Efficacy of Certain Plant Extracts Against Stonebrood Pathogen (*Aspergillus flavus*) in Honeybee in Vitro

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ABSTRACT

A laboratory study to test the effect of three different concentrations (250, 500 and 1000 ppm) of each of the petroleum ether, hexane, chloroform, acetone, ethanol and distilled water extracts of caraway, clove, fenugreek, ginger, marjoram, santonica, and thymus on growth of *Aspergillus flavus* (the fungus that caused stonebrood disease in honey bee apiaries) in potato-dextrose agar medium (PDA) was carried out. The growth of fungus was completely inhibited with all the solvent extracts of clove at all the concentrations except chloroform extracts at 250 and 500 ppm. It decreased the growth of the fungus as compared with the control (untreated check). The extracts of the petroleum ether, hexane and acetone of santonica and thymus plants at the concentration 1000 ppm inhibited completely the growth of the fungus. However, aqueous extracts of the tested plants were less effective when compared with the other ones.

Key Words: *Apis mellifera*, Stone brood disease, *Aspergillus flavus*, Essential oils, Plant extracts, Caraway, Clove, Fenugreek, Marjoram, Santonica, Thyme, Ginger.

INTRODUCTION

Stonebrood of honey bees (*Apis mellifera*) is a fungal disease caused by the fungus, *Aspergillus* spp. Ali (2001) isolated five species of fungi associated with the honey bee they were (*A. flavus, A. niger, Ascosphaera apis, Tieghumiomyces* sp., and *Curvularia* sp.), among the fungi isolated; *A. flavus* and *As. apis* had pathogenic effects. Meanwhile, Shoreit and Bagy (1995) isolated *Aspergillus niger, A. flavus, A. fumigatus, A. oryzae* and *Penicillium funiculosum* from diseased and healthy colonies in Egypt.

Lack of effective chemical control agent for stonebrood has resulted in an increased interest in the investigation of alternative control strategies. Essential oils of many plants are known to exhibit significant antimicrobial activity against a wide spectrum of microorganisms (Davis and Ward, 2003). The antifungal activities of different essential oils that inhibit the development and destroy *A. flavus* have been studied by many investigators; caraway (Regina *et al.* 1992 and Soliman and Badea, 2002); clove (Thompson and Cannon, 1986; Farag, 1990; Abul-Khair *et al.* 1995; Montes and Carvajal 1998 and Guynot *et al.* 2003); fenugreek (El-Shayeb and Mabrouk 1984); ginger (Tantaoui and Baraoud, 1994 and Yin and Cheng, 1998); and thymus (Thompson and Cannon, 1986; Farag, 1990; Tantaoui and Baraoud, 1994; Amvam *et al.* 1998; Montes and Carvajal, 1998; Soliman and Badea, 2002 and Guynot *et al.* 2003). Ali and Abo El-Enain (2005) evaluated extracts of clove, fenugreek, marjoram and thyme in petroleum ether, hexane, acetone, ethanol, chloroform and aqueous against growth of *Ascosphaera apis.* They concluded that the growth of fungus was completely inhibited with all the solvents extracts of clove except water extract and acetone extract of thyme. Fenugreek extracts were less effective on growth of the fungus. All the extracts had no effect on honey bees when treated under laboratory conditions in cages.

The present work aims to evaluate the efficacy of the extracts of Caraway, Clove, Fenugreek, Marjoram, Thyme, Santonica, and Ginger plants in petroleum ether, hexane, acetone, ethanol, chloroform and aqueous on the growth of the fungus (*Aspergillus flavus* Link et Gary) in vitro.

MATERIALS AND METHODS

1. Isolation and identification of *Aspergillus flavus*

Naturally infected honey bee brood (mummies) was collected from infected honey bee colonies of the apiary of the Faculty of Agriculture, Ain Shams University in 2003. Isolation, purification and identification were carried out according to Ali (2001).

2. Plant extracts

Seven plants were used in this study, namely; Caraway (*Carum carvi*), Clove (*Eugenia aromatic*), Fenugreek (*Trigonella foenumgraecum*), Ginger (*Zingiber officinale*), Marjoram (*Organium majorana*), Santonica (*Artemisia cinae*), and Thyme

(*Thymus vulgaris*). Extraction process involved; seeds of caraway and fenugreek, floral buds of clove, all parts of santonica, marjoram and thyme, and the roots of ginger. The solvents; petroleum ether, hexane, chloroform, acetone, ethanol, and distilled water were applied for each plant. Extraction procedures were carried out in the Department of Plant Protection. One kilogram from each material was cleaned by removing any impurities. Clove buds and ginger were ground at high speed micromil. The petroleum ether extract was performed at 40 - 60^{0}C and filtered through anhydrous sodium sulfphate. Collected extracts were evaporated under controlled conditions and maintained in sterilized glasses (Afifi *et al.* 1988).

3. Effect of plant extracts on growth of *Aspergillus flavus*

Six extracts of each of the caraway, clove, fenugreek, ginger, marjoram, santonica and thyme plants were tested for their effects on growth of *A. flavus* in vitro. The tested concentrations of each extract were 250, 500 and 1000 ppm. The extracts were added to flasks containing potato dextrose agar medium (PDA) before solidifying and rotated to ensure an equal distribution of extracts, and then poured in sterilized Petri-dishes. A mycelial disk (9 mm diameter) taken from 7 days old culture of the fungus and transferred to the center of the dish. Inoculated dishes were incubated at 28°C until full growth was noticed in any plate, (9 days) from the control treatment (extract free). Four plates were applied as replicates for each treatment. The average diameter of fungal growth was measured after 3, 6 and 9 days from inoculation (Abdulsalam and Mohamed 1989). The percentage of growth reduction was calculated as compared to the control treatment.

Data was statistically analyzed using the "F" test and the value of LSD (P= 0.05) was calculated according to Cochran and Cox (1957).

Growth reduction (%) =

$$\frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

RESULTS AND DISCUSSION

Results illustrated in Tables (1-7) demonstrated that essential oils at the different concentrations (250, 500 and 1000 ppm) of different extracts of caraway, clove, fenugreek, ginger, santonica, marjoram and thymus with different solvents of petroleum ether, hexane, chloroform, acetone, ethanol and distilled water decreased with the growth of the fungus *A. flavus* different ratios when compared with untreated check. The data also showed that the growth of the fungus was greatly reduced with increasing the concentration of each extract. In general, the essential oil extracts of clove, santonica, marjoram and thymus were most effective for reducing the growth of the fungus when compared with other extracts.

1. Effect of caraway extractions

Data in Table (1) show that the extracts did not affect the growth reduction of the fungus as compared with untreated check. Only the ethanol extracts at concentration 1000 ppm a gave little effect, where it caused 10.13 and 20.37% growth reductions of the fungus after 3 and 6 days of inoculation, respectively.

2. Effect of clove extractions

Table (2) shows that all the concentrations of essential oils inhibited the growth of the fungus, where the growth reduction was 100%, except the chloroform extract at concentration 250 and 500 ppm, which gave moderate growth reductions, 0.15, 39.58 and 25.78 and 85.07, 59.76 and 60.56 % after 3, 6 and 9 days, respectively. Distilled water extract did not affect the growth at any tested concentration.

3. Effect of fenugreek extractions

Data in Table (3) show that the growth of the fungus did not decrease by the extracts at any concentration. Only, petroleum ether extract, at 1000 ppm gave a slight affect on the growth after 3 days (13.93%), and hexane extract at all concentrations gave also a slight effect on the growth. The growth reductions were 18.24, 18.74 and 21.23 % and 30.23, 31.25 and 31.82 % for the concentrations 250, 500 and 1000 ppm after 3 and 6 days of inoculation, respectively.

4. Effect of ginger extractions

Table (4) shows that all the concentrations gave a slight effect on the growth of the fungus. The highest percentage of growth reduction however was obtained by hexane extract after 6 days, where they were 31.55, 31.55 and 33.38 % for 250, 500 and 1000 ppm., respectively.

5. Effect of marjoram extractions

Data in Table (5) show that the effect of petroleum ether extract at 1000 ppm inhibited completely the growth of the fungus, where the growth reduction was (100 %). Hexane, acetone and ethanol extracts at 1000 ppm ranked second, where the growth reduction was 85.07% for the fore-

mentioned extracts after 3 days, respectively. The other extracts varied in growth reduction from slight to moderate. On the other hand, the distilled water extract did not reduce the growth of the fungus when compared with the other solvents extracts.

6. Effect of santonica extractions

Growth of the fungus was inhibited completely with the petroleum ether, hexane and acetone extracts at the concentration 1000 ppm, where the growth reduction was 100% Table (6). The other concentrations varied in their effects, where they ranged from moderate to high percentages. The distilled water extract was less effective when compared with other solvents extracts.

7. Effect of thymus extractions

Data in Table (7) show the effect of essential oil extracts of thymus at different concentrations of different solvents on the growth and growth reduction of *A. flavus*. Growth of the fungus was inhibited completely with the petroleum ether, hexane and acetone extracts at the concentration 1000 ppm, where the growth reduction was 100%. Hexane extracts at 250 and 500 ppm came in the second order for inhibiting the fungus growth, where they were 85.07, 88.13 and 100 % after 3, 6 and 9 days, respectively. The other concentrations varied their effects, where they ranged between slight and moderate percentages. The distilled water extract did not effect the growth of the fungus as compared with other solvents extracts.

Obtained results proved, in general, that using chemical solvents for extracting any of the tested medicinal plants was necessary to attain potential impact against *A. flavus* under laboratory conditions than using distilled water. Yin and Cheng (1998) found no inhibitory effect against *A. flavus* when they examined the effects of water-soluble extracts of some aromatic plants on its growth. Also, Ali and Abo El-Enain (2005) recorded that the growth of *Ascosphaera apis* was inhibited at various degrees with the extracts of clove, fenugreek, marjoram and thyme with petroleum ether, hexane, acetone, ethanol and chloroform. The obtained results revealed also that all the tested plant extracts had antifungal activity and this activity was positively increased by the increase of concentration. The findings of El-Shayeb and Mabrouk (1984); Thompson and Cannon (1986), Abul-Khair *et al.* (1995) and Schollenberger and Zamorski (1997) were in agreement with the obtained results.

The highest growth reduction of *A. flavus* was recorded with clove, santonica, thymus and

Table (1): Fungal area (Cm^2) and growth reduction of *Aspergillus flavus* colonies in Petri dishes following the application of Caraway (*Carum carvi*) extracts with different concentrations.

Solvents	Days after inoculation	Growth diameter (cm) at the following concentrations				Growth reduction (%)		
		Untreated Check	250 ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm
Petroleum ether	3	3.06 ± 0.06	2.96 ± 0.04	2.90 ± 0.04	2.75 ± 0.03	3.27	5.23	10.13
	6	6.53 ± 0.02	6.41 ± 0.06	6.39 ± 0.05	5.50 ± 0.07	1.84	2.14	15.77
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	0.00	0.00
Hexane	3	3.06 ± 0.06	2.93 ± 0.04	2.85 ± 0.01	2.80 ± 0.07	4.25	6.86	8.50
	6	6.53 ± 0.02	6.35 ± 0.05	6.25 ± 0.02	5.90 ± 0.11	2.76	4.29	9.65
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	0.00	0.00
Chloroform	3	3.60 ± 0.60	3.05 ± 0.06	2.95 ± 0.03	2.70 ± 0.15	0.33	3.59	11.76
	6	6.53 ± 0.02	6.28 ± 0.08	6.22 ± 0.01	5.99 ± 0.02	3.83	4.75	8.27
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	0.00	0.00
Acetone	3	3.60 ± 0.60	2.98 ± 0.04	2.90 ± 0.02	2.90 ± 0.09	2.61	5.23	5.23
	6	6.53 ± 0.02	6.27 ± 0.09	6.15 ± 0.06	6.00 ± 0.22	3.98	5.82	8.12
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	0.00	0.00
Ethanol	3	3.06 ± 0.06	2.88 ± 0.08	2.86 ± 0.03	2.75 ± 0.10	5.88	6.54	10.13
	6	6.53 ± 0.02	5.85 ± 0.15	5.80 ± 0.01	5.20 ± 0.01	10.41	11.18	20.37
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	0.00	0.00
Distilled water	3	3.06 ± 0.06	2.99 ± 0.04	2.99 ± 0.03	2.99 ± 0.13	2.29	2.29	2.29
	6	6.53 ± 0.02	6.35 ± 0.05	6.32 ± 0.06	6.20 ± 0.11	2.76	3.22	5.05
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	0.00	0.00

L.S.D at 5% Treat. 0.9 Conc. 0.4 Time 0.3 Interactions 1.3

Maximum area of fungi in Petri dishes was 9 cm.

Table (2): Fungal area (Cm^2) and growth reduction of *Aspergillus flavus* colonies in Petri dishes following the application of Clove (*Eugenia aromatic*) extracts with different concentrations.

Solvents	Days after inoculation	Growth diameter (cm) at the following concentrations				Growth reduction (%)		
		Untreated Check	250 ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm
Petroleum ether	3	6.03 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
	6	7.58 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
	9	9.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
Hexane	3	6.03 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
	6	7.58 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
	9	9.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
Chloroform	3	6.03± 0.06	1.08 ± 0.01	0.90 ± 0.00	0.00 ± 0.00	70.15	85.07	100
	6	7.58 ± 0.04	4.58 ± 0.13	3.05 ± 0.15	0.00 ± 0.00	39.58	59.76	100
	9	9.00 ± 0.00	6.68 ± 0.11	3.55 ± 0.05	0.00 ± 0.00	25.78	60.56	100
Acetone	3	6.03 ± 0.60	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
	6	7.58 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
	9	9.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
Ethanol	3	6.03 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
	6	7.58 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
	9	9.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
Distilled water	3	6.03 ± 0.06	5.90 ± 0.05	5.70 ± 0.11	5.65 ± 0.04	2.16	3.48	6.30
	6	7.58 ± 0.04	7.08 ± 0.11	6.73 ± 0.02	6.50 ± 0.12	6.60	11.21	14.25
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	00.00	00.00

L.S.D at 5% Treat. 0.8 Conc. 0.2 Time 0.2 Interactions 1.5
Maximum area of fungi in Petri dishes was 9 cm.

Table (3): Fungal area (Cm^2) and growth reduction of *Aspergillus flavus* colonies in Petri dishes following the application of Fenugreek (*Trigonella foenumgraecum*) extracts with different concentrations.

Solvents	Days after inoculation	Growth diameter (cm) at the following concentrations				Growth reduction (%)		
		Untreated Check	250 ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm
Petroleum ether	3	6.03 ± 0.06	6.03 ± 0.16	6.03 ± 0.11	5.19 ± 0.13	0.00	0.00	13.93
	6	8.80 ± 0.01	8.80 ± 0.02	8.80 ± 0.01	8.80 ± 0.23	0.00	0.00	0.00
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	0.00	0.00
Hexane	3	6.03 ± 0.06	4.93 ± 0.07	4.90 ± 0.01	4.75 ± 0.07	18.24	18.74	21.23
	6	8.80 ± 0.01	6.14 ± 0.05	6.05 ± 0.11	6.00 ± 0.06	30.23	31.25	31.82
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	0.00	0.00
Chloroform	3	6.03 ± 0.06	5.03 ± 0.13	5.00 ± 0.03	4.00 ± 0.09	16.58	17.08	33.66
	6	8.80 ± 0.01	8.80 ± 0.04	8.80 ± 0.11	8.80 ± 0.03	0.00	0.00	0.00
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	0.00	0.00
Acetone	3	6.03 ± 0.06	6.00 ± 0.01	5.88 ± 0.12	5.80 ± 0.14	0.50	2.49	3.81
	6	8.80 ± 0.01	8.80 ± 0.11	8.80 ± 0.03	8.50 ± 0.01	0.00	0.00	3.41
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	0.00	0.00
Ethanol	3	6.03 ± 0.06	6.03 ± 0.03	5.95 ± 0.05	5.90 ± 0.03	0.00	1.33	2.16
	6	8.80 ± 0.01	8.80 ± 0.01	8.15 ± 0.13	8.10 ± 0.04	0.00	7.39	7.95
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	0.00	0.00
Distilled water	3	6.03 ± 0.06	6.03 ± 0.06	6.03 ± 0.03	6.03 ± 0.12	0.00	0.00	0.00
	6	8.80 ± 0.01	8.80 ± 0.06	8.80 ± 0.05	8.80 ± 0.03	0.00	0.00	0.00
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	0.00	0.00

L.S.D at 5% Treat. 2.0 Conc. 1.2 Time 0.8 Interactions 2.9
Maximum area of fungi in Petri dishes was 9 cm.

Table (4): Fungal area (Cm^2) and growth reduction of *Aspergillus flavus* colonies in Petri dishes following the application of Ginger (*Zingiber officinale*) extracts with different concentrations.

Solvents	Days after inoculation	Growth diameter (cm) at the following concentrations				Growth reduction (%)		
		Untreated Check	250 ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm
Petroleum ether	3	3.06 ± 0.06	3.06 ± 0.01	2.95 ± 0.03	2.83 ± 0.11	00.00	3.59	07.52
	6	6.53 ± 0.02	6.21 ± 0.04	6.00 ± 0.01	5.06 ± 0.31	04.90	8.12	23.43
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	7.32 ± 0.16	00.00	0.00	18.67
Hexane	3	3.06 ± 0.06	3.06 ± 0.15	3.06 ± 0.01	3.00 ± 0.18	00.00	0.00	01.96
	6	6.53 ± 0.02	4.47 ± 0.03	4.47 ± 0.13	4.35 ± 0.26	31.55	31.55	33.38
	9	9.00 ± 0.00	6.69 ± 0.10	6.60 ± 0.04	6.55 ± 0.07	25.67	26.67	27.22
Chloroform	3	3.60 ± 0.60	2.93 ± 0.04	2.88 ± 0.02	2.75 ± 0.20	04.25	05.88	10.13
	6	6.53 ± 0.02	6.38 ± 0.07	6.35 ± 0.02	6.13 ± 0.06	02.30	02.76	06.12
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	7.85 ± 0.12	00.00	00.00	12.78
Acetone	3	3.60 ± 0.60	2.62 ± 0.04	2.55 ± 0.01	2.25 ± 0.20	14.38	16.67	26.47
	6	6.53 ± 0.02	4.86 ± 0.10	4.77 ± 0.04	4.65 ± 0.30	25.57	26.95	28.79
	9	9.00 ± 0.00	6.78 ± 0.09	6.67 ± 0.06	6.50 ± 0.24	24.67	26.00	27.78
Ethanol	3	3.06 ± 0.06	2.58 ± 0.05	2.50 ± 0.11	2.45 ± 0.18	15.69	18.30	19.93
	6	6.53 ± 0.02	4.54 ± 0.07	4.50 ± 0.05	4.30 ± 0.29	30.47	31.09	34.15
	9	9.00 ± 0.00	6.70 ± 0.05	6.59 ± 0.01	6.10 ± 0.30	25.55	26.78	32.22
Distilled water	3	3.06 ± 0.06	2.96 ± 0.07	2.90 ± 0.01	2.90 ± 0.08	03.27	05.23	05.23
	6	6.53 ± 0.02	6.41 ± 0.04	6.21 ± 0.02	6.20 ± 0.05	01.84	04.90	05.05
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	00.00	00.00	00.00

L.S.D at 5% Treat. 0.8 Conc. 0.5 Time 0.3 Interactions 1.5

Maximum area of fungi in Petri dishes was 9 cm.

Table (5): Fungal area (Cm^2) and growth reduction of *Aspergillus flavus* colonies in Petri dishes following the application of Marjoram (*Organium majorana*) extracts with different concentrations.

Solvents	Days after inoculation	Growth diameter (cm) at the following concentrations				Growth reduction (%)		
		Untreated Check	250 ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm
Petroleum ether	3	6.03 ± 0.06	4.40 ± 0.04	3.22 ± 0.16	0.00 ± 0.00	27.03	46.60	100.0
	6	8.80 ± 0.01	6.23 ± 0.08	4.38 ± 0.01	0.00 ± 0.00	29.20	50.23	100.0
	9	9.00 ± 0.00	9.00 ± 0.00	7.00 ± 0.03	0.00 ± 0.00	00.00	22.22	100.0
Hexane	3	6.03 ± 0.06	3.08 ± 0.04	2.99 ± 0.01	0.90 ± 0.00	48.92	50.41	85.07
	6	8.80 ± 0.01	6.12 ± 0.07	5.77 ± 0.12	4.28 ± 0.09	30.45	34.43	47.95
	9	9.00 ± 0.00	9.00 ± 0.00	8.22 ± 0.11	6.55 ± 0.18	00.00	08.67	27.22
Chloroform	3	6.03 ± 0.06	4.43 ± 0.07	4.02 ± 0.08	3.75 ± 0.17	26.53	33.33	37.81
	6	8.80 ± 0.01	6.09 ± 0.04	5.88 ± 0.01	5.23 ± 0.05	30.79	33.18	40.57
	9	9.00 ± 0.00	9.00 ± 0.00	8.88 ± 0.11	6.75 ± 0.10	00.00	01.53	25.00
Acetone	3	6.03 ± 0.06	3.19 ± 0.06	2.94 ± 0.02	0.90 ± 0.00	47.10	51.24	85.07
	6	8.80 ± 0.01	5.43 ± 0.04	5.40 ± 0.01	5.00 ± 0.21	38.29	42.73	43.18
	9	9.00 ± 0.00	6.09 ± 0.02	6.02 ± 0.01	5.99 ± 0.01	32.33	33.11	33.44
Ethanol	3	6.03 ± 0.06	4.44 ± 0.04	4.00 ± 0.01	0.90 ± 0.00	26.37	33.67	85.07
	6	8.80 ± 0.01	6.13 ± 0.04	5.99 ± 0.02	5.75 ± 0.10	30.34	31.93	34.66
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	6.35 ± 0.20	00.00	00.00	29.44
Distilled water	3	6.03 ± 0.06	5.56 ± 0.03	5.55 ± 0.11	5.53 ± 0.17	07.79	07.96	08.79
	6	8.80 ± 0.01	8.80 ± 0.11	8.80 ± 0.22	8.80 ± 0.10	00.00	00.00	00.00
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	00.00	00.00	00.00

L.S.D at 5% Treat. 1.9 Conc. 0.9 Time 0.7 Interactions 2.0

Maximum area of fungi in Petri dishes was 9 cm.

Table (6): Fungal area (Cm^2) and growth reduction of *Aspergillus flavus* colonies in Petri dishes following the application of Santonica (*Artemisia cinae*) extracts with different concentrations.

Solvents	Days after inoculation	Growth diameter (cm) at the following concentrations				Growth reduction (%)		
		Untreated Check	250 ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm
Petroleum ether	3	6.03 ± 0.06	0.90 ± 0.00	0.90 ± 0.00	0.00 ± 0.00	85.07	85.07	100
	6	7.58 ± 0.04	3.50 ± 0.02	2.50 ± 0.05	0.00 ± 0.00	53.82	67.02	100
	9	9.00 ± 0.00	4.07 ± 0.03	3.99 ± 0.09	0.00 ± 0.00	54.78	55.67	100
Hexane	3	6.03 ± 0.06	0.90 ± 0.00	0.90 ± 0.00	0.00 ± 0.00	85.07	85.07	100
	6	7.58 ± 0.04	2.38 ± 0.33	1.15 ± 0.02	0.00 ± 0.00	68.60	84.83	100
	9	9.00 ± 0.00	3.02 ± 0.02	2.65 ± 0.01	0.00 ± 0.00	66.44	70.56	100
Chloroform	3	6.03 ± 0.60	4.04 ± 0.02	3.88 ± 0.03	3.50 ± 0.09	33.00	35.66	41.96
	6	7.58 ± 0.04	3.60 ± 0.05	3.50 ± 0.03	3.45 ± 0.15	52.51	53.82	54.49
	9	9.00 ± 0.00	6.08 ± 0.04	5.97 ± 0.15	5.68 ± 0.18	32.44	33.67	36.89
Acetone	3	6.03 ± 0.06	3.12 ± 0.04	2.97 ± 0.01	0.00 ± 0.00	48.26	50.75	100
	6	7.58 ± 0.04	4.02 ± 0.04	3.50 ± 0.02	0.00 ± 0.00	46.96	53.82	100
	9	9.00 ± 0.00	6.05 ± 0.03	5.82 ± 0.01	0.00 ± 0.00	32.78	35.33	100
Ethanol	3	6.03 ± 0.06	3.34 ± 0.07	3.01 ± 0.03	0.90 ± 0.00	44.61	50.08	85.07
	6	7.58 ± 0.04	3.62 ± 0.06	3.38 ± 0.08	3.22 ± 0.07	52.24	55.41	57.52
	9	9.00 ± 0.00	6.15 ± 0.05	6.00 ± 0.13	5.90 ± 0.17	31.67	33.33	34.44
Distilled water	3	6.03 ± 0.06	4.40 ± 0.07	4.32 ± 0.05	4.30 ± 0.08	27.03	28.36	28.69
	6	7.58 ± 0.04	6.99 ± 0.06	6.62 ± 0.05	5.99 ± 0.19	7.78	12.66	20.98
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00	00.00	00.00

L.S.D at 5% Treat. 1.3 Conc. 0.5 Time 0.5 Interactions 1.3

Maximum area of fungi in Petri dishes was 9 cm.

Table (7): Fungal area (Cm^2) and growth reduction of *Aspergillus flavus* colonies in Petri dishes following the application of Thymus (*Thymus vulgaris*) extracts with different concentrations.

Solvents	Days after inoculation	Growth diameter (cm) at the following concentrations				Growth reduction (%)		
		Untreated Check	250 ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm
Petroleum ether	3	6.03 ± 0.06	3.06 ± 0.03	2.15 ± 0.01	0.00 ± 0.00	49.25	64.34	100
	6	7.58 ± 0.04	5.29 ± 0.12	4.00 ± 0.02	0.00 ± 0.00	30.21	47.23	100
	9	9.00 ± 0.00	9.00 ± 0.00	7.22 ± 0.11	0.00 ± 0.00	00.00	19.78	100
Hexane	3	6.03 ± 0.06	0.90 ± 0.00	0.90 ± 0.00	0.00 ± 0.00	85.07	85.07	100
	6	7.58 ± 0.04	0.90 ± 0.00	0.90 ± 0.00	0.00 ± 0.00	88.13	88.13	100
	9	9.00 ± 0.00	0.90 ± 0.00	0.90 ± 0.00	0.00 ± 0.00	100.0	100.0	100
Chloroform	3	6.03 ± 0.60	3.05 ± 0.04	2.99 ± 0.03	2.55 ± 0.03	44.42	50.41	57.71
	6	7.58 ± 0.04	5.00 ± 0.06	5.00 ± 0.01	4.50 ± 0.04	34.04	34.04	40.63
	9	9.00 ± 0.00	9.00 ± 0.00	7.03 ± 0.00	6.73 ± 0.01	0.00	21.89	25.22
Acetone	3	6.03 ± 0.06	4.02 ± 0.11	2.47 ± 0.40	0.00 ± 0.00	33.33	95.03	100
	6	7.58 ± 0.04	6.06 ± 0.03	4.00 ± 0.01	0.00 ± 0.00	20.05	47.23	100
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	0.00 ± 0.00	0.00	00.00	0.00
Ethanol	3	6.03 ± 0.06	4.48 ± 0.02	3.99 ± 0.01	2.91 ± 0.07	25.70	33.83	51.74
	6	7.58 ± 0.04	6.04 ± 0.04	5.55 ± 0.02	4.43 ± 0.05	20.32	26.78	41.56
	9	9.00 ± 0.00	9.00 ± 0.00	7.00 ± 0.10	6.63 ± 0.09	00.00	22.22	26.33
Distilled water	3	6.03 ± 0.06	6.07 ± 0.07	6.00 ± 0.01	5.95 ± 0.04	00.50	00.50	1.33
	6	7.58 ± 0.04	7.55 ± 0.03	7.52 ± 0.10	7.50 ± 0.02	00.00	00.79	1.05
	9	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	00.00	00.00	0.00

L.S.D at 5% Treat. 1.2 Conc. 0.6 Time 0.6 Interactions 1.2

Maximum area of fungi in Petri dishes was 9 cm.

marjoram extracts. In contrast, the lowest inhibition rates were recorded by caraway, fenugreek and ginger extracts. These results were also, in agreement with those registered by Farag (1990); Amvam *et al.* (1998) and Montes and Carvajal (1998) who found that essential oils of clove and thyme caused a strong antifungal activity against *A. flavus*. On the other hand, Tantaoui and Baraoud (1994) stated that thyme was able to stop growth of *Aspergillus* sp. at 1% concentration. Also, Ali and Abo El-Enain (2005) found that fenugreek extracts in petroleum ether, hexane, acetone, ethanol and chloroform were less effective on growth of *Ascosphaera apis* than clove, marjoram or thyme extracts.

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