus , Rizoctonea solani , Cladosporium sphaerospermum)* ,While one colony(*Aspergillus fumigates*) gave off a red color from the very first moment at a high concentration Evidence of high concentration of toxins Ceclopiazonic acid. As in (figure:4)

Fig 4: Testing the ability of fungal isolates to produce toxins CPA
a: Red Color b , c : Purple Color

And 10 fungal isolates gave a yellow colo *(Astenphylium botryosum ،A.oryzae، A.fumigates ،A.terreus، A.fumigates ، Cladosporium sphaerospermum، A.flavus Fusarium poae ، Cladosporium herbarum ،Astenphylium globuliferum)* Evidence of its production of alkaloids While, the remaining 23 fungal isolates did not give any color reaction when Ehrlich's reagent was added to them, indicating that they are free of these toxins, as shown in (Figure: 5).

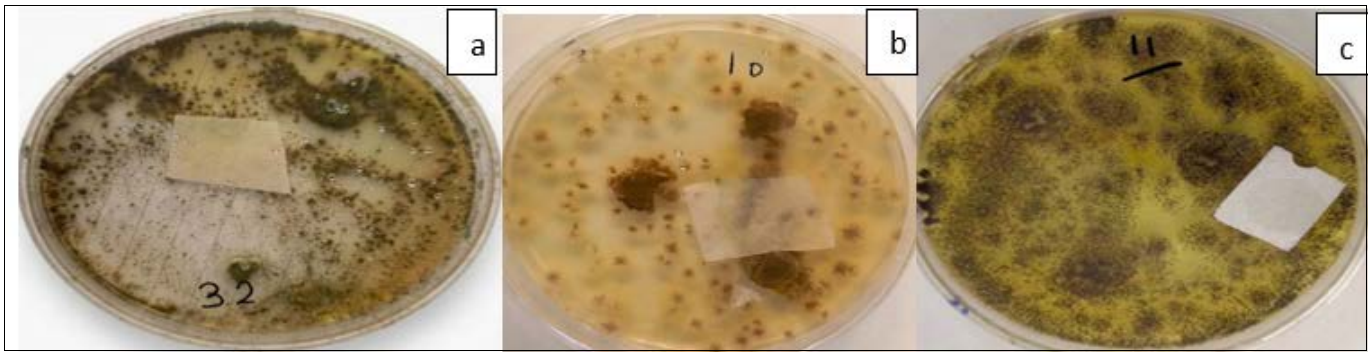


Fig 5: Testing the ability of fungal isolates to produce toxins Alkaloids
a, b : Yellow Color c: Colorless

Comparison of the ability of fungal isolates to produce cyclopiazonic acid and other alkaloids and the extent to which these toxins are present in barley malt samples

The results showed a marked variation in the color response of both the barley malt samples and the fungal isolates at different percentages, as shown in the (figure: 6)

The red color appeared in the malt beverage samples in a 27.5%, and this was the highest percentage, where 10 samples R18 ,R 24 ,R26 ,R21 ,R28 ,R30 ,R31 ,R32 ,R33 ,R3 It gave a reddish-brown color reaction Evidence of its heavy contamination with toxins cyclopiazonic acid When compared to the fungi isolated from it, it was found that the fungus *A.fumigatus*

A red color reaction was observed Immediately the indicator was added to it 2.38% When compared to the same isolated barley malt sample R 12 , it was found that the sample gave Strong response to the detector It appeared in the same shade of red This confirms that this sample of barley malt beverage is contaminated with toxins Cyclopiazonic acid and This fungus *A.fumigatus* is what produced these toxins During manufacturing and storage until consumption . As for the fungi that gave a different color reaction than the red color that appeared in the samples They are fungi *Astenphylium botryosum* isolated from a sample R24 and *Cladosporium herbarum* isolated from a sample R32 that gave a yellow color reaction Evidence of the presence of alkaloid toxins This can be explained by the fact that the red drink sample contains cyclopiazonic acid and other toxins The possibility of the fungus producing it in high concentrations Or the presence of other toxins in the drink originating from raw materials contaminated with toxins While a single fungal isolate, A. flavus isolated from sample R18 gave a purple color Evidence that the fungus was the cause of this sample's contamination with toxins during manufacturing and storage until consumption The remaining fungi isolated from the same samples that did not give any color reaction are non-toxic fungi includes Derchslera australiensis isolated from R21 and Cladosporium macrocarpum , Alternaria alternata from R26 and Cladosporium macrocarpum from R28 and Cladosporium sphaerospermum from R30 and Cladosporium sphaerospermum from R31, The reason for the presence of cyclopiazonic acid in the malt beverage samples was The result of barley contamination during field and storage before production.

While 19% of the fungal isolates produced a purple color from the first moments of adding the reagent to them and 7.5% of the malt beverage samples gave the same color Evidence of the presence of toxins Cyclopiazonic acid and When comparing the toxin-producing fungi with the beverage samples isolated from it and contaminated with cyclopiazonic acid Show fungi *P.expansum* and *Fusarium oxysporum* isolated from R13 and *A.flavus* isolated from R 19 and *Cladosporium sphaerospermum* isolated from R29 These fungi are the ones that produced toxins cyclopiazonic acid And it contaminated the malt beverage During manufacturing and storage until consumption. Some fungi produced a purple color and isolated drink samples from them showed a different color reaction like *Rhizoctonia solani* isolated from R20 It produced a yellow color, indicating the presence of alkaloid toxins depending on the storage conditions. And *A.flavus* isolated from R18 It gave a reddish-brown color reaction It was mentioned previously and P.verrucosum isolated from R14 and Tricoderma harzianum from R37 Both samples of malt beverage were free of these toxins This is evidence of the lack of suitable production conditions during storage and manufacturing. As for the pink color we observe its appearance in barley malt samples in 2.5% Evidence of cyclopiazonic acid toxins And its absence in fungi isolated from it Evidence that the barley used in manufacturing was previously contaminated with these toxins It was previously contaminated with these toxins, so it gave off a small percentage of the poison in the drink If the contamination were a result of manufacturing and storage then the contaminating fungi would also be toxin-producing. The fungal isolates that produced alkaloids (yellow color)were in 23.80% While in the malt beverage samples it was at a rate of 30% and When comparing the fungi that produce them with the drink samples isolated from them and contaminated with alkaloids the A.flavus isolated from R22 and A . fumigatus isolated from R10 Cladosporium sphaerospermum isolated from R27 and Fusarium poae isolated from R15 These fungi produced alkaloids during manufacturing and storage until consumption , and One fungal isolate produced a color different from that of a yellow malt beverage sample it is *Rhizoctonia solani* isolated from R20 It was mentioned previously. Samples that were contaminated with alkaloids and their fungal isolates were pure an d did not produce these toxins includes *A.fumigates, A.niger* isolated from R11 and A. flavus isolated from R8 and A.niger from R17 and A.fumigates isolated from R23 The presence of alkaloid toxins in the malt beverage samples was due to contamination in the field and during storage prior to production (Raw materials contaminated with toxins) . The remaining fungal isolates representing 54.76% And samples of malt beverages amounting to 32.50% It did not give any color reaction This indicates that it is free from cyclopiazonic acid toxins and other alkaloids. The samples include malt beverages and fungi isolated from it that are free from contamination. *Alternaria infectoria , Cladosporium herbarum ,P.chrysogenum, A.niger* isolated from R2 and *A. Flavus, A.niger* isolated from R3 and *Trichoderma hazianum , Trichoderma viride* isolated from R37 *and Stemphylium globuliferum* from R38 .There are samples although they are free of contamination Its fungal isolates show a reaction with the reagent Evidence of its production of cyclopiazonic acid and other alkaloids includes *A.fumigates, A.terreus* isolated from R7 and *A.oryzae* from R5 and *Cladosporium macrocarpum* from R39 Produced alkali toxins . The reason these toxins did not appear in the beverage samples was that the appropriate conditions were not present During storage and manufacturing to the production of these toxins and There was one drink sample Whose fungal isolate was toxin_Producing Cyclopiazonic acid it is P.verrucosum isolated from R14 It was mentioned previously.

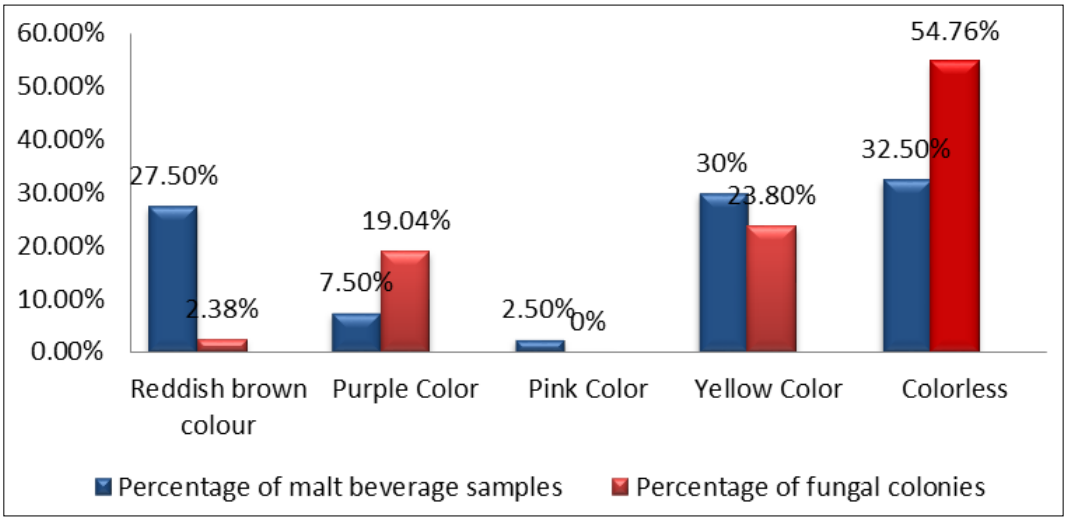


Fig 6: compares the percentages of fungal colonies and malt beverage samples, and their production of cyclopiazonic acid and other alkaloids.

Testing the potential of thyme oil to inhibit the growth of toxin-producing fungi Developed on PDA medium by using different concentrations of oil for fungi *Aspergillus fumigatus* and *Penicillium expansum*

The results showed that the fungi A.fumigatus and Penicillium expansum were completely inhibited (100%) at a concentration of 0.5% as in the (figure: 7).

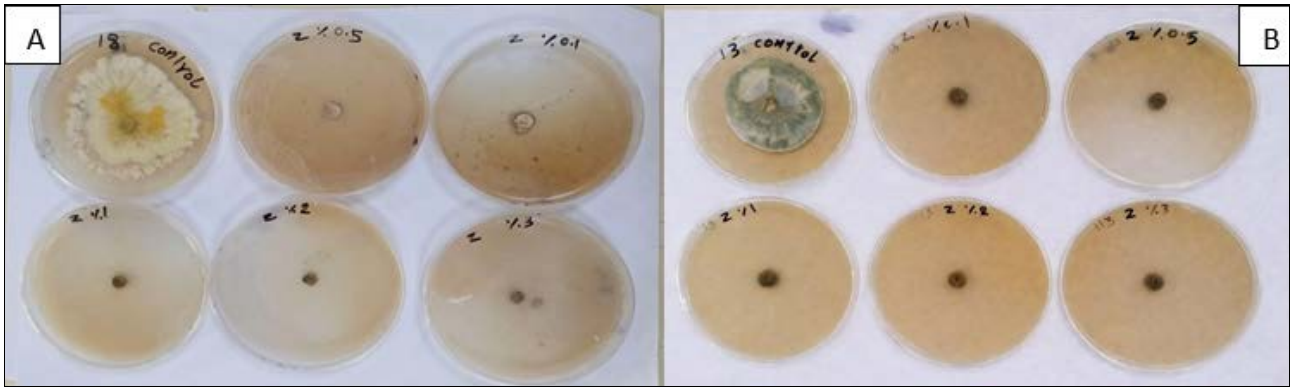


Fig 7: inhibits the growth of the fungi A. fumigatus and Penicillium expancum using thyme oil

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