*Egypt. J. Chem.* **Vol. 67**, No. 7 pp. 57 - 70 (2024)

**Egyptian Journal of Chemistry**

**http://ejchem.journals.ekb.eg/**

# Detection of Mycotoxigenic Fungi Associated with Pomegranate Fruits (*Punica Granatum* L.) and Their Control Using Garden Cress Meal Extract

**Engy M. Akl[1], Embaby E. M. [2], Marwa A. Younos[3*]**

[1]*Fats and Oils Department, Food Industries and Nutrition Research Institute, National Research Centre, Dokki, Postal Code 12622, Giza; Egypt*

[2]*Plant Pathology Dept., Agriculture and Biological Research Institute, National Research Centre, Dokki, Postal Code 12622, Giza; Egypt*

[3]*Food Toxicology and Contaminants Department, Food Industries and Nutrition Research Institute, National Research Centre, Dokki, Postal Code 12622, Giza; Egypt*

**Abstract**

Pomegranate fruits (*Punica granatum* L.) are susceptible to fungal infection and subsequent contamination with mycotoxins. So the current work aimed at the detection of mycotoxigenic fungi associated with pomegranate fruits and their control using polyphenolic acetonic extract of garden cress meal (GCM). The occurrence of mycotoxigenic fungi was assessed. The polyphenolic compounds of the acetonic extract of GCM were elucidated. Each of its antioxidant activity and antifungal activity against isolated mycotoxigenic fungi were evaluated. The obtained data indicated that, 388 fungal isolates were detected. *Rhizoctonia solani* and *Penicilliumexpansum* were the most abundant fungi. Alternariol toxin was produced by *Alternariaalternata* (isolate No. 3) from location A, Aflatoxins were produced by *A. parasiticus* (isolate No.2) from location C and Patulin toxin was produced by *Penicilliumexpansum* (isolate No.13) from location B. Furthermore, the acetonic extract exhibited the highest phenolic compounds and antioxidant activity compared with other solvent extracts from GCM. Gallic acid was the major identified phenolic compound. The polyphenolic acetonic extract reduced significantly the fungal growth and spore viability of the mycotoxigenic fungi at all concentrations used compared with the control. Also, the growth inhibition and the spore viability reduction were increased by increasing the concentration used. It could be concluded that the polyphenolic acetonic extract of GCMhad a significant antifungal effect against mycotoxigenic fungi attacks pomegranate fruits.

***Keywords:*** Pomegranate fruits, Mycotoxigenic fungi, Mycotoxins, Garden cress seeds extract, Polyphenolic compounds, Antioxidant activity, Antifungal activity.

## 1. Introduction

The pomegranate (*Punica granatum* L.) is an important fruit that belongs to the *Punicaceae* family [1]. It is primarily deciduous and can grow in various agro-climatic conditions, allowing it to be cultivated worldwide [2]. Pomegranate fruit is known for its exceptional sensory and nutritional properties, making it highly valuable in terms of nutrition and medicine [3]. It is rich in carbohydrates and minerals and contains calcium, iron, and sulfur [4]. However, pomegranate fruits are prone to attack by pathogens, resulting in significant post-harvest losses for farmers [5]. Previous studies have identified *Penicillium* spp., *Alternaria alternata,* and *Aspergillus niger* as the main causal pathogens of heart rot in pomegranate fruits [6; 7]. These fungi typically infect the fruit during bloom/fruit set and spread to the interior, causing "black heart" or "heart rot" [8]. Previous

*Corresponding author e-mail: marwayounos@yahoo.com;
EJCHEM use only: Received date 26 September 2023; revised date 30 December 2023; accepted date 08 January 2024
DOI: 10.21608/EJCHEM.2024.239167.8674

studies have demonstrated that several species of *Penicillium* and *Aspergillus* involved in the postharvest decay of fruits can produce mycotoxins [9; 10]. Samson and Pitt [11] have reported *Penicillium funiculosum* as a mycotoxin producer, and *Aspergillus niger* produces mycotoxins such as oxalic acid crystals, kojic acid, and malformins, while Aflatoxins can be produced by *Aspergillus flavus* and *A. parasiticus* [12]. Additionally, *A. alternata* isolated from pomegranate fruits can produce Alternariol (AOH), Tenuazonic acid (TA), and Altenuene (ALT) [13]. Currently, synthetic fungicides are being used to combat postharvest rots in pomegranates [5], but this can result in chemical residues that may pose a risk to human health and cause environmental pollution. Therefore, there is a growing interest in biological control methods as a safer and more environmentally friendly alternative [14]. Many plant products derived from forage, pasture, aromatic, medicinal, and wild plants have been successfully used to inhibit the growth and sporulation of various mold fungi, thereby reducing plant pathogenic microorganisms [15]. Garden cress (*Lepidium sativum* Linn.) is an annual herb belonging to the *Brassicaceae* family. It is commonly referred to as garden cress and is known for its fast growth as an edible plant. The seeds, roots, and leaves of garden cress have economic value, with a particular focus on the cultivation of seeds. Garden cress exhibits various pharmacological effects, including antibacterial, antifungal, antioxidant, cytotoxic, diuretic, and hepatoprotective activities [16]. The seeds of GC are especially noteworthy due to their composition. They contain several nutraceutical components such as proteins, fats, carbohydrates, and fibers, as well as important minerals including iron, calcium, potassium, and magnesium [17; 18]. Mucilage in GCM exhibited a protective effect against enter-colitis in rats [19]. Garden cress seeds, along with other medicinal and herbal plants, are recognized as rich sources of beneficial compounds like polyphenols, phenolic acids, flavonoids, and various phytochemicals [20]. Plant extracts obtained from GCM contain a range of biocomponents, including caffeic acid, ferulic acid, carnosic acid, rosmarinic acid, salvianolic acid, sagerinic acid, and many others. These phytochemicals are present in both enzymatic and solvent extracts of garden cress seeds [18; 21].Therefore, the aims of this study were (i) to identify the main postharvest fungal pathogens attacks pomegranate fruits and their associated mycotoxins, and (ii) to evaluate the antioxidant activity and antifungal activity of the polyphenolic acetonic extract of Garden cress meal (GCM) against mycotoxigenic fungi associated with pomegranate fruits.

## 2. Material and Methods:

### 2.1. Collection of samples:

Randomly five fresh samples of Pomegranate fruits were collected from each of four different Governorates (A= Cairo, B= Giza, C= Qalubia & D= Gharbia) in Egypt during (2022). Each sample was collected in sterile polyethylene bags and conveyed to the microbiology laboratory for analysis within 24 hours of collection

### 2.2. Plant material:

Garden cress (*Lepidium sativum*) seeds were purchased from a local market and then subjected to defatting by soxhlet apparatus and normal hexane as the defatting solvent, then allowed to air-dry in a fume hood to remove residual hexane. The resulting defatted meal was ground in a coffee mill to obtain a finely divided material suitable for extraction studies as described in Younos and Akl, [18].

### 2.3. Chemicals:

All chemicals were obtained from Sigma-Aldrich.

### 2.4. Culturing Procedure:

Naturally infected samples were washed with distilled water and then, disinfected by immersing them in ethanol 70% for 2 min. Sterilized fruits were washed twice with sterile double–distilled water (5min each), and allowed to dry for 1 hr. in laminar flow. An appropriate size of spoilt Pomegranate fruit was carefully cut with of sterile blade. Sliced portions (0.5 cm) were then plated on a sterile Potato Dextrose Agar (PDA) medium complemented with 2% tetracycline to inhibit bacterial growth. Three replicates of each tested sample were inoculated with 4 pieces for each plate. Incubation was done at 28 ± 2°C for 5 days until fungal growth was noticed. The different isolates were sub-cultured on freshly prepared (PDA) to obtain their pure culture as described in Khan et al., [5].

**2.5. Identification Procedure:**

All obtained fungal colonies were examined morphologically and microscopically and then identified according to Domsch et al., [22]; Pitt and Hocking, [23]; Raper and Fennel, [24]; Samson, [25].

**2.6. Determination of mycotoxins production:**

All isolated mycotoxigenic fungi (*Alternariaalternata, Aspergillus flavus, A. parasiticus, Fusarium* sp., and *Penicilliumexpansum*)were tested for mycotoxins production. Production of Alternariol toxin was done by culturing *Alternariaalternata*on rice medium according to Torres et al., [26], and determined by chromatography analysis according to Nawaz et al., [27]. All *A. flavus,* and *A. parasiticus* isolates were propagated as pure culture in 100 ml yeast extract sucrose (YES) medium to be tested for Aflatoxins production as described in Munimbazi and Bullerman, [28]; Younos and Akl, [18], and extracted and determined according to Kumar et al., [29]; Rubert et al., [30]. Production of Fumonisin $B_1$ was done by culturing *Fusarium* sp. on corn medium according to Bailly et al., [31], and its extraction and determination were performed as described by Le Bars et al., [32]; Ndube et al., [33], while Patulin production was done by culturing *Penicilliumexpansum* on Malt Yeast Extract agar medium (MEA, and extracted as described by Neri et al., [34], and determined by HPLC according to Christian, [35].

**2.7. Preparations of garden cress meal (GCM) phenolic extracts by different solvents**

The dry garden cress defatted meal was extracted as described by Akl et al., [19]. Briefly, the meal (one gram) was extracted with 90% of each solvent (methanol, ethanol, propanol, butanol, acetone, and distilled water), then mixed for 1 hour in a crest ultrasonic water bath at 38.5 kHz. The mixtures were centrifuged to give supernatant A. The precipitate (macerated) or re-dissolved in another quantity of solvent then repeats the first process twice to give supernatants B, and C. Collected supernatants were used for the determination of Total Phenolic Contents (TPC) and its antioxidant activity. Prepare a considerable amount of acetonic extract for determination of polyphenolic by HPLC and for antifungal evaluation. The watery extract is concentrated by a rotary evaporator and directly freeze-dried by (Crest Alpha 1-4 LSC plus Germany). The freeze-dried phenolic extract is kept in the refrigerator until used.

**2.8. Determination of Total Phenolic Compounds (TPC)**

The content of phenolic compounds was determined according to the method of Fu et al., [36]. In detail, 200 µL of the sample was completed with 3ml distilled water. 2mL of 10% folin reagent was added and then shaken well for 5 minutes. 1ml of 7.5% sodium carbonate was added then shakes. The mixture was left for one hour in the dark then the absorbance at 765 nm was measured using a spectrophotometer (T80 UV vis spectrophotometers).

**2.9. Evaluation of the antioxidant activity of all soluble phases by two methods**

**2.9.1.DPPH (2,2-diphenyl-picrylhydrazyl) radical-scavenging**

The method described by De Ancos et al., [37] was utilized to determine the DPPH radical-scavenging. The reduction of the DPPH radical was measured at 517 nm. Results were expressed as percentage inhibition of the DPPH using the following equation:

$$\text{Inhibition of DPPH (\%)} = \frac{\text{absorbance control} - \text{absorbance sample}}{\text{absorbance control}} \times 100$$

Where the absorbance of control is the absorbance of the DPPH solution without extract

**2.9.2. Ferric reducing antioxidant power (FRAP)**

The reducing power of each extract was determined according to Zhao et al., [38]. The absorbance was measured spectrophotometrically at 700 nm. The measurement was compared to the standard curve of a prepared BHT solution. The final results were expressed as milligrams of BHT equivalents per gram based on dry weight.

**2.10. Analysis of Phenolic Compounds by HPLC**

HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an autosampler and a diode-array detector as described in Younos and Akl, [18].

**2.11. *In vitro* antifungal activity of the polyphenolic acetonic extractof GCM**

#### 2.11.1. Effect on mycelial growth of mycotoxigenic fungi

The polyphenolic acetonic extract of GCM was tested on the mycelial growth of mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus, Fusarium* sp., and *Penicilliumexpansum*) *in vitro*. The tested extract was mixed with sterilized Potato Dextrose Agar (PDA) medium at different concentrations ((0.5 %, 1 %, and 2 %) (v/v)) in separately sterilized Petri dishes. All Petri dishes were inoculated separately at the center with 5mm-disc inoculums 7-day old of each of the tested isolated fungi using a sterilized cork borer. Three plates were used as replicates for each treatment and then incubated at 28 ± 2°C. Colony diameter was measured after 7 d of the incubation period [39]. Medium-free extract was used as a control. Growth inhibition percent was calculated according to Jabeen et al., [40] by using a formula:-

$$\text{Growth inhibition (\%)} = \frac{(C - T)}{C} \times 100$$

Where C = growth in control, T = growth in treatment

#### 2.11.2. Effect on spore viability of mycotoxigenic fungi

The polyphenolic acetonic extract of GCM was tested on spore viability of mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus, Fusarium* sp., and *Penicilliumexpansum*) using various concentrations (0.5 %, 1 %, and 2 %). A disc of 0.5 cm diameter of each fungal culture (7 days old) on PDA was placed at the center of each Petri dish and then incubated at 28 ± 2°C for 5 days. To harvest the produced spores, 9 ml of sterile water was flooded over the grown fungal mycelium and separated using a drawing brush, and then spore suspension was filtered through a muslin cloth. The concentration of collected spore suspension was adjusted to 1 $x10^2$ conidia /ml with the aid of a hemocytometer slide. Each of the PDA plates with extract (0.5 %, 1 %, and 2 %) and free of the extract was inoculated with 1 ml of spore suspension (containing 1 $x10^2$ conidia/ml.)and then spread evenly over the plate. Each treatment had three replicate plates then incubated at 28 ± 2°C. After 48 h of incubation, the proportion of spores that had germinated was calculated according to Meena and Mariappan, [41].

### 2.12. Statistical Analysis

Data obtained in this study were analyzed using software (IBM SPSS Statistics v.16. USA). Statistical significance was performed using a one-way Analysis of Variance (ANOVA) test. A value of $p<0.05$ was considered statistically significant. The least significant difference (LSD) was calculated at $P \leq 0.05$ according to Gomez and Gomez, [42].

## 3. Results

### 3.1. Total fungal isolates associated with pomegranate fruits

Since most fruits and vegetables such as pomegranate fruits contain high levels of water and nutrients, they serve as good substrates that support the growth of pathogenic microorganisms. Isolation of mycoflora associated with pomegranate fruits from four different locations resulted 388 fungal isolates as shown in **Table 1.** On the other hand, data showed that location A had the highest total fungal isolates (120 isolates (30.93%)) followed by location B (103 isolates (26.55%)), and location C (91 isolates (23.45%)), while location D had the least fungal isolates which recorded 74 isolates (19.07%).

**Table 1:** Total fungal isolates associated with pomegranate fruits

| Samples | | Location | | | | Total |
|---|---|---|---|---|---|---|
| | | A | B | C | D | |
| 1 | T.I | 27 | 20 | 14 | 11 | 72 |
| | % | 6.96 | 5.15 | 3.61 | 2.84 | 18.56 |
| 2 | T.I | 29 | 13 | 22 | 16 | 80 |
| | % | 7.47 | 3.35 | 5.67 | 4.12 | 20.62 |
| 3 | T.I | 19 | 24 | 20 | 9 | 72 |
| | % | 4.90 | 6.19 | 5.15 | 2.32 | 18.56 |
| 4 | T.I | 32 | 9 | 18 | 15 | 74 |
| | % | 8.25 | 2.32 | 4.64 | 3.87 | 19.07 |
| 5 | T.I | 13 | 37 | 17 | 23 | 90 |
| | % | 3.35 | 9.54 | 4.38 | 5.93 | 23.20 |
| T.I | | 120 | 103 | 91 | 74 | 388 |
| % | | 30.93 | 26.55 | 23.45 | 19.07 | 100 |

T.I = Total Isolates, A= Cairo, B= Giza, C= Qalubia & D= Gharbia

### 3.2. Fungal frequencies associated with pomegranate fruits

Identification of fungal species associated with pomegranate fruits indicated that eleven fungal species belonging to nine fungal genera were identified as shown in **Fig. 1 and Table 2.** These are *Alternariaalternata, Aspergillus niger, A. flavus, A. parasiticus, Colletotrichumgloeosporioides, Fusarium* sp., *Geotrichum* sp., *Mucor* sp., *Penicilliumexpansum, Rhizoctoniasolani* and *Rhizopusstolinifer*. On the other hand,

*Rhizoctoniasolani* had the highest fungal frequency (23.20%), followed by *Penicilliumexpansum* (19.59%), *Aspergillus niger* (19.07 %), *Rhizopusstolinifer* (11.86 %), *Mucor* sp. (10.82%), *Geotrichum* sp. (5.15 %), *Colletotrichumgloeosporioides* (4.12 %), *Fusarium* sp. (2.58 %), *Altarnaria* sp. (2.06 %), and *A. flavus* (1.03 %). Less fungal frequency was recorded with *A. Parasiticus* which gave 0.52%.

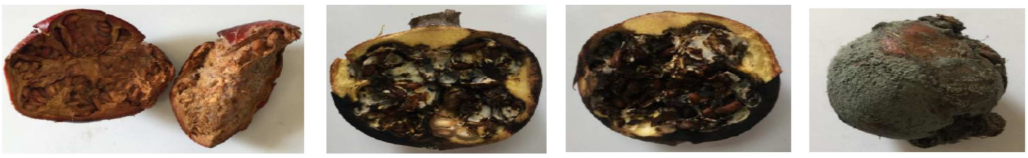

**Fig. 1:** Natural fungal contamination of pomegranate fruits

**Table 2:** Fungal frequencies associated with pomegranate fruits

| Fungi | | Location | | | | Total |
|---|---|---|---|---|---|---|
| | | A | B | C | D | |
| *Alternariaalternata* | T.I | 3 | NF | 4 | 1 | 8 |
| | % | 0.77 | - | 1.03 | 0.26 | 2.06 |
| *Aspergillus niger* | T.I | 20 | 7 | 30 | 17 | 74 |
| | % | 5.15 | 1.80 | 7.73 | 4.38 | 19.07 |
| *A. flavus* | T.I | 2 | 1 | 1 | NF | 4 |
| | % | 0.52 | 0.26 | 0.26 | - | 1.03 |
| *A. parasiticus* | T.I | 1 | NF | 1 | NF | 2 |
| | % | 0.26 | - | 0.26 | - | 0.52 |
| *Colletotrichumgloeosporioides* | T.I | 5 | NF | 8 | 3 | 16 |
| | % | 1.29 | - | 2.06 | 0.77 | 4.12 |
| *Fusarium* sp. | T.I | 3 | NF | 5 | 2 | 10 |
| | % | 0.77 | - | 1.29 | 0.52 | 2.58 |
| *Geotrichum* sp. | T.I | 7 | NF | 10 | 3 | 20 |
| | % | 1.80 | - | 2.58 | 0.77 | 5.15 |
| *Mucor* sp. | T.I | 14 | 9 | 12 | 7 | 42 |
| | % | 3.61 | 2.32 | 3.09 | 1.80 | 10.82 |
| *Penicilliumexpansum* | T.I | 23 | 18 | 20 | 15 | 76 |
| | % | 5.93 | 4.64 | 5.15 | 3.87 | 19.59 |
| *Rhizoctonia solani* | T.I | 28 | 45 | NF | 17 | 90 |
| | % | 7.22 | 11.60 | - | 4.38 | 23.20 |
| *Rhizopusstolinifer* | T.I | 14 | 23 | NF | 9 | 46 |
| | % | 3.61 | 5.93 | - | 2.32 | 11.86 |
| Total | T.I | 120 | 103 | 91 | 74 | 388 |
| | % | 30.93 | 26.55 | 23.45 | 19.07 | 100 |

T.I = Total Isolates, NF= Not Found, A= Cairo, B= Giza, C= Qalubia & D= Gharbia

### 3.3. Determination of mycotoxins production

Mycotoxigenic fungi constitute a significant subset of the fungal population associated with pomegranate fruit rots and are responsible for fruit deterioration. Determination of mycotoxins produced by mycotoxigenic fungi (*Alternariaalternata*, *Aspergillus flavus*, *A. parasiticus*, *Fusarium* sp., and *Penicilliumexpansum*) isolated from pomegranate fruits resulted in,*Alternaria alternata* (isolate No. 3) which isolated from location A was found to produce 2.13 µg/ml of Alternariol toxin, Aflatoxins were produced by *A. parasiticus* (isolate No. 2) from location C with a concentrations of 0.02 µg/ml (0.01AFB2 and 0.01AFG2) and *Penicillium expansum* (isolate No.13) from location B was found to produce 5.09 µg/ml of Patulin toxin. None of *A. flavus* and *Fusarium* sp. isolates was mycotoxins producer, as shown in **Table 3**.

**Table 3:** Determination of mycotoxins production

| L | Mycotoxigrnic fungi | Isolate No. | Mycotoxins (µg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Alternariol | Aflatoxins | | | | | Fumonisin $B_1$ | Patulin |
| | | | | $AFB_1$ | $AFG_1$ | $AFB_2$ | $AFG_2$ | Total | | |
| A | *Alternaria alternata* | 3 | 2.13 | - | - | - | - | - | - | - |
| | *Aspergillus flavus* | - | - | ND | ND | ND | ND | ND | - | - |
| | *A. parasiticus* | - | - | ND | ND | ND | ND | ND | - | - |
| | *Fusarium* sp. | - | - | - | - | - | - | - | ND | - |
| | *Penicillium expansum* | - | - | - | - | - | - | - | - | ND |
| B | *Aspergillus flavus* | - | - | ND | ND | ND | ND | ND | - | - |
| | *Penicillium expansum* | 13 | - | - | - | - | - | - | - | 5.09 |
| C | *Alternaria alternata* | - | ND | - | - | - | - | - | - | - |
| | *Aspergillus flavus* | - | - | ND | ND | ND | ND | ND | - | - |
| | *A. parasiticus* | 2 | - | ND | ND | 0.01 | 0.01 | 0.02 | - | - |
| | *Fusarium* sp. | - | - | - | - | - | - | - | ND | - |
| | *Penicillium expansum* | - | - | - | - | - | - | - | - | ND |
| D | *Alternaria alternata* | - | ND | - | - | - | - | - | - | - |
| | *Fusarium* sp. | - | - | - | - | - | - | - | ND | - |
| | *Penicillium expansum* | - | - | - | - | - | - | - | - | ND |

ND= Not detected, A= Cairo, B= Giza, C= Qalubia & D= Gharbia

### 3.4. Total phenolic compounds and antioxidant activity of GCM extracts

In this study, garden cress meal was subjected to different solvents. The effect of solvents on the extraction yield of total phenolic compounds and their antioxidant activities were investigated. 90% of the following solvents (methanol, ethanol, isopropanol, butanol, acetone, and distilled water) were studied. **Fig. 2**showed that various solvents have a significant effect on total phenolic compound yield. The results showed that 90% acetone (9.77mg/g) and distilled water (11.59 mg/g) exhibited the highest yield than 90% isopropanol (6.93mg/g) and 90% butanol (6.39 mg/g) which have the lowest yield. Antioxidant activities of the various solvents extracts from garden cress meal were evaluated by DPPH and FRAP methods as shown in **Table 4**. The polyphenolic acetonic extract exhibited the highest antioxidant activity (94.60 %) and (11.96 mg/g) followed by distilled water (92.64%) and (14.93mg/g) when measured by DPPH and FRAP methods respectively then the other solvents showed lower activities.

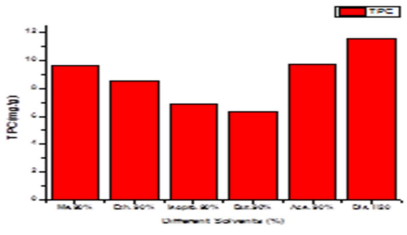


**Fig. 2:** Effect of different solvents on the extraction yield of total phenolic compounds (TPC) from garden cress seed meal (Me. Methanol, Eth. Ethanol, Isopro. Isopropanol, But. Butanol, Ace.Acetone, and Dis. $H_2O$ Distilled water)

**Table 4:** Antioxidant activity of different solvents extracted from GCM

| GCM | DPPH % | FRAP mg/g |
|---|---|---|
| Methanol 90% | 89.52±0.5 | 14.09±0.2 |
| Ethanol 90% | 88.92±0.4 | 14.80±0.1 |
| Isopropanol 90% | 86.10±0.2 | 13.19±0.2 |
| Butanol90% | 77.13±0.1 | 10.76±0.1 |
| Acetone 90% | 94.60±0.3 | 11.96±0.2 |
| Distilled $H_20$ | 92.64±0.4 | 14.93±0.3 |

Results are mean values of three replicates ± standard deviation

### 3.5. Polyphenolic compounds of the acetonic extract of GCM determined by HPLC

Table 5 illustrates the polyphenolic compounds determined by HPLC. It showed that gallic acid, chlorogenic acid, syringic acid, rutin, and ferulic acid are the major identified phenolic compounds in the polyphenolic acetonic extract, and appreciable amounts of propyl gallate, naringenin, quercetin, and cinnamic acid, which described by HPLC chromatograms in **Fig. 3**.

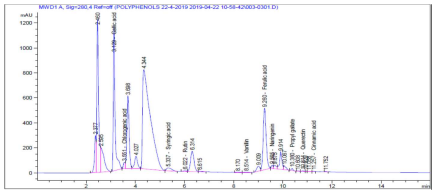

**Fig. 3:** HPLC chromatograms of acetonic extract of GCM

**Table 5:** HPLC of polyphenolic compounds determined in acetonic extract of GCM

| Polyphenolic compounds | Acetonic extract Conc. (µg / g ) |
|---|---|
| Gallic acid | 22956.28 |
| Chlorogenic acid | 2828.68 |
| Catechin | 0.00 |
| Coffeic acid | 0.00 |
| Syringic acid | 856.30 |
| Rutin | 413.33 |
| Ellagic acid | 0.00 |
| Coumaric acid | 0.00 |
| Vanillin | 73.29 |
| Ferulic acid | 6867.07 |
| Naringenin | 237.92 |
| Propyl Gallate | 137.53 |
| Quercetin | 108.22 |
| Cinnamic acid | 8.67 |

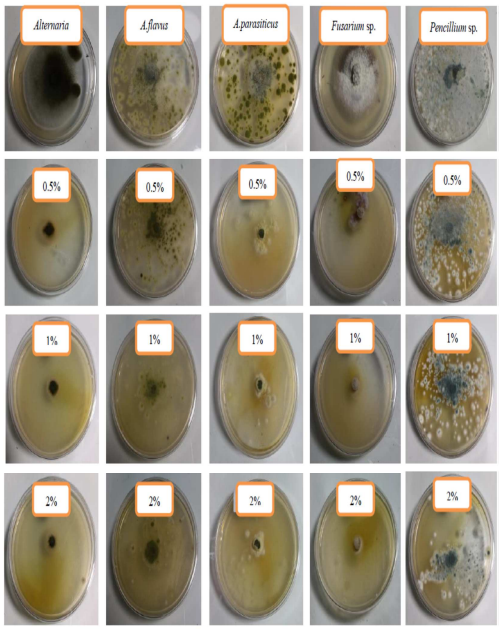


**Fig. 4:** Effect of the polyphenolic acetonic extract of GCM at different concentrations (0.5, 1, 2 %) on mycelial growth of mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus, Fusarium* sp., and *Penicilliumexpansum*)**.**

### 3.6. *In vitro* antifungal activity of the polyphenolic acetonic extractof GCM

#### 3.6.1. Effect on mycelial growth of mycotoxigenic fungi

The effect of the polyphenolic acetonic extract of GCM on mycelial growth of mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus, Fusarium* sp., and *Penicilliumexpansum*) was evaluated by employing various concentrations (0.5%, 1%, and 2%). Data in **Table 6** and **Fig. 4** indicated that the polyphenolic acetonic extract of GCM reduced significantly ($P < 0.05$) the fungal growth of the tested fungi at all concentrations used compared with the untreated control. Also, the growth inhibition was increased significantly ($P < 0.05$) by increasing the concentration used. On the other hand, higher reduction percent of the mycelial growth was detected with *Alternaria alternate*, where it gave 77.14, 73.33 and 66.67% reduction percent, followed by *Fusarium* sp. which recorded 77.27, 68.18& 50.00%, *A. parasiticus* (70.00, 53.33& 40.00%), and *A. flavus* (65.63,53.13& 40.00%) at 2 %, 1 % & 0.5 % respectively. Less reduction percent was detected with *Penicilliumexpansum*, which gave 40.63, 25.00&12.50 % at the same concentrations respectively.

#### 3.6.2. Effect on spore viability of mycotoxigenic fungi

The effect of the polyphenolic acetonic extract of GCM on spore viability of mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus, Fusarium* sp., and *Penicilliumexpansum*) was evaluated by employing various concentrations (0.5%, 1%, and 2%). Data in **Table 7** indicated that the polyphenolic acetonic extract of GCM reduced significantly ($P < 0.05$) the spore viability of the tested fungi at all concentrations used compared with the untreated control. Also, the reduction percentage was increased significantly ($P < 0.05$) by increasing the concentration used. Also, data in this table showed that, the highest reduction percent was recorded with *A. parasiticus* and *Penicilliumexpansum*, where it gave 89.04, 81.74 & 63.01% inhibition percent for *A. parasiticus*, and 82.58, 73.33&60.00 % for *Penicilliumexpansum*, followed by *A. flavus* and *Fusarium* sp. which recorded 83.06, 74.86& 50.27 % for *A. flavus* and 78.22, 72.00& 52.44% for *Fusarium* sp. at 2%, 1%& 0.5% respectively. While the least reduction percent was recorded with *Alternariaalternata*, (75.98, 63.69& 42.46% at the same concentrations respectively).

**Table 6:** Effect of the polyphenolic acetonic extract of GCM on mycelial growth of mycotoxigenic fungi

| Plant Extract | Conc. % | *Alternaria alternata* | | *Aspergillus flavus* | | *A. parasiticus* | | *Fusarium* sp. | | *Penicillium expansum* | | LSD 5% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | linear growth (mm) | R % | linear growth (mm) | R % | linear growth (mm) | R % | linear growth (mm) | R % | linear growth (mm) | R % | |
| The polyphenolic acetonic extract of GCM | 0.5 % | 17.5 ± 0.01 $^{ab}$ | 66.67 | 65.0 ± 0.35 $^{c}$ | 18.75 | 45.0 ± 0.47 $^{bc}$ | 40.00 | 27.5 ± 0.10 $^{c}$ | 50.00 | 70.0 ± 0.24 $^{bc}$ | 12.50 | 1.083 A |
| | 1 % | 14.0 ± 0.10 $^{ab}$ | 73.33 | 37.5 ± 0.11 $^{ab}$ | 53.13 | 35.0 ± 0.50 $^{ab}$ | 53.33 | 17.5 ± 0.19 $^{ab}$ | 68.18 | 60.0 ± 0.25 $^{ab}$ | 25.00 | |
| | 2 % | 12.0 ± 0.20 $^{a}$ | 77.14 | 27.5 ± 0.26 $^{a}$ | 65.63 | 22.5 ± 0.20 $^{a}$ | 70.00 | 12.5 ± 0.14 $^{a}$ | 77.27 | 47.5 ± 0.37 $^{a}$ | 40.63 | |
| Control | | 52.5 ± 0.42 $^{c}$ | | 80.0 ± 0.74 $^{d}$ | | 75.0 ± 0.10 $^{d}$ | | 50.0 ± 0.25 $^{d}$ | | 80.0 ± 0.65 $^{d}$ | | 0.815 B |
| LSD 5% | | 0.629 A | | 0.950 C | | 0.852 BC | | 0.612 AB | | 0.787 D | | |

R% = Reduction percent, GCM= Garden Cress Meal, Results are mean values of three replicates ± standard deviation. The different letters in each column indicate significant differences at P<05.

**Table 7:** Effect of the polyphenolic acetonic extract of GCM on spore viability of mycotoxigenic fungi

| Plant Extracts | Conc. | *Alternariaalternata* | | *Aspergillus flavus* | | *A. parasiticus* | | *Fusarium* sp. | | *Penicilliumexpansum* | | LSD 5% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Viable spores x$10^2$ | R % | Viable spores x$10^2$ | R % | Viable spores x$10^2$ | R % | Viable spores x$10^2$ | R % | Viable spores x$10^2$ | R % | |
| The polyphenolic acetonic extract of GCM | 0.5 % | 103.00 ± 0.80 $^{c}$ | 42.46 | 91.00 ± 0.80 $^{c}$ | 50.27 | 81.00 ±0.62 $^{c}$ | 63.01 | 107.00 ± 0.90 $^{c}$ | 52.44 | 186.00 ± 0.34 $^{c}$ | 60.00 | |
| | 1 % | 65.00 ± 0.30 $^{b}$ | 63.69 | 46.00 ± 0.10 $^{b}$ | 74.86 | 40.00 ±0.55 $^{b}$ | 81.74 | 63.00 ± 0.25 $^{ab}$ | 72.00 | 124.00 ±0.10 $^{b}$ | 73.33 | 23.587A |
| | 2 % | 43.00 ± 0.60 $^{a}$ | 75.98 | 31.00 ± 0.23$^{a}$ | 83.06 | 24.00 ± 0.60 $^{a}$ | 89.04 | 49.00 ±0.22 $^{a}$ | 78.22 | 81.00 ± 0.33 $^{a}$ | 82.58 | |
| Control | | 179.00 ± 0.60 $^{d}$ | | 183.00 ± 0.81 $^{d}$ | | 219.00 ± 0.63 $^{d}$ | | 225.00 ± 0.40 $^{d}$ | | 465.00 ± 0.25 $^{d}$ | | 61.481B |
| LSD 5% | | 20.696 B | | 24.539 AB | | 29.854 A | | 26.998 AB | | 63.084 A | | |

R% = Reduction percent, GCM= Garden Cress Meal, Results are mean values of three replicates ± standard deviation. The different letters in each column indicate significant differences at P<05.

## 4. Discussion

Fungal infections are a significant contributor to postharvest losses and economic decline in pomegranate crops. Many of these fungi infect the pomegranate fruits during the blooming stage and remain dormant until the fruits ripen. Additionally, some fungal infections occur during harvest and postharvest handling, usually through injuries in the fruit's rind [43]. A total of 388 fungal isolates belonging to eleven different fungal species were obtained from pomegranate fruit samples from four different locations. The identified species included *Alternariaalternata, Aspergillus niger, A. flavus, A. parasiticus, Colletotrichumgloeosporioides, Fusarium* sp., *Geotrichum* sp., *Mucor* sp., *Penicilliumexpansum, Rhizoctoniasolani,* and *Rhizopusstolinifer*. On the other hand, *Rhizoctoniasolani*, and *Penicilliumexpansum* were the most abundant fungi, which recorded 23.20& 19.59 respectively. Similar results were obtained by Snowdon, [44], who mentioned that; *Aspergillus flavus* has the potential to spoil various fresh fruits, including pomegranate. Grigoryan et al., [45] isolated fifteen species of microscopic fungi from pomegranate samples and found that approximately 80% of the isolates belonged to the genus *Penicillium* spp. Other fungal genera such as *Aspergillus, Fusarium*, and *Mucor* were also identified. Anonymous, [46] mentioned that pomegranate rot disease is primarily caused by *Rhizoctoniasolani*. Nallathambi and Umamaheswari, [47] isolated *Alternaria* spp., *Aspergillus parasiticus*, *A. flavus*, *Penicillium* spp., and *A. niger* from damaged pomegranate fruits in India. In another study by

Adaskaveg, [48], it was reported that *Aspergillus niger, Penicillium digitatum*, and *Alternaria alternate* were the most significant fungal pathogens known to infect pomegranate. According to Palou et al.,[49], pomegranate fruit decay is caused by various fungal pathogens, including *Alternaria* spp., *Botrytis cinerea, Aspergillus niger*, *Aspergillus* spp., *Colletotrichum gloeosporioides, Coniella* spp., *Nematospora* spp., *Pilidiella granati, Penicillium* spp., and *Rhizopus* spp. Ilgın and Karaca, [50] found that, among the 12 fungi species isolated from pomegranate orchards, *Alternaria alternate, Aspergillus niger, Penicillium* sp., *Cladosporium herbarum*, and *Colletotrichum gloeosporioides* were the most common in fruit samples. Less frequently isolated fungi included *Fusarium semitectum, Botrytis cinerea, Epicoccum nigrum*, *Fusicoccum aesculi, Coniella granati, Pleospora herbarum*, and *Trichothecium roseum*. Mincuzzi et al., [51] reported that the main etiological agents of postharvest pomegranate fruit diseases were various species of *Penicillium* spp., *Alternaria* spp., *Coniella granati*, and *Botrytis cinerea*, while *Aspergillus niger*, *Colletotrichum acutatum*, *Aspergillus* spp., and *Cytospora punicae* were less prevalent. Mincuzzi and Ippolito, [43] mentioned that gray and blue molds caused by *Botrytis* spp. and *Penicillium* spp., respectively, were the main postharvest fungal diseases of pomegranates. Other diseases included black heart and black spot caused by *Alternaria* spp., anthracnose related to species in the *Colletotrichum* genus, and Coniella rot due to *Coniella granati*. The diversity of fungal species can be influenced by climatic conditions, including rainfall and temperature. Temperature and humidity play vital roles in the growth of fungal pathogens on fruits and greatly affect the occurrence and severity of fungal diseases in plants [52].

Mycotoxigenic species not only make up a considerable portion of the fungi causing rot in pomegranate fruits, leading to their deterioration, but they also present a potential risk to the health of individuals consuming products derived from pomegranate[53]. Determination of the mycotoxins produced by mycotoxigenic fungi (*Alternariaalternata*, *Aspergillus flavus, A. parasiticus, Fusarium* sp., and *Penicilliumexpansum*) isolated from pomegranate fruits resulted that, *Alternaria alternata* (isolate No. 3) from location A produced Alternariol toxin with a concentrations of 2.13 µg/ml, and aflatoxins were produced by *A. parasiticus* (isolate No. 2) from location C with a concentrations of 0.02 µg/ml, while *Penicillium expansum* (isolate No.13) from location B produced 5.09 µg/ml of Patulin toxin. None of *A. flavus* and *Fusarium* sp. isolates was mycotoxins producer. Similar findings were obtained by Nallathambi and Umamaheswari, [47], who reported that three pathogenic isolates of *Aspergillus parasiticus* and one isolate of *A. flavus*, obtained from damaged pomegranate fruit samples in India, were found to produce a range of aflatoxins. The *A. parasiticus* isolates synthesized aflatoxins B2, G1, and G2 in a culture medium (YESA) and only aflatoxin G2 in pomegranate arils. The *A. flavus* isolate produced only aflatoxin B2 in both the culture medium and infected arils. Ammar and El-Naggar [13] found that *A. alternata* isolated from pomegranate fruits produced Alternariol (AOH), Tenuazonic acid (TA), and Altenuene (ALT). They also found that *Penicillium funiculosum* produced Patulin (PAT) and Penicillic acid (PAC). According to Kanetis et al., [53], 89% of the Alternaria isolates associated with pomegranate fruit decay in Greece and Cyprus produced AOH and AME in vitro, while only 43.9% produced TEN. Additionally, all Alternaria species (*A. alternata, A. tenuissima*, and *A. arborescens*) from Greece exhibited higher average concentrations of AOH compared to the respective species isolates from Cyprus. Myresiotis et al., [54] conducted a study testing six different *A. alternata* isolates obtained from pomegranate samples sold in Greece for the presence of Alternariol (AOH) and Alternariol monomethyl ether (AME) both in vitro (using PDA cultures) and in vivo (in pomegranate fruits). They found that all isolates produced AOH and AME in PDA cultures. In artificially inoculated fruits, it was observed that all isolates produced AOH and AME on pomegranates. Additionally; Elhariry et al., [55] reported that all isolated *Alternaria* strains, including *Alternaria alternate* and *A. tenuissima*, from the inner infected tissues of pomegranate fruits, exhibited substantial production of alternariol toxin in vitro using rice as a substrate. The differences in mycotoxin production can be attributed to several factors, including environmental conditions like temperature, pH, water activity, and relative humidity, as well as strain specificity and the composition of the substrate.

The seeds of garden cress (*Lepidium sativum* L.) are an excellent source of dietary fiber, protein, omega-3 fatty acids, iron, and other vital elements, in addition to phytochemical compounds [17; 18]. Various solvents used in this study, which have different polarities, have a significant effect on polyphenolic compound yield. Distilled water and acetone were both higher in polarity than the other solvents used as butanol and isopropanol. Rafińska et al., [56] studied the effect of solvent and found that 96% ethanol as co-solvent was the best antimicrobial activity. The methanolic extract of GCM included alkaloids, flavonoids, tannins, Phenols, terpenoids, glycosides, and saponins. These findings showed that it contains a large number of chemical compounds that may be the cause of the various pharmacological actions connected to the secondary metabolites already present that support distinctive antibacterial activities [57; 58]. Antioxidant activities of the various solvents extracts from GCM were evaluated by DPPH and FRAP methods. The polyphenolic acetonic extract exhibited the highest antioxidant activity followed by distilled water than the other solvents used because of its content of phenolic compounds; it acts as an in vivo and in vitro antioxidant [59]. Garden cress' ethanolic extract demonstrates that it has significant antioxidant activity and could be a source of natural antioxidant chemicals [60]. It also lessens structural damage to the liver and hepatic injuries by reducing inflammation, oxidative stress, and liver cell death [61].

The polyphenolic compounds of acetonic garden cress extract determined by HPLC showed that gallic acid is the most abundant phenolic compound. These results are in agreement with those confirmed by Martins et al., [21]; El-Salam et al., [17]. However, Vanillin exists only in the polyphenolic acetonic extract and not found in a mixture of enzyme extracts determined by Younos and Akl, [18].In addition to Syringenic, Cinnamic, Caffeic, Catechol, Pyrogallol, Salicylic, Benzoic, and Ellagic found in ethanolic and methanolic extracts determined by Ahmed et al.,[62]. The differentiations in the polyphenolic were due to the different polarities of each solvent that are capable of liberating various compounds. These polyphenolic compounds include anthraquinones, flavonoids, xanthones, anthocyanins, and tannins [63]. The solvent extraction provided approximately pure extract rather than watery extract. This is because water can dissolve protein and carbohydrates than other organic solvents.

Numerous plant-based compounds have been recognized for their antifungal properties and have been investigated for their potential use in reducing postharvest decay of fruits and vegetables [64]. Evaluation of antifungal activity of the polyphenolic acetonic extract of GCM using various concentrations (0.5 %, 1 %, and 2 %) on mycelial growth and spore viability of mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus, Fusarium* sp. and *Penicilliumexpansum*) resulted that, the polyphenolic acetonic extract of GCM reduced significantly the fungal growth and spore viability of the tested fungi at all concentrations used compared with the untreated control. Also, the growth inhibition and the reduction percent of spore viability were increased by increasing the concentration used. Similar results were obtained by Sharma et al., [65] who conducted a study on the ethanolic extract of Garden cress seeds to evaluate its antifungal activity against *Alternaria alternate, Aspergillus flavus*, and *Fusarium equiseta* by employing various concentrations (2 - 8%), and it was observed that all concentrations inhibited fungal growth. According to Berehe and Boru [66], the crude extract from Ethiopian *Lepidium sativum* seeds showed antifungal properties against tested fungi, including *A. niger, F. oxysporum,* and *F. solani*. George et al., [67] found that, the methanolic extract of *L. sativum* seeds had a significant antifungal activity against *Aspergillus flavus*, *Aspergillus fumigatus, Candida albicans, Fusarium* sp., *Microsporum* sp., *Penicillium* sp., *Penicillium marneffi*, and *Rhizopus* sp. Tayel et al., [68] reported that the *Lepidium sativum* seed extract exhibited a significant antifungal activity against the phytopathogenic fungi *P. digitatum* and *P. italicum*. Omer et al., [57] reported that the crude extract of garden cress seeds displayed antifungal activity against two fungal species, *Aspergillus niger*, and *C. albicans*. George and Thankamani [69] conducted a study to test various extracts of *Lepidium sativum* seeds (Hexane, n-Butanol, Ethyl Acetate, Methanol, and Aqueous) against several fungal species including *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Cryptococcus* sp., *Candida albicans, Curvularia* sp., *Epidermophyton, Fusarium* sp., *Microsporum* sp., *Penicillium* spp., and *Rhizopus* sp. They found that the methanol extract exhibited

the highest potency, followed by the butanol and ethyl acetate extracts, where the methanol extract completely inhibited the growth of all fungi except *Curvularia* sp. and *A. niger*, which showed weak growth towards the end of the incubation period. In another study by Adera et al.,[70], the antifungal activity of *L. sativum* seed and leaf oil extracts was evaluated. Both extracts demonstrated antifungal activity against *Aspergillus niger* and *C. albicans*, whereas the leaf oil extract exhibited stronger antifungal activity. The antifungal potential of the polyphenolic acetonic extract of GCM in this study can be attributed to its bioactive compounds, including gallic acid, chlorogenic acid, syringic acid, ferulic acid, quercetin, and vanillin, which are responsible for its antifungal activity. Where Sharma et al., [65] reported that the presence of constituents like flavonoids and tannins in the extract could contribute to its antifungal activity. Similarly, Hussaini et al.,[71] suggested that the antifungal activity of plant extracts may be attributed to the presence of phenolic acids and flavonoid compounds.

## 5. Conclusion

The current study demonstrated that, various mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus, Fusarium* sp., and *Penicilliumexpansum*) were associated with pomegranate fruits, and produced several mycotoxins that caused potential health hazards to humans. Using the polyphenolic acetonic extract of garden cress meal could be a valuable source of bioactive compounds with substantial biological activities including antioxidant and antifungal activities. It can be concluded that it exhibited a significant impact on inhibiting the growth and spore viability of mycotoxigenic fungi contaminating pomegranate fruits. Moreover, the application of various plant extracts can serve as an eco-friendly approach to managing fungal diseases in pomegranate fruit production. In addition, these extracts are characterized by their environmental safety, economic feasibility, absence of residual issues, and cost-effectiveness, enabling them to be valuable competitors to synthetic products.

## Acknowledgements

The authors wish to thank to Food Toxicology and Contamination Dept. as well as the Plant Pathology Dept., National Research Center (NRC), Egypt for their help and encouragement during this study.

## Declaration of interest

No conflicts of interest.

## Data Availability

The data that supports this work is available upon reasonable request.

## Ethical approval

This study was approved by the Medical Research Ethics Committee at the National Research Center, Egypt. (No. 08421223).

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

## References

[1] Mercure EW (2007) The pomegranate: a new look at the fruit of paradise. J. Hortic. Sci. 42: 1088–1092.
[2] Levin GM (2006) Pomegranate roads: a Soviet botanist's exile from Eden.p. 15– 183. In: B.L. Baer (eds.), Floreat Press, Forestville, CA.
[3] Viuda-Martos M, Fernandez-Lopez J, Perez-Alvarez JA (2010) Pomegranate and its many functional components as related to human health: A review. Compr. Rev. Food Sci. F., 9, 635–654.
[4] Waskar DP (2006) Pomegranate (*Punica granatum* L.)Advances in arid Horticulture, 2, 375-94.
[5] Khan AA, Habib A, Khan WA, Arif MU (2021) Management of Grey mold of Pomegranate (*Punica granatum* L.) through essential oils.*Life Sci J*; 18(11):2126.
[6] Michailides TJ, Morgan DP, Quist M, Reyes HC (2012) Infection pomegranate by *Alternaria* sp. causing blackheart.Phytopathol Z. 98:S105.
[7] Zhang L, McCarthy MJ (2012) Black heart characterization and detection in pomegranate using NMR relaxometry and MR imaging. Postharvest Biol. Technol., 67: 96–101.
[8] Yehia HM (2013) Heart rot caused by *Aspergillus niger*through splitting in leathery skin of pomegranate fruit. Afr. J. Microbiol. Res., 7: 834-837.
[9] Moslem MA, Yassin MA, El-Samawaty AMA, Sayed SRM, Amer OE (2013) Mycotoxin-producing Penicillium species Involved in Apple Blue Mold. J Pure Appl Microbiol,7:187-193.
[10] Tancinová D, Barboráková Z, Kacinová J, Mašková Z, Volcková M (2013) The occurrence of micromycetes in apple and their potential ability to produce mycotoxins. J. Microbiol. Biotechnol. Food Sci., 2: 1800-1807.

[11] Samson RA, Pitt JI (1990) Modern Concepts in *Penicillium* and *Aspergillus* Classification. NATO ASI Series, Life Sciences, volume 185.
[12] Blumenthal CZ (2004) Production of toxic metabolites in *Aspergillus niger, Aspergillus oryzae*, and *Trichoderm areesei* : justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. Regul.Toxicol. Pharmacol.,39: 214–228.
[13] Ammar MI, El-Naggar MA (2014) Screening and Characterization of Fungi and their associated Mycotoxins in some Fruit Crops.Int. j. adv. res., Volume 2, Issue 4, 1216-1227.
[14] Xingqi L, Xinyue L, Yiheng H, Mengting D, Yanrong L (2020) Identification the Pathogens Causing Rot Disease in Pomegranate (*Punica granatum* L.) in China and the Antifungal Activity of Aqueous Garlic Extract, Forests, 11, 34.
[15] Petróczy M, Nagy G, Bánátfy R, Palkovics L (2006) In vitro antifungal activity of essential oils on pathogens .University of Budapest, Department of Plant Pathology Aromax Inc. Budapest, Hungary.
[16] Baregama C, Goyal A (2019). Phytoconstituents, pharmacological activity, and medicinal use of *Lepidium sativum*Linn.: A review. *Asian J Pharm Clin Res*, *12*(4), pp.45-50.
[17] El-Salam A, Kholoud H, Toliba AO, El-Shourbagy GA, El-Nemr SE (2019) Chemical and functional properties of garden cress (*Lepidium sativum* L.) seeds powder. Zagazig J. Agri Res., 46(5), pp.1517-1528.
[18] Younos MA, Akl EM (2022) Evaluation of enzymatic phenolic extract from Garden Cress seed meal against aflatoxigenic fungi isolated from Eggplant fruits. Egypt. J. Chem. 65(4):287–299.
[19] Akl EM, Taha FS, Mohamed SS, Mohamed RS (2021). Characterization of garden cress mucilage and its prophylactic effect against indomethacin-induced enter-colitis in rats.Biointerface Res. Appl. Chem., 11(6), 13911-13923.
[20] Bhatia P, Sharma A, George AJ, Anvitha D, Kumar P, Dwivedi VP, Chandra NS (2021) Antibacterial activity of medicinal plants against ESKAPE: An update. Heliyon, 7, e06310.
[21] Martins N, Barros L, Santos-Buelga C, Henriques M, Silva S, Ferreira IC (2015) Evaluation of bioactive properties and phenolic compounds in different extracts prepared from *Salvia officinalis* L. Food Chem., 170, 378–385.
[22] Domsch KH, Gams W, Anderson TH (1980).Compendium of Soil Fungi. New York, Academic Press., London .
[23] Pitt JI, Hocking AD (1997) Fungi and Food Spoilage. Blackic Academic and Professional , London.
[24] Raper KB, Fennel DI (1965) The Genus Aspergillus. Baltimore, Williams and Wilkins, Pp.686.
[25] Samson RA (1979) The Genus *Aspergillus* Described Since 1965. Stud. Mycol., 18, 80.
[26] Torres A, González H, Etcheverry M, Resnik S, Chulze S (1998) Production of alternariol and alternariol mono-methyl ether by isolates of *Alternaria* spp. from Argentinian maize, Food Addit. Contam., 15:1, 56-60.
[27] Nawaz S, Scudamore KA, Rainbird SC (1997) Mycotoxins in ingredients of animal feeding stuffs: I. Determination of alternaria mycotoxins in oilseed rape meal and sunflower seed meal. Food Addit. Contam. 14, 249-262.
[28] Munimbazi C, Bullerman L (1998) High Performance Liquid Choromatographic method for the determination of moniliformin in corn. J. AOAC Int., 81, 999-10.
[29] Kumar A, Shukla R, Singh P, Dubey N (2010) Chemical Composition, Antifungal and Anti Aflatoxigenic Activities of *Ocimum Sanctum* l. Essential Oil and Its Safety Assessment As Plant Based Antimicrobial. Food Chem Toxicol. 48:539-54.
[30] Rubert J, Soler C, Mañes J (2012) Application of an HPLC–MS/MS method for mycotoxin analysis in commercial baby foods. Food Chem.; 133(1):176-183.
[31] Bailly JD, Querin A, Tardieu D, Guerre P (2005) Production and purification of fumonisins from a highly toxigenic *Fusarium verticilloides* strain, Revue Méd. Vét, 156, 11, 547-554.
[32] Le bars J, Le bars P, Dupuy J, Boudra H (1994) Biotic and a biotic factors in fumonisin B1 production and stability. J. Assoc. Off. Anal. Chem. Int., 77, 517-521.
[33] Ndubea N, der Westhuizena L, Greenb IR, Shepharda GS (2011) HPLC determination of fumonisin mycotoxins in maize: A comparative study of naphthalene-2,3-dicarboxaldehyde and o-phthaldialdehyde derivatization reagents for fluorescence and diode array detection, J. Chromatogr. B, 879 2239– 2243.
[34] Neri F, Donati I, Veronesi F, Mazzoni D, Mari M (2010) Evaluation of *Penicillium expansum* isolates for aggressiveness, growth and patulin accumulation in usual and less common fruit hosts. Int J Food Microbiol 143: 109-117.
[35] Christian G (1990) Hplc tips and tricks. Great britain at the iden press. Oxford .pp 608.
[36] Fu R, Zhang Y, Guo Y, Liu F, Chen F (2014). Determination of phenolic contents and antioxidant activities of extracts of Jatropha curcas L. seed shell, a by-product, a new source of natural antioxidant. Ind Crops Prod 58:265–270.
[37] De Ancos B, Sgroppo S, Plaza L, Cano MP (2002). Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. J Sci Food Agric 82(8):790–796.
[38] Zhao H, Fan W, Dong J, Lu J, Chen J, Shan L, Lin Y, Kong W (2008). Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties.Food Chem., 107(1), pp.296-304.
[39] Singh S, Srivastava S, Mishra J, Raaj R, Sina A (2014) Evaluation of Some Plant Extract Against Predominant Seed Mycoflora Of Mungbean

*Vigna Radiata* (L.) Wilczek Seed. *Life sci. leafl*.,(51) 83- 89.

[40] Jabeen N, Ahmed M, Shaukat S, Salam I (2013) Allelopathic Effects of Weeds On Wheat (*Triticum Aestivum* L.) Germination and Growth. Pak. J. Bot, 45(3), 807-811.

[41]Meena S, Mariappan V (1993) Effect of plant products on seed borne mycoflora of *sorghum Madras Agnl .J* , 80: 383-387.

[42] Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research.John wiley & sons.

[43] Mincuzzi A, Ippolito A (2023) Pomegranate: Postharvest Fungal Diseases and Control, New Adv. Postharvest Technol.

[44] Snowdon AL (1990) A color atlas of Post-harvest diseases and disorders of fruits and vegetables 1. General introduction and fruits, London: Wolfe Scientific.

[45] Grigoryan KM, Hayrapetyan HG, Sarkisyan M, Hakobyan L (2006) Pomegranate (*Punica granatum*) rot causing microscopic fungi, their occurrence in pomegranate juice and toxigenic ability, Toxicol. Lett.164S, S1–S324.

[46] Anonymous (2008) Studies on important diseases of pomegranate. *Annu. Rep*., 2007-08, National Research Centre on Pomegranate Solapur, India, pp24-46.

[47] Nallathambi P, Umamaheswari C (2009) Detection of aflatoxins in pomegranate arils infected by *Aspergillus* species,Indian Phytopath. 62 (2): 178-182.

[48] Adaskaveg EJ (2012) Postharvest Decays of California Pomegranates: Developing Export Market for California; Producers. Department of Plant Pathology, University of California. Riverside.

[49] Palou L, Taberner V, Guardado A, del Rio MA, Monstesinos-Herrero C (2013) Incidence and etiology of postharvest fungaldiseases of pomegranate (*Punica granatum cv. Mollar de Elche*) in Spain. Phytopathol.Mediterr., 52, 478–489.

[50] Ilgın T, Karaca G (2016) Fungal Agents Causing Diseases on Pomegranates Grown in Antalya, Turkey Asian .Agri. Food Sci., 04(06), 286.

[51] Mincuzzi A, Sanzani SM, Palou L, Ragni M, Ippolito A (2022) Postharvest Rot of Pomegranate Fruit in Southern Italy: Characterization of the Main Pathogens. J. Fungi, 8, 475.

[52] Rodrigues BB, Kakde UB (2019) Post-harvest fungi associated with *Solanum lycopersicum* (Tomato) fruits collected from different markets of Mumbai. Int. Multidiscip. Res. J., 9, (01).

[53] Kanetis L, Testempasis S, Goulas V, Samuel S, Myresiotis C, Karaoglanidis GS (2015) Identification and mycotoxigenic capacity of fungi associated with pre and postharvest fruit rots of pomegranates in Greece and Cyprus. Int. J. Food Microbiol., 208, 84–92.

[54] Myresiotis CK, Testempasis S, Vryzas Z, Karaoglanidis GS, Papadopoulou-Mourkidou E (2015) Determination ofmycotoxins in pomegranate fruits and juices using a QuEChERS-based method, Food Chem., vol. 182, pp. 81–88.

[55] Elhariry HM, Khiralla GM, Gherbawy Y, Abd Elrahman H (2016) Natural occurrence of Alternaria toxins in pomegranate fruit and the influence of some technological processing on their levels in juice. Acta Aliment., Vol. 45 (3), pp. 380–389.

[56] Rafińska K, Pomastowski P, Rudnicka J, Krakowska A, Maru´ska A, Narkute M, Buszewski B (2019) Effect of solvent and extraction technique on composition and biological activity of *Lepidium sativum* extracts. Food Chem., 289, 16–25.

[57] Omer AB, Nour AH, Ali MM, Ishag OA, Erwa IY, Ali MA (2020). Phytochemical screening, antimicrobial and antioxidant activity of *Lepidium sativum* seeds extract. South Asian Res. J. Nat. Prod, 3(1), pp.10-17.

[58] Solomon GB, Aman DB (2014). Phytochemical screening and antimicrobial activities of crude extract of *Lepidium sativium* seeds grown in Ethiopia. I.J. Pharmaceutical Sci. Res. 5(10):4182-4187.

[59] Singh CS, Paswan VK (2017). The potential of garden cress (*Lepidium sativum* L.) seeds for development of functional foods. Advances in Seed Biology.

[60] Malara J, Chairmanb K, Singh AR, Vanmathid JS, Balasubramaniana A, Vasanthie K (2014). Antioxidative activity of different parts of the plant *Lepidium sativum* Linn. Biotechnol Rep. 3:95-8.

[61] Raish M, Ahmad A, Alkharfy KM, Ahamad SR, Mohsin K, Al-Jenoobi FI, Al-Mohizea AM, Ansari MA (2016). Hepatoprotective activity of *Lepidium sativum* seeds against D-galactosamine/lipopolysaccharide induced hepatotoxicity in animal model. BMC complementary and alternative medicine, 16,1-11.

[62] Ahmed MEM, El danasoury M, El-khamissi HA, Taie HAA (2023). Phytochemicals, Phenolic Compounds and Antioxidant Activity of Garden cress (*Lepidium sativum* L.) seeds. . J. Agric. Res., Feb.19.

[63] Czapecka E, Mareczek A, Leja M (2005). Antioxidant activity of fresh and dry herbs of some Lamiaceae species. Food Chem,93:223-6.

[64] El-Ghaouth A, Wilson CL (1995) Biologically-based technologies for the control of postharvest diseases.Postharvest News Inf 6:5–11.

[65] Sharma RK, Vyas K, Manda H (2012) Evaluation of antifungal effect on ethanolic extract of *Lepidium sativum* L. seed. Int J Phytopharmacol 3:117–120.

[66] Berehe SG, Boru AD (2014) Phytochemical screening and antimicrobial activities of crude extract of *Lepidium sativum* seeds grown in Ethiopia. Int. J. Pharm. Sci. Res. 5 (10), 4182–4187.

[67] George RE, Thomas SK, Kunjumon M, Thankamani V (2015) Analysis of phytoconstituents and *in vitro* antifungal evaluation of methanolic extract of *Lepidium sativum* Linn.Seeds.Int J Pharm Bio Sci;6:490-7.

[68] Tayel AA, Moussa SH, Salem MF, Mazroua KE, El-Trasc WF (2016) Control of citrus molds using bioactive coatings incorporated with fungal chitosan/plant extracts composite J Sci Food Agric; 96: 1306–1312.

[69] George RE, Thankamani V (2021) Antimicrobial Profiling of *Lepidium sativum* Seed –A Comparative Study with Different Solvent Extracts IOSR, J. pharm. biol. sci. (IOSR-JPBS), 16 (5) Ser. II (Sep.–Oct. 2021), PP 20-24.

[70] Adera F, Yusuf Z, Desta M (2022) Physicochemical Properties and Biological Activities of Garden Cress (*Lepidium sativum* L.) Seed and Leaf Oil Extracts Can. J. Infect. Dis. Med. Microbiol. Article ID 2947836, 8 pages.

[71] Hussaini J, Rehman N, Khan A, Ali L, Kim J, Zakarova A, AL-Harrasi A, Shinwar Z (2014) Phytochemical and biological assessment of medicinally important plant *ochradenus arabicus*.Pak. J. Bot.: 46(6): 2027-2034