



EVALUATION OF THE ACTIVITY OF THE ALCOHOL EXTRACTS, NANOMOLECULES AND GREEN SILVER NANOPARTICLES OF THE PLANT *SALVIA OFFICINALIS* AND *CINNAMON* IN TREATING AFLATOXIN B1 FROM *ASPERGILLUS FLAVUS* IN CONTAMINATED POULTRY FEED

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Abstract

This study included obtaining isolate of AFB1 producing *Aspergillus flavus* from the Department of Plant Protection, College of Agricultural Engineering Sciences of the University of Baghdad. The inhibitory activity of *Salvia officinalis* (Sage) leaves and *cinnamon* alcoholic extract at (5 and 10%) as individually pattern against the fungi isolate as well as the inhibitor activity of Sage nanomolecules (SNMs) and Cinnamon nanomolecules (CNMs) at concentration of 5µg/100 ml produced from mechanical grinding of the plants powder, green silver nanoparticles of sage leaf extract (SAGNPs) at a concentration of 208.75 ppm and green silver nanoparticles of the cinnamon extract (CAGNPs) at concentration of 252.3 ppm. Results exhibited greater ($P < 0.01$) growth inhibition with 100, 100, 83.53, 89.41, 97.53 and 99.77% respectively as compared with the control group. Feed that contaminated with AFB1 were treated with either sage (10%) or Cinnamon (5%) leaves alcoholic extract as well as with SNMs (5 µg/Kg feed), CNMs (5 µg/Kg feed), SAGNPs (208.75 ppm), CAGNPs (252.3 ppm) Four weeks incubation period. Detection of AFB1 was done using a high performance liquid chromatography technique (HPLC). Results observed absence of AFB1 in treated samples as compared with the control group (presence of 38 ppb AFB1). It can be concluded that plant extracts and green silver nanoparticles have highly inhibited activity against *A.flavus* that can be used as good alternatives of harmful fungicides to public health.

Key words : Activity, cinnamon and sage, nanoparticles green, aflatoxinB1, poultry feed.

Introduction

Feed contamination of crops or their industrial residues with mycotoxins is difficult to avoid, especially aflatoxins, the most common and dangerous in the ranges of impact (Bennett *et al.*, 2003). Aflatoxins are highly toxic mycotoxins that cause many cancers such as hepatocellular carcinoma, lung cancer, stomach, intestines etc. Aflatoxins are secondary metabolites produced during the metabolism of some genus *Aspergillus* as *Aspergillus flavus*, *A. parasiticus* and *Penicillium puberulum*, It can be found in legumes, nuts (walnuts, almonds, cashews,

pistachios), peanuts, soybeans, grains such as corn, rice, and barley when suitable conditions for their growth, especially in tropical and subtropical areas. (Gratz *et al.*, 2004). To control the growth of isolates secreting mycotoxins through the use of different methods such as physical, chemical and biological methods, where the biosynthesis of AFB1 can be inhibited by a number of natural compounds found in most medicinal plants such as cinnamon, sage leaves and others, because they contain many active compounds And aflatoxin production (Mahamoud, 1994). It is also known that there are many techniques have a role in the treatment of the effects of food contaminants such as aflatoxin, including the latest

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nanotechnology (nanoparticle technology or nanotechnology) and this technique is measured in nanometers, and is part of the millionths of a millimeter, it should be noted that this technique is used in. the present research is aimed to evaluate the effectiveness of alcoholic extract, particles and silver nanoparticles of cinnamon powder and sage leaves in inhibiting the growth of *A. flavus* and inhibiting the toxicity of AFB1 when add them to contaminated poultry feed.

Materials and methods

Preparation of isolation *Aspergillus flavus*

The isolation of aflatoxin-producing *Aspergillus flavus* (B1) (diagnostic isolation) was obtained from the Mycotoxic Laboratory Plant Protection Department / College of Agricultural Engineering sciences / University of Baghdad isolated from Iraqi rice Isolated on the dietary medium potato Dextrose Agar (PDA) and incubated at 25 ° C for 10 Days and then kept the farms under cooling until use.

Preparation of the basic concentrations of alcoholic extract, Nano molecules and silver nanoparticles of sage leaves and cinnamon

Concentrations of solutions were prepared according to the method reported by Al-Tamimi (2001) with some modifications as follows:

- A. Concentrations of alcoholic extract of sage leaves and cinnamon were prepared individually at 1, 5 and 10% concentrations by dissolving (0.1, 0.5 and 1) grams of dry powder of alcoholic extract in 10 mL sterile distilled water.
- B. The nanoparticles of sage leaves and cinnamon were prepared separately in concentrations (2, 5, 10) 5 µg / 100ml respectively by dissolving (0.2, 0.5, 1) 5 µg respectively of the nanoparticle powder of both plants in 10 mL water Sterile distilled.
- C. Green Silver nanoparticle solutions of sage leaves and cinnamon were prepared individually at concentrations (2, 5 and 10)% equivalent (83.5,208.75, 417.5) and (100.9, 252.3 and 504.6) ppm, respectively, by dissolving (0.2, 0.5, 1) mL respectively of silver nanoparticle extract prepared in 10 ml sterile distilled water.

Detection of inhibitory activities of alcohol extracts, nanoparticles, silver nanoparticles prepared of sage leaves and cinnamon of *A. flavus* fungus. (in vitro)

All the concentrations prepared in the above mentioned part were taken separately from cinnamon and sage leaves individually and added to 100 ml of the agricultures medium (PDA) and poured in to 9 cm dishes

and left to harden the PDA (25 ± 2 °C) and then the readings were taken after (3, 5, 7) Lap days and compare them by calculating the growth of both *A. flavus* fungus. The vertical diameters of the mushroom colony were estimated to calculate the inhibition ratio according to the following equation:

$$\text{Inhibition \%} =$$

$$\frac{\text{Compared to the surrounding average colonizati on - Colony Treatment average colonizati on}}{\text{Colonial comparision average colonizati on}} \times 100$$

Contamination of feed by AFB1 and the adding of alcoholic extracts, nanoparticles and green silver nanoparticles to cinnamon and sage leaves

Feed ingredients were mixed and contaminated at the Poultry Field of the College of Agricultural Engineering science / University of Baghdad. by adding fungal isolate *A. flavus* producing AFB1 to the feed and incubated for four weeks. The feed contaminated with AFB1 was treated by alcoholic extracts of cinnamon and sage leaves at concentrations 5 and 10% respectively as well as SNMs, CNMs, SAgNPs and CagNPs 5 µg/100ml, 5 µg/100ml, 208.75 ppm, 252.3 ppm respectively, using a high-performance liquid chromatography device (HPLC) for detection AFB1. Feed samples contaminated with fungus *A. flavus* product of aflatoxin toxin compared with control samples (untreated) which exhibited the presence of AFB1 at a concentration of 38 ppb.

Statistical analysis

SAS (2012) was used study the effect of different treatment on the studied characteristics according to a complete random design (CRD). The Mathematical model was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

µ: Mean, T_i:The effect of the transaction, e_{ij}: Standard error

Results and Discussion

Effectiveness of alcoholic extracts of cinnamon and sage leaves in inhibiting the growth of *A. flavus*:

(Table 1) shows the inhibitory activity of alcoholic extracts of sage and cinnamon leaves. The highest inhibition rate was 96.25 and 100% respectively at concentration 5%, with the average growth rate of fungi 0.33, zero cm, and the lowest inhibition rate at 1%. The average fungal growth rate were 1.63 and 0.62 cm was 80.82 and 92.71%, respectively, as compared with the control treatment where the inhibition ratio was zero and the average diameter of the fungal growth (8.5 cm). The concentration of 5% was the lowest to kill the fungi of cinnamon extract, while the lowest concentration to kill the sage extract was 10% (Fig. 1).

The results observed significant differences ($P < 0.001$) in the inhibition percentage of the concentrations 5 and 10% of the alcoholic extract for cinnamon plant and sage leaves respectively on the concentration of 1% on the one hand and the control treatment on the other hand and significantly higher concentration of 1%. Non-significant differences were observed among the three concentrations in the average diameter of the fungal growth, while they were superior to the control treatment. The results indicate that the average diameter of fungal growth and inhibition rates exceeded the alcoholic extract of both plants over the powder for the same plants. This superiority can be attributed to the fact that the active compounds present in the plants are in a state of association with the sugary and protein compounds which leads to reduce or determine the effectiveness of the active compounds while in In the case of the extract there is disintegration and release of these active compounds from binding to other carbohydrates and protein, allowing them to concentrate and increase effectiveness (Al-Zubaidi, 2005).

The inhibitory activity of the plants extracts may be due to the fact that they contain various active compounds such as alkaloids, phenols and glycosides that inhibit the growth of many pathogenic microorganisms, including fungus under study, as well as the containment of the cinnamon extract on flavonoids and steroids as well as the effect of other active groups such as glycosides Alkaloids, resins, soaps, coumarins and turbines may make it have a higher inhibitory activity than the alcoholic extract of sage leaves that lacked flavonoids and steroids in Specific chief effective groups. These results are consistent with Shawkat *et al.*, (2012). It showed that the alcoholic extract of sage leaves at a concentration of 5% gave the highest rate of inhibition of fungi the water

extract gave the highest inhibition at 15% concentration. Our results agree with Hashim *et al.*, (2008) who pointed out that the inhibitory activity of fungi at the high concentration of oil extract of cinnamon may be due to containing basic chemicals such as: turbines and flavones and pointed to the presence of more than one substance may inhibit fungal growth and the production of aflatoxin. In addition, the plant extracts contain phenolic compounds that have antimicrobial activity in general and fungi in particular (Simi *et al.*, 2004). also reported that the antimicrobial activity of several plants, including cinnamon *C. zeylanicum*, whose oil showed the most potency for containing the essential ingredient Trans-cinnamaldehyde. Other studies suggested that phenolic compounds penetrate the cell membrane and bind at active sites with enzymes within the cell and close them and thus make them unable to bind to the base materials. With adenosine triphosphate group (ATP), which are oxidizing agents, they bind to them and stop working, making energy weak within the microbial cell, whether bacterial or fungal, reducing its effectiveness and leading to killing (Ultee *et al.*, 2002). This results in loss of membrane integrity, inhibition of cell growth and reduced cell wall rigidity, thereby inhibiting growth of the fungus (Avanço *et al.*, 2017).

This corresponds to what Yin *et al.*, (2015) noted in his study to verify the inhibitory effect of two phenolic compounds, carvacrol (CR) and cinnamaldehyde (cinnamaldehyde), on the growth of *A. flavus* and *A. parasiticus* and the production of (AFB1). The two compounds reduced the growth of *A. flavus* and *A. parasiticus* in addition to the reduction of AFB1 production by at least 60% compared to control group.

Effectiveness of nanoparticles and green silver nanoparticles in inhibiting the growth of *A. flavus* fungus

Table 1: Inhibitory effectiveness of alcohol powder and cinnamon extracts of cinnamon and Sage leaves in *A.flavus* growth.

Inhibition%	Average fungal growth diameter (cm)	Concentration %	Transactions	No.
h 0.00 ± 0	8.5±0.26a	—	Control	1
c 80.82 ± 3.59	c 1.63 ± 0.02	1	Alcoholic extract of sage leaves	2
ab 96.25 ± 3.78	c 0.33 ± 0.01	5		
a 100 ± 0.00	c 0.00 ± 0	10		
b 92.71 ± 3.42	c 0.62 ± 0.05	1	Alcohol extract of cinnamon	3
a 100 ± 0.00	c 0 ± 0.00	5		
a 100 ± 0.00	c 0 ± 0.00	10		
**	**		Moral level	

**($P < 0.01$).

(Table 2) noticed the results of the inhibitory activity of SNMs and CNMs on the growth of fungus *A. flavus* The optimal inhibitory activity of SNMs and CNMs was at a concentration of 5 µg / ml100 inhibition ratio (83.53 and 89.41)% respectively and at the average fungal growth diameter (1.4 and 0.9) respectively. While the concentration of 2 µg / ml100 was the lowest inhibition and reached (68.24 and 76.47)% respectively at the average fungal growth rate (2.7 and 2) cm respectively compared to the control treatment by zero inhibition and at the average diameter of the fungal

Table 2: The inhibitory activity of nanoparticle powder particles and green nanoparticle minutes in the growth of *A. flavus* fungus.

Inhibition%	Average fungal growth diameter (cm)	Concentration %	Transactions	No.
f 0±0.00	8.5±0.26 a	—	control	1
e 68.24±2.73	b 2.7±0.04	2 µg/100ml	Nanomolecules of sage plant powder	2
c 83.53±3.07	cd 1.4±0.01	5 µg/100ml		
a 100±0.00	0±0.000	10 µg/100ml		
d 76.47±2.61	c 2.0±0.01	2 µg/100ml	Nanomolecules of Cinnamon powder	3
b 89.41±4.21	d 0.9±0.02	5 µg/100ml		
a 100±0.00	0±0.00	10 µg/100ml		
90.82±3.71 b	d 0.78±0.02	83.5 ppm	The green silver nanoparticles of sage leaves	4
97.53±3.40 a	e 0.21±0.01	208.75 ppm		
100±0.00 a	0±0.00	417.5 ppm		
98.59±2.85 a	f 0.12±0.01	100.9 ppm	The green silver nanoparticles Cinnamon nanoparticles	5
99.77±0.41 a	g 0.02±0.004	252.3 ppm		
100±0.00 a	0±0.00	504.6 ppm		
**	**		Moral level	

** (P<0.01).

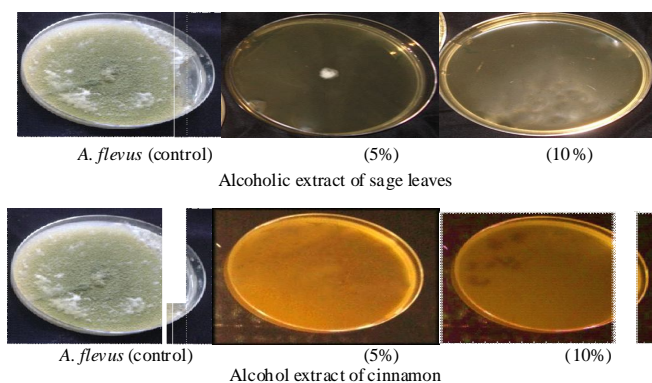


Fig. 1: Effect of alcoholic extract of cinnamon and sage leaves on *A. flavus* growth.

growth 8.5 cm. (Fig. 2).

The green silver nanoparticles SAgNPs and CAgNPs also gave inhibition activity of 100% fungal growth at concentrations (417.5 and 504.6) ppm, respectively, while the lowest inhibition was (90.82 and 98.59)% respectively at (83.5 and 100.9) ppm respectively, compared with the control group in which the inhibition ratio (Zero) and the average diameter of the fungal growth (8.5) cm (Fig. 2). The results of the statistical analysis showed significant differences at the probability level ($P < 0.001$) in the inhibition ratio and the average diameter of the fungal growth among the three concentrations of the nanomolecules of (SNMs) and (CNMs) on the one hand and the control treatment on the other hand. SAgNPs and CAgNPs were highly significant at ($P < 0.01$) among

83.5, 208.75, 100.9 and 252.3 ppm respectively in average fungal growth and inhibition rate compared with control group. For both plants the concentration was 5 µg / ml100, as for the green silver nanoparticles of sage and cinnamon The optimum inhibitory efficacy was at concentrations (208.75 and 252.3) ppm, respectively. These concentrations were added to contaminated feed.

These results were in agreement with Abdulrahman and Hussein (2017), where the results showed that the effectiveness of silver nanoparticles (AgNPs) in inhibition of *A. flavus* mushrooms was 100% at concentration (175, 200) ppm, while 95% at 150 ppm concentration. Al-Othman *et al.*, (2014) also noted that the adding of silver nanoparticles reduces the size of fungi growth as well as their production of aflatoxin by (81.1-95.5)%. They caused damage and destroys in the

growth and shape of cell walls, unusual swelling and rupture. Research has shown the mechanism of action of inhibiting silver ions of microorganisms. After the addition of the extract with cationic silver and its addition as a therapeutic substance, DNA loses its ability to replicate (Feng *et al.*, 2000), affecting the functioning of some cellular proteins and the basic enzymes of ATP. Ag + can affect the function of enzymes associated with the cell membrane, such as those found in the gastrointestinal tract and can also stimulate necrotic cell death or apoptosis in fungi. (Yamanaka *et al.*, 2005; Sharon *et al.*, 2009).

Detection of AFB1 using High Performance Liquid Chromatography (HPLC)

The high performance liquid chromatography (HPLC) technique used to detect AFB1 in contaminated feed samples was treated with the addition of alcohol extracts of sage leaves (5%) and cinnamon at (10%), nanomolecules of sage and cinnamon leaves powder at 5 µg/kg feed, green silver nanoparticles of sage leaves (SAgNPs) and cinnamon (CAgNPs) at concentration of 208.75 and 252.3 ppm/kg feed respectively. The results showed that all these samples were free of AFB1 as compared to the positive control treatment Non-treated feed with AFB1 which gave AFB1 the level of concentration of 38 ppb (Fig. 3 and 4).

This finding is consistent with those reported by Nabawy *et al.*, (2014) who assessed the antifungal potential of the biological construction of ZnO nanoparticles and Fe₂O₃ nanoparticles compared to

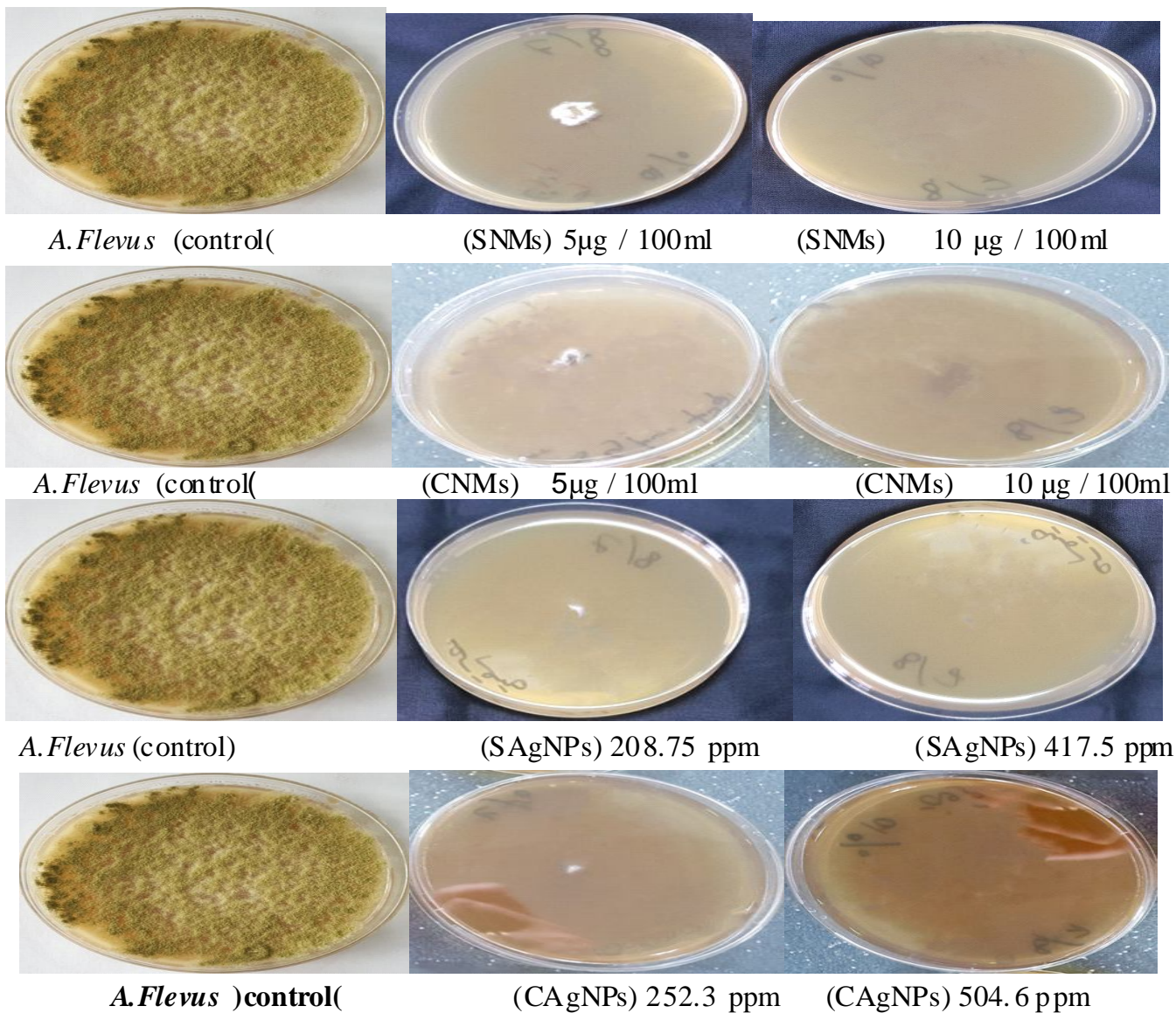


Fig. 2: Effect of Nanomolecules and green silver nanoparticles of cinnamon and sage leaves on *A. flavus* growth.

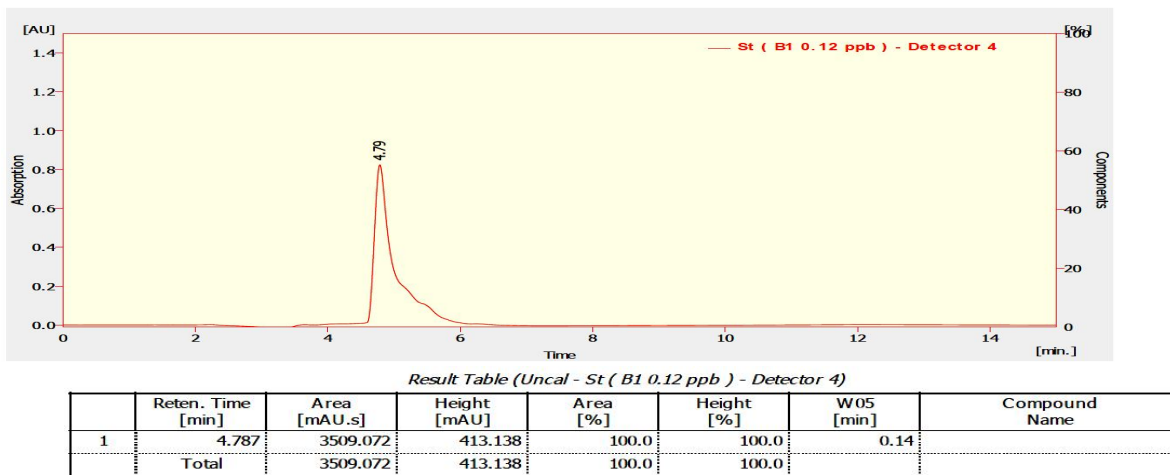


Fig. 3: Shows the results of the aflatoxin standard model test for B1 using HPLC technology.

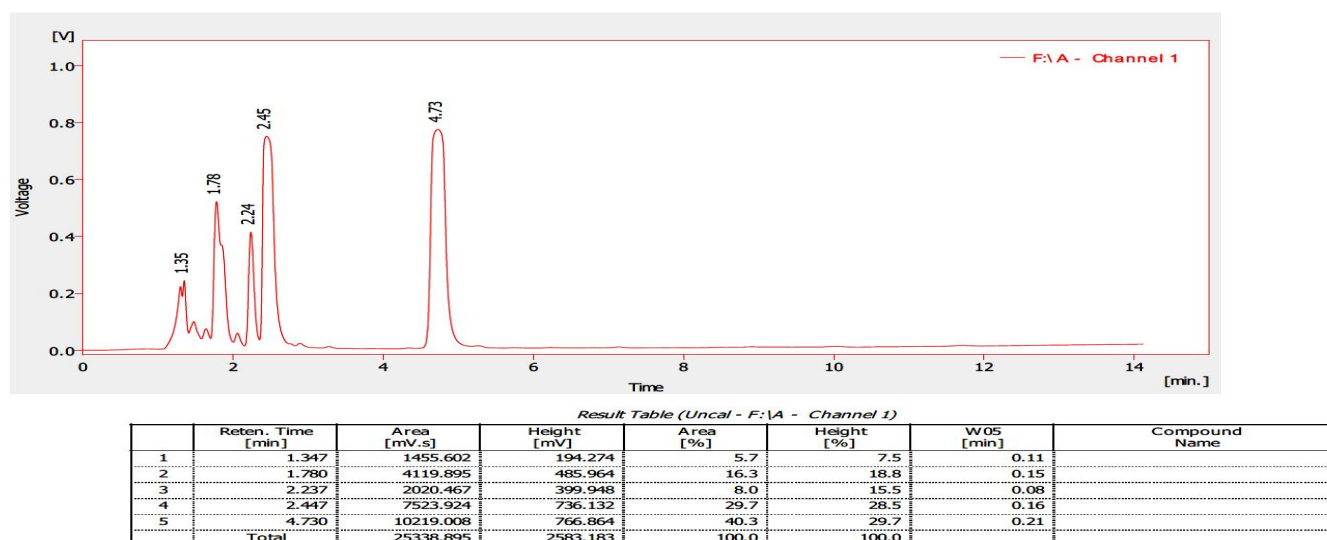


Fig. 4: Shows the results of the model inspection of the feed sample contaminated with AFB1 poison using HPLC technique.

antifungal feed additives (probiotic, propionic acid and clove oil) at different concentrations ranging from 25 to 250 ppm in growth inhibition. *A. flavus* strains of AFB1 toxin. The production of AFB1 by *A. flavus* was affected by all nanoparticles and other fungi inhibitors, where *A. flavus* growth inhibition zone appeared at concentrations lower 50g/ ml than ZnO and Fe₂O₃ nanoparticles, while similar effects of conventional antifungals require a relatively higher concentration of (100-200) ppm. The results of Abdulrahman and Hussein (2017) also showed that the silver nanoparticles inhibited the production of IFLA B1 in corn grains and showed 100% inhibition of fungal growth in the fungus-contaminated corn sample.

It can be concluded that increasing the efficiency of herbal extracts and medicinal plants by converting them into nanoparticles and into green nanoparticles against molds and fungi, including molds producing fungal toxins as (aflatoxin), including AFB1 and reducing its toxic effects in broiler diet compared to positive control treatment (contaminated feed without treatment).

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