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# Phytochemical Analysis and Antifungal Effects of the Extracts and Oils of Harjel (*Solenostemma argel*) Basil (*Ocimum basilicum*) and Parsley (*Petroselinum crispum*) on the Fungus Aspergillus flavus Growth and Aflatoxin Production, Sudan

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## Abstract

Sudan is one of the largest countries in Africa and is rich in many valuable plants that produce materials important for human and animal health. Some of these were used in traditional medicine. The present study aims to investigate the antifungal activities and the phytochemical composition of the extracts and oils of three plants (Hargel et al.). The study was conducted at the Food Microbiology Laboratory, Faculty of Engineering and Technology, University of Gezira. The mycelial growth and spore germination methods were used for the antifungal tests, using a selective medium, while thin-layer chromatography was used for phytochemical tests and aflatoxin analysis. The effects of clove oil and some amino acids were used for comparison. The results of the fungal growth showed that the leaf extracts of Hargel were highly effective and gave complete inhibition (0.0 cm radial growth), while the stem extracts and oil of Hargel were less effective, given (0.3 mm). Rehan (Basil) oil was the most effective (0.0 cm) compared to (0.4cm) and (0.5cm) for the extracts of the leaf and stem, respectively. The oil, leaf, and stem extracts of Parsley and clove oil were all highly effective, giving (0.0 cm) radial growth. The results of the effects of the amino acids on radial growth indicated that they were all highly effective, giving complete (0.0 cm) at a higher concentration (100mg/ml). The results of the effect on mycelial weights showed that the oils of the three plants, as well as that of clove, gave complete inhibition (0.0 mg weight); in contrast, the extracts of leaf of Hargel, Parsley, and Basil showed (1.74, 3.26 and 3.34, mg respectively) while, the extracts of the stems gave (3.29, 1.86 and 1.86, mg respectively). Among the amino acids tested, only methionine, leucine, and arginine were adequate (7.0, 7.4, and 7.4 mg, respectively). Concerning the effects of the spore germination, it was found that all the oils tested gave complete inhibition (0.0 %, pore germination) and the stem extracts of the three plants. However, only the leaf extracts of Parsley were highly influential; those of Harjel and Rehan were less effective (7.69% and 15.0 % germination, respectively). All the amino acids tested were less effective, giving about (10.0 % germination). The leaf and stem extracts of Harjel and both Parsley and Clove oils inhibited aflatoxin production by the fungus at all concentrations. The stem extracts of Rehan, the leaf extracts of Parsley, and the oil of Harjel inhibited aflatoxin production at higher concentrations only. The stem extracts and Rehan's oil were found to be unable to inhibit aflatoxin production at all tested concentrations. The chemical analysis of the tested plants' extracts and oils revealed that they contained saponins, tannins, flavonoids, alkaloids, and phenolics but not Steroids. However, glutamic acid was the only amino acid that inhibited aflatoxin production at all concentrations. From the results, it could be concluded that the oils of the plants were more effective than the extracts, and the leaf and stem extracts of Parsley were better among the extracts. It could be recommended that more plant extracts be tested since some plants may be more effective.

## Keywords

Phytochemical Analysis, Antifungal, Aflatoxin Production.

# INTRODUCTION

Fungal deterioration and mycotoxin contamination of various food and feedstuffs are significant problems in the tropics and subtropics, where climatic conditions and storage practices favor fungal growth (Quiroga *et al*., 2009; Shukla *et al.*, 2009; Salari *et al*., 2012). The risk of mycotoxin, particularly aflatoxin contamination, is a food safety concern for grains and other field crops worldwide (Kumar *et al*., 2007; Reddy *et al*., 2009). The Food and Agriculture Organization (FAO) estimated that around 25% of the world's cereals were contaminated by mycotoxins, including aflatoxins (Dowling, 1997).

Aflatoxin B1 is one of the most common and dangerous mycotoxins produced by *A.flavus* (Manafi & Khosravinia, 2013). Aflatoxins were found in a variety of food commodities such as maize, groundnut, and other cereals worldwide, and it was reported that about 4.5 billion people in developing countries were systematically exposed to uncontrolled amounts of aflatoxin; Shukla *et al*., (2008). Medicinal plants display antioxidant and antimicrobial properties that protect the human body against cellular oxidation reactions and pathogens.

Thus, it is essential to characterize different types of medicinal plants for their antioxidant and antimicrobial potential (Mothana & Lindequist, 2005; Bajpai *et al*., 2005; Wojdylo *et al*., 2007). Medicinal plants produce specific bioactive molecules responsible for their antimicrobial properties (Rios & Recio, 2005; Kuete *et al*., 2008; Kuete, 2010). The substances that inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. On the other hand, indiscriminate use of commercial antimicrobial drugs in treating infectious diseases has resulted in Multiple-drug resistance to many human pathogenic microorganisms.

The antioxidant properties of plant extracts have been attributed to their polyphenol contents, so plants containing high levels of polyphenols are of great importance as natural antioxidants. Many byproducts and wastes generated by agro-industries contain polyphenols with potential application as food antioxidants and preventive agents against some diseases (Torres *et al*., 2002). This situation has necessitated a more radical approach in the search for new antioxidant and antimicrobial substances from various sources that could be used as novel antioxidant and antimicrobial chemotherapeutic agents. While 25 to 50% of the current pharmaceuticals were derived from plants, none were used as antimicrobials.Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. The structure and antimicrobial properties of the phytochemicals were also addressed. Since many of these compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians must consider the consequences of patients' self-medicating with these preparations.

Many studies have been done to control aflatoxin poisoning and prevent the aflatoxin-producing fungi's growth. The most important of them were essential oils and plant extracts. Plant extracts of many species (Hargel-Rehan and Parsley) were reported to have antimicrobial activities. This paper aimed to investigate the effects of different concentrations of some essential oils on inhibiting the fungal growth of *A. flavus* and aflatoxin production. The effects of different concentrations of the leaf and stem extracts were also tested against the growth of the fungus *A. flavus* and aflatoxin production, and the determination of the effects of some amino acids against *A. flavus* growth and aflatoxin production was also compared.

# MATERIALS AND METHODS

## *Source of materials*

### Plants source

Different plant parts and oils, Hargel *(Solenotemma argel)* Rehan, *(Ocimum basilicum),* and Parsley *(Carum Petroselinum),* used in this study, were obtained from the Wad Medani local market. However, clove oil was used in the present study for comparison.

### Microorganisms source

The groundnut sample seeds were collected from the Wad Medani market and used to isolate the fungus (*Aspergillus flavus*). Isolation was made in the Food Sciences and Technology Laboratory Faculty of Sciences and Technology University of Gezira, using PDA media.

### Isolation of the fungus

Groundnut-infected seeds were collected from the market**.** The groundnut samples were washed several times with sterile distilled water and then dried on filter paper to remove residual water. Pieces (7 to 8) of these dried groundnut seeds were distributed in a Petri dish on a solid PDA medium's surface occulated plates were incubated in an incubator at 37°C. The plates were investigated daily for fungal growth. Colonies identified as *A. flavus* (green color) were detected, and percent infection was calculated. Contamination with fungi other than *A. flavus* was also estimated.

## *Preparation of plant part extracts*

The plant parts were washed in tap water, dried at room temperature for 15 days, and blended into a powder using a mortar and pestle. For the preparation of the extracts of each of the dried plant parts, 50 g were added to 500 ml distilled water, left overnight, and then filtered through sterile filter paper in a Buchner fennel for half an hour before being passed through a membrane filter (0.22µm) and kept in dark bottles before being used.

### Preparation of different concentrations

Five concentrations (0.0, 25.0, 50.0, 75.0, and 100.0 mg/ ml) were made by diluting different extracts with the medium in the flasks. All solutions were sterilized in an autoclave at 121$^{0}$C (15/b/in2) for 15 minutes and then cooled to room temperature. Five amino acids (glutamic acid, alanine, methionine, arginine, and leucine) were obtained from the Food Department laboratory and were also prepared similarly.

## *Media used*

### Potato Dextrose Agar (PDA)

This medium was used for the isolation and maintenance of fungi and other experiments whenever needed. The medium consists of the following (g/L):

| | |
|---|---|
| Potato (peeled and diced) | 200 |
| D-Glucose | 20 |
| Agar | 15 |
| Distilled water | 1000ml |

## *Preparation of the medium*

Two hundred grams of the peeled and diced potato were boiled for 1 hour in one liter of distilled water; then, the extract was filtered and made up to 1 liter. The D-glucose and agar were added and dissolved by steaming and steering. Then, the medium was dispensed in 100 ml samples in conical flasks covered with cotton plugs and aluminum foil before being sterilized in the autoclave at 1210C (15 Ib/in2) for 15 minutes. It was then stored at 40C in a refrigerator. If needed, the medium was melted in a water bath and poured into sterilized Petri dishes.

### Potato Dextrose Broth

The medium was used when a liquid culture was needed, mainly for the mycelial weight experiments, for the growth and maintenance of fungi, and other experiments, whenever needed. The medium consists of the following (g/L):

| | |
|---|---|
| Potato peeled and diced | 200 |

| | |
|---|---|
| D-Glucose | 20 |
| Distilled water | 1000ml |

After preparation, the medium was dispensed in 250 ml flasks (each containing 50 ml) and autoclaved at 1210 C before use.

### The spore germination medium

The medium used for the spore germination experiments was prepared as described by Abdel-Rhim and Arbab (1985). It consists of the following materials :( g/l)

| | |
|---|---|
| Glucose | 10.0 |
| Glutamic acid (Monosodium salt) | 5.0 |
| $KH_2PO_4$ | 1.0 |
| $Mgso_4.7H_2O$ | 0.25 |
| $Cu\ so_4.5H_2O$ | 0.02 |
| $Feso_4.7H_2O$ | 0.063 |
| $Znso_4.7H_2O$ | 0.011 |
| $Mncl_2.4H_2O$ | 0.035 |
| $Cacl_2$ | 0.067 |
| $(NH_4)_2\ SO_4$ | 1.98 |
| Distilled water | 1000 |

The pH of the medium was adjusted to 4.5 by adding drops of a concentrated Hcl acid before being dispensed in the conical flask and sterilized in the autoclave at 121ºc (15/b/in2) for 15 minutes. However, glucose was sterilized, separated by filtration, and then added to the medium under aseptic conditions.

## *Effects of the extracts and oils on fungal growth*

### Effects on radial growth

The medium used for fungal radial growth was the PDA, as was mentioned in section 3.3.1. Five concentrations under test were prepared by serially diluting the extract and mixing it with the medium. The media containing the different concentrations of each extract were then sterilized, poured into a Petri dish, and left to solidify at room temperature (28 - 30º C). Each solidified Petri dish was inoculated by a fungal growth disc cut by a sterile cork borer (5.0 mm diameter) from the edge of an actively growing culture of *A. flavus* grown on PDA. The inoculated Petri dishes were then incubated at room temperature for eight days. All treatments were done in triplicates. The diameter of growth was measured every 48 hours by taking the average of two crossed dimensions for each disc in a Petri dish. The radial growth was calculated as a percentage of the diameter of the discs.

### Effects on mycelial weight

The potato dextrose broth medium (PDB) described above was prepared and dispensed in 100 ml conical flasks (50 ml in each flask). The five concentrations (0.0, 25.0, 50.0, 75.0, and 100.0 mg/ ml) made by serial diluting the extract with the medium were dispensed in different flasks. All solutions were sterilized in an autoclave at 121ºc (15/b/in2) for 15 minutes and then cooled at room temperature. Each flask was inoculated by three discs made by a sterile cork-borer (5.0 mm in diameter) from an edge of an actively growing culture of (*A.flavus*) grown on a solidified PDA medium. Inoculated flasks were incubated at room temperature (28- 30º C) for eight days. After incubation, the culture was filtered through sterilized Wattman NO. 1 filter papers, and the mycelia mats were collected, weighed (fresh weight), and dried at 80oc for 24 hrs before being reweighed (dry weight). All treatments were done in triplicates.

## *Effects on spore germination*

### Preparation of conidial suspension

The culture of *A. flavus* used for the present experiment was grown in solidified PDA medium slopes in a screw cotton plugged bottle at room temperature for four days. Their spores were collected by covering the culture with sterile distilled water and brushed gently with a sterile inoculation loop. The spore suspension was filtered through a sintered glass funnel to remove hyphae and conidiophores. The spores were finally suspended in sterile deionized water, shaken in a Griffin flask, shaken to dispense spore clumps, and diluted with sterile distilled water. It has been reported that the spores of almost all fungi failed to germinate in free, sterile, and distilled water. Therefore, the medium described by Abdel-Rahim and Arbab (1985), mentioned in section 3.3.3, was used for this test.

### The procedure

To study the effect of plant extracts on the spore germination of *A.flavus*, the medium was prepared and dispensed in test tubes (10 ml in each test tube).

The concentrations (0.0, 25.0, 50.0, 75.0, and 100.0 mg/ ml) were prepared for the extract and sterilized. Each clean sterilized slide received 0.5 ml from the abovementioned concentrations and was inoculated with 0.1 ml from the conidial suspension prepared as in section 3.5.1. The slides were placed in Petri dishes with moist filter papers and then incubated at 30. Reading was taken after 6,12,18,24 hours of incubation, and the germination was stopped by adding tiny drops of cotton blue in lacto phenol to each slide. Germination percentage was assessed by examining 100 spores in a microscopic field. All treatment was done in triplicates.

## *Effects on aflatoxin production*

### Toxin extraction

From the filtrate obtained from the mycelial dry weight experiment, only 20ml were taken and placed into a 250ml separation funnel; 50 ml of chloroform was then added, and the separating funnels were stopped with a rubber plug coated with aluminum foil to protect the rubber from being attacked by the chloroform. The flasks were shaken on a Griffin shaker for 30 minutes to ensure a good extraction. The contents of each flask were separated by removing the lower layer of the filtrate into a conical flask; the chloroform was then evaporated to dryness in a water bath (70), as described by Abdel-Rahim *et al*. (2002).

### Chromatographic plate preparation

Silica gel (kiesl gel "G") coats 20 × 20cm glass plates. This type of silica gel separates aflatoxins B and G into separate zones. However, it will not separate B into $B_1$ and $B_2$ or G into G1 and G2.

Forty grams of the silica gel were weighed and mixed with 80 ml of distilled water, which was used to coat five plates. The plates were covered with a 0.5 cm thick layer of silica gel. The coating was spread by using an automatic leveler and spreader apparatus. Coated plates were left in a dust-free atmosphere for 10 minutes and heated in an oven at $70^0$C for two hours. The plates were then cooled in the dust-free atmosphere for 30 minutes before being placed into a plate cabinet.

### Spotting of the extraction

The dried extracted samples were washed in a known volume of chloroform. For spotting of the extraction, an amount of 5 to 25 u ml of the solution was spotted on the prepared TLC paper by a micro syringe. The papers were then dried before being developed in a chromatic tank.

### Development of the chromatographic plates

The loaded chromatographic plates were developed in a diethyl ether solution in a chromatographic tank. The RF value of aflatoxin in this solvent is actually (zero over a 10cm path length from the primary

line). Plates were allowed to dry before being redeveloped in a chloroform –Methanol (95:5) solution as was described by Jones (1972) and Abdel-Rahim *et al*., (1989).

The solvent was allowed to move 10cm above the baseline. The plates were then dried and examined in a dark room under an ultraviolet lamp (peat emission 366mm, Philips HPW 125 watts type) at a distance of about 30cm from the lamp. The visible fluorescence of the sample was compared with the standard aflatoxin. If the spots are too intense compared with the standard, they must be diluted and re-chromatographed. The concentration of aflatoxins in the sample can be calculated as follows: -

$$\text{Aflatoxin content (mg/Kg)} = \frac{S \times Y \times V}{W \times Z}$$

Whereas:

S = Volume in ml of the standard of equivalent intensity.
Y = Concentration of B1 standard in mg/ml.
Z = Volume in ml of sample required to give fluorescence.
V = Volume in ml of solvent required to dilute final extract.
W = Weight in grams of the original sample in the final.

## *Photochemical screening*

Photochemical screening is a significant step in isolating new and novel compounds. Kinds of plants such as (Rehan – Hargel, and Parsley), different parts of each plant, such as (leaf and stem) were selected for photochemical screening to identify the different classes of secondary metabolites. Solvents extract the plant material with the help of different solvents (Chloroform et al.).

Photochemical analysis of the extract revealed the presence of Saponin, Tannin, Flavonoid, Alkaloid, Steroids, and Phenolic compounds.

### Test for Saponin

A known weight (20g) of the dried powder of each part of the mentioned plants was extracted with (250ml) ethanol and filtered. Aliquots of the alcoholic extracts (10ml) each were evaporated to dryness under reduced pressure. The residue was dissolved in distilled water (2ml) and filtered; then the filtrate was vigorously shaken; if a voluminous fourth was developed and persisted for almost one hour, this indicates the presence of saponin (Harborne, 1998).

### Test for Tannin

The dried powder of each part of the mentioned plant, weight (20g), was extracted with ethanol (250 ml) and filtered. Ferric chloride reagent (0.5 ml) was added, and the appearance of a green color, which changes to a bluish-black or precipitate, indicates the presence of tannin (Balbaa1974).

### Test for Flavonoids

A known weight (5g) of the dried powder of each part (leaf and stem) of the mentioned plants was macerated in 1% hydrochloric acid (100ml) overnight and filtered. The filtrate was subjected to the following test: A known volume (2ml) from each filtrate was rendered alkaline with sodium hydroxide (10 w/v) (2ml) if a yellow color was formed that might indicate the presence of flavonoids.

### Test for Steroids

A known weight (5g) was extracted with petroleum ether (100ml) and filtrated. The filtrate was evaporated to dryness, and the residue was dissolved in chloroform (10ml). Aliquots of chloroform extract (3ml) were mixed with concentrated acetic acid anhydride (3ml), and a few drops of sulphuric acid were added. The formation of a reddish violet ring at the junction of the two layers showed the presence of unsaturated sterols and or triterpenes (Harborne, 1974).

## Test for Alkaloids

A known weight (20g) was extracted with ethanol (250ml) and filtered. Aliquots from ethanolic extract (10ml) each were mixed with hydrochloric acid (20ml 10%v/v) and filtered. The filter was alkaline with ammonium hydroxide and extracted with successive portions of chloroform. The combined chloroform extract was evaporated to dryness; the residue was dissolved in hydrochloric acid (2 ml 10%v/v) and tested with Mayer's reagent and Dragendorffs reagent, respectively; if a precipitate was formed, it is an indication for the presence of alkaloids (Balbae,1974).

## Test for Phenolic compound

The phenolic compound resulted from adding a 1% ferric chloride solution to the extract, which caused it to become intense green, purple, blue, or black.

## Statistical analysis

The Statistical analysis methods used in this work were ANOVA and Duncan's multiple range tests.

| **Hargel leaf Concentration (100mg/ml)** | **Incubation period (days) Radial growth (cm)** | | | | **Mean** |
|---|---|---|---|---|---|
| | **2** | **4** | **6** | **8** | |
| **0** | 1.3 | 2.0 | 2.3 | 2.7 | 2.1 a ± 0.6 |
| **25.0** | 1.5 | 1.7 | 1.8 | 1.8 | 1.7 b ± 0.1 |
| **50.0** | 1.0 | 1.5 | 1.8 | 2.0 | 1.6 b ± 0.4 |
| **75.0** | 1.0 | 1.5 | 1.5 | 2.0 | 1.5 b ± 0.04 |
| **100.0** | 0.3 | 1.0 | 1.5 | 1.5 | 1.1 c ± 0.06 |
| SE± | | | | | 0.10 |
| CV% | | | | | 12.68 |

**Table (1):** Effect of different concentrations of Hargel leaf extracts on *A.flavus* radial growth
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

| **SOV** | **DF** | **SS** | **MS** | **F-cal** | **F-tab $_{0.05}$** | **P-value** |
|---|---|---|---|---|---|---|
| **Period** | 3 | 2.6575 | 0.8858 | 21.92 | 3.4903 | 0.0001 |
| **Concentration** | 4 | 2.0830 | 0.5208 | 12.88 | 3.2592 | 0.0003 |
| **Error** | 12 | 0.4850 | 0.0404 | | | |
| **Total** | 19 | 5.2255 | | | | |

ANOVA table

| **Hargel stem Concentration (100 mg/ml)** | **Incubation period (days) Radial growth (cm)** | | | | **Mean** |
|---|---|---|---|---|---|
| | **2** | **4** | **6** | **8** | |
| **0** | 1.5 | 1.7 | 1.8 | 2.0 | 1.8$^{a}$ ± 0.2 |
| **25.0** | 1.5 | 1.5 | 1.5 | 1.5 | 1.7 $^{a}$ ± 0.0 |
| **50.0** | 1.5 | 1.5 | 2.0 | 2.5 | 1.5 $^{a}$ ± 0.5 |
| **75.0** | 1.0 | 1.3 | 1.8 | 2.0 | 1.5$^{a}$ ± 0.5 |
| **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0$^{b}$ ± 0.0 |
| SE± | | | | | 0.01 |
| CV% | | | | | 18.04 |

**Table (2):** Effect of different concentrations of Hargel stem extracts on *A. flavus* radial growth
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 0.7540 | 0.2513 | 4.36 | 3.4903 | 0.0269 |
| **Concentration** | 4 | 9.2370 | 2.3092 | 40.10 | 3.2592 | 0.0001 |
| **Error** | 12 | 0.6910 | 0.0576 | | | |
| **Total** | 19 | 10.6820 | | | | |

ANOVA table

| Hargel oil Concentration (100mg/ml) | Incubation period (days) Radial growth (cm) | | | | . |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 0.9 | 1.4 | 1.4 | 1.5 | 0.4 $^{a}$ ± 0.1 |
| **25.0** | 0.7 | 0.7 | 0.7 | 0.7 | 2.5 $^{a}$ ± 1.8 |
| **50.0** | 0.6 | 0.6 | 0.6 | 0.6 | 2.4 $^{a}$ ± 1.5 |
| **75.0** | 0.5 | 0.5 | 0.5 | 0.5 | 1.6 $^{b}$ ± 0.8 |
| **100.0** | 0.3 | 0.3 | 0.3 | 0.3 | 1.1 $^{b}$ ± 0.7 |
| SE± | | | | | 0.12 |
| CV% | | | | | 45.06 |

**Table (3):** Effect of different concentrations of Hargel oil on *A. flavus* radial growth

* Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 14.1520 | 4.7173 | 9.3061 | 3.4903 | 0.0019 |
| **Concentration** | 4 | 11.9570 | 2.9892 | 5,8970 | 3.2592 | 0.0073 |
| **Error** | 12 | 6.0830 | 0.5069 | | | |
| **Total** | 19 | 32.1920 | | | | |

ANOVA table

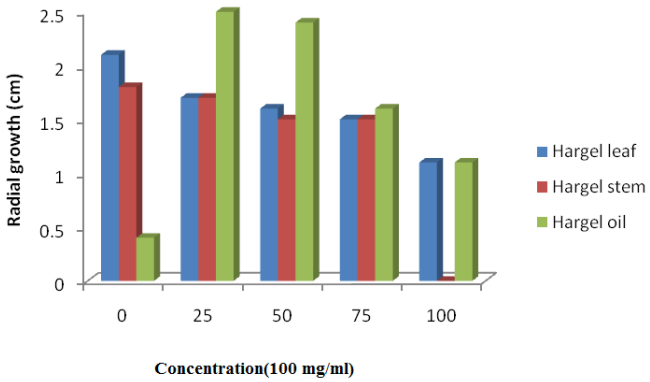


**Fig (2):** The effect of the extracts of different partsof Hargel on radial growth (cm) of A. flavus.

| Rehan leaf Concentration (100mg/ml) | Incubation period (days) Radial growth (cm) | | | | Mean |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 1.5 | 1.7 | 2.5 | 3.0 | 2.2$^{a}$ ± 0.7 |
| **25.0** | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 $^{a}$ ± 0.0 |
| **50.0** | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 $^{ab}$ ± 1.1 |
| **75.0** | 1.0 | 1.0 | 1.0 | 1.0 | 1.5$^{b}$ ± 0.0 |
| **100.0** | 0.5 | 1.0 | 1.0 | 1.0 | 1.0$^{b}$ ± 0.4 |
| SE± | | | | | 0.01 |
| CV% | | | | | 13.14 |

**Table (4):** Effect of different concentrations of Rehan leaf extract on *A. flavus* radial growth

* Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 2.7380 | 0.9126 | 4.1200 | 3.4903 | 0.0001 |
| **Concentration** | 4 | 4.8930 | 1.2232 | 1.8401 | 3.2592 | 0.0003 |
| **Error** | 12 | 2.6590 | 0.2215 | | | |
| **Total** | 19 | 10.2880 | | | | |

ANOVA table

| Rehan stem Concentration (100mg/ml) | Incubation period (days) Radial growth (cm) | | | | Mean |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 1.5 | 1.6 | 2.0 | 2.5 | 1.9 $^{a}$ ± 0.5 |
| **25.0** | 1.5 | 1.5 | 2.0 | 2.3 | 1.9 $^{a}$ ± 0.5 |
| **50.0** | 1.2 | 1.3 | 1.7 | 1.7 | 1.5 $^{b}$ ± 0.3 |
| **75.0** | 1.0 | 1.3 | 1.5 | 1.5 | 1.3 $^{b}$ ± 0.2 |
| **100.0** | 0.4 | 1.3 | 1.5 | 1.5 | 1.2 $^{b}$ ± 0.5 |
| SE± | | | | | 0.01 |
| CV% | | | | | 13.14 |

**Table (5):** Effect of different concentrations of Rehan stem extracts on A. flavus radial growth.
* Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 1.9615 | 0.6538 | 15.67 | 3.4903 | 0.0002 |
| **Concentration** | 4 | 1.7670 | 0.4417 | 10.59 | 3.2592 | 0.0007 |
| **Error** | 12 | 0.5010 | 0.0417 | | | |
| **Total** | 19 | 4.2295 | | | | |

ANOVA table

| Rehan oil Concentration (100mg/ml) | Incubation period (days) Radial growth (cm) | | | | Mean |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 1.5 | 1.5 | 1.8 | 2.3 | 1.8 $^{a}$ ± 0.4 |
| **25.0** | 1.3 | 1.3 | 1.7 | 2.0 | 1.6 $^{a}$ ± 0.3 |
| **50.0** | 1.5 | 1.5 | 1.7 | 1.9 | 1.7 $^{a}$ ± 0.2 |
| **75.0** | 1.0 | 1.3 | 1.5 | 0.0 | 1.0 $^{a}$ ± 0.7 |
| **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 $^{ab}$ ± 0.0 |
| SE± | | | | | 0.04 |
| CV% | | | | | 34.14 |

**Table (6):** Effect of different concentrations of Rehan oil on *A.flavus* Radial growth
* Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 0.2340 | 0.0780 | 0.4712 | 3.4903 | 0.7071 |
| **Concentration** | 4 | 8.7030 | 2.1757 | 13.1462 | 3.2592 | 0.0002 |
| **Error** | 12 | 1.9810 | 0.1655 | | | |
| **Total** | 19 | 10.9180 | | | | |

ANOVA table

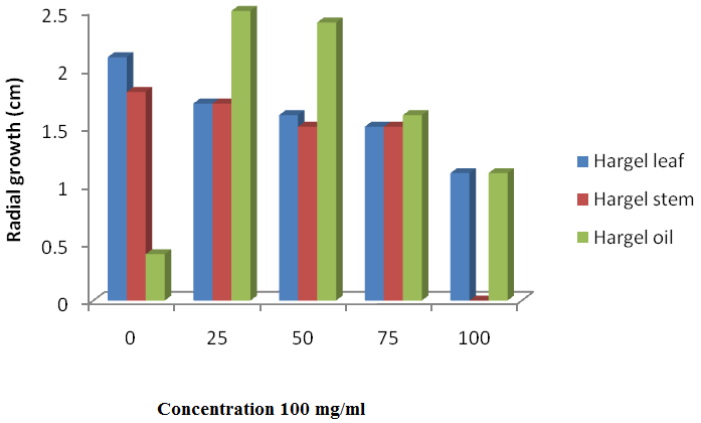


**Fig (3):** The effect of the extracts of different parts of Rehan on radial growth (cm)

| Parsley leaf Concentration (100 mg/ml) | Incubation period (days) Radial growth (cm) | | | | Mean |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 1.5 | 1.7 | 2.0 | 2.5 | 1.9 $^{a}$ ± 0.4 |
| **25.0** | 1.0 | 1.3 | 1.5 | 1.7 | 1.4 $^{a}$ ± 0.3 |
| **50.0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 $^{c}$ ± 0.0 |
| **75.0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 $^{c}$ ± 0.0 |
| **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 $^{c}$ ± 0.0 |
| SE± | | | | | 0.01 |
| CV% | | | | | 31.51 |

**Table (7):** Effect of different concentrations of Parsley leaf extracts on *A. flavus* radial growth
* Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 0.3160 | 0.1053 | 2.4375 | 3.4903 | 0.1152 |
| **Concentration** | 4 | 13.6730 | 3.4182 | 79.1250 | 3.2592 | 0.0001 |
| **Error** | 12 | 0.5190 | 0.0432 | | | |
| **Total** | 19 | 14.5080 | | | | |

ANOVA table

| Parsley stem Concentration (100 mg/ml) | Incubation period (days) Radial growth (cm) | | | | Mean |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 1.5 | 1.8 | 2.0 | 2.5 | 2.0$^{a}$ ± 0.4 |
| **25.0** | 1.0 | 1.3 | 1.7 | 2.5 | 1.5$^{b}$ ± 0.6 |
| **50.0** | 1.0 | 1.0 | 1.5 | 2.3 | 1.6$^{b}$ ± 0.7 |
| **75.0** | 0.0 | 0.5 | 1.3 | 2.0 | 1.0 $^{bc}$± 0.9 |
| **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0$^{c}$ ± 0.0 |
| SE± | | | | | 0.03 |
| CV% | | | | | 29.06 |

**Table (8):** Effect of different concentrations of Parsley stem extracts on *A. flavus* radial growth.
* Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 3.8695 | 1.2898 | 1.7898 | 3.4903 | 0.0010 |
| **Concentration** | 4 | 9.1720 | 2.2930 | 19.0132 | 3.2592 | 0.0001 |
| **Error** | 12 | 1.4480 | 0.1206 | | | |
| **Total** | 19 | 14.4895 | | | | |

ANOVA table

| Parsley oil Concentration (100mg/ml) | Incubation period (days) Radial growth (cm) | | | | Mean |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 2.0 | 2.5 | 2.7 | 3.0 | 2.6 $^{a}$ ± 0.4 |
| **25.0** | 1.5 | 1.7 | 2.0 | 2.1 | 1.8 $^{a}$ ± 0.3 |
| **50.0** | 1.0 | 1.2 | 1.5 | 1.7 | 1.4 $^{ab}$ ± 0.3 |
| **75.0** | 1.0 | 1.0 | 1.0 | 1.0 | 1.0$^{c}$ ± 0.0 |
| **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0$^{c}$ ± 0.0 |
| SE± | | | | | 0.00 |
| CV% | | | | | 14.39 |

Table (9) Effect of different concentrations of Parsley oil on *A. flavus* radial growth

* Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 0.5975 | 0.1991 | 5.3093 | 3.4903 | 0.0146 |
| **Concentration** | 4 | 14.4420 | 3.6105 | 96.280 | 3.2592 | 0.0001 |
| **Error** | 12 | 0.4500 | 0.0375 | | | |
| **Total** | 19 | 15.4895 | | | | |

ANOVA table

| Clove oil Concentration (100 mg/ml) | Incubation period (days) Radial growth (cm) | | | | Mean |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 2.5 | 4.0 | 6.7 | 8.0 | 5.3$^{a}$ ± 2.5 |
| **25.0** | 1.0 | 1.5 | 2.0 | 2.5 | 1.8$^{b}$ ± 0.6 |
| **50.0** | 1.0 | 1.0 | 1.5 | 1.7 | 1.3$^{bc}$ ± 0.4 |
| **75.0** | 0.5 | 0.7 | 0.7 | 0.8 | 0.7$^{bc}$ ± 0.1 |
| **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0$^{c}$ ± 0.0 |
| SE± | | | | | 0.26 |
| CV% | | | | | 56.97 |

**Table (10):** Effect of different concentrations of Clove oil on *A. flavus* radial growth.

* Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 7.7695 | 2.5898 | 2.4494 | 3.4903 | 0.1139 |
| **Concentration** | 4 | 68.0320 | 17.0080 | 16.0862 | 3.2592 | 0.0001 |
| **Error** | 12 | 12.6880 | 1.0573 | | | |
| **Total** | 19 | 88.4895 | | | | |

ANOVA table

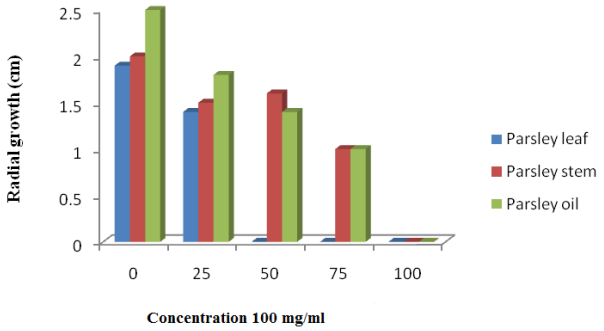


**Fig (4):** The effect of the extracts of different parts of Parsley on radial growth (cm) of *A. flavus*.

| Glutamic acid Concentration (100 mg/ml) | Incubation period (days) Radial growth (cm) | | | | Mean |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 5.58 | 6.39 | 7.20 | 8.19 | $6.8^{a} \pm 1.2$ |
| **25.0** | 4.68 | 5.13 | 6.03 | 7.38 | $5.8^{b} \pm 0.5$ |
| **50.0** | 4.32 | 4.50 | 4.95 | 5.40 | $4.8^{c} \pm 1.3$ |
| **75.0** | 2.25 | 3.51 | 4.59 | 5.13 | $3.9^{d} \pm 0.8$ |
| **100.0** | 0.00 | 0.90 | 1.35 | 1.80 | $1.0^{e} \pm 0.0$ |
| SE± | | | | | 1.88 |
| CV% | | | | | 8.46 |

**Table (11):** Effect of different concentrations of glutamic acid on A. flavus radial growth
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 13.6177 | 4.53 | 32.35 | 3.4903 | 0.0001 |
| **Concentration** | 4 | 79.2690 | 19.81 | 141.5 | 3.2592 | 0.0001 |
| **Error** | 12 | 1.7115 | 0.14 | | | |
| **Total** | 19 | 94.5982 | | | | |

ANOVA table

| Alanine Concentration (100mg/ml) | Incubation period (days) Radial growth (cm) | | | | Mean |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 3.24 | 3.69 | 3.87 | 4.23 | $3.8^{a} \pm 0.6$ |
| **25.0** | 1.89 | 2.70 | 2.97 | 3.15 | $2.7^{b} \pm 0.2$ |
| **50.0** | 1.26 | 1.53 | 1.53 | 1.71 | $1.5^{c} \pm 0.3$ |
| **75.0** | 0.72 | 1.17 | 1.35 | 1.53 | $1.2^{Cd} \pm 0.8$ |
| **100.0** | 0.00 | 0.90 | 1.35 | 1.80 | $1.0^{d} \pm 0.0$ |
| SE± | | | | | 0.11 |
| CV% | | | | | 11.21 |

**Table (12):** Effect of different concentrations of alanine on *A. flavus* radial growth
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 3.0532 | 1.017 | 19.94 | 3.4903 | 0.0001 |
| **Concentration** | 4 | 21.6529 | 5.431 | 106.49 | 3.2592 | 0.0001 |
| **Error** | 12 | 0.6220 | 0.051 | | | |
| **Total** | 19 | 25.3282 | | | | |

ANOVA table

| Methionine Concentration (100 mg/ml) | Incubation period (days) Radial growth (cm) | | | | Mean |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 0.9 | 2.88 | 3.24 | 3.96 | $2.7^{a} \pm 0.6$ |
| **25.0** | 0.72 | 1.26 | 1.80 | 2.16 | $1.5^{b} \pm 0.5$ |
| **50.0** | 0.63 | 1.26 | 1.80 | 1.17 | $1.2^{bc} \pm 0.3$ |
| **75.0** | 0.36 | 0.54 | 0.90 | 0.90 | $0.7^{C} \pm 0.8$ |
| **100.0** | 0.00 | 0.90 | 1.35 | 1.80 | $1.0^{bc} \pm 0.0$ |
| SE± | | | | | 0.22 |
| CV% | | | | | 32.03 |

**Table (13):** Effect of different concentrations of methionine on *A. flavus* radial growth
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 6.5071 | 2.17 | 10.85 | 3.4903 | 0.0012 |
| **Concentration** | 4 | 10.0909 | 2.52 | 12.60 | 3.2592 | 0.0004 |
| **Error** | 12 | 2.5061 | 0.20 | | | |
| **Total** | 19 | 19.1042 | | | | |

ANOVA table

| Arginine Concentration (100 mg/ml) | Incubation period (days) Radial growth (cm) | | | | Mean |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 1.80 | 1.98 | 2.70 | 3.78 | 2.6 $^{a}$ ± 0.3 |
| **25.0** | 0.90 | 1.17 | 1.35 | 1.71 | 1.3$^{b}$ ± 0.6 |
| **50.0** | 0.45 | 0.72 | 0.90 | 1.80 | 1.0$^{bc}$ ± 0.3 |
| **75.0** | 0.36 | 0.72 | 0.90 | 0.90 | 0.7$^{C}$ ± 0.8 |
| **100.0** | 0.00 | 0.90 | 1.35 | 1.80 | 1.0$^{bc}$ ± 0.0 |
| SE± | | | | | 0.16 |
| CV% | | | | | 24.51 |

**Table (14):** Effect of different concentrations of arginine on *A. flavus* radial growth

*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 4.5242 | 1.50 | 15 | 3.4903 | 0.0003 |
| **Concentration** | 4 | 8.5187 | 2.12 | 21.2 | 3.2592 | 0.0001 |
| **Error** | 12 | 1.2368 | 0.10 | | | |
| **Total** | 19 | 14.2798 | | | | |

ANOVA table

| Leucine Concentration (100 mg/ml) | Incubation period (days) Radial growth (cm) | | | | Mean |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | |
| **0** | 1.08 | 1.26 | 1.80 | 1.98 | 1.5 $^{a}$ ± 0.3 |
| **25.0** | 0.72 | 1.08 | 1.26 | 1.44 | 1.1$^{b}$ ± 0.3 |
| **50.0** | 0.36 | 0.63 | 0.90 | 0.90 | 0.7$^{bc}$ ± 0.2 |
| **75.0** | 0.18 | 0.18 | 0.36 | 0.54 | 0.3$^{d}$ ± 0.8 |
| **100.0** | 0.00 | 0.90 | 1.35 | 1.80 | 1.0$^{bc}$ ± 0.0 |
| SE± | | | | | 0.12 |
| CV% | | | | | 36.55 |

**Table (15):** Effect of different concentrations of leucine on *A. flavus* radial growth

*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 2.1546 | 0.7183 | 11.64 | 3.4903 | 0.0007 |
| **Concentration** | 4 | 3.3477 | 0.8369 | 13.56 | 3.2592 | 0.0002 |
| **Error** | 12 | 0.7411 | 0.0617 | | | |
| **Total** | 19 | 6.2434 | | | | |

ANOVA table

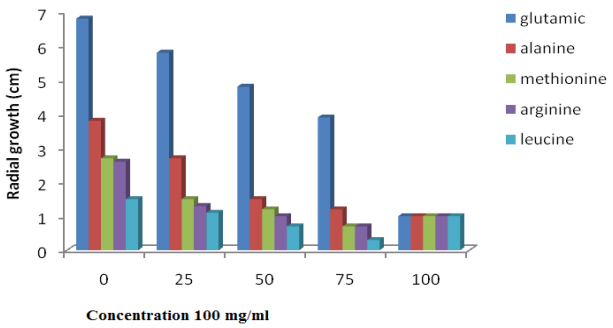


**Fig (5):** The effect of the different concentrations of amino acids on radial growth (cm) of *A. flavus*.

| Concentration 100 mg/ ml | Mycelial fresh weight (mg) | | |
|---|---|---|---|
| | Hargel | Rehan | Parsley |
| **0.0** | 4.23 | 8.16 | 10.37 |
| **25.0** | 3.72 | 8.12 | 8.83 |
| **50.0** | 3.60 | 6.60 | 7.23 |
| **75.0** | 2.60 | 6.00 | 6.10 |
| **100.0** | 1.74 | 3.34 | 3.25 |

**Table (16):** Comparison between the effects of leaf extracts of some plants on *A.flavus* mycelial fresh weight

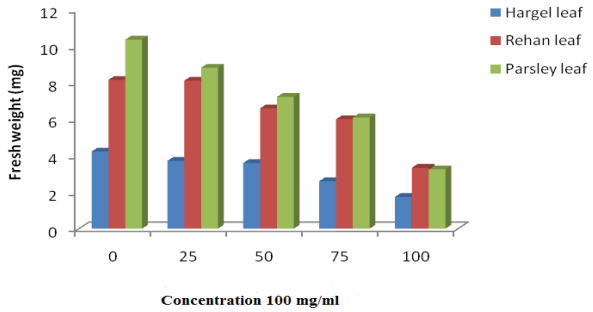


**Fig (6):** The effects of concentration of leaf extracts of different plants on *A. flavus* mycelial fresh weight

| Concentration 100 mg/ml | Mycelial dry weight (mg) | | |
|---|---|---|---|
| | Hargel | Rehan | Parsley |
| **0.0** | 0.49 | 0.49 | 0.49 |
| **25.0** | 0.39 | 0.39 | 0.39 |
| **50.0** | 0.27 | 0.35 | 0.35 |
| **75.0** | 0.27 | 0.25 | 0.25 |
| **100.0** | 0.20 | 0.25 | 0.25 |

**Table (17):** Comparison between the effects of leaf extracts of some plants on *A.flavus* mycelial dry.

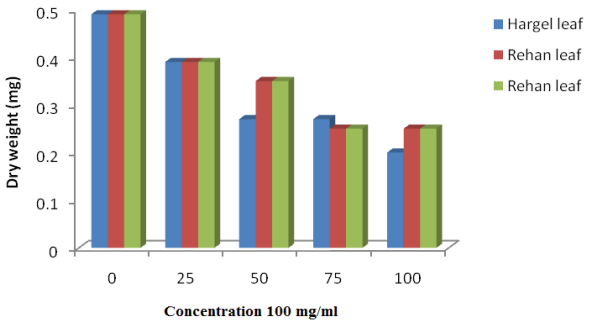


**Fig (7):** The effects of different concentrations of leaf extracts of different plants on *A.flavus* mycelial dry weight

| Concentration 100 mg/ml | Mycelial fresh weight (mg) | | |
|---|---|---|---|
| | Hargel | Rehan | Parsley |
| **0.0** | 7.35 | 7.35 | 7.35 |
| **25.0** | 7.01 | 5.95 | 7.04 |
| **50.0** | 6.77 | 5.32 | 6.87 |
| **75.0** | 6.70 | 4.99 | 6.36 |
| **100.0** | 3.42 | 3.29 | 1.86 |

**Table (18):** Comparison between the effects of stem extracts of some plants on A.flavus mycelial fresh weight.

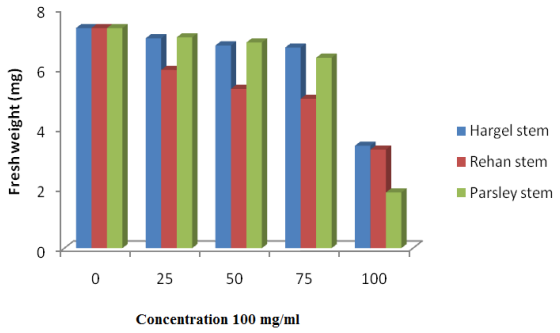


**Fig (8):** The effects of different concentrations of stem extracts of different plants on *A.flavus* mycelial fresh weight.

| Concentration 100mg/ml | Mycelial dry weight (mg) | | |
|---|---|---|---|
| | Hargel | Rehan | Parsley |
| **0.0** | 0.45 | 0.45 | 0.45 |
| **25.0** | 0.41 | 0.43 | 0.36 |
| **50.0** | 0.33 | 0.38 | 0.36 |
| **75.0** | 0.25 | 0.30 | 0.34 |
| **100.0** | 0.25 | 0.20 | 0.19 |

**Table (19):** Comparison between the effects of stem extracts of some plants on *A.flavus* mycelial dry weigh.

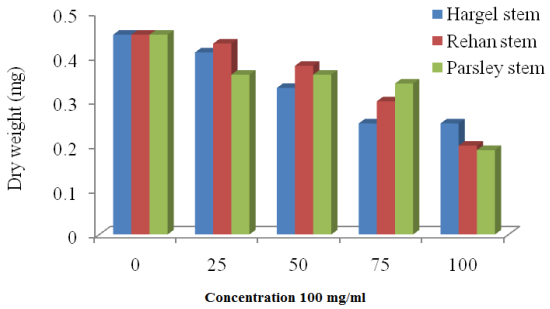


**Fig (9):** The effects of different concentrations of stem extracts of different plants on *A.flavus* mycelial dry weight

| Concentration 100 mg/ml | Mycelial fresh weight (mg) | | | |
|---|---|---|---|---|
| | Hargel | Rehan | Parsley | Clove |
| **0.0** | 4.60 | 4.60 | 4.60 | 4.60 |
| **25.0** | 1.90 | 2.20 | 2.00 | 1.36 |
| **50.0** | 1.30 | 1.73 | 1.14 | 0.80 |
| **75.0** | 1.10 | 1.77 | 0.77 | 0.68 |
| **100.0** | 0.8 | 0.00 | 0.00 | 0.00 |

**Table (20):** Comparison between the effects of oils of some plants on *A. flavus* mycelial fresh weight.

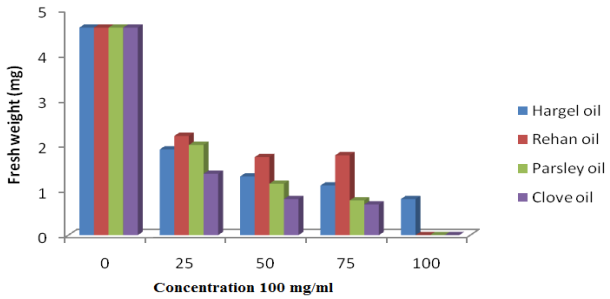


**Fig (10):** The effects of different concentrations of oils of different plants on *A.flavus* mycelial fresh weight.

| Concentration 100 mg/ml | Mycelial dry weight (mg) | | | |
|---|---|---|---|---|
| | Hargel | Rehan | Parsley | Clove |
| **0.0** | 0.90 | 0.90 | 0. 90 | 0.90 |
| **25.0** | 0.80 | 0.77 | 0.60 | 0.03 |
| **50.0** | 0.30 | 0.60 | 0.40 | 0.02 |
| **75.0** | 0.10 | 0.50 | 0.10 | 0.02 |
| **100.0** | 0.00 | 0.00 | 0.00 | 0.00 |

**Table (21):** Comparison between the effects of oils of some plants on *A. flavus* mycelial dry weight

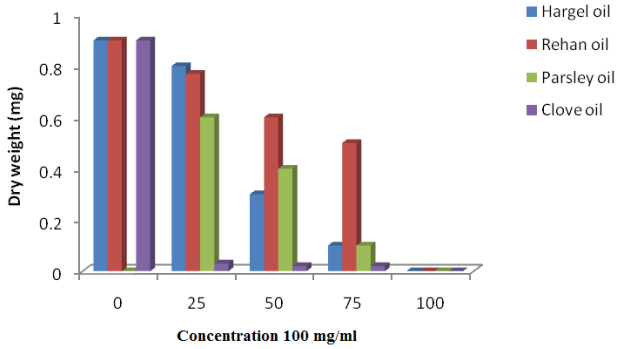


**Fig (11):** The effects of different concentrations of oils of other plants on *A.flavus* mycelial dry weight

| Concentration 100mg/ml | Mycelial dry weight (mg) | | | | |
|---|---|---|---|---|---|
| | Glutamic acid | Alanine | Methionine | Arginine | Leucine |
| **0.0** | 100 | 77.2 | 40.2 | 56.7 | 46.3 |
| **25.0** | 77.2 | 33.0 | 25.0 | 41.5 | 27.1 |
| **50.0** | 25.2 | 21.5 | 17.0 | 17.1 | 17.0 |
| **75.0** | 13.1 | 10.2 | 7.0 | 7.3 | 7.4 |
| **100.0** | 27.4 | 27.4 | 27.4 | 27.4 | 27.4 |

**Table (22):** Effects of amino acid on *A. flavus* mycelial dry weight

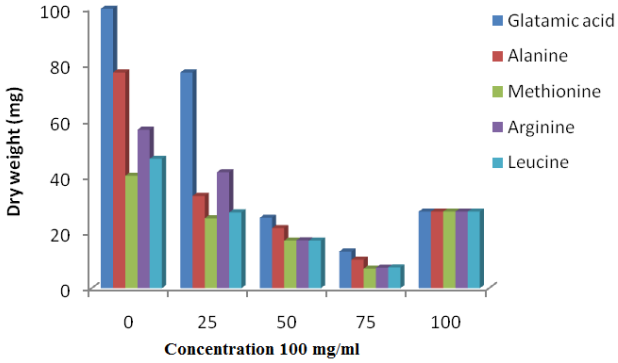


**Fig (12):** The effects of different concentrations of some amino acids on *A. flavus* mycelial dry weight

| Hargel leaf Concentration (100mg/ml) | spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 25.00 | 50.00 | 88.7 | 100.00 | $65.9^{a} \pm 34.7$ |
| **25.0** | 00.00 | 43.75 | 52.38 | 69.23 | $41.3^{ab} \pm 29.5$ |
| **50.0** | 42.85 | 54.54 | 66.66 | 69.23 | $58.3^{bc} \pm 12.1$ |
| **75.0** | 37.50 | 57.14 | 63.63 | 16.66 | $43.7^{c} \pm 21.2$ |
| **100.0** | 00.00 | 6.00 | 6.25 | 7.69 | $5.0^{c} \pm 3.4$ |
| SE± | | | | | 35.69 |
| CV% | | | | | 35.66 |

**Table (23):** Effect of different concentrations of Hargel leaf extracts on *A. flavus* spore germination
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 3648.1 | 1216.03 | 3.31 | 3.4903 | 0.0569 |
| **Concentration** | 4 | 8834.3 | 2208.57 | 6.02 | 3.2592 | 0.0068 |
| **Error** | 12 | 4399.0 | 366.58 | | | |
| **Total** | 19 | 16881.6 | | | | |

ANOVA table

| Hargel stem Concentration (100mg/ml) | spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 20.00 | 60.00 | 80.00 | 100.00 | 65.0 $^{a}$ ± 34.2 |
| **25.0** | 00.00 | 42.00 | 45.00 | 49.60 | 34.2 $^{ab}$ ± 23.0 |
| **50.0** | 33.00 | 33.00 | 33.00 | 33.00 | 33.0$^{b}$ ± 0.0 |
| **75.0** | 00.00 | 00.00 | 00.00 | 00.00 | 0.0$^{b}$ ± 0.0 |
| **100.0** | 00.00 | 00.00 | 00.00 | 00.00 | 0.0$^{b}$ ± 0.0 |
| SE± | | | | | 38.44 |
| CV% | | | | | 43.85 |

**Table (24):** Effect of different concentrations of Hargel stem extracts on *A. flavus* spore germination
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 1897.2 | 632.4 | 2.38 | 3.4903 | 0.1206 |
| **Concentration** | 4 | 11949.9 | 2987.4 | 11.25 | 3.2592 | 0.0005 |
| **Error** | 12 | 3187.0 | 265.5 | | | |
| **Total** | 19 | 17034.2 | | | | |

ANOVA table

| Hargel oil Concentration (100mg/ml) | spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 25.0 | 35.7 | 80.0 | 100.0 | 60.2 $^{a}$ ± 35.7 |
| **25.0** | 30.6 | 32.0 | 40.5 | 45.1 | 37.1 $^{b}$ ± 6.9 |
| **50.0** | 20.0 | 28.1 | 32.4 | 35.0 | 28.9$^{bc}$ ± 6.6 |
| **75.0** | 17.0 | 20.3 | 24.0 | 25.0 | 21.6$^{c}$ ± 3.7 |
| **100.0** | 00.00 | 00.00 | 00.00 | 00.00 | 0.0$^{c}$ ± 0.0 |
| SE± | | | | | 65.42 |
| CV% | | | | | 78.33 |

**Table (25):** Effect of different concentrations of Hargel oil on *A.flavus* spore germination
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 1636.3 | 545.4 | 2.62 | 3.4903 | 0.0983 |
| **Concentration** | 4 | 7725.5 | 1931.3 | 9.30 | 3.2592 | 0.001 |
| **Error** | 12 | 2492.2 | 207.6 | | | |
| **Total** | 19 | 11854.2 | | | | |

ANOVA table

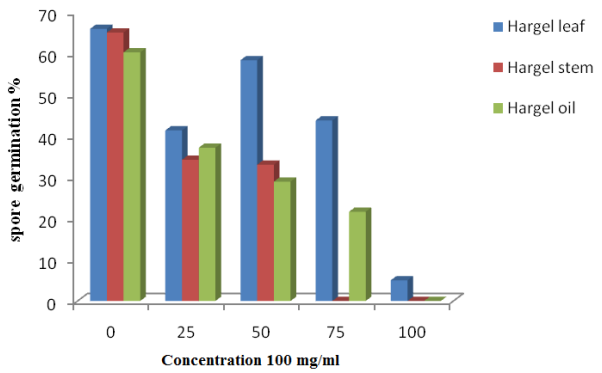


**Fig (13):** The effects of different Hargel plant extracts on the spore germination of *A.flavus.*

| Rehan leaf Concentration (100mg/ml) | spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 32.0 | 39.7 | 70.0 | 100.0 | 60.4[a] ± 31.1 |
| **25.0** | 25.0 | 30.0 | 42.0 | 55.5 | 38.1 [a] ± 13.6 |
| **50.0** | 18.0 | 25.0 | 45.0 | 56.0 | 36.1 [a] ± 17.6 |
| **75.0** | 15.0 | 20.0 | 22.0 | 22.7 | 19.9 [a] ± 3.5 |
| **100.0** | 10.10 | 12.00 | 15.00 | 15.00 | 13.0 [a] ± 2.4 |
| SE± | | | | | 5.00 |
| CV% | | | | | 16.48 |

**Table (26):** Effect of different concentrations of Rehan leaf extracts on *A. flavus* spore germination
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 2716.9 | 905.6 | 6.34 | 3.4903 | 0.0080 |
| **Concentration** | 4 | 5424.4 | 1356.1 | 9.50 | 3.2592 | 0.0011 |
| **Error** | 12 | 1713.3 | 142.7 | | | |
| **Total** | 19 | 9854.6 | | | | |

ANOVA table

| Rehan stem Concentration (100 mg/ml) | spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 30.0 | 45.0 | 60.0 | 100.0 | 58.8 [a] ± 30.1 |
| **25.0** | 29.1 | 30.0 | 31.3 | 55.0 | 36.4[b] ± 12.5 |
| **50.0** | 22.0 | 25.0 | 28.8 | 35.0 | 27.7 [b] ± 5.6 |
| **75.0** | 16.0 | 18.0 | 20.3 | 20.1 | 18.6 [b] ± 2.0 |
| **100.0** | 00.00 | 00.00 | 00.00 | 00.00 | 0.0 [b] ± 0.0 |
| SE± | | | | | 42.79 |
| CV% | | | | | 49.90 |

**Table (27):** Effect of different concentrations of Rehan stem extracts on *A. flavus* spore germination
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 1446.1480 | 482.0 | 3.13 | 3.4903 | 0.0655 |
| **Concentration** | 4 | 7549.3720 | 1887.3 | 12.27 | 3.2592 | 0.0003 |
| **Error** | 12 | 1845.3520 | 153.7 | | | |
| **Total** | 19 | 10840.8720 | | | | |

ANOVA table

| Rehan Oil Concentration (100mg/ml) | spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 20.00 | 40 | 75 | 100 | 58.5 [a] ± 35.7 |
| **25.0** | 00.00 | 25 | 40 | 63 | 32.0 [b] ± 26.4 |
| **50.0** | 00.00 | 10 | 18 | 22 | 12.5 [b] ± 9.7 |
| **75.0** | 00.00 | 00.00 | 00.00 | 00.00 | 0.0 [b] ± 0.0 |
| **100.0** | 00.00 | 00.00 | 00.00 | 00.00 | 0.0 [b] ± 0.0 |
| SE± | | | | | 42.35 |
| CV% | | | | | 63.84 |

**Table (28):** Effect of different concentrations of Rehan oil on *A. flavus* spore germination
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 3059.3500 | 1019.7 | 3.89 | 3.4903 | 0.0372 |
| **Concentration** | 4 | 9998.8000 | 2499.7 | 9.55 | 3.2592 | 0.0010 |
| **Error** | 12 | 3140.4000 | 261.7 | | | |
| **Total** | 19 | 16198.5500 | | | | |

ANOVA table

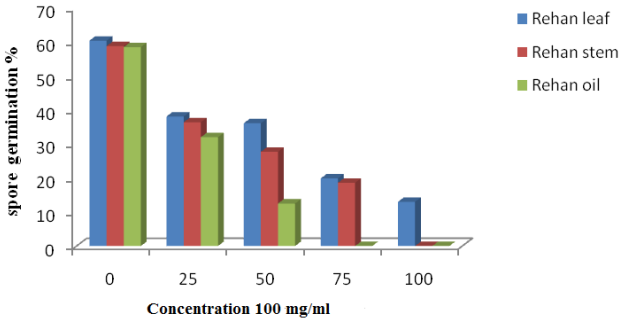


**Fig (14):** Shows the effects of different concentrations of Rehan plant extracts on the spore germination of *A. Flavus.*

| Parsley leaf Concentration (100mg/ml) | spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 40.00 | 40.00 | 40.00 | 40.00 | 40.0$^{a}$ ± 0.0 |
| **25.0** | 37.00 | 37.00 | 37.00 | 37.00 | 37.0 $^{a}$ ± 0.0 |
| **50.0** | 33.33 | 33.33 | 33.33 | 33.33 | 33.3 $^{a}$ ± 0.0 |
| **75.0** | 12.50 | 22.50 | 33.33 | 33.33 | 25.4 $^{a}$ ± 1.0 |
| **100.0** | 00.00 | 00.00 | 00.00 | 00.00 | 0.0$^{b}$ ± 0.0 |
| SE± | | | | | 0.37 |
| CV% | | | | | 5.61 |

**Table (29):** Effect of different concentrations of Parsley leaf extracts on *A. flavus* spore germination.
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 60.1177 | 20.0392 | 3.64 | 3.4903 | 0.0113 |
| **Concentration** | 4 | 4162.4693 | 1040.6173 | 26.25 | 3.2592 | 0.0001 |
| **Error** | 12 | 18.19 | 1.51 | | | |
| **Total** | 19 | 4462.4693 | | | | |

ANOVA table

| Parsley stem Concentration (100mg/ml) | spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 30.00 | 34.00 | 73.00 | 100.00 | 59.3$^{a}$ ± 33.4 |
| **25.0** | 00.00 | 25.00 | 28.57 | 33.33 | 21.7$^{b}$ ± 14.9 |
| **50.0** | 00.00 | 18.75 | 30.00 | 46.15 | 23.7 $^{c}$ ± 19.4 |
| **75.0** | 00.00 | 22.22 | 33.33 | 50.00 | 26.4$^{d}$ ± 21.0 |
| **100.0** | 00.00 | 00.00 | 00.00 | 00.00 | 0.0$^{e}$ ± 0.0 |
| SE± | | | | | 0.03 |
| CV% | | | | | 8.46 |

**Table (30):** Effect of different concentrations of Parsley stem extracts on *A. flavus* spore germination.
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 4402.2701 | 1467.4 | 8.57 | 3.4903 | 0.0026 |
| **Concentration** | 4 | 7219.7095 | 1804.9 | 10.54 | 3.2592 | 0.0007 |
| **Error** | 12 | 2054.0103 | 171.1 | | | |
| **Total** | 19 | 13675.9899 | | | | |

ANOVA table

| Parsley oil Concentration (100mg/ml) | spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 100.00 | 34.7 | 65.00 | 80.00 | 69.93 $^{a}$ ± 27.5 |
| **25.0** | 50.00 | 00.00 | 00.00 | 14.28 | 12.50 $^{b}$ ± 25.0 |
| **50.0** | 43.75 | 00.00 | 00.00 | 00.00 | 14.51 $^{b}$ ± 20.6 |
| **75.0** | 20.00 | 00.00 | 00.00 | 00.00 | 5.00 $^{b}$ ± 10.0 |
| **100.0** | 00.00 | 00.00 | 00.00 | 00.00 | 0.00 $^{b}$ ± 0.0 |
| SE± | | | | | 6.51 |
| CV% | | | | | 63.84 |

**Table (31):** Effect of different concentrations of Parsley oil on *A. flavus* spore germination.
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 3689.18 | 1229.72 | 7.26 | 3.4903 | 0.0049 |
| **Concentration** | 4 | 12812.70 | 3203.17 | 18.91 | 3.2592 | 0.0001 |
| **Error** | 12 | 2032.99 | 169.41 | | | |
| **Total** | 19 | 18534.88 | | | | |

ANOVA table

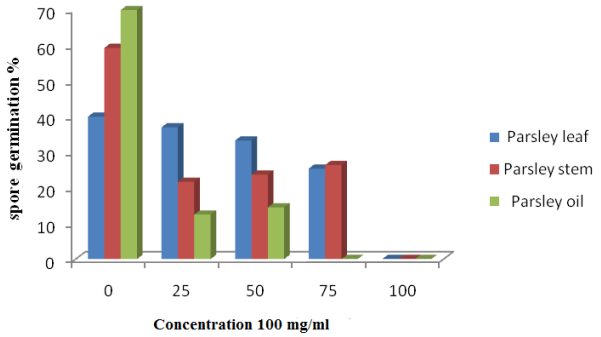


**Fig (15):** The effects of different Parsley plant extracts on the spore germination of *A. flavus.*

| Clove oil Concentration (100mg/ml) | spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| 0 | 100.00 | 100.00 | 100.00 | 100.00 | 100.0$^{a}$ ± 2.2 |
| 25.0 | 00.00 | 04.00 | 04.54 | 04.76 | 3.3$^{b}$ ± 2.2 |
| 50.0 | 00.00 | 04.28 | 04.35 | 04.75 | 3.3$^{b}$ ± 2.2 |
| 75.0 | 00.00 | 03.00 | 04.10 | 05.00 | 3.0$^{b}$ ± 0.0 |
| 100.0 | 00.00 | 00.00 | 00.00 | 00.00 | 0.0$^{c}$ ± 0.0 |
| SE± | | | | | 0.61 |
| CV% | | | | | 5.61 |

Table (32): Effect of different concentrations of Clove oil on *A. flavus* spore germination.
* Means followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 26.1093 | 8.70 | 5.67 | 3.4903 | 0.0113 |
| **Concentration** | 4 | 30499.1866 | 7624.79 | 5049.52 | 3.2592 | 0.0001 |
| **Error** | 12 | 18.1922 | 1.51 | | | |
| **Total** | 19 | 30543.4881 | | | | |

ANOVA table

| glutamic acid Concentration (100mg/ml) | spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 74.5 | 76.0 | 90.9 | 100 | 85.4 $^{a}$ ± 22.8 |
| **25.0** | 52.1 | 60.0 | 88.6 | 100 | 75.2$^{ab}$ ± 12.9 |
| **50.0** | 50.0 | 57.5 | 71.4 | 86.0 | 66.2$^{b}$ ± 11.3 |
| **75.0** | 40.1 | 44.3 | 50.7 | 65.8 | 50.2c ± 1.8 |
| **100.0** | 5.3 | 6.2 | 7.8 | 9.3 | 7.2$^{d}$ ± 0.0 |
| SE± | | | | | 3.95 |
| CV% | | | | | 13.91 |

**Table (33):** Effect of different concentrations of glutamic acid on *A. flavus* spore germination.
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 2406.7015 | 802.23 | 12.83 | 3.4903 | 0.0005 |
| **Concentration** | 4 | 14999.6950 | 3749.923 | 59.98 | 3.2592 | 0.0001 |
| **Error** | 12 | 750.1210 | 62.5100 | | | |
| **Total** | 19 | 18156.5175 | | | | |

ANOVA table

| Alanine Concentration (100mg/ml) | spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 19.9 | 45.3 | 77.0 | 80.6 | 55.7$^{a}$ ± 28.7 |
| **25.0** | 12.8 | 35.1 | 67.7 | 72.1 | 46.9$^{a}$ ± 28.1 |
| **50.0** | 0.0 | 14.2 | 23.0 | 26.1 | 15.8$^{b}$ ± 11.7 |
| **75.0** | 0.0 | 10.3 | 15.3 | 20.1 | 11.4$^{b}$ ± 8.6 |
| **100.0** | 6.9 | 7.4 | 8.8 | 9.8 | 8.2$^{b}$ ± 1.3 |
| SE± | | | | | 6.16 |
| CV% | | | | | 44.63 |

**Table (34):** Effect of different concentrations of alanine on *A.flavus* spore germination.
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 3647.1880 | 1215.73 | 8.00 | 3.4903 | 0.0034 |
| **Concentration** | 4 | 7754.9420 | 1938.74 | 12.75 | 3.2592 | 0.0003 |
| **Error** | 12 | 1823.4820 | 151.95 | | | |
| **Total** | 19 | 13225.6120 | | | | |

ANOVA table

| Methionine Concentration (100mg/ml) | Spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 17.9 | 43.7 | 81.0 | 81.7 | 56.1$^{a}$ ± 31.0 |
| **25.0** | 12.7 | 32.2 | 65.4 | 70.1 | 45.1$^{a}$ ± 27.4 |
| **50.0** | 0.0 | 16.2 | 22.0 | 24.1 | 15.6$^{b}$ ± 10.9 |
| **75.0** | 0.0 | 9.8 | 24.8 | 28.2 | 15.7$^{b}$ ± 13.2 |
| **100.0** | 0.0 | 8.3 | 9.7 | 10.3 | 7.1$^{b}$ ± 4.8 |
| SE± | | | | | 5.80 |
| CV% | | | | | 41.61 |

**Table (35):** Effect of different concentrations of methionine on *A. flavus* spore germination.
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 4469.4535 | 1489.817 | 11.04 | 3.4903 | 0.0009 |
| **Concentration** | 4 | 7296.3870 | 1824.096 | 13.52 | 3.2592 | 0.0002 |
| **Error** | 12 | 1618.3090 | 134.859 | | | |
| **Total** | 19 | 13384.1495 | | | | |

ANOVA table

| Arginine Concentration (100mg/ml) | Spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0** | 6.2 | 20.2 | 35.7 | 40.1 | $25.6^{a} \pm 15.5$ |
| **25.0** | 0.0 | 12.2 | 25.3 | 38.2 | $18.9^{ab} \pm 16.5$ |
| **50.0** | 0.0 | 7.3 | 20.4 | 35.0 | $15.7^{b} \pm 15.4$ |
| **75.0** | 0.0 | 6.0 | 20.3 | 23.5 | $12.5^{bc} \pm 11.3$ |
| **100.0** | 0.0 | 6.3 | 8.7 | 9.7 | $6.2^{c} \pm 4.4$ |
| SE± | | | | | 2.77 |
| CV% | | | | | 35.15 |

**Table (36):** Effect of different concentrations of arginine on *A. flavus* spore germination.
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 2314.1695 | 771.389 | 25.15 | 3.4903 | 0.0001 |
| **Concentration** | 4 | 834.7870 | 208.696 | 6.80 | 3.2592 | 0,0042 |
| **Error** | 12 | 368.0530 | 30.671 | | | |
| **Total** | 19 | 3517.0095 | | | | |

ANOVA table

| Leucine Concentration (100mg/ml) | Spore germination % Incubation period (hrs) | | | | Mean |
|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | |
| **0.0** | 0.9 | 32.3 | 41.2 | 43.0 | $29.4^{a} \pm 19.5$ |
| **25.0** | 0.0 | 23.0 | 33.4 | 35.5 | $23.0^{ab} \pm 16.3$ |
| **50.0** | 0.0 | 11.5 | 22.3 | 28.0 | $15.5^{b} \pm 12.4$ |
| **75.0** | 0.0 | 9.9 | 20.3 | 20.7 | $12.7^{bc} \pm 9.8$ |
| **100.0** | 0.0 | 7.3 | 7.9 | 9.8 | $6.3^{c} \pm 4.3$ |
| SE± | | | | | 3.124 |
| CV% | | | | | 36.01 |

**Table (37):** Effect of different concentrations of leucine on *A. flavus* spore germination.
*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

| SOV | DF | SS | MS | F-cal | F-tab $_{0.05}$ | P-value |
|---|---|---|---|---|---|---|
| **Period** | 3 | 2274.7140 | 758.24 | 19.41 | 3.4903 | 0.0001 |
| **Concentration** | 4 | 1295.4050 | 323.85 | 8.29 | 3.2592 | 0.0019 |
| **Error** | 12 | 468.6510 | 39.05 | | | |
| **Total** | 19 | 4038.7700 | | | | |

ANOVA table

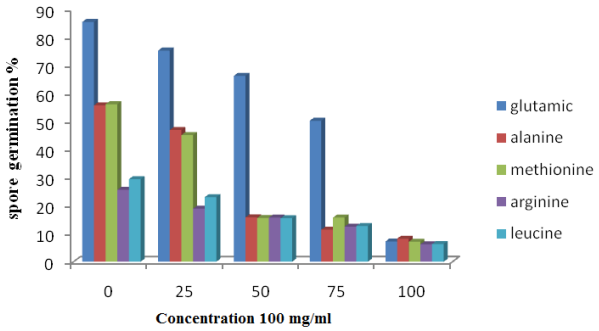


**Fig (16):** The effects of different amino acid concentrations on the spore germination of *A.flavus*.

| Aflatoxin production | Concentration (100mg/ml) | | |
|---|---|---|---|
| | 50.0 | 100.0 | Standard |
| **Hargel leaf** | - | - | + |
| **Hargel stem** | - | - | + |
| **Rehan leaf** | + | + | + |
| **Rehan stem** | + | - | + |
| **Parsley leaf** | + | - | + |
| **Parsley stem** | + | - | + |
| **Hargel oil** | + | - | + |
| **Rehan oil** | + | + | + |
| **Parsley oil** | - | - | + |
| **Clove oil** | - | - | + |

**Table (38):** Effect of extracts of some plants on aflatoxin production

**(+) = Positive, (-) =Negative

| Concentration Ml / 100ml | Aflatoxin production | | | | |
|---|---|---|---|---|---|
| | Glutamic acid | Alanine | Methionine | Leucine | Arginine |
| **0.0** | - | - | - | - | - |
| **25.0** | + | - | - | - | - |
| **50.0** | + | - | - | - | - |
| **75.0** | + | - | - | - | - |
| **100.0** | + | - | - | - | - |

**Table (39):** Effect of the different Amino Acids on aflatoxin production by *A.flavus.*

**(+) = Positive, (-) =Negative

| Class | Hargel leaf | Hargel stem | Rehan leaf | Rehan stem | Parsley leaf | Parsley stem |
|---|---|---|---|---|---|---|
| **Saponins** | - | + | ++ | + | ++ | + |
| **Tannins** | - | - | + | - | + | - |
| **Flavonoids** | - | - | + | + | + | + |
| **Alkaloids** | - | - | - | - | + | - |
| **Steroids** | - | - | - | - | - | - |
| **Phenolics** | - | - | + | - | + | - |

**Table (40):** The qualitative Phytochemical screening of the presence of the main classes on some part plants.

**(++) indicated the presence of the class
(+) indicated the presence of the class in relatively high concentration
(-) indicated that the class was not tested

## RESULTS & DISCUSSION

Fungal deterioration and mycotoxin contamination of various food and feedstuffs are significant problems in the tropics and subtropics, where climatic conditions and storage practices are favorable to fungal growth (Quiroga *et al*., 2009; Shukla *et al*., 2009; Salari *et al*., 2012). The risk of mycotoxin, particularly aflatoxin contamination, is a significant food safety concern for grains and other field crops worldwide (Kumar *et al*., 2007; Reddy *et al*., 2009). The Food and Agriculture Organization (FAO) estimated that around 25% of cereals are contaminated by mycotoxins, including aflatoxins (Dowling, 1997).

Aflatoxin B1 is one of the most common and dangerous mycotoxins produced by *A.flavus* (Manafi and Khosravinia. 2013). The aflatoxins were found in a variety of food commodities such as maize, groundnut, and other cereals worldwide. However, it is reported that about 4.5 billion people in developing countries are systematically exposed to uncontrolled amounts of aflatoxin (Shukla *et al*., 2008). Plants may represent a potential source of antibiotics, as evidenced by the massive number of studies dealing with antimicrobial activities (Alderman & Marth, 1976; Pratt, 1977; Pelcazar *et al*., 1977). The use of plants and their extracts as remedies for curing many diseases has stimulated studies to investigate

the presence of effective antimicrobial substances in them (Ahmed, 2004; Abdel Daim, 2001; Sulieman *et al*., 2008; Abdel-Rahim & Idris, 2010). Many studies have been done to control aflatoxin poisoning and prevent the aflatoxin-producing fungi's growth. The most important of them was the use of essential oils and plant extracts. Plant extracts of many species of plants were reported to have antimicrobial activities (Abdel-Rehim *et al*., 1997). However, Hargel-Rehan and Parsley were not tested against *A. flavus* and aflatoxin production.

The present study investigated the biological activity of the extracts of Hargel, Rehan, and Parsley plant parts and oils against the fungus (*Aspergillus flavus*). It also investigated the effect of amino acids on (Aspergillus flavus) and the phytochemical compounds of the extracts. The antifungal activity was made on mycelia growth (radial growth and fresh and dry weights of mycelia). The plate count and the cup plate inhibition zone methods were used for the spore germination test. The effects of the leaf and stem extracts and oil of Hargel on the radial growth of *A. flavus* showed that the leaf extracts of Hargel were highly effective, giving complete inhibition in reducing radial growth of A. flavus; the radial growth decreased with increasing concentrations of the leaf extracts.

However, all the concentrations of the leaf extracts were significantly ($P \leq 0.05$) effective in inhibiting growth compared to the control. The effect of the stem extracts of Hargel on radial growth of *A. flavus*. was almost similar to that of the leaf extracts, i.e., all the concentrations of the stem extract were significantly effective in inhibiting growth compared to the control. The effect of the oil was better than all the part extracts, especially at the higher concentrations (75-100mg/ml). The oils were highly effective at their higher concentration (100mg/ml).

On the other hand, the leaf and stem extracts were effective at the two higher concentrations (75,100 mg/ml). The oil of Rehan was the most effective compared to the extracts of Rehan leaf and stem; the oil, the leaf, and stem extracts of Parsely and clove oil were all highly effective on the radial growth of *A. flavus*. Abdel-Rahim *et al*. (2012a) found that the higher concentration (100mg/ml) of some plant extracts was highly effective in reducing the radial growth of *A. niger* compared to *P. italicum*. Elsaeim (2002) reported that pomegranate is very effective against worms and good against decent. Zainal *et al*. (1988) reported a pronounced effect of the leaf litter extracts of mesquite on *A. niger* and *C. albicans*. Mohamed *et al.* (2012) found that the dried fruits of *Acacia nilotica* were reported to be active against *C. albicans* and used to treat oral candidiasis. Methanolic extract of the plant was also found to be active against two animal viruses, Newcastle Disease and Fowlpox Viruses. Saini (2008) examined the comparative antimicrobial studies of different *Acacia* species; he found that A. nilotica exhibited the highest activity against the three tested bacterial (Escherichia et al. aureus and *Salmonella typhi*) and the two fungi tested (*Candida albicans* and *Aspergillus niger*).

Basil or Rehan extracts, on the other hand, have been used as a flavoring and seasoning agent in foods and beverages and therapeutically for centuries (Charalambous, 1994). In folk medicine, basil is used due to its carminative, stimulant, and antispasmodic properties. Basil has shown antioxidant, antimicrobial, and antitumor activities due to its phenolic acids and aromatic compounds (Hussain *et al*., 2008). The medicinal value of any plant is based on bioactive phytochemical components like phenolics, alkaloids, flavonoids, tannins, and saponins because these components produce specific physiological actions in the human body (Hanif *et al*., 2011). More than 280 plant species have been investigated for their inhibitory effect on toxigenic Aspergilli, and nearly 100 of these plants had some activity on growth or toxin production by fungi (Montes & Carvaial, 1998). Mabrouk and Shayeb (1980) reported that Clove oil completely inhibited the mycelial growth of *A.flavus* and aflatoxin formation. Although the plant extracts of the three plants used here have not been tested as antifungals, the Clove oil inhibited the mycelial growth of *A. flavus* and aflatoxin production effectively (Reddy *et al.*, 2009).

Essential oils and concentrated liquid herbal extracts inhibit the growth of a wide variety of pathogenic microorganisms, and many have great potential as antimicrobial agents (Gupta, 2008). The antimicrobial activity of essential oils has been recognized for many years. However, few investigations have compared large numbers of oils and extracts using directly comparable methods. Foodborne illnesses resulting from bacteria have been of concern to public health. Controlling pathogenic bacteria

would reduce foodborne outbreaks and assure consumers of a safe, wholesome, nutritious food supply (Reddy *et al*., 2009). Regarding the spore germination tests, the leaf extracts and oil of Hargel were the least effective, while the stem extracts were highly effective at the two concentrations (75,100mg/ml).

From the results of the extracts of the different parts of Rehan on spore germination of *A. flavus*, it is clear that the leaf and stem extracts were less effective, except they were effective at a higher concentration(100mg/ml). At the same time, the oil was highly effective at the two higher concentrations (75 and 100mg/ml). The effect of the different part extracts of Parsley is mainly on the spore germination of A. flavus. However, different part extracts were significantly better. The leaf extracts were the least effective, followed by the stem extracts, while the oil (was highly effective at all concentrations). Based on the study results, it could be concluded that the oils were more effective against the spore germination of *A. flavus*. However, Abdel-Rahim et al. (2012b) reached the same conclusion. Similar results were also obtained by different investigators (Abdel-Rahim et al., 1989; Al-Jali et al., 1997; Abdel-Rahim & Mohammed Ali, 2002; Sulieman et al., 2008; Abdel-Rahim et al., 2010) using different plant part extracts and oils. The effects of the different amino acids tested on the spore germination of *A. flavus* showed that Glutamic acid at all of its concentrations was the most effective amino acid, followed by alanine but only at its two lowest concentrations (0.0 and 25.0mg/ml). On the other hand, Methionine, leucine, and arginine were found to be the least effective, even at their higher concentrations. All microorganisms require nitrogen for their growth. Most of them utilize organic nitrogenous compounds such as proteins and their derivative products, e.g., amino acids, as sources of nitrogen. Fungi may take amino acids without modification, while high molecular weight compounds, such as proteins, cellulose, and starch, need first to be broken down by hydrolase enzymes (proteinases, cellulases, amylases, etc.).

However, the other reactions are mediated by oxidation and reduction (Moreau,1979). The leaf and stem extracts of the Hargel, Parsley oil, and Clove oil inhibited aflatoxin production at all concentrations. In contrast, Rehan stems, Parsley leaf, Parsley stem extracts, and Hargel oil inhibited aflatoxin production completely but at their higher concentration (100mg/ml) only. However, Rehan leaf extracts and Rehan oil are tested ineffective even at their higher concentration. On the other hand, glutamic acid was the only amino acid among the amino acids tested to be effective in inhibiting aflatoxin production by *A. flavus*. Domain and Drew (1977) and Bullerman *et al.* (1984) found that the aflatoxin-producing fungi (A. flavus and A. parasitics) need amino acids for their growth and can hydrolyze proteins to amino acids, which would be used as nitrogen sources.

The breakdown of proteins and carbohydrates by molds sometimes renders flour unfit for manufacturing bread. Lipolysis in various seeds, e.g., in groundnuts, may occur by some molds, e.g., A. flavus, A. fumigatus, and *A. niger*. The lipolytic activities of these fungi cause rancidity of the oil (Abdallah, 2001). Aflatoxin is a name given to a group of toxins produced by two fungi, Aspergillus flavus and Aspergillus parasiticus. Aflatoxin occurs naturally in a wide range of commodities (Abdel-Rahim, 2005). Aflatoxins were designated as B and G according to the color they show on the chromatographic plates after being viewed under Ultraviolet light (B for blue and G for green).

Each comprises two compounds, B1, B2, and G1, G2 (Fig1). However, M1 and M2 are hydroxylated derivates of B1 and B2, respectively, and are excreted in milk, urine, and feces as metabolic products. However, another type of aflatoxin, P1, is particularly found as a secondary metabolite in monkeys belonging to the Rhesus family family (Abdel-Rahim & Suleiman, 2009). The short-term toxicity of aflatoxin exposure of humans to these compounds in foods, leading to liver cancer, has been well established. Due to food safety problems, aflatoxin-contaminated commodities cannot be sold above 20 parts per billion (ppb) in food and feed and 0.5 ppb in milk and eggs (Abdel-Rahim, 2005). Phytochemical compounds were found on the extracts of the different parts extracts of the different plants tested. Saponins were found in all tested plant parts extracts except the Hargel leaf extracts at different levels. However, the saponins were high in the Rehan leaf and Parsley leaf extracts. Tannins were found on the Rehan or Basil leaf and Parsley leaf extracts, whereas not on the Hargel leaf, Hargel stem, Rehan stem, and Parsley stem extracts. Flavonoids were found on the Rehan leaf, Rehan stem,

Parsley leaf, and Parsley stem extracts but not on the Hargel leaf and Hargel stem extracts. Alkaloids were only found in parsley leaf extracts. Phenolic compounds were found in the Rehan leaf and the Parsley leaf extracts but not in the Hargel leaf, the Hargel stem, the Rehan stem, and the Parsley stem extracts.

However, Steriods were not detected in any of the part extracts of the plants tested. sHavsteen (1983) and Gabor (1988) found that *A. nilotica* is a medicinal plant with flavonoids as a constituent. Flavonoids are benzo-γ-pyrone derivatives that are widespread in plants. About 4000 individual flavonoids have been isolated. Flavonoid constituents of the diet were first identified as Vitamin C and were found to be essential in maintaining capillary wall integrity and capillary resistance. The dried peels of the Punica granatum fruits contain about 26% tannins. The bark can also be used as a source of tannins. The root and bark contain about 22% tannins, and a jet-black ink can be made from it (Gupta, 2000).

## CONCLUSIONS

The leaf extracts of Hargel, oil of Basil or Rehan, the oil, the leaf and stem extracts of Parsley, and the clove oil were all highly effective against the radial growth of *A. flavus*, while the other extracts and oils were less effective. The effects of the amino acids on radial growth indicated that they were all highly effective, giving complete at a higher concentration (100mg/ml). The oils of the three plants and that of the clove were highly effective in reducing the mycelial weights of the fungus A. flavus; the extracts of the Hargel, Parsley, and Basil leaves were less effective. Only methionine, leucine, and arginine were adequate among the amino acids tested against mycelial weight. All the oils tested and the three pants' stem extracts wholly inhibited spore germination. All the amino acids tested were less effective against spore germination. The oils of the plants were more adequate in inhibiting aflatoxin production than the extracts, and the leaf and stem extracts of Parsley were the better among the other extracts. The chemical analysis of the extracts and oils of the tested plants found that they contained saponins, tannins, flavonoids, alkaloids, and phenolics but not steroids.

## RECOMMENDATIONS

The effective extracts and oils of the tested plants can be used as antifungal agents after investigations. The active compounds in these extracts and oils need to be verified in further studies.

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