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A STUDY ON THE USE OF SOME NATURAL EXTRACTS AS ANTI- FUNGI TO PROLONG THE CONSERVATION OF WHEAT AND ITS PRODUCTS.

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ABSTRACT

Different extracts of carrot seeds, leaves, and roots were evaluated for their effectiveness inhibition on some groups of microorganism fungal growth and reducing the production of aflatoxins. Ethanol, Chloroform, and water showed effects against some groups of microorganisms which contaminated wheat products. Water and chloroform extracts of yellow carrot leaves showed the most effect against feacal group. Water, ethanol and chloroform extracts from carrot seeds, leaves, and root reduced the fungal growth rate of *Penicillum funiculosum, Fusarium compactum, fusarium chlamydosporum, Fusarium monilliforme, fusarium roseum, Aspergillas niger, Aspergillus fumagatus, Fusarium solani*. No aflatoxin was produced by fungi in wheat samples treated by extracts of carrot seeds, herbs, and roots.

Keywords: Carrots,fungal growth, preservation and aflatoxins

INTRODUCTION

Wheat is the world's most important food grain. In general, wheat is mainly consumed in bakery products manufactured from endosperm flour, which forms 83% of the kernel, and has unique technological properties for creating superior, consumer appealing product quality in terms of flavor and texture. (Jacobs *et al*., 1998, Liu *et al*., 2000, Pereira *et al*., 2002). Dietary fiber and other functional food products with specific physiological functions of health, which are concentrated in the outer layers of the grains (bran), and forms 14% of kernel. However, additions of cereal bran, especially in such amounts that health benefits can be expected, cause several problems in the flavor and texture. In addition, bran fraction is rich in spoilage organisms such as rope forming bacteria *Bacillus subtilis* which increases the challenges to retain microbiological safety of wheat breads supplemented with bran.

It is estimated that between 5 and 10% of the world’s food production is lost due to fungal deterioration.

In Western Europe, mould spoilage of bread alone is estimated to cause than £200 million (Legan, 1993).

Aflatoxins, a group of naturally occurring toxins produced by the molds *Aspergillus flavus* and *Aspergillus parasiticus*, sometimes contaminate a variety of foods and feeds. Several investigators have demonstrated that Aflatoxin are acutely toxic, carcinogenic, teratogenic and mutagenic. Aflatoxin B1 (AF B1) is the most toxic of the group and appears naturally in the largest quantities (Applebaum and Marth, 1982).Toxins that could be produced by some members of the genus fusarium such as F. verticillioides, are toxic

substances to human and animals (Chen and Zhang, 1987, Gelderblom *et al*., 1998).

It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Mitscher *et al*., 1987). Also, the resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required. Extracts from many of plant species have been screened for the presence of antimicrobial substances that can be used in controlling plant diseases. Medicinal and some indigenous plants are important sources of biologically active compounds against phytophathogenic fungi and bacteria as well as plant diseases (Wiader and Lamer, 1996, EL-Kazzaz *et al*., 2003).

Some investigators tried to decrease the production of mycotoxins by introducing plant extracts or essential oils to the fungal growing media or the food commodity. Growth of A. flavus and aflatoxin production was also inhibited by citrus peel extracts (Abdalla *et al*., 2001). Chamomile, cinnamon and pepper have the potential to decrease the growth of fungi as well as formation and accumulation of mycotoxins. (Abdelhamid *et al*., 1995). Chloroform extract of carrot root tissue inhibited Aflatoxin production by *A. parasiticus* but did not affect the mycelia weight of culture. The inhibitory activity was PH dependent and inhibition was lost when the initial pH was 6.0 or higher. (Batt *et al*., 1980). Purified ethanolic extracts of peeled and shredded carrots showed an antimicrobial effect against a range of food-borne, micro-organisms. (Babic *et al*., 1994).

Cultivars of carrot differ in their essential oil contents and leaves have higher amount of essential oils than the roots. (Senalik and Simoin, 1987; Kainulainen *et al*., 1998, 2002 ;Habegger and Schnitzler, 2000). The principal compounds in the leaves were β-myrcene, α-asarone, methyl isoeugenol, β-caryophyllene, (E)-β-farnesene, limonene and sabinene. While, in roots, the main compounds were α-pinene, sabinene, β- pinene, β-myrcene, limonene, total monoterpenes and (E)- β-farnesene.

This study was done in the laboratory to evaluate the efficiency of two cultivated (red and yellow) carrot seed, leaves, and root extracts against some plant pathogenic fungi. The possibility of reducing the production of aflatoxin and fumonisin by the most effective extracts was also studied.

MATERIALS AND METHODS

The present work was carried out in the Regional Center for Food and Feed and Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

- Wheat variety Giza 168 was obtained from Wheat Research Department, Field Crops Research Institute, ARC. Giza. Egypt.
- Two varieties of fresh carrot (red and yellow) and yellow carrot seeds were purchased from local market.

1. Preparation of carrot leaves, root and seeds powders:

Carrot leaves and root powders were washed and chopped fairly thinly. The materials were dried in cabinet dryer at 60 - 65º C temperature 80 m /h air flow rate for 24 h. After drying the pieces of leaves and root and seeds were ground separately using a Maxy Hermetic Mill Grinder, patent N: 53985 B, Italy.

2- Preparation of carrot leaves, roots and seeds extracts:

Water, ethyl alcohol (96% V/V) and chloroform was used to extract the antimicrobial compounds in red or yellow carrot leaves, roots and seeds. The extracts were prepared according to the method given by Ashour (1989) as follows: 50 gm plants or seeds powder were mixed with 500 ml solvent, water only was used hot (90°C). Mixtures were kept in dark at room temperature for four days intermittent stirring (3-4 times for 5 min. daily) and filtered through whatman No. 40 filter paper. Extracts were evaporated to dryness (by rotary evaporator) to determine the weight of the extracted material before transferred to 3% extract (3 gm of extracted material) in 100 ml measuring flask using the same solvent. The extracts were kept in refrigerator at 4-7°C.

3- Preparation of wheat samples :

1-Wheat was sampled into sixteen samples (500gm).

2-Wheat samples conditioning: in this process water is added to temper wheat samples to 15% moisture, and allowed to stand for up to 24 hours in closed cans to secure maximum toughening of the bran with optimum mellowing of the starchy endosperm. The quantity of added water was calculated from the follow equation:

Added water %= Wheat sample weight X(15-% wheat sample moisture/100–14)

3- Wheat samples milling: wheat samples were milled in a standard Brabender Duisburg laboratory mill, Type 279002, Germany, Break and reduction flours were collected and sieved into (flour, fine bran, coarse bran, brown shorts and white bran).

4-Chemical analysis:

Wheat and its mill product samples were analyzed for moisture, ether extract, ash, protein and crude fiber according to the methods described in the A.O.A.C. (1995). Carbohydrates were determined by difference.

5-Bacteriological experiments:

The obtained extracts were evaluated for their efficiency in reducing the microbial load and growth of molds under study in wheat and its mill products (whole grain, flour, fine bran, coarse bran, brown shorts and white shorts). Wheat was divided into sixteen samples treated with different extracts as the follows:

1. Three of wheat samples were treated with a known volume 5ml (each 1 ml give the required concentration 0.03/100 gm wheat) of ethanol extracts of yellow carrot (leaves, roots or seeds) .
2. Two of wheat samples were treated with 5 ml of ethanol extracts of red carrot leaves or roots.
3. Three of wheat samples were treated with 5 ml of chloroform extracts of yellow carrot (leaves, roots or seeds).
4. Two of wheat samples were treated with 5 ml of chloroform extracts of red carrot (leaves or roots).

5. Two of wheat samples were treated with 5 ml of water extracts of yellow carrot (leaves or roots).
6. Two of wheat samples were treated with 5 ml of water extracts of red carrot (leaves or roots).
7. One samples was treated with 0.03% sodium propionate (as a positive control)
8. The last sample was treated with water only (as a negative control).

All extracts were added to water, which was used during wheat samples conditioning, then, the samples were milled. Wheat and its products samples were analyzed for its moisture contents poured into bottles at room temperature for bacteriological analysis after months and 6 months.

Table (1): Average of daily temperature and specific humidity during the experiment months

Parameters \ Months	April	May	June	July	August	September	October
Average of daily temperature C^o	25.3	26.7	28.5	29.4	30.4	29.0	24.1
Average of specific humidity	43.3	48.0	54.0	63.0	61.0	60.0	65.0

6- Bacteriological evaluation:

Appropriate dilutions prepared from each sample were used for inoculating different nutrient and selective media. The microbial determinations were applied as follows:

A) Total aerobic viable counts:

Aerobic bacterial counts were estimated on glucose yeast extract nutrient agar medium as the method reported by (APHA, 1990) using pouring plate technique. Suitable plates were counted after incubation at 37C^o for 48 hours.

B) Coliform and faecal coliform counts

Coliform and faecal coliform counts were estimated on MacConkey ager (APHA, 1990) using pouring plate technique. Suitable plates were counted after 24 hours at 37C^o and 44.5C^o for total coliform and faecal coliform counts, respectively.

C) Determination of total counts of fungi

Total counts of fungi were determined on potato dextrose agar days and the counts of fungi (cfu /g) were determined as described in American Public Health, Association (1981) and Oxoid Manual (2000). Isolation and identification of fungi.

Developed colonies on PDA medium were transferred to PDA slants and purified using the single spore technique (Hansen, 1926) and / or hyphal tip technique (Riker and Riker, 1936). Purified isolates were identified according to their morphological and microscopical characters and confirmed by Dept., Plant Pathology institute ARC, Egypt.

D) Mycotoxins analysis:

All standards of mycotoxins were purchased from Sigma company, U.S.A. All chemicals and solvents used were of ACS grade. Thin layer chromatography (TLC) was performed using 20 × 20 cm TLC aluminum plates recoated with 0.25 mm silica gel 60 (Merck). Aflatoxins were extracted

by B.F. method as described in AOAC (1998). Extracts were dissolved in 200 μL chloroform and vortexed, then 20 μL aliquot and 10 μL of the standards were spotted on TLC plates and developed in dark room with ethyl ether: methanol: water (96: 3:1). After drying, the spots were examined with U.V at a wave length of 365 nm (AOAC 1998).

RESULTS AND DISCUSSION

***Chemical composition of raw materials:**

The chemical composition of whole grain, flour, fine bran, coarse bran, brown shorts and white shorts was studied and the obtained results are shown in Table (1). It could be noticed that moisture, protein, fat, crude fiber and ash contents of fine bran, coarse bran, brown shorts and white shorts were higher than wheat flour. White shorts has the highest percent of moisture and protein, while wheat flour has highest percent of total carbohydrates. Protein content increased in the out layers of wheat grain. Coarse bran has the highest percent of crude fiber (11.4%), follows by fine bran (8.5%) and brown shorts (5.5%). These results are in agreement with those reported by(Faheid and Hegazy,1991).

The moisture contents of the wheat products after mill processing are presented in Table (3).

From the results in Table (3) it be noticed that coarser bran samples had the highest amount of moisture contents, while wheat flour samples had the lowest amount of moisture contents.

Total aerobic plate counts (T.A.C) total Coliforms counts (T.C.C), Fecal coliforms counts (F.C.C), Molds counts, total aflatoxins levels and isolated fungi of some wheat products were determined as seen in Table (4). The highest number of isolated fungi was obtained from most wheat products which were averaged between 2×10^{-4} to 7×10^{-3}(CFU/g).

Table (2): Chemical composition of whole grain, flour, fine bran, coarse bran, brown shorts and white shorts.

Samples	Moisture %	As a dry basis				
		Protein %	Fat %	Crude fiber%	Ash %	Total carbohydrates%
Whole grain of wheat	10.5	15.5	1.3	2.4	1.8	79.08
Wheat flour	12.5	10.8	0.35	0.43	0.55	87.87
Fine bran	13.0	15.1	3.2	8.5	3.9	69.3
Coarse bran	13.0	13.7	4.0	11.4	4.7	66.2
Brown shorts	14.0	14.8	4.5	5.5	3.8	71.4
White shorts	14.5	17.5	3.7	2.1	2.7	74.0

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Table (3): Moisture contents of wheat products after mill processing.

Treatments		Type of extract	Moisture content of Wheat products %				
			Red shorts	White shorts	Flour	Fin bran	Courser bran
Red carrot	Herb	Water extract	13.8	13.3	12.8	14.8	15.1
		Ethanol extract	13.8	13.4	12.8	14.7	15.0
		Chloroform extract	14.0	13.6	13.1	14.8	15.0
	Root	Water extract	13.8	13.5	12.9	14.6	14.8
		Ethanol extract	13.5	13.1	12.5	14.3	14.4
		Chloroform extract	14.3	13.9	13.1	15.0	15.3
Yellow carrot	Herb	Water extract	13.9	13.1	12.6	14.7	15.1
		Ethanol extract	13.9	13.6	13.3	14.7	15.1
		Chloroform extract	14.4	13.7	13,4	15.3	15.3
	Root	Water extract	14.4	13.7	13.2	15.0	15.0
		Ethanol extract	14.3	13.7	13.2	15.2	15.8
		Chloroform extract	14.8	13.6	13.0	15.7	18.1
		Ethanol extract	14.4	14.0	13.8	16.3	17.2
		Chloroform extract	14.4	13.5	13.0	15.5	16.5
Sodium propionate			14.9	14.5	13.6	15.8	16.5
Water			13.6	13.3	13.3	14.7	15.3

Table (4): Initial total Aerobic plate counts, total coliform, fecal coliform, molds counts, total aflatoxins and Isolated fungi of examined, wheat and wheat product.

Sample	TAC (CFU g^{-1})	TCC (CFU g^{-1})	FCC (CFU g^{-1})	Mold (CFU g^{-1})	Isolated fungi	Total Aflatoxin content (µg / Kg)
Whole grain of wheat	14×10^{-1}	13×10^{-1}	N.D	7×10^{-3}	*Penicillum funiculosum* *fusarium compactum0* *fusarium chlamydosporum*	0.0
Wheat flour	6×10^{-3}	13×10^{-3}	N.D	8×10^{-4}	*Aspergillus Niger* *Aspergillus Aculeatus* *Aspergillus flavus* *Penicillium digitatum*	0.0
Brown shorts	7×10^{-2}	15×10^{-1}	N.D	2×10^{-4}	*Fusarium pallidoroseum* *Penicillium digitatusm*	0.0
White shorts	5×10^{-2}	25×10^{-3}	N.D	2×10^{-4}	*Fusarium pallidoroseum* *Penicillium digitatusm*	0.0
Fine bran	50×10^{-2}	11×10^{-2}	N.D	3×10^{-3}	*Aspergillus niger* *Fusarium rosum* *Fusarium solani*	0.0
Coarse bran	10×10^{-1}	13×10^{-2}	35×10^{-2}	4×10^{-3}	*Fusarium monilliforme* *Fusarium rosum* *Aspergillus fumagatus* *Ochraceus sp.*	0.0

(*CFU)= Cell forming unit.

Isolated fungi were identified as *Penicillum funiculosum*, *Fusarium compactum, Fusarium chlomydosporum, Fusarium monilliforme, Fusarium rosum, Aspergillus fumagatus, Ochraceus sp., Aspergillus niger, Aspergillus*

flavus, Penicillium digitatum, Fusarium solani, and *Fusarium pallidoroseum.* All investigated samples were free of aflatoxins as seem in the same Table(4).

The microbial load of the wheat products samples indicated that the T.A.C were ranged from $6\times10^{-3}$to 10×10^{-1}CFU/g, while the F.C.C were 0.0 CFU/g in all samples except it was 35×10^{-2} CFU/g in coarser bran sample, and T.C.C levels were ranged from 13×10^{-3} to 15×10^{-1} CFU/g in the same samples.

The initial total Aerobic plate 60 mts, total coliform, fecal coliform, molds, total aflatoxins and isolated fungi of examined carrots products were shown in Table (5) cont.

Table (5): Initial total Aerobic plate counts, total coliform, fecal coliform, mold, total aflatoxins and isolated fungi of examined carrots product.

Sample	ATC (CFU g^{-1})	TCC (CFU g^{-1})	FCC (CFU g^{-1})	Mold (CFU g^{-1})	Isolated fungi	Total Aflatoxin content (µg / Kg)
Yellow carrot roots	83×10^{-4}	15×10^{-4}	N.D	2×10^{-3}	*Alternaria Sp. Alternaria teruis sp*	0.0
Red carrot roots	83×10^{-4}	15×10^{-4}	N.D	2×10-3	*Alternaria Sp. Alternaria tenuis Sp.*	0.0
Yellow carrot seeds	6×10^{-2}	3×10^{-2}	N.D	1×10^{-2}	*Aspergillus sydowii Alternaria Sp. Alternaria tenuis Sp.*	0.0
Yellow carrot leaves	175×10^{-4}	190×10^{-4}	N.D	2×10^{-2}	*Chlamydos porum Alternaria Sp. Aspergillus sydowii*	0.0
Red carrot leaves	166×10^{-4}	174×10^{-4}	N.D	5×10^{-2}	*Chlamydos porum Alternaria Sp. Aspergillus sydowii*	0.0

A.T.C = total aerobic counts. **T.C.C= total coliforms counts.**
F.C.C= fecal coliforms counts. **C.F.U= cell forming unit.**

Table (6,7 and 8) showed that the effect of water, ethanol and chloroform extracted of red and yellow carrot (leaves, roots or seeds) on the microbial load, fungal growth and its aflatoxin production for some wheat products after 1 month of storage.

The obtained data indicated that the addition of water extracted of red carrot roots to wheat products samples was more effective for reducing the molds counts, which recorded numbers of 1×10^{-1} CFU/g. Molds counts were 0.0 CFU / g for fine and course bran by using water extracted carrot leaves. The counts of molds in wheat products were ranged from 1×10^{-1} to 2×10^{3}, 1×102 to 9×10^{3}, and 1×10^{2} to 1×10^{3} for the treatments with ethanol extracted of red carrot root, yellow carrot root, and yellow carrot seeds, respectively. The given data showed that the best treatment was achieved by using chloroform extracted yellow carrot seeds with fine bran which recorded 0.0 CFU/g.

The obtained data indicated that the addition of ethanol extracted of red carrot herbs was more effective for reducing the total aerobic bacteria which recorded the number of 16×10^2 to 10×10^1 CFU / g, while it was 4×10^2 to 55×10^3 CFU/g, with the treatment of yellow and red carrot root. Suing yellow carrot herbs did not showed any effective for the numbers of total aerobic bacteria. Treatments with water as sodium propionate had no effect too.

Total coliform counts were determined in all wheat products samples treated by the extracted carrot products samples treated by the extracted carrot products. Data showed that the addition of all extracted yellow and red carrot herbs was more effective for reducing numbers of total coliform, which recorded 0.0 CFU / g in the most type of samples. Some results were achieved by using of all extracted of carrot roots. Extracted carrot seeds had no effective for the numbers of total coliforms, while treatment with sodium propionate showed less effective for total coliform counts which recorded rang of 7×10^1 to 20×10^1 FCU/ g. It was ranged from 17×10^2 to 25×10^2, in case of treatment with water only.

Table (6): Effect of water extracted of red and yellow carrot (leaves and roots) on the microbial load and fungal growth on some wheat products after 1 month of storage.

Treatments		Microbial count	Wheat products				
			Red shorts	White shorts	Flour	Fin bran	Courser bran
Red carrot	Leaves	T.A.C.	30×10^{-3}	45×10^{-3}	80×10^{-3}	N.D	N.D
		T.C.C.	16×10^{-2}	N.D	4×10^{-1}	N.D	N.D
		F.C.C.	5×10^{-1}	N.D	N.D	N.D.	N.D.
		Mold	1×10^{-3}	3×10^{-3}	6×10^{-1}	1×10^{-1}	N.D
	Root	T.A.C.	4×10^{-2}	14×10^{-2}	37×10^{-2}	32×10^{-3}	17×10^{-3}
		T.C.C.	2×10^{-2}	10×10^{-2}	18×10^{-1}	22×10^{-1}	15×10^{-2}
		F.C.C.	5×10^{-1}	9×10^{-1}	7×10^{-1}	9×10^{-1}	11×10^{-1}
		Mold	4×10^{-1}	1×10^{-1}	1×10^{-1}	2×10^{-1}	2×10^{-1}
Yellow carrot	Leaves	T.A.C.	75×10^{-3}	30×10^{-3}	65×10^{-2}	97×10^{-3}	75×10^{-3}
		T.C.C.	N.D	N.D	4×10^{-1}	N.D	3×10^{-1}
		F.C.C.	N.D	N.D	N.D	N.D	N.D
		Mold	1×10^{-3}	1×10^{-2}	2×10^{-2}	1×10^{-2}	1×10^{-1}
	Root	T.A.C.	35×10^{-3}	11×10^{-3}	8×10^{-3}	35×10^{-2}	55×10^{-3}
		T.C.C.	15×10^{-2}	17×10^{-2}	25×10^{-2}	27×10^{-2}	24×10^{-3}
		F.C.C.	16×10^{-2}	10×10^{-2}	11×10^{-2}	10×10^{-2}	12×10^{-3}
		Mold	1×10^{-3}	1×10^{-2}	2×10^{-2}	1×10^{-2}	1×10^{-1}
Sodium propionate		T.A.C.	37×10^{-3}	35×10^{-2}	30×10^{-1}	25×10^{-1}	9×10^{-2}
		T.C.C.	N.D.	7×10^{-1}	20×10^{-1}	7×10^{-1}	N.D.
		F.C.C.	N.D.	N.D.	N.D.	N.D.	N.D.
		Mold	5×10^{-2}	1×10^{-3}	2×10^{-2}	1×10^{-1}	1×10^{-1}
Water		T.A.C.	24×10^{-3}	38×10^{-3}	27×10^{-3}	60×10^{-3}	85×10^{-3}
		T.C.C.	17×10^{-2}	22×10^{-2}	18×10^{-2}	20×10^{-3}	25×10^{-2}
		F.C.C.	12×10^{-2}	9×10^{-2}	9×10^{-2}	15×10^{-3}	16×10^{-2}
		Mold	6×10^{-1}	8×10^{-1}	3×10^{-2}	1×10^{-2}	2×10^{-1}

A.T.C = total aerobic counts. T.C.C= total coliforms counts.
F.C.C= fecal coliforms counts.

Table (7): Effect of Ethanol extracted of red and yellow carrot (leaves, roots and seeds) on the microbial and fungal growth on some wheat products after 1 month of storage.

Treatments		Microbial count	Wheat products				
			Red shorts	White shorts	Flour	Fin bran	Courser bran
Red carrot	Leaves	T.A.C.	22×10^{-2}	10×10^{-1}	18×10^{-1}	45×10^{-1}	11×10^{-2}
		T.C.C.	N.D.	N.D	N.D.	N.D	2×10^{-2}
		F.C.C.	N.D.	N.D	N.D	N.D.	N.D.
		Mold	2×10^{-2}	2×10^{-3}	1×10^{-1}	3×10^{-1}	4×10^{-2}
	Root	T.A.C.	3×10^{-1}	7×10^{-3}	9×10^{-2}	7×10^{-3}	6×10^{-3}
		T.C.C.	N.D.	N.D.	3×10^{-1}	N.D.	N.D.
		F.C.C.	N.D.	N.D	N.D.	N.D.	N.D.
		Mold	2×10^{-2}	12×10^{-3}	1×10^{-3}	1×10^{-2}	1×10^{-2}
Yellow carrot	Leaves	T.A.C.	12×10^{-2}	3×10^{-2}	11×10^{-2}	11×10^{-2}	19×10^{-2}
		T.C.C.	N.D	N.D	N.D.	N.D	10×10^{-1}
		F.C.C.	N.D	N.D	N.D	N.D	N.D
		Mold	2×10^{-2}	3×10	14×10^{-3}	1×10^{-3}	1×10^{-3}
	Root	T.A.C.	3×10^{-2}	23×10^{-3}	3×10^{-2}	28×10^{-2}	21×10^{-2}
		T.C.C.	1×10^{-2}	N.D.	1×10^{-2}	N.D.	N.D.
		F.C.C.	N.D.	N.D.	4×10^{-1}	N.D.	N.D.
		Mold	7×10^{-2}	8×10^{-2}	4×10^{-2}	1×10^{-2}	4×10^{-2}
	Seed	T.A.C.	5×10^{-2}	3×10^{-3}	8×10^{-2}	3×10^{-3}	9×10^{-3}
		T.C.C.	N.D.	N.D.	N.D.	N.D.	N.D.
		F.C.C.	N.D.	N.D.	N.D.	N.D.	N.D.
		Mold	1×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-2}	1×10^{-3}
Sodium propionate		T.A.C.	37×10^{-3}	35×10^{-2}	30×10^{-1}	25×10^{-1}	9×10^{-2}
		T.C.C.	N.D.	7×10^{-1}	20×10^{-1}	7×10^{-1}	N.D.
		F.C.C.	N.D.	N.D.	N.D.	N.D.	N.D.
		Mold	5×10^{-2}	1×10^{-3}	2×10^{-2}	1×10^{-1}	1×10^{-1}
Water		T.A.C.	24×10^{-3}	38×10^{-3}	27×10^{-3}	60×10^{-3}	85×10^{-3}
		T.C.C.	17×10^{-2}	22×10^{-2}	18×10^{-2}	20×10^{-3}	25×10^{-2}
		F.C.C.	12×10^{-2}	9×10^{-2}	9×10^{-2}	15×10^{-3}	16×10^{-2}
		Mold	6×10^{-1}	8×10^{-1}	3×10^{-2}	1×10^{-2}	2×10^{-1}

A.T.C = total aerobic counts. **T.C.C= total coliforms counts.**
F.C.C= fecal coliforms counts. **C.F.U= cell forming unit**

On the other hand, fecal coliforms counts were effected by addition of ethanol and chloroforms extracted carrots (root and herb), compared with extracted water which caused more contamination (5×10^{1} to 12×10^{3} CFU/g).

The addition of water to wheat products showed more counts of fecal coliforms which recorded 9×10^{-2} to 15×10^{-3} CFU/g.

The effect of water, ethanol and chloroform extracted of red and yellow carrot (herbs and roots) on the microbial load of wheat products samples compared with that treated by water or sodium propionate as seen in the same table.

Regarding the addition of carrots extracts, data indicated that the effects on the numbers of total aerobic bacteria was achieved as a result of inhibition effect of antimicrobial compounds of carrot. The obtained data are similar with that mentioned by (Babic *et al* 1994), who reported that the antimicrobial compounds of carrot play a major role in the resistance to microbial load. An antimicrobial activity was detected in purified carrot extract. Phenolic compounds, falacarindiol, free saturated fatly acid (dedecanoic acid) and methyl esters of satured fatty acids (of dedecanoic and pentadecanoic acids).

Table (8): Effect of chloroform extracted of red and yellow carrot (leaves, roots and seeds) on the microbial load and fungal growth on some wheat products after 1 month of storage.

Treatments		Microbial count	Wheat products				
			Red shorts	White shorts	Flour	Fin bran	Courser bran
Red carrot	Leaves	T.A.C.	10×10^{-2}	10×10^{-1}	75×10^{-1}	30×10^{-1}	16×10^{-1}
		T.C.C.	2×10^{-1}	2×10^{-1}	19×10^{-1}	7×10^{-1}	N.D.
		F.C.C.	N.D.	N.D.	9×10^{-1}	N.D.	N.D.
		Mold	1×10^{-2}	4×10^{-1}	1×10^{-2}	1×10^{-1}	1×10^{-3}
	Root	T.A.C.	33×10^{-3}	16×10^{-3}	17×10^{-2}	24×10^{-2}	16×10^{-3}
		T.C.C.	N.D	N.D	4×10	N.D	10×10^{-2}
		F.C.C.	N.D	N.D	N.D	N.D	2×10^{-2}
		Mold	5×10^{-1}	3×10^{-2}	2×10^{-3}	2×10^{-2}	1×10^{-1}
Yellow carrot	Leaves	T.A.C.	12×10^{-2}	90×10^{-3}	35×10^{-3}	1×10^{-1}	5×10^{-2}
		T.C.C.	32×10^{-3}	25×10^{-3}	4×10^{-3}	N.D	N.D
		F.C.C.	5×10^{-3}	N.D	N.D	N.D	N.D
		Mold	25×10^{-3}	1×10^{-2}	1×10^{-3}	6×10^{-2}	1×10^{-3}
	Root	T.A.C.	7×10^{-2}	35×10^{-1}	4×10^{-3}	25×10^{-2}	26×10^{-2}
		T.C.C.	N.D	N.D	5×10^{-1}	N.D	N.D
		F.C.C.	N.D	N.D	N.D	N.D	N.D
		Mold	6×10^{-2}	2×10^{-2}	1×10^{-2}	2×10^{-1}	2×10^{-1}
	Seed	T.A.C.	80×10^{-1}	90×10^{-3}	43×10^{-1}	45×10^{-1}	15×10^{-2}
		T.C.C.	52×10^{-1}	75×10^{-2}	23×10^{-1}	3×10^{-1}	N.D
		F.C.C.	3×10^{-1}	70×10^{-1}	16×10^{-1}	N.D	N.D
		Mold	2×10^{-3}	1×10^{-3}	6×10^{-3}	N.D	2×10^{-2}
Sodium propionate		T.A.C.	37×10^{-3}	35×10^{-2}	30×10^{-1}	25×10^{-1}	9×10^{-2}
		T.C.C.	N.D.	7×10^{-1}	20×10^{-1}	7×10^{-1}	N.D.
		F.C.C.	N.D.	N.D.	N.D.	N.D.	N.D.
		Mold	5×10^{-2}	1×10^{-3}	2×10^{-2}	1×10^{-1}	1×10^{-1}
Water		T.A.C.	24×10^{-3}	38×10^{-3}	27×10^{-3}	60×10^{-3}	85×10^{-3}
		T.C.C.	17×10^{-2}	22×10^{-2}	18×10^{-2}	20×10^{-3}	25×10^{-2}
		F.C.C.	12×10^{-2}	9×10^{-2}	9×10^{-2}	15×10^{-3}	16×10^{-2}
		Mold	6×10^{-1}	8×10^{-1}	3×10^{-2}	1×10^{-2}	2×10^{-1}

A.T.C = total aerobic counts. **T.C.C= total coliforms counts.**
F.C.C= fecal coliforms counts. **C.F.U= cell forming unit**

These compounds exhibited an inhibitory effect on several food- borne bacteria (Eklund, 1985) and (Moir and Eyles 1992).

(Kurosaki and Nishi ,1983), Showed that 6- methoxymellein had an inhibitory effect against a range of food- borne microorganisms.

The carrot extracts was more active against bacteria than against the yeast, and was more active against gram positive than against gram negative bacteria (Kabara, 1987).

Our results were in agreement with (Batt *et al.,* 1980) who investigated that the chloroform extracted carrot roots was no inhibition of fungi. On contrary, Batt *et al.* 1980 did not observe any delay in fungi growth when various levels of chloroform extract from carrot root were added.

(Alderman and Morth,1976) reported that carrot extracted inhibited both fungi growth and aflatoxin production.

As shown in Table(9) that the water extract of the seeds of the yellow carrot effective in reducing both the TAC, TCC, FCC in all wheat products during storage for 7 months. the treatment by alcohol extract of the seeds of the Yellow carrot as well as led to reduce the FCC in all wheat products to zero. in all products It is noted that most of the extracts had a strong impact on reducing the proportion of FCC except alcohol and aqueous extract of the roots of the yellow carrot.

Also notes that most of the extracts had a strong impact on reducing the proportion of TCC except chloroform extract of the red carrot leaves and aqueous extract of the yellow and red roots and sodium propionate.

While the rest of the extracts effects given appropriate and effective to cut all TAC, TCC, FCC through the period of storage for 7 months in most wheat products .

As shown in Table 10, the strongest effect on the fungi for treatment of water extract of to the seeds of Yellow carrot.

As shown in Table (10) free of all treated wheat products from mycotoxins during storage for 7 months. Despite the isolation of certain fungi producing the mycotoxins Such as a *Aspergillus flavus*. It is noted that Aspergillus is more presence of fungi during the storage period. Also, the chloroform extract of red and yellow carrot products was with a clear impact on the growth of fungi during storage period as well as sodium propionate.

Table (10): Effect of water, ethanol, and chloroform extracted of red and yellow carrot (leaves, roots or seeds) on the fungal growth and its aflatoxin production in some wheat products after 7 months of storage.

Treatments		Type of extract	Flour	Brown shorts	White shorts	Fine bran	Coarse bran	Isolated fungi	Aflatoxin content Mg/Kg
Red carrot	leave	Water extract	11×10^{-2}	6×10^{-1}	8×10^{-1}	7×10^{-3}	10×10^{-3}	*Aspergillus aculeatus&Penicillium digitatum*	0.0
		Ethanol extract	9×10^{-2}	4×10^{-1}	6×10^{-1}	6×10^{-3}	8×10^{-2}	*Aspergillus niger*	0.0
		Chloroform extract	8×10^{-3}	5×10^{-1}	12×10^{-2}	1×10^{-1}	5×10^{-2}	*Aspergillus spp.*	0.0
	Root	Water extract	2×10^{-1}	2×10^{-1}	1×10^{-1}	1×10^{-1}	4×10^{-1}	*Aspergillus aculeatus&Penicillium digitatum*	0.0
		Ethanol extract	4×10^{-2}	3×10^{-2}	6×10^{-2}	2×10^{-2}	3×10^{-2}	*Aspergillus niger & Penicillium digitatum*	0.0
		Chloroform extract	3×10^{-1}	3×10^{-2}	6×10^{-3}	8×10^{-2}	5×10^{-1}	*Aspergillus niger &Aspergillus versicolor*	0.0
Yellow carrot	leave	Water extract	6×10^{-1}	7×10^{-2}	9×10^{-2}	3×10^{-2}	3×10^{-2}	*Aspergillus sydowii Alternaria SPP.*	0.0
		Ethanol extract	8×10^{-2}	14×10^{-3}	33×10^{-3}	7×10	6×10^{-2}	*Aspergillus aculeatus& Aspergillus flavus Aspergillus niger &Penicillium digitatum*	0.0
		Chloroform extract	12×10^{-3}	9×10^{-2}	13×10^{-3}	11×10^{-2}	5×10^{-2}	*Aspergillus niger &Aspergillus versicolor*	0.0
	Root	Water extract	3×10^{-1}	2×10^{-2}	6×10^{-2}	4×10^{-2}	15×10^{-3}	*Aspergillus aculeatus&Penicillium digitatum*	0.0
		Ethanol extract	9×10^{-2}	7×10^{-2}	9×10^{-2}	18×10^{-2}	15×10^{-2}	*Aspergillus niger &Aspergillus versicolor*	0.0
		Chloroform extract	5×10^{-1}	3×10^{-1}	3×10^{-2}	4×10^{-2}	13×10^{-2}	*Aspergillus niger &Aspergillus versicolor*	0.0
	Seed	Ethanol extract	11×10^{-3}	4×10^{-2}	3×10^{-2}	3×10^{-3}	4×10^{-3}	*Aspergillus niger &Aspergillus flavus Fusarium verticillioides*	0.0
		Chloroform extract	3×10^{-2}	2×10^{-3}	13×10^{-3}	3×10^{-3}	8×10^{-3}	*Aspergillus flavus &Fusarium verticillioides & Aspergillus spp.*	0.0
Sodium propounate			2×10^{-1}	5×10^{-1}	4×10^{-2}	5×10^{-3}	2×10^{-1}	*Aspergillus niger &Aspergillus versicolor*	0.0
Water			7×10^{-1}	5×10^{-2}	7×10^{-2}	18×10^{-1}	13×10^{-1}	*Aspergillus flavus Aspergillus niger & Penicillium digitatum*	0.0

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دراسة إمكانية الاستفادة من بعض المستخلصات الطبيعية كمضادات للنموات الفطرية لإطالة مدة حفظ القمح و منتجاته.

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تم إستخلاص عروش وجذور وبذور الجزر الأصفر والأحمر بواسطة الماء أو الإيثانول أو الكلورفورم ثم أجريت اختبارات للتعرف على تأثير تلك المستخلصات المختلفة على النمو الفطري وإنتاج الأفلاتوكسينات والحمل الميكروبي في القمح ومنتجاته مثل الدقيق والسن الأحمر والأبيض والردة الخشنة والردة الناعمة.

وقد أظهرت تلك المستخلصات تأثير اختزالي للعدد الفطري وأعداد البكتيريا الهوائية وبكتيريا القولون بنسبة إختلفت من مستخلص لآخر. وقد تمت مقارنة النتائج باستخدام الماء فقط أو الماء المضاف إليه مثبط فطري وهو بروبيونات الصوديوم.

أظهرت النتائج أن أفضل المعاملات الإختزالية كانت باستخدام مستخلصات الإيثانول والكلوروفوروم بالمقارنة باستخدام المستخلص المائي.

من أكثر الفطريات التي تأثرت باستخدام تلك المستخلصات هي فطريات *Penicillum funiculosum*, *Fusarium compactum, Fusarium chlomydosporum, Fusarium monilliforme, Fusarium rosum, Aspergillus fumagatus, Ochraceus sp., Aspergillus niger, Aspergillus flavus, Penicillium digitatum, Fusarium solani*, and *Fusarium pallidoroseum.* البنسيليوم فيونيكلم- فيوزاريوم كومباكتم- فيوزاريوم روزم- اسبرجلس- بنجر- اسبرجلس فيونيوجاتس- اسبرجلس فلافس- فيوزاريوم سولاني- فيوزاريوم مونيليوفورمكذلك لم تستطع الفطريات إفراز أفلاتوكسينات في كل العينات المختبرة.

قام بتحكيم البحث

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Table (9): Effect of water, ethanol, and chloroform extracted of red and yellow carrot (leaves, roots or seeds) on the microbial load in some wheat products after 7 months of storage.

Treatments		Type of extract	T.A.C.					T.C.C.					F.C.C.				
			Flour	Brown shorts	White shorts	Fine bran	Coarse bran	Flour	Brown shorts	White shorts	Fine bran	Coarse bran	Flour	Brown shorts	White shorts	Fine bran	Coarse bran
Red carrot	leave	Water extract	16×10^{-2}	55×10^{-3}	7×10^{-2}	N.D	N.D	8×10^{-1}	6×10^{-1}	N.D.	N.D.	N.D.	N.D.	15×10^{-1}	N.D.	N.D.	N.D.
		Ethanol extract	67×10^{-1}	4×10^{-1}	71×10^{-1}	5×10	33×10^{-2}	N.D.	N.D	N.D.	N.D.	2×10^{-2}.	N.D.	N.D.	N.D.	N.D.	N.D.
		Chloroform extract	10× 10	40×10^{-2}	6×10	16×10	32×10^{-1}	4× 10	32×10^{-1}	21×10^{-1}	11×10^{-1}	3×10^{-3}	N.D.	N.D.	N.D.	N.D.	N.D.
	Root	Water extract	7×10^{-1}	8×10^{-2}	26×10^{-2}	52×10^{-3}	4×10^{-2}	38×10^{-1}	7×10^{-2}	30×10^{-2}	42×10^{-1}	35×10^{-2}	22×10^{-1}	12×10^{-1}	17×10^{-1}	12×10^{-1}	23×10^{-1}
		Ethanol extract	15×10^{-2}	7×10^{-1}	12×10^{-3}	11×10^{-3}	16×10^{-3}	5×10^{-1}	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
		Chloroform extract	33×10^{-2}	7×10^{-2}	35×10^{-3}	4×10^{-1}	31×10^{-3}	9×10^{2}	N.D.	N.D.	N.D.	17×10^{-2}	N.D.	N.D.	N.D.	N.D.	7×10^{-2}
Yellow carrot	leave	Water extract	11×10^{-1}	40×10^{-2}	55×10^{-3}	18×10^{-2}	15×10^{-2}	8×10^{-1}	3×10^{-3}	1×10^{-3}	N.D.	6×10^{-1}	1×10^{-2}	2×10^{-3}	4×10^{-3}	N.D.	N.D.
		Ethanol extract	23×10^{-2}	21×10^{-2}	7×10^{-2}	2×10^{-1}	33×10^{-2}	N.D.	N.D.	N.D.	N.D.	21×10^{-1}	N.D.	N.D.	N.D.	N.D.	N.D.
		Chloroform extract	7×10^{-2}	25×10^{-2}	18×10^{-2}	3×10^{-1}	1×10^{-1}	14×10^{-3}	13×10^{-2}	45×10^{-3}	N.D.	3×10^{-3}	N.D.	12×10^{-3}	N.D.	N.D.	N.D.
	Root	Water extract	16×10^{-3}	72×10^{-3}	21×10^{-3}	8×10^{-1}	11×10^{-2}	35×10^{-2}	33×10^{-2}	32×10^{-2}	5×10^{-1}	44×10^{-3}	21×10^{-2}	24×10^{-2}	21×10^{-2}	18×10^{-2}	2×10^{-2}
		Ethanol extract	6×10^{-2}	20×10^{-1}	3×10^{-2}	48×10^{-2}	42×10^{-2}	3×10^{-2}	4×10^{-2}	N.D.	N.D.	N.D.	7×10^{-1}	65×10^{-1}	21×10^{-2}	23×10^{-2}	18×10^{-3}
		Chloroform extract	9×10^{-3}	16×10^{-2}	65×10^{-1}	55×10^{-2}	52×10^{-2}	15×10^{1}	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D
		Ethanol extract	17×10^{-2}	12×10^{-2}	3×10^{-1}	8×10^{-3}	19×10^{-3}	33×10^{-1}	N.D.	19×10^{-1}	9×10^{-1}	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
		Chloroform extract	83×10^{-1}	15×10	17×10^{-2}	60×10^{-1}	33×10^{-2}	53×10^{-1}	62×10^{-1}	17×10^{-1}	7×10^{-1}	N.D.	24×10^{-1}	5×10^{-1}	88×10^{-1}	N.D.	N.D.
Sodium propionate			54×10^{-1}	71×10^{-3}	63×10^{-2}	44×10^{-1}	17×10^{-2}	35×10^{-1}	23×10^{-2}	15×10^{-1}	18×10^{-1}	3×10^{-4}	N.D.	28×10^{-4}	N.D.	N.D.	N.D.
Water			35×10^{-3}	54×10^{-3}	77×10^{-3}	12×10^{-2}	17×10^{-2}	23×10^{-2}	25×10^{-2}	38×10^{-2}	5×10^{-2}	5×10^{-1}	28×10^{-2}	2×10^{-1}	19×10^{-2}	34×10^{-3}	36×10^{-2}

A.T.C = total aerobic counts.
T.C.C= total coliforms counts.
F.C.C= fecal coliforms counts.