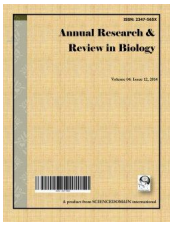

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Oil-bioactive Films as an Antifungal Application to Save Post-harvest Food Crops

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Authors' contributions

This work was carried out in collaboration between all authors. Author MGS designed the study and performed the statistical analysis. While authors AGAR and ANB wrote the protocol and the first draft of the manuscript. Authors AGAR, MGS and ANB managed the analyses of the study. Authors MMH and HAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Agricultural wastes were rich in many components, which may considered as a source of natural and active materials. This study was targeted to apply a non-traditional method to safe grains against toxigenic fungi and its mycotoxins.
Materials and Methods: Three extracts of immature fig fruit (ImFF), fig leaves (FL), and pomegranate husks (POH) were collected, the antimicrobial and antifungal characters of the extracts were evaluated, and it was tested to reduce mycotoxigenic fungi and mycotoxin. The toxicity of these extracts were determined using brine shrimp bioassay. Jojoba oil used as a carrier for concentrate of those extracts.
Results and Discussion: Total phenolic and flavonoid content for the three extracts were varied. Immature fig extract (ImFF) showed the best results either in antimicrobial or in antifungal effect, its

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toxicity on brine shrimp was low, also it has highly ability as aflatoxins reducing in liquid media (52.7%) followed by fig leaves extract (20%). In addition; jojoba have higher Induction period, as ImFF dissolved in jojoba, the oxidative stability of the oil turned to be the highest value comparing to oil with other two extracts. The application of biofilm coat on the soybean grain using ImFF dissolved in jojoba oil recorded as the best way to save the grains against mycotoxigenic fungi and toxin producing.

Conclusion: Agricultural wastes could be one of the novel source for bioactive components, ImFF was the best extract which give the best results as an application in postharvest grain safety operation. The application of oil-extract film was a novel method saving grains against mycotoxins.

Keywords: Agricultural and Mediterranean food wastes; antioxidant aflatoxins; detoxification; jojoba oil.

1. INTRODUCTION

The Mediterranean plants like fig fruit *(Ficus carica),* olive *(Olea europaea) and* pomegranate *(Punica granatum)* have a high level of natural antioxidants such as carotenoids, phenolic, flavonoid, organic acids, vitamin E, and C scavenge free radicals, which can prevent the oxidative stress and related disease (cardiovascular diseases and cancer) [1]. Phenolic compounds are common plant secondary metabolites which have not only physiological functions but also health benefits because they can act as antioxidants. It may assist this purpose by decreasing or donating hydrogen to other compounds, scavenging free radicals, and quenching singlet oxygen [2-4]. Beside that they have anti-carcinogenic, anti-inflammatory, or anti-mutagenic [5,6]. Bioactive components extracted from agro-industrial waste display physiological and functional properties that make them promising ingredients for the functional food industry and for health applications [7-9].

Satish et al. [10] studied the antifungal characters for a group of plant extracts versus toxigenic fungi. Eight different species of *Aspergillus* that isolated from food grains were investigated contra more than fifty aqueous extracts plants. These extracts used in that experiment was varied in its effect on fungal growth from susceptibility in some strain to highly resistance in the others. For those plants just eight out of the sum extracts had positive results on fungal growth reducing with especially best record on mycelia growth decreased, the most susceptible strain was *Aspergillus niger*. Otherwise, in a followed study, a good record for plant extraction was record [11].

Infection by *Aspergillus* sp. and other mycotoxigenic fungi is an iterative process in food stores especially in cereal grains [12,13]. Also, it grows and contaminate many food commodities such as peanut, maize, nut fruits, and milk products [8,14]. Four types of aflatoxins (B_1, B_2, G_1, and G_2) are common to produce by *Aspergillus* fungi [15]. Aflatoxins are exceedingly the highest hazardous health' enemies for human and animal, it can cause mutagenicity and carcinogenesis effects through feed and food contamination [16]. Many food materials have a highly chance to attack by fungal contamination with especially to toxigenic fungi species [17], thus could rise the chance of mycotoxin excretes on food commodities in which aflatoxins group are a harmful part of it [18]. In comparison to other contaminants mycotoxins, particularly aflatoxins, comes as a great source of severity and exposure to chronic diseases foundation, cancers had a great relation to mycotoxin presence in body fluids [19].

Jojoba oil (JOO), the magical waxy material, which had a lot of best characteristics, it consists of a long chain fatty acid (gadoleic C20:1) conjugated to mono chain alcohol with good properties as waxy spread material by ability to be as a carrier for active components [20]. Jojoba oil also had a good antifungal effect on some strains of toxigenic fungi [21]. In many areas of Africa and America, there was a great loss in cereals and grain which reach 25 to 50% of the quantities harvested. Cereals losses were also high since the climate change conditions accelerate the growth of molds, mites, and toxigenic fungi which provide an optimum situation for its development out of the most year times. There is a lack of an authoritative data for the loss in soybean post-harvest due to contaminated level. It is possible to increase the amount of soybeans obtainable for marketing without raising the number of feddan devoted to this cereals. One of the way to reducing soybeans post-harvest losses by improving the

current post-harvest operation. This including reducing the risk of mycotoxins that causing yield losses and harmful health impacts. Another is rising yield by an implementation of a novel application that is able to reduce the risk hazard of food or feed toxicology and contaminants.

Therefore, this study was aimed at evaluating anti-bacterial, anti-fungal, detoxification of toxigenic fungi and antioxidant activities of agricultural wastes, including immature fig fruits (ImFF); fig leaves (FL), and pomegranate husk (POH) extracts. Also it was targeted to apply the concentrated of those extracts after freeze-drying as a biofilm loaded on a jojoba oil to evaluate its ability to reduce both toxigenic fungal growth and retard the aflatoxin production. To our knowledge, the detoxification properties of FL extract, ImFF extract of *Ficus carica* on aflatoxigenic fungi had not been evaluated. This enforces the need for investigating the inhibitory effects and detoxification properties of *Ficus carica* extracts and further identification of the biologically active chemical ingredients.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Agricultural wastes or by-products which included ImFF; FL, and POH were collected from a small pilot plant for jams and juices industrial, located in Borg El Arab area, Alexandria, Egypt. Each material labeled, numbered, a noted with the date of collection, and their localities were recorded.

2.2 Preparation of Plant Extract

One hundred gram of agricultural waste material was dried in hot air oven (45°C), the resultant dried material was collected, grinding, and preserved in sealed bags and stored in refrigerator until used. The content of each bag was extracted using ultrasonic assisted possesses (at 42°C for 45 min) with eco-friendly solvent system [7]. The aqueous isopropyl solution was added at ratio of 5:1 (v/w), pH was adjusted to 5-6.5 [22]. The extraction process was done triple times; finally the gain was collected and centrifuged at 4000 Xg for 20 minutes. The supernatant containing the extract was transferred to a pre-weighed flask and concentrated by rotary evaporator at 50°C. The crude extract was weighed and dissolved in a known volume of dimethyl sulphoxide (DMSO) to obtain a final concentration of 5 mg/1 µl.

2.3 Selection of Bacterial and Fungal Strains

In order to suggest methodologies for screening the natural extracts antimicrobial activity, two different qualitative methods were evaluated as follows: agar diffusion test, employing two different types of reservoirs (filter paper disc impregnated with extracts–test and wells in dishes). Bacteria and fungi strains were prepared and reactivate from a lyophilized media of each strain, bacterial strains are divided to Gram positive strain (*Staphylococcus aureus* and *Bacillus cereus*) and Gram negative strain (*Campylobacter jejuni* and *Salmonella typhi*) cultured on tryptic soy agar; whereas the toxigenic fungal strain under investigation were *Aspergillus flavus, Aspergillus parasiticus, Fusarium solani, and Alternaria sp*., these microorganisms were cultured on potato dextrose agar media.

2.4 Detection of Antimicrobial Activity

2.4.1 Determination of minimal inhibition concentration of extracts

The minimal inhibitory concentration (MIC) can be determined as the lowest concentration at which no growth occurs, it is done as follows: nutrient medium will be prepared and sterilized in universal bottles, each containing 10 mL medium. Different amounts of the tested extract will be added to the broth medium to give the following concentration: 6.25 mg/ml to 100 mg/ml. The microorganism suspension of 50 µl was added to the broth dilutions. These were incubated for 18 hours at 37°C. The MIC value is determined as the lowest concentration of plant extract that did not give any visible bacterial growth. Each assay should be carried out in triplicate. The MIC test will be quantified the antimicrobial activity of plant extract [23].

2.4.2 Determination of minimal fungicidal concentration of extracts

The hypha growth inhibition test can be used to determine the antifungal activity of the plant extract against fungal strains as previously described by Picman [24]. Briefly, dilutions of the test solutions dissolved in vehicle will be added to sterile melted potato dextrose agar (PDA) at 45°C to give final concentrations of 100, 10, 1, 0.8, 0.6, 0.4, 0.2, and 0.1 mg/mL of plants extracts. The resultant solution will be thoroughly mixed and approximately 15 mL will be poured

onto the Petri plate. Sterile discs of 1 mm of fungal mycelium cut from the edge of actively growing colonies will be inoculated in the center of the agar plate and then incubated in a humid chamber at 25°C. Three replicates will be used for each concentration. The concentration required to give 50% inhibition of hyphen growth IC_{50} will be calculated from the regression equation. Nystatin can be used as a positive control.

2.5 Preparation of Standards for Aflatoxin

For Aflatoxin standards received as dry films or crystals to container of dry aflatoxins B_1, B_2, G_1, G_2 add volume of one of the following solvents: acetonitrile, benzene – acetonitrile (98+2), methanol or toluene – acetonitrile (9+ 1) calculated to give a concentration as ng/ml.

2.6 HPLC Chromatography

One hundred microliter of the samples were injected into the HPLC column heated to 40°C. The mobile phase was an acetonitrile: water: acetic acid solution (51:47:2, v/v). The flow rate was 1 ml/min. For fluorescent detection of ochratoxin A the excitation wavelength will 333 nm, and the emission wavelength will 443 nm.

2.7 Detoxification Methods

The extracts from agricultural wastes were prepared by using aqueous isopropyl, the extracted materials were concentrated to near dried then freeze-dried were used to convert it to concentrated materials, 5mg of each extract concentrate was dissolved in 0.5 mL in dimethyl-sulphoxide (DMSO), this value used to inject in the plate wells or in the plate disks as a detoxification application against either pathogenic bacteria or toxigenic fungi.

2.8 Preparation of Extracts Brine Shrimp Lethality Test

Concentrated parts of plants was steeped consecutive in dichloromethane followed by iso-propanol: water (1:1) each for 24 hours, extracts were gains from the materials. The extracts were filtered and solvents removed by a rotary evaporator at a temperature of 45°C. Furthermore, the extracts were dried using freeze dryer to remove any residues of water and stored under -20°C until use. At the test steps, freeze-dried extracts were dissolved in DMSO at different concentrations, a triplicate of 50 ml vials was used to each concentration, the vials were incubated with brine shrimp larvae in a volume of 10 ml [25]. Ten brine shrimp were then placed in each of the triplicate vials as ten eggs for each vial. Otherwise, a mixture of DMSO and sea water was used as a negative treatment (control). Cytoxan (an active material is cytophosphane) which used as drug was used as a positive control in this experiment. After 24h the larval were examined versus a white background, by the magnifying lenses, the average of survived larvae was determined. The mean mortality proportion was incurred versus the logarithm of concentrations and the concentration killing fifty percent of the shrimp larvae (LC_{50}) [26].

2.9 *In-vitro* Evaluation of Anti-radical

2.9.1 DPPH radical scavenging activity

The scavenging activity of samples was estimated according to the procedure described by Shimada [27] with some modifications. Firstly, an aliquot of 1 ml of sample extracts at different concentrations (25, 50, 75, 100 $\mu g.mL^{-1}$) were added to test tubes with 1 ml of 0.078 µM DPPH radical in ethanol. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. The reaction mixture was determined at 515 nm with UV-vis spectrophotometer. Extraction solvent was used as blank while mixture without extract served as control. Ascorbic acid was used as a standard. The scavenging effect was calculated based on the following equation:

$$\text{Scavenging effect (\%)} = [(Abs_{control} - Abs_{sample})]/ (Abs_{control}) \times 100$$

Where, $A_{control}$ = Absorbance of the control solution; A_{sample} = Absorbance of the test extract. IC_{50} value (mg extract/mL) is the inhibition concentration of the test content at which the DPPH radicals were scavenged by 50% and was calculated interpolation from linear regression analysis.

2.9.2 ABTS radical scavenging activity

The free radical-scavenging activity of samples was determined by ABTS radical Cation decolorization assay described by Re Pellegrini [28]. The stock solutions included 7 Mm ABTS solution and 4.9 potassium per-sulfate solutions. Working solution was prepared by mixing the two stock solutions in equal proportions and allowing

them to react for 12-16 hours in dark place at room temperature. Before use, the solution was diluted with ethanol to obtain absorbance between 0.800 and 1.000 nm, mixed with sample (25 to 100 $\mu g.mL^{-1}$) or standard solutions. A control containing methanol and $ABTS^{\bullet}$+ solution was also realized. The absorbance was read at 734 nm after 30 min incubation at 25°C. The percentage inhibition of free radical ABTS was calculated from the following equation:

$$\% \text{ of inhibition} = ((Abs_{control} - Abs_{test}) / Abs_{control}) \times 100$$

Then, curves were constructed by plotting percentage of inhibition against concentration in µg/mL. The equation of this curve allowed to calculate the IC_{50} corresponding to the sample concentration that reduced the initial $ABTS^{\bullet}$+absorbance of 50%.

2.10 Determination of Total Phenolic Content

Phenolic compounds in the sample extracts was estimated by using Folin-Ciocalteu assay, based on procedures described by Olajire and Azeez [29]. One milliliter of sample from aqueous or ethanol extract was mixed with 1 ml of Folin and Ciocalteu's phenol reagent (1:9; Folin-Ciocalteu reagent: distilled water). After 5 min, 1 ml of 13% sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min and its absorbance was read at 725 nm. A calibration curve was constructed with different concentrations of gallic acid as standard. The results were expressed as mg of gallic acid equivalents (GAE) per gram of extract.

2.11 Determination of Flavonoid Compound

The Aluminum tri-chloride ($AlCl_3$)method was used for determination of the total flavonoid content of the sample extracts according to the method described by Jagadish [30] with some modifications. An aqueous or ethanol extracts (250µl) was mixed with 1.25 ml of deionized distilled water (ddH_2O) and 75 µl of 5% $NaNO_2$. After five min incubation at room temperature, 150 µl of 10% $AlCl_3$ solution was added. Half milliliter of 1M NaOH was added after the next 6 min. The mixture was vigorously shaken on orbital shaker for 5 min at 200 rpm and the absorbance was read at 510 nm against a blank. Catechol with different concentrations was used as a standard. The extracts from agricultural wastes were prepared and tested against some pathogenic bacteria and toxigenic fungi, an application were done by dissolving the agricultural waste materials in jojoba oil waxes.

2.12 Grain Preservation Application

The soybean grain for each treatment, toxin free, were sterile use sodium hypo- chlorite 3%, dried well using a sterile filter paper and soft tissues, drying seeds were used in a trial to examine the antifungal powerful of each concentrated extracts of ImFF, FL, and POH. A constant weight of those concentrated extract material (0.2mg) were dissolved in 2 mL of a waxy spread material with a character to make a thin film (Jojoba oil). Jojoba oil play an important role as a carrier agent besides using it as a treatment alone. The concentrated material in oil give a colloidal solution using to covering soybean grains as a preventive material against mycotoxigenic fungi. The application was done by brushing the 2 mL solution all over the half kilogram of seeds.

2.12.1 Soybean grain biofilm preservation against fungal growth

Half kilogram of sterile dried soybean grain were divided into five groups (four treatments and control), treatment groups were done as: 1– Jojoba oil; 2– Oil + ImFF; 3– Oil + FL; 4 – Oil + POH, and control which was considered as the last treatment. All groups were inoculated using a toxigenic *Aspergillus* strain, then it was filled in a sterile plastic bags, incubated at 23±1ºC for two weeks, at the end of the experiment the grains were inspected for fungal growth.

2.12.2 Application of soybean coating film as a novel anti-aflatoxigenic

One kilogram of soybean grains were used for each applied treatment, the soybean seed were examined firstly to determine its content of aflatoxin. A high producing strain of *Aspergillus Parasiticus* was utilized as a forced infection to evaluate the biofilm coating ability to stop aflatoxin excretion on soybean grains. One milligram of each concentrated material from agricultural wastes extracts (ImFF, FL, and POH) were dissolved in 10 ml of jojoba oil, along using oil only and the control as additional two treatments. JOO alone and mixed with the extracts were examined to oxidative stability by Rancimat method (at 100ºC and air flow rate of 20 L/h). The oil film alone and the loaded oils were spread

well each on its treatment using a soft brush. All treatment groups were incubated for 12 days, at 30ºC under the suitable condition of toxin production [31].

2.13 Statistical Analysis

Data were statistically analyzed with SPSS software [32]. One-way analysis of variance (ANOVA) was used to study significant difference among means, with a significance level at p = 0.05, all data inside tables and figures were represented as values ±SD.

3. RESULTS AND DISCUSSION

3.1 Antifungal Activity of Agro-by Products Extracts

The potential effects of several plant extracts and bio-control agents on the fungal growth and aflatoxins production have been examined by numerous authors [7,15,33]. The antifungal activity of Agro-by products extracts against toxigenic fungi was evaluated in the study (Fig. 1). The growth of toxigenic fungi (*Aspergillus flavus*; *Aspergillus niger*; *Penicillium* sp.; *Fusarium moniliforme)* were effectively controlled using agricultural wastes extracts. Those extracts compared with the growth of the untreated media contained fungi as a control. Three treatments using extracts (ImFF, FL, and POH) beside the control were applied against four different toxigenic fungal strains, ImFF extract was the most antifungal activity against fungal growth all over the total days of the experiment, whereas, the impact of POH extract comes next to it, and the power of FL to reduce the growth comes as a last one.

3.2 Antioxidant Activity in Some Agricultural by-Products Waste

The antioxidant potential was assessed using two generally used colorimetric procedures specifically, free radical scavenging activity DPPH and ABTS colorimetric test, it is important to use different assays, instead of depend on a

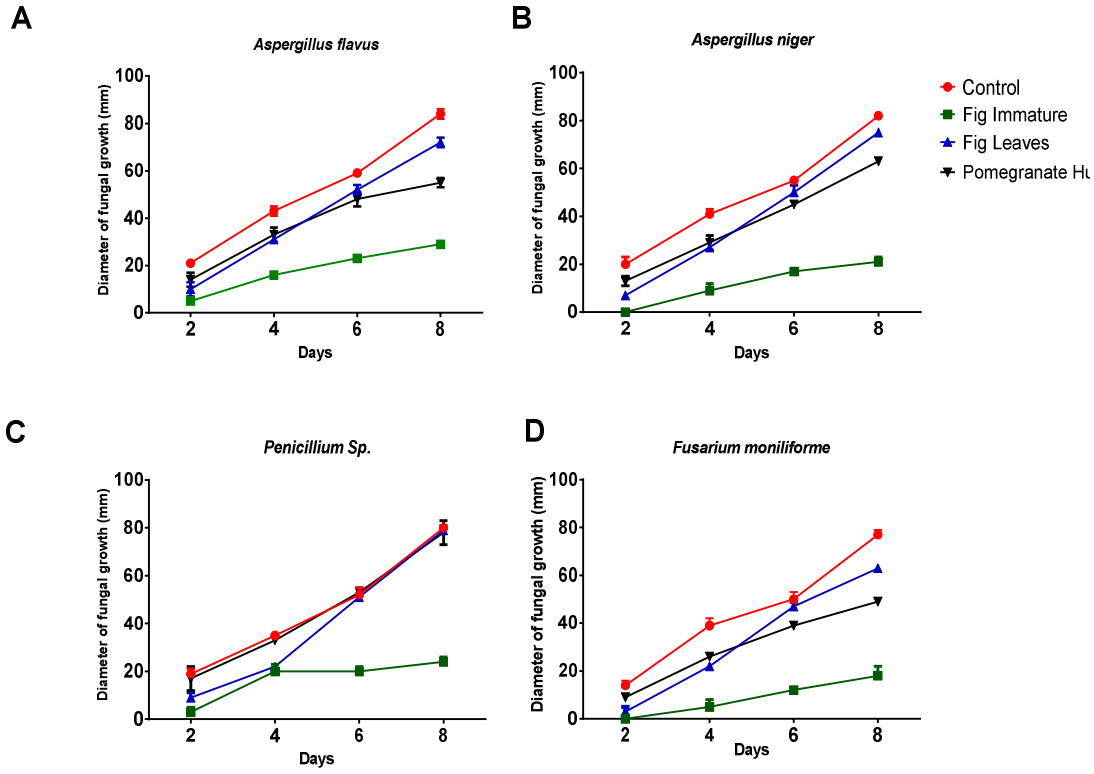

Fig. 1. Antimycotic properties of agricultural by-product materials against toxigenic fungi (A) *Aspergillus flavus* (B) *Aspergillus niger* (C) *Penicillium* Sp. (D) *Fusarium moniliforme*

single assay to assess and compare the antioxidant capacity. The selection of end-points and the expression of results even within the same method, so that comparison between the values quantified by different laboratories can be quite difficult [34]. Analytical data presented in (Fig. 2) indicated that the scavenging capacity against $DPPH^{\bullet}$ differed significantly between the ImFF and FL. The high antiradical effect was achieved by FL exhibited stronger DPPH scavenging activity than ImFF, with mean values of 76.45 and 67.97%, respectively. Although the percent inhibition values of *F. carica* leaves extract were not much greater than the standard antioxidant (ascorbic acid).

Extracts of pomegranate husk and fig leaves has been reported in previous studies as one of the strongest antioxidants, even higher than some synthetic antioxidants like butylated hydroxyl anisole (BHA) or butylated hydroxyl toluene (BHT) [35]. The strong activity of pomegranate husk may be due to the presence of phenolic, flavonoid and anthocyanin, which is known for its antioxidant activity. In the DPPH assay, the highest antioxidant activity (expressed as IC_{50} µg/ml) was observed for ImFF (61.47±4.27), POH (56.91± 9.51), followed by FL (41.19 ±5.14) with respect to ascorbic acid (14 ± 0.28).

The abilities of extracts from FL extract, ImFF extract, and POH extract, assayed to be scavenging the $ABTS^{\bullet}+$ radical in comparison with ascorbic acid, are shown in Fig. 3. The scavenging effect of FL was observed to be higher than other extracts. The FL showed a linear increase in $ABTS^{\bullet}+$ radical-scavenging activity with increasing concentration, reaching 86.54±3.36% scavenging activity at a concentration of 100 µg/ml. The scavenging activities of ImFF extract and POH extract showed radical-scavenging activity 85.30±1.28 and 82.18±2.88%, respectively; whereas, ascorbic acid (92.66±0.27) at the same concentration.

In the $ABTS^{\bullet}+$ assay, the antioxidant activity was quantified in terms of reduction in ABTS+ radicals by antioxidants and it ranged (IC_{50} µg/ml) between 32.55±1.08 µg/ml for ImFF extract; 32.88±3.66 µg/ml for FL and 41.26±1.84 µg/ml for POH extract with respect to ascorbic acid (14.43±0.39) as a standard material. The decolorization inspect, using free blue-green ABTS•+ and DPPH radicals were appeared to be a highly helpful gadget in efficiently standardizing the antioxidant activity of each chemical component or mixed extracts. Those procedures can help to speedy total antioxidant activity as ascorbic acid equipollent to antioxidant capacity [27,28].

3.3 Total Phenolic and Flavonoids Content

The main phytochemical such as phenolic compounds have been the major source for the antioxidant activity of fruits. The total phenolic levels of ImFF extract, FL extract and POH extract were determined using the Folin-Ciocalteu assay. The total phenolic content of the ImFF extract, FL extract and POH extract were recorded at values of 1066.25±0.90, 262.08±1.37, and 709.66±0.76mg GAE/100 g, respectively (Fig. 4). In this study, the examined values obtained for ImFF extract were higher than documented in the literature. Thus, Bey [36] reported that the total phenolic of figs, cultivated in Algeria, ranging between 482.62 and 644.11 mg GAE /100 g or Jokić [37] who reported values ranging between 3.90 mg and 1.9 mg GAE/100 g in different varieties of fig extracts.

The total flavonoid content is mentioned in Fig. 4 are expressed in terms of (mg catechol /g). The POH extract showed the highest concentration of flavonoids (132.06±1.1 mg catechol/g). Mean-while, in FL extract flavonoids were concentrated (55.633±0.77 mg catechol /g) and the flavonoid value of ImFF extract were recorded as (124.33±0.76 mg catechol/g). Agreed with the previous investigations reported that; total phenolic of pomegranate varied from 80.5 to 274 mg/g and total flavonoids was appeared to reach 56.4 mg/g [38]. It is well known that, Flavonoids are important for human health because of their high nutraceutical activities as free radical scavenging activity [39]. The variability in total phenolic and flavonoids among studied samples could be partially attributed to fruit varieties. Polyphenols are secondary metabolites which are derivatives of the pentose phosphate, shikimate and phenyl- propanoid pathways in plants [40]. *F. carica* leaves and fruits are excellent source of phenolic compounds, phenolic contents in our study were highest than the sum of the determined phenolic compounds registered by Mahmoudi [41] on ten Algerian *Ficus carica L.* varieties leaves. On the other hand, Shiban [38] reported that pomegranate husk was the rich on phenolic compounds (91.2 ± 09.5) mg GAE/g dry matter.

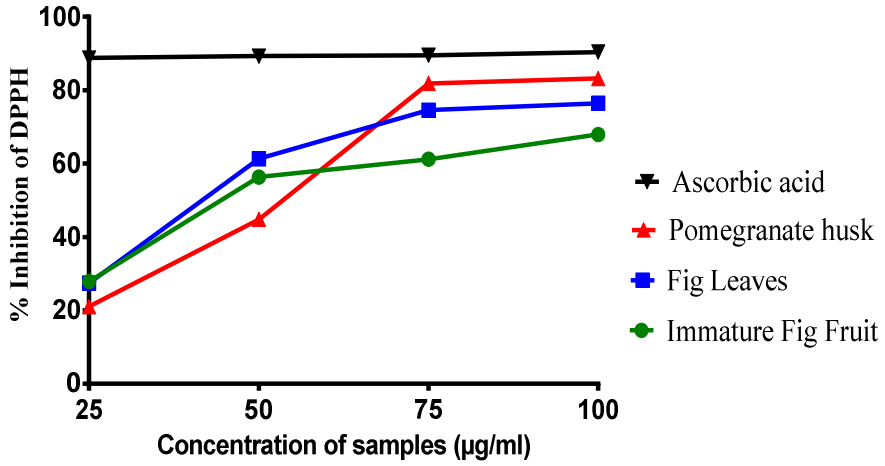

Fig. 2. Scavenging effects of by-product extracts from immature fig fruits, fig leaves and pomegranate husk on DPPH•+ radicals

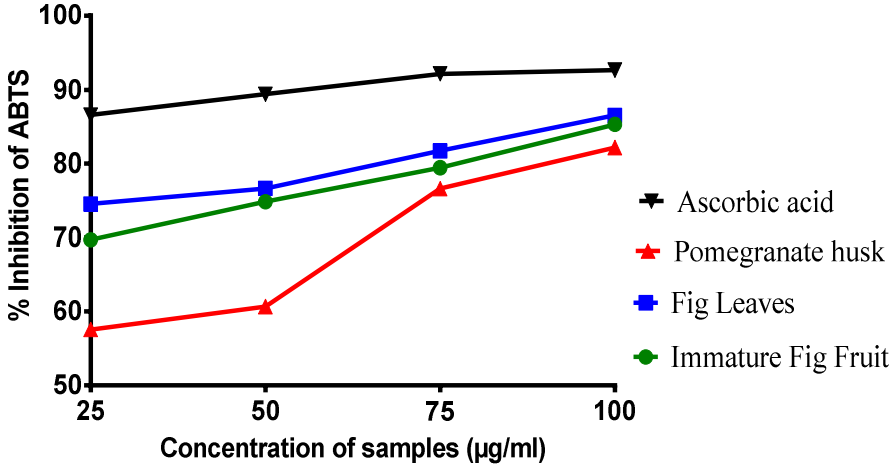

Fig. 3. Scavenging effects of by-product extracts from immature fig fruits, fig leaves and pomegranate husk on ABTS•+ radicals

3.4 Cytotoxicity Study

In the present study, the brine shrimp lethality assay of extracts of ImFF, FL, POH extract and cytoxan as standard were determined using the procedure of Sahgal [42]. The results of the Brine Shrimp Lethality Assay using the by-product extracts and the LC_{50} values obtained for extracts of these extracts and that of the positive control, cytoxan are given in Table 1.

The study shows maximum LC_{50} values took place at a concentration of >1000 µg/mL. In the present examination, varying degrees of lethality were observed with display to the test samples. The extract of ImFF and FL appear to be more effective as it showed an LC_{50} value of >1000 and 870 µg/ml respectively which can be considered to be comparable to the standard, cytoxan (LC_{50} value of 10 µg/ml). Brine Shrimp Lethality An assay shows cytotoxicity as well as a wide range of other nutraceutical benefits such as antimicrobial, antioxidant and anticancer activity (Anderson et al. 1988).

3.5 Antimicrobial Activity

The results were representing the antimicrobial activity of agricultural wastes extracts, those wastes were for ImFF extract, FL extract, POH

extract along with standards compounds (Tetracycline as antibiotic and Nystatin as antifungal), and the data were appeared in Table 2. A constant weight (900 µg) of the freeze-dried wastes extract (concentrated material) was taken from each material of ImFF, FL, and POH; the concentrate was dissolved in 1 mL DMSO until completely dissolved. Then, concentration was used for the dose of antibacterial and antifungal assay. The highest activity was recorded for ImFF extract as 28 mm diameter of zone inhibition against *Pseudomonas aeruginosa* followed by 26 mm diameter of zone inhibition against *Escherichia coli* and *Salmonella typhimurium* at the concentration of 900 µg /ml (Table 2). On the other hand, the lowest activity of plant extract was 5 mm diameter of zone inhibition observed for fig leaves against *Aspergillus fumigates* at the concentration of 900 µg /ml. In the comparison to reference standard tetracycline showed 37 mm diameter of zone inhibition against *Salmonella typhimurium* at 16 µg /ml. From the results that recorded in Table 2, it was found that; the ImFF extract showed the best antimicrobial activity against the Gram-positive bacteria, the Gram-negative bacteria and toxigenic fungi. Many authors reported antimicrobial activity of different plant extracts [43-45], and our present investigation supported the previous findings.

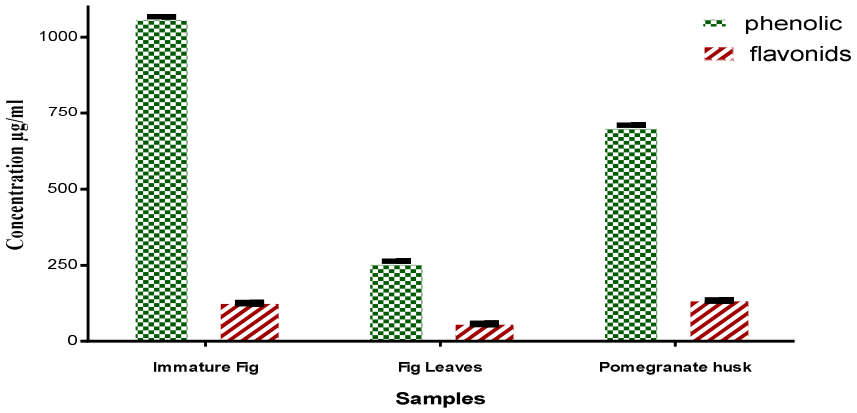

Fig. 4. Total phenolic, flavonoids and ascorbic acid of by- product extracts (means ± S.D.)

Table 1. LC_{50} values in brine shrimp lethality assay of by-product extracts

	Immature fig fruit extract	Fig leaf extract	Pomegranate husk extract	Cytoxan
Dichloromethane	>1000	870	550	10
Isopropanol: Water	>1000	540	490	15

- *LC_{50} of the extracts were: µg/mL*

Table 2. The inhibition zones (mm) produced by agricultural wastes extracts antimicrobial activity comparing to standard antibiotics (tetracycline and Nystatin) on agar well assay

Harmful microorganisms strains	Immature fig fruit extract	Fig leave extract	Pomegranate husk	Tetracycline (standard antibacterial)	Nystatin (standard antifungal)
Pseudomonas aeruginosa	28	16	22	36	17
Listeria monocytogenes	25	17	23	37	15
Escherichia coli	26	17	22	33	16
Salmonella typhimurium	26	13	20	37	11
Aspergillus parasiticus	15	3	9	-	31
Aspergillus fumigates	17	5	14	-	33
Penicillium notatum	22	9	15	-	32
Penicillium expansum	18	7	14	-	30

- *Tetracycline utilized as a standard antibiotic at 16 µg/ml.*
- *Nystatin utilized as a standard antifungal at 32 µg/ml.*
- *Inhibition zone are represented as millimeter diameter*

3.6 Antifungal Activity of Agricultural Waste Materials

The bioactive components which included in the agricultural wastes materials were extracted as a trial for gained a value-add to those wastes, thus will enhance the re-use of these wastes by eco-friendly applications. The antifungal characters of those agricultural wastes extracts were determined for ImFF extract, FL extract and POH extract. Otherwise, those extracts were comparable to a standard antifungal (Nystatin) to examine its antifungal abilities at different concentrations on four types of toxigenic fungi (Table 3). The Fungicidal Inhibitory concentration was 8-9 mg/ml for ImFF extract, this result was observed against the toxigenic fungi strains under the study, followed by 75 mg/ml for POH. Furthermore, the lowest activity was 130 mg/ml for FL extract against pathogenic fungi. Different plant extracts have been reported for their antifungal properties [44,46,47], which supports our present findings. Overall, the ImFF extract showed high activity against all the tested toxigenic fungi.

3.7 Minimum Inhibition Concentration and Minimum Bactericidal Concentration of Agricultural Waste Extracts

Minimum inhibition concentration (MIC) and Minimum Bactericidal concentration (MBC) of agricultural waste extracts. MIC is defined as the lowest concentration of the by- product extracts that inhibit the growth of organisms. Measurement of the MIC is necessary for diagnostic laboratories because it assists in confirming resistance of the microorganism to an antimicrobial agent and it observes the activity of novel antimicrobial agents. The concentration of plant extract that completely killed the bacteria and fungi was taken as MBC and MFC, sequentially. Furthermore, it was remarked that; most of the antimicrobial characteristics of various plant part extractions represent a value of MBC which is nearly twice higher than their identical MIC [48].

The MIC and MBC concentrations of each extract against *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* and *Salmonella typhimurium* were evaluated in the present study (Table 4). The results indicated that immature fig extract was the effective material against gram positive and negative bacteria. The MIC value was of 0.4-0.5 mg/mL (Table 4). On the other hand, the MBC value of 0.7 mg/mL for ImFF extract.

Young and Cha [49] reported that the antibacterial activity of fig leaves extract exhibited inhibitory activities against *Staphylococcus aureus* (MIC, 2.5 to 20 mg/mL; MBC, 5 to 20 mg/mL). The phytochemical components show that the aqueous extract of ripe dried fruit of fig includes flavonoids, coumarins, terpenes, alkaloids and saponins. Some phenolic compounds, with described pharmacological properties have already been separated from fig leaves, specifically phenolic acids like ferrulic acid, and also phytosterols like taraxasterol, flavonoids like quercetin, luteolin and rutin [50,51].

3.7.1 Anti-aflatoxigenic properties of agricultural wastes extracts

Agricultural waste extracts were used as anti-aflatoxigenic materials against aflatoxin production in liquid media, 0.2 g of each (ImFF, FL, and POH) concentrated extracts were dissolved in 1L of autoclaved yeast extract sucrose (YES) media, each flask was inoculated with *Aspergillus parasiticus* which producing the four types of aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2), the results were appeared in Fig. 5. The ImFF extract material showed the best reducing impact on the four types of aflatoxins, which resultant as a great reducing impact on total aflatoxin ratio, the POH extract material had a reducing impact which comes in the second order after ImFF extract. Otherwise, the impact of FL extract material appeared as the last one in reducing aflatoxin amount. The all over effect of the three agricultural waste extracts can ordered as: ImFF>POH > FL. Quantification of AFB_2 in the present of ImFF extract revealed a reduction of over 60% of AFB_1suggesting that the aqueous extract of ImFF extract degraded the toxin (Fig. 5).

Detoxification of aflatoxin by agricultural wastes have been reported by many authors [7,52-54]. Moreover, many plant compounds have been shown to have ameliorative effects against AFB_1-induced toxicity in animals [55-58]. In the present study, agricultural wastes extracts obtained from ImFF, FL, and POH were evaluated for their ability to detoxify the AFB_1; AFB_2; AFG_1; AFG_2 and Total aflatoxins. Of the various plant extracts, ImFF extract showed the maximum reduction of all aflatoxins treatment after incubation for 24 h at 37ºC.

Table 3. Fungicidal Inhibitory concentration of agricultural wastes extracts comparing to standard antifungal compound

Fungi strains	Immature fig fruit extract (mg/ml)	Fig leaf extract (mg/ml)	Pomegranate husk extract (mg/ml)	Nystatin (mg/ml) (standard antifungal)
Aspergillus parasiticus	9	130	75	0.032
Aspergillus fumigates	8	130	75	0.032
Penicillium notatum	8	130	75	0.032
Penicillium expansum	8	130	75	0.032

**The minimal fungicidal concentration was done against four types of toxigenic fungi.*
** Raw material concentration were calculate as mg/mL.*
** Nystatin utilized as a standard antifungal material*

Table 4. Determination of the Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of agricultural waste extracts

By-product extracts	Gram positive bacteria		Gram negative bacteria	
	Staph. aureus	***Listeria monocytogenes***	***Escherichia coli***	***Salmonella typhimurium***
Minimum Inhibition Concentration (MIC)				
Immature Fig	0.5	0.4	0.4	0.4
Fig Leave	22	21	22	22
Pomegranate. Husk	13	13	13	13
Tetracycline	0.016	0.016	0.016	0.016
Minimum Bactericidal Concentration (MBC)				
Immature Fig	0.7	0.7	0.7	0.7
Fig Leave	50	50	45	50
Pomegranate Husk	30	30	30	30
Tetracycline	0.016	0.016	0.016	0.016

- *MIC and MBC values were determined against four pathogenic bacterial strains (Gram+ and Gram -).*
- *Tetracycline was used as a standard inhibit material.*
- *Concentrations of raw materials and the standard were represented in (mg/ml)*

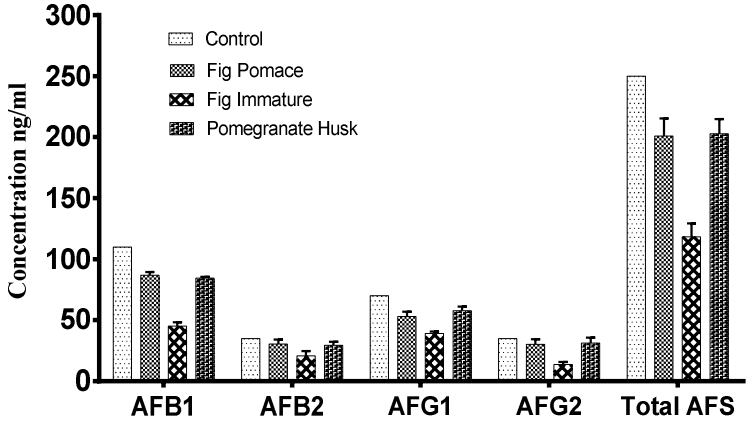

Fig. 5. Degradation of AFB_1; AFB_2; AFG_1; AFG_2 and Total AF_S in the presence of difference by product extracts of fig leaves, fig immature fruit, and pomegranate husk extract incubation at 30°C/10 days

3.8 Oxidative Stability (Rancimat Method)

It was found that JOO had the higher oxidative stability (129.2 h). However, in the case of its supplementation by agricultural wastes extracts, this improving the oil oxidative stability. Concerning the mixing ImFF extract to JOO enhance the induction period (IP) to reach around 192 h, followed by POH which recorded IP value at 190.8 h, and finally the IP value of FL was recorded as 177.6 h. The higher IP (129.2 h) of JO is referring to the resonance impact along linear chain of 38 to 44 carbons atoms. Noah et al. [21] found that gadoleic acid (20:1) reached to 71.3% in JOO. Gadoleic mono unsaturated fatty acid, had many benefits, the double bonds are widely separated and are more or less equidistant from the central ester linkage. These bonds are considered inaccessible and their shared electrons are well protected against oxidation.

3.9 Anti-mycotic and Anti-aflatoxigenic Application Using Agricultural Waste Extract

Depending on our results, the data showed good antifungal and antimicrobial characters of the agricultural waste extracts under the study, the antifungal effect was clearly on four types of fungi (*Aspergillus flavus; Aspergillus niger; Penicillium Sp.; Fusarium moniliforme*), this supported the utilized of natural antifungal components from agricultural waste extracts as a novel application targeted to preserve cereal crop and increase its safety. The extracts were use individually to hold on jojoba oil as the best carrier material. The art of the application here was by using a waxy material that able to distribute well and could carry the natural components. The carrier material here plays several functions as follow: 1– As a spreader that can use to coat grains. 2–As a good carrier that are able to dissolve active components along the seed film. 3– Somewhat, had antimicrobial characters that may help to increase the safety degree of the grains if the dissolved material not reached an area on a grain.

Coated grains of soybean with either jojoba oil only or oil loaded by the concentrated extracts of agricultural wastes showed a good resistance against *Aspergillus* fungal growth. After two weeks of incubation for soybean grains at 23±1ºC, the grain treated by jojoba oil loaded by ImFF which give the best result of fungal growth inhibition, followed by the grain treated by oil loaded by POH concentrated extract. The grain treated by oil loaded by FL extract ordered as third one, while coating the soybean grains by jojoba oil only give a result as the fourth order comparing to the control.

3.10 Soybean Anti-aflatoxigenic Application using an Oil Biofilm

Five groups of soybean grains were prepared as 1 Kg for each, and initial aflatoxin content were determined. The treatment for the groups were (control, grains filmed by jojoba oil only, grains filmed by Oil +ImFF, grains filmed by Oil + FL, grains filmed by Oil + POH) (Fig. 6). The concentrate of each agricultural wastes extracts was dissolved individually in jojoba oil, the suspended concentrate – oil was utilized to distribute all over the grains of each treatment, the resultant grains in each group were completely covered by the oil or oil holding extracts except for control, treated grains were inoculated by a high producing toxin strain (*Aspergillus parasiticus*).

As the results shown in Fig. 6, after the storing time of infected grains, either the oil coated or the oil-suspended coated grain, coating grains by ImFF extract solved in jojoba oil give a result as the best treatment to save the grain against *Aspergillus* toxigenic fungi. Also, the soybean grains coated by jojoba- POH extract was free from fungal growth, that indicate it was a good coated film to extend the safety of the stored grains ordered as an alternative method for using ImFF extract. The coated grains by jojoba- FL extract comes in the third order which appeared in good grains case. Finally for the soybean grains coated just by jojoba oil showed a very little fungal growth, control grains were highly contaminated by the fungi, it was appeared in a high fungal growth contaminated grains.

Infected samples were incubated for 12 days then the amount of aflatoxins levels were recorded as showed in Table 5. Aflatoxin level were varied from not detected (ND) to contamination by aflatoxin B_1 and aflatoxin G_1, treated samples with oil +ImFF, oil+ POH recorded as ND sample for aflatoxins presence; otherwise, the Oil + FL coated sample was found to contain aflatoxin B_1 at levels of 37 ng/kg with ND of aflatoxin G_1, sample coated by jojoba oil only had aflatoxin amount of 41 ng/kg and 13 ng/kg for aflatoxin B_1 and aflatoxin G_1. While the aflatoxin B_1 and G_1 of control were recorded as 198 ng/kg and 74 ng/kg, respectively.

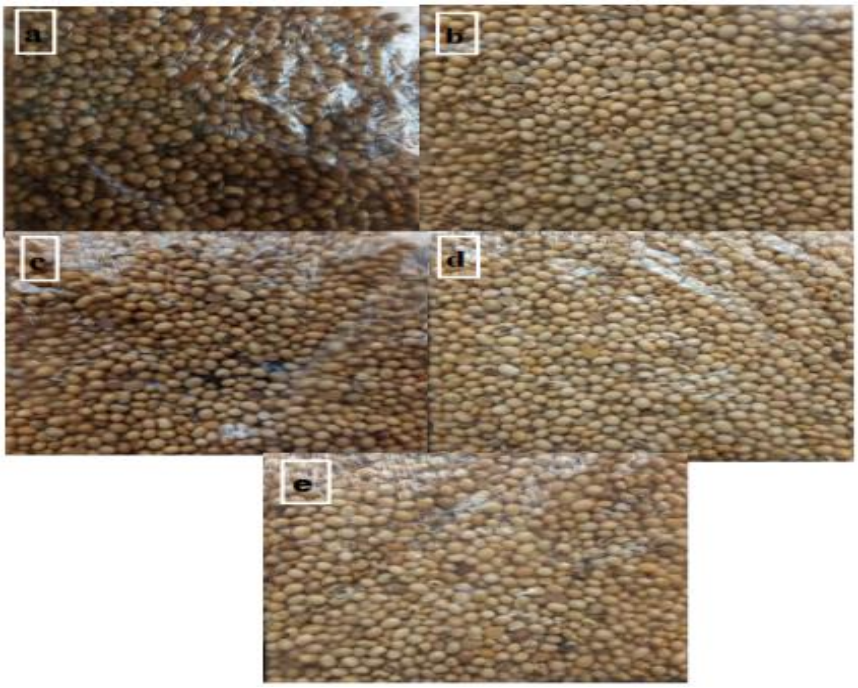

Fig. 6. Stored soybean grains for 15 days/30ºC after coated by jojoba oil and agricultural wastes extracts dissolved in oil as an anti-aflatoxigenic materials. (a) Control, (b) ImFF extract +JOO, (c) JOO, (d) POH+JOO, (e) F.L extract + JOO

Table 5. Stored soybean grains for 15 days/30ºC after coated by jojoba oil loaded with agricultural wastes extracts as an anti-aflatoxigenic materials

	Aflatoxin amount ng/kg			
	Before		After	
	AFB_1	AFG_1	AFB_1	AFG_1
Control	ND	ND	198	74
Grains coated with Jojoba oil	ND	ND	41	13
Grains +oil+ ImFF	ND	ND	ND	ND
Grains + oil + FL	ND	ND	37	ND
Grains +oil+ POH	ND	ND	ND	ND

** ImFF = Immature fig fruit; FL= Fig leaves; POH= Pomegranate husks*

4. CONCLUSION

The agricultural wastes, like ImFF, FL, and POH and their extracts is a great source of active components and polyphenols, it also had many components with a better impact as antimicrobial and antifungal characters. Agricultural waste extracts had a good reducing effect on aflatoxins. Jojoba oil, beside its waxy spread character, it had a good antimicrobial effect. Jojoba oil is a good carrier for a concentrate of agricultural wastes material, the extracts which carried on oil were applied as a bio-film to coat soybean, this help to protect it against fungal contamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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