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# The Potent Activity of Pomegranate Peel Extract Irradiated with Gamma Radiation against Rice Weevil, *Sitophilus oryzae* (L.)

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**Abstract:** Fruit peel extracts of irradiated *Punica granatum* were inspected under laboratory conditions for their insecticidal activities against the Rice weevil, *Sitophilus oryzae* adults and compared with the extract obtained from unirradiated peels. Both the unirradiated and the irradiated extract caused different mortality, where the irradiated extract was the highest effect. Obtained results revealed that the highest mortality of unirradiated extract at concentration of 8% was 36.7% after 96 hours while irradiated extract with 10 KGy showed highly mortality 93.3% at same concentration and time. The data indicated that unirradiated extract had attractive effect. The results showed the opposite results when filter paper treated with irradiated pomegranate extract, the repellent effect increased with increasing the extract concentration. The highest effect was demonstrated after 2 hours, which the repellency percentage reached 93.3%. The effect of 10KGy of gamma radiation on total phenolic content and antioxidant activity of pomegranate peel powder was studied. Comparing irradiated pomegranate peel powder to unirradiated peel, a substantial rise in the percentage of total phenolic content and antioxidant activity were observed. Also, the components of the ethanolic extract from unirradiated and irradiated pomegranate peel were identified by HPLC analysis.

**Keywords:** *Sitophilus oryzae*, Gamma radiation, Pomegranate peels extract, Repellent, Toxicity, Total phenolic content, antioxidant activity, HPLC.

## 1 Introduction

Stored agricultural products are considered the main target of many pests. It could be attacked by mites, insects, and microbial diseases. Estimated global losses from stored grain insects range from 9% to more than 20% in both developed and developing countries **[**1**].** Rice weevil, *Sitophilus oryzae* is primary major insect infests grains in field and when they are being stored. It has a wide host rang such as wheat, maize, sorghum and many other products. This weevil feeds on grain, which makes it unsuitable for human consumption. This reduces their nutritional quality causing huge losses to farmers **[**2**].** Sharma et al. **[**3**]** estimated that the loss rate in stored rice and grains is 30% and may reach 50% during storage. Larvae and adults of *S. oryzae* are the destructive stages by feeding on rice that causes qualitative and quantitative losses **[**4**],** additionally, lower the product's market worth **[**5**].** Tripathi **[**6**]** asserts that the larvae develops and matures inside the grain, consuming what is inside and leaving irregular holes in all the infected grains.

Chemical insecticides are efficient at reducing stored insect pests, but they damage the environment and could put the amount of natural enemies at risk. Also, insecticidal applications have been done to try and completely control the stored grain pests but in vain. Fumigation, the most commonly used method, is unsafe for humans, animals, and the environment, as well as birds and beneficial insects **[**7**].** In contrast, products derived from specific medicinal plants can be used safely without harming non-target organisms and may even benefit humans **[**8**].** Along with the frequent use of pesticides, numerous insect pests became more tolerable **[**9,10 **].** Therefore, the main focus of scientists around the world is to find safe controlling ways in order to lessen these negative impacts.

Plants are a rich source of bioactive chemicals and may

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offer alternative insect control options, especially for integrated pest management [11, 12]. Plant extracts and pure substances can harm insects in multiple ways such as toxicity, mortality, antifeedant, growth inhibition, suppression of reproductive behavior, decreased fecundity and fertility. Yang and Tang [13] conducted a review of plants used for controlling pest insects. They found a significant correlation between medicinal plants and pesticidal plants.

*Punica granatum*, also known as pomegranate, has garnered attention from researchers for its various applications in medicine and food industry [14,15]. Numerous studies have confirmed the antimicrobial and antifungal properties of extracts from various parts of trees, including bark, leaves, fruit, and fruit peel Al-Zoreky [16] and Voravuthikunchai et al. [17]. Tripathi and Singh [18] and Gandhi et al. [19] have reported on the molluscicidal and insecticidal effects of these extracts. The fruit peels account for approximately 50% of the total weight and are commonly thrown away as waste [20]. According to Yasoubi et al. [21] *P. granatum's* peel extract contains substantial amounts of polyphenols such gallic acid, ellagic acid, and ellagic tannins. After conducting a phytochemical screening, it was discovered that the aqueous extract from pomegranate peels contains flavonoids, tannins, and alkaloids. These positive results suggest that the extract may contain biologically active components that could explain its traditional use [22]. Johnson [23] prepared seven different extracts from pomegranate peel using various solvents (ethanol, methanol, either alone or in combination with acid, acetone and water). The study measured the content of punicalagins and ellagic acid in pomegranate peel extracts using different solvents. The highest amounts were detected using ethanol-acid extract (13.86% and 17.19% w/v respectively), while the lowest levels were obtained with acetone and water extracts.

Food irradiation involves using controlled ionizing radiation, such as gamma radiation, x-rays, and electron beams, to improve hygiene, safety, and reduce the amount of microbes in perishable food products. This process helps to extend the shelf life of these food items. Gamma radiation can cause the radiolysis for water, resulting in the formation of reactive oxygen species (ROS) such as OH•, H•, O•2, and HO•2. These ROS, especially OH•, can damage DNA and other significant molecules, leading to the death of microorganisms. However, they can also impact a plant's antioxidant and ROS levels, modify bioactive components, and cause the accumulation of phenolic compounds [ 24, 25, 26]. Variyar et al. [27] found that after being exposed to gamma radiation (10 KGy), cinnamon and clove showed an increase in phenolic acid content, while nutmeg remained unchanged.

The hope underlying these experiments was to find out if the toxicity of irradiated pomegranate peel extract with gamma radiation as a sustainable natural product for controlling rice weevil, *Sitophilus oryzae.*

## 2 Materials and methods

### *2.1 Insect Stock Culture*

Rice weevil, Sitophilus oryzae were obtained from Entomology laboratory, Faculty of Agriculture at Ain Shams University. Colonies were reared for many generations in plastic jars (600 ml) supplied with about 200g of sterilized healthy wheat grains and approximately 50 pairs of freshly emerged adults were introduced, then kept under constant condition (27 ◦C ±1, 65%±5% RH). The females were permitted oviposition their eggs in the grains, then removed after 24 hours, leaving the egg plugs on the wheat grains. To obtained adults with age ranged 1-7 days, the infested grains with (1 day old) eggs incubated until adult emergence and separated for the experiments.

### *2.2 Preparation of pomegranate peel extracts*

Pomegranates of the Manfaluti cultivar were obtained from a local market, washed, and manually peeled. The peels were dried in shade and ground into a coarse powder to prepare the extracts About 200 grams of powder were extracted by stirring 200 milliliters of 60% ethanol concentration (in 40% water) for 24 hours at 25℃ using a magnetic stirrer. The extract was filtered through filter paper to remove peel particles. The ethanol was left to evaporate at room temperature for 48 hours before diluting the extract to 10% for use.

### *2.3 Irradiation Treatment*

Wight of 200g dried pomegranate peel was irradiated with 10KGy which is recommended for microbial decontamination according to McDonald et al. [28] The experiment was conducted using a Gamma Cell (Co60 source) irradiation unit Model 220 located at the National Center for Radiation Research and Technology (NCRRT). The dose rate during the experiment was 1.106 KGy/hour.

### *2.4 Toxicity bioassay*

Wheat grains (25g) were treated with different pomegranate extract concentrations with distilled water (2, 4, 6 and 8%), for both unirradiated and irradiated extract. Treated grains were submerged in extract for 20 seconds then kept in dry air till dry. Three replicates were used to evaluate the efficacy of plant extract, each replicate consist of 10 adults. Grains were dipped in distilled water only as control. Adults were examined continuously; mortality was recorded each 24 hours for 3 days.

### *2.5 Repellent bioassay*

The effectiveness of pomegranate extract as a repellant was tested using both unirradiated and irradiated samples on a 9 cm diameter filter paper that was split in half. A micropipette was used to evenly distribute 0.5ml of extract at each concentration uniformly (2, 4, 6, and 8%) over one half of the filter paper. For the control group, 0.5ml of distilled water was used in the second half. The filter paper was left to dry for 10 minutes and then placed in a 9cm diameter Petri dish. Ten adult were positioned in the center of the paper. Concentrations were replicated three times. The settled adults were counted after 15, 30 and 120 min. The percentage repellency (PR) formula, as stated by McDonald et al. [28] was used as:

$$\textbf{Eq. (A.1):}\quad PR = \frac{Nc - Nt}{Nc + Nt} \times 100$$

To calculate the average repellency value of each extract, we used the variables Nc (number of insects in the control group) and Nt (number of insects in the treated test group). These values were then assigned to different repellency classes, ranging from class 0 (PR ≤ 0.1%) to class V (PR = 80.1 - 100%). Specifically, class I represented PR values between 0.1% and 20%, class II represented values between 20.1% and 40%, class III represented values between 40.1% and 60%, and class IV represented values between 60.1% and 80%.

## *2.6 Total Phenolic Content*

To determine the total phenolic compounds present in both the unirradiated and irradiated extracts, Folin-Ciocalteu reagent was utilized. The method described by Shahidi and Naczk [29] involved adding 2.5 ml of 10% Folin Ciocalteu reagent and 2 ml of Na CO (2% w/v) to 0.5 ml of each plant extract solution sample (1 mg/ml). The mixture was incubated at 45℃ with shaking for 15 minutes. The absorbance was measured at 725 nm using UV/visible spectrophotometer (Jasco V530, Japan) against a blank sample. The phenolic content of each extract was measured in mg of gallic acid equivalent per gram of extract.

## *2.7 Antioxidant activity*

The antioxidant activity of both plant extracts (irradiated and unirradiated) and the standard were determined based on the radical scavenging effect of stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical activity, according to Gulluce et al. **[**30**].** The working solutions of the test extracts were prepared by diluting them in methanol. As a standard, ascorbic acid was used. A 0.004% DPPH solution was prepared in methanol. Then, 1 ml of the sample solution and 1 ml of the standard solution were mixed separately with 1 ml of the DPPH solution. After being kept in the dark for 20 minutes, the solution mixtures were measured for optical density at 517 nm using a Spectrophotometer. Methanol (1 mL) and DPPH solution (0.004%, 1 mL) were mixed and used as a blank. The optical density was recorded, and the % inhibition was calculated using the given formula:

Eq. (A.2):

$$\textit{Percent (\%) inhibition of DPPH activity} = \frac{A-B}{A} \times 100$$

Where A = optical density of the blank and B = optical density of the sample.

## *2.8 HPLC conditions*

HPLC analysis was conducted on an Agilent 1260 series with a separation using an Eclipse C18 column of dimensions 4.6 mm x 250 mm i.d. and 5 µm. The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A) ; 15-16 min (82% A) and 16-20 (82%A). The detector was monitored at 280 nm and the sample solutions were injected with a 5 µl volume. The column temperature was maintained at 40 ℃.

## *2.9 Stastical Analysis*

The percentage of adult mortality was analyzed using SAS 2001 [31] program. LSD means comparisons were conducted with the Duncan option, and significant differences were determined by one-way analysis of variance. Control mortality was corrected by using Abbot [32] formula as follows:

$$\text{Eq. (A.3):}\quad \textit{\% corrected mortality} = \frac{T-C}{100-C} \times 100$$

Where: T: % mortality in treatment, C: % mortality in check (control).

# 3 Results

## *3.1 Feeding toxicity*

Results of mortality percentages of *Sitophilus oryzae* adult were displayed in Figures (1&2). Unirradiated and irradiated extract caused different mortality, but the irradiated extract was the highest effect. The obtained results showed that the lowest mortality percent when using unirradiated extract with concentration of 2% where as it was 3% after 24 hours and the highest percent was 6.6% after 96 hours from the exposure. The highest mortality was noticed at the concentration of 8% at the same previous exposure periods, where it was recorded 30.0 and 36.6 % after 24 and 96 hours, respectively Figure (1). On the other hand for irradiated extract, substantial mortality was achieved than the unirradiated ones. The mortality percentages recorded at the four exposure periods were 56.7, 73.3, 86.7 and 93.3% at irradiated extract with concentration of 2, 4, 6 and 8% respectively Figure (2). The obtained results were supported with the statistical analysis

where it demonstrated significant differences between the four extract concentrations Table (1).

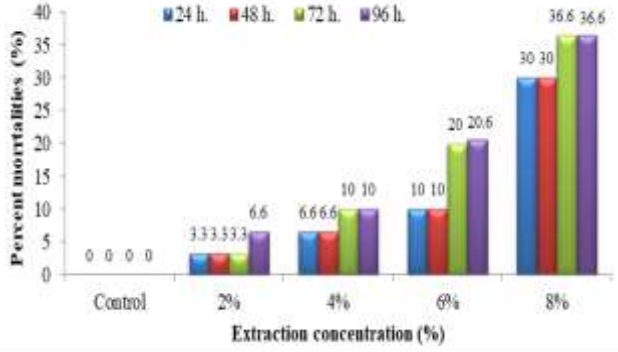


**Fig. 1:** Effect of unirradiated pomegranate extract on *Sitophilus oryzae* adults mortalities during different exposure periods.

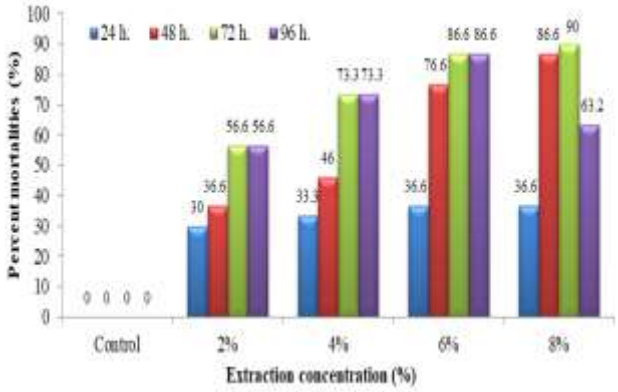


**Fig. 2:** Effect of irradiated pomegranate extract on *Sitophilus oryzae* adults mortalities during different exposure periods.

**Table 1:** Toxicity of unirradiated and irradiated pomegranate extract against *Sitophilus oryza*e adults recorded at four exposure periods.

| Concentration (%) | Mean number of adults mortality ± SE | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Exposure periods | | | | | | | |
| | Unirradiated extract | | | | Irradiated extract | | | |
| | 24 h. | 48 h. | 72 h. | 96 h. | 24 h. | 48 h. | 72 h. | 96 h. |
| Control | 0.0±0.0$^{a}$ | 0.0±0.0$^{a}$ | 0.0±0.0$^{a}$ | 0.0±0.0$^{a}$ | 0.0±0.0$^{a}$ | 0.0±0.0$^{a}$ | 0.0±0.0$^{a}$ | 0.0±0.0$^{a}$ |
| 2% | 0.3±0.3$^{a}$ | 0.3±0.3$^{a}$ | 0.3±0.3$^{a}$ | 0.7±0.3$^{a}$ | 3.0±0.0$^{b}$ | 3.7±0.3$^{b}$ | 5.7±0.5$^{b}$ | 5.7±0.5$^{b}$ |
| 4% | 0.7±0.5$^{a}$ | 0.7±0.5$^{a}$ | 1.0±0.5$^{ab}$ | 1.0±0.5$^{a}$ | 3.3±0.3$^{b}$ | 4.7±0.3$^{b}$ | 7.3±0.3$^{bc}$ | 7.3±0.3$^{c}$ |
| 6% | 1.0±0.0$^{a}$ | 1.0±0.0$^{a}$ | 2.0±0.0$^{b}$ | 2.7±0.3$^{b}$ | 3.7±0.3$^{b}$ | 7.7±0.7$^{c}$ | 8.7±0.7$^{c}$ | 8.7±0.3$^{d}$ |
| 8% | 3.0±0.5$^{b}$ | 3.0±0.5$^{b}$ | 3.7±0.3$^{c}$ | 3.7±0.3$^{b}$ | 3.7±0.3$^{b}$ | 8.7±0.3$^{c}$ | 9.0±0.4$^{c}$ | 9.3±0.3$^{d}$ |
| F test | 7.8 | 7.8 | 19.7 | 17.3 | 36.2 | 53.4 | 40.4 | 89.9 |
| L.S.D$_{5\%}$ | 1.3 | 1.3 | 1.1 | 1.2 | 0.8 | 1.5 | 1.8 | 1.2 |

- *Same letters in the same column indicated no significant differences between extract concentrations.
- The original number of adults who were irradiated and those who were not irradiated was 30 adults/concentration.

## *3.2. Repellent activity*

The repellency effect of unirradiated and irradiated pomegranate extracts against *Sitophilus oryzae* adults is demonstrated in Table (2). Data indicated that non-irradiated extract had attractive effect at all used concentrations except at 2%, where it the highest attractive effect was observed after 2 hours. The percentage of repellency reached 13.3, -20.0, -46.7, -53.3 and -73.3% when the filter paper treated with concentrations of 2, 4, 6 and 8% respectively. Opposite results were observed when filter paper was treated with irradiated pomegranate extract, where with an increase in extract concentration the repellent effect increased. The lowest effect was demonstrated after 15 min. (26.7% at concentration of 2%), while the greatest effect was recorded after 120 min (93.35 % at concentration of 8%). These results may be due to change the chemical composition of pomegranate extract.

**Table 2:** Repellent effects of unirradiated and irradiated pomegranate peel extracts against *Sitophilus oryzae* adults at three exposure periods

| Exposure periods (%) / Concentration | 15 min | | 30 min | | 120 min | | 15 min | | 30 min | | 120 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unirradiated pomegranate extracts | | | | | | Irradiated pomegranate extracts | | | | | |
| | Rp (%) | Rc | Rp (%) | Rc | Rp (%) | Rc | Rp (%) | Rc | Rp (%) | Rc | Rp (%) | Rc |
| 2% | 0.0 | 0 | 6.7 | I | 13.3 | I | 26.7 | II | 33.3 | II | 53.3 | III |
| 4% | -66.7 | 0 | -46.7 | 0 | -20.0 | 0 | 33.3 | II | 60.0 | III | 66.7 | IV |
| 6% | -53.3 | 0 | -53.3 | 0 | -53.3 | 0 | 40.0 | II | 60.0 | III | 80.0 | IV |
| 8% | -66.7 | 0 | -86.7 | 0 | -73.3 | 0 | 40.0 | II | 66.7 | IV | 93.3 | V |

- Rp= Repellency percentage, Rc= Repellency classes
- The original number of adults who were irradiated and those who were not irradiated was 30 adults/concentration.

## *3.3. Total phenolic and antioxidant contents.*

The effect of 10kGy gamma radiation on pomegranate peel extract's total phenolic content is displayed in Table 3 and Figure 3. According to the obtained results, irradiated pomegranate peel extract had a considerably higher proportion of total phenolic content (1.07 mg/g FW) than the unirradiated peel (0.939 mg/g FW). In addition, the antioxidant content has increased from 93.26% on unirradiated peel to 94.05% on irradiated peel (Table 3 and Figure 4).

**Table 3:** The impact of gamma radiation (10kGy) on the total phenolic and antioxidant levels in pomegranate peel powder**.**

| Treatments | Phenolic content (mg/g FW) | Antioxidant content (%) |
|---|---|---|
| Control | 0.939 | 93.26 |
| 10kGy | 1.07 | 94.05 |

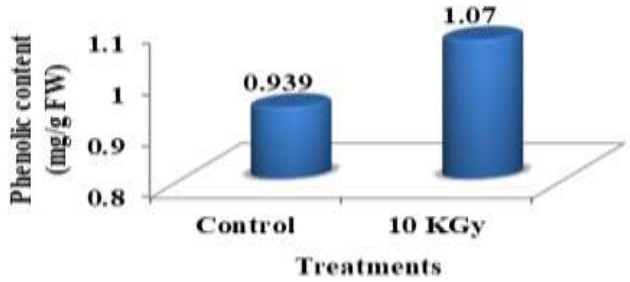


**Fig. 3:** Mean values of Phenolic content in unirradiated and irradiated pomegranate extract.

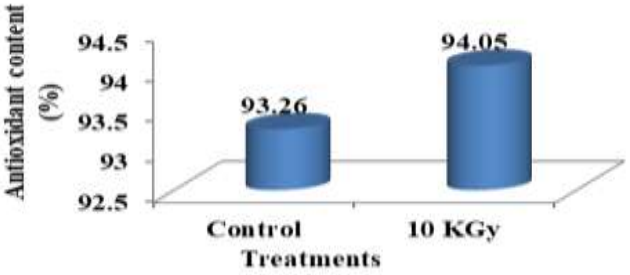


**Fig. 4:** Antioxidant content percent of unirradiated and irradiated pomegranate extract.

## *3.4. Identification of the bioactive compounds of unirradiated and irradiated pomegranate peel extract by HPLC chromatograms*

Identification and quantitative analysis of polyphenolic and flavonoid compounds from pomegranate peel was performed using HPLC. The ethanolic extracts of unirradiated and irradiated pomegranate peel were fractionated into 16 peaks using HPLC. Figures 5-7 and Table 4 display the components.

In the current study, we identified nine phenolic compounds and seven flavonoids in pomegranate peel extract, both irradiated and unirradiated. Table 4 and Figures 5-7 showed that the highest amount of phenolic compounds in both extracts (unirradiated and irradiated ) was Gallic acid (602.19 and 868.12 µg/ml) followed by Ellagic acid (241.92 and 213.44 µg/ml) respectively. Also, the highest amount of flavonoids in both extracts was the Querectin (478.97 and 689.76 µg/ml) followed by Apigenin (348.99 and 530.48 µg/ml), Hesperetin (220.09 and 283.43 µg/ml) and Catechin (165.19 and 194.69 µg/ml) respectively. After analyzing the data, it was observed that a dose of 10 KGy led to an increase in the levels of five phenolic compounds - Gallic acid, Chlorogenic acid, Methyl gallate, Pyro catechol, and Cinnamic acid - compared to the unirradiated extracts. However, the levels of the remaining phenolic compounds decreased. On the other hand all the flavonoids increased in the irradiated pomegranate peel extracts than in the unirradiated extracts except Daidzein (Table 4).

**Table 4:** HPLC chromatograms of the bioactive compounds of unirradiated and irradiated pomegranate peel extract with 10 kGy.

| No. | Compounds | Type | $R_t$ (min) | Standards | | Unirradiated (0 KGy) | | Irradiated (10 kGy) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Area | Conc. (µg/ml) | Area | Conc. (µg/ml) | Area | Conc. (µg/ml) |
| 1 | Gallic acid | Ph. | 3.370 | 173.71 | 15 | 6973.64 | 602.19 | 10053.30 | 868.12 |
| 2 | Chlorogenic acid | Ph. | 4.188 | 365.12 | 50 | 239.27 | 32.77 | 462.07 | 63.28 |
| 3 | Catechin | Fl. | 4.597 | 302.86 | 75 | 667.07 | 165.19 | 786.16 | 194.69 |
| 4 | Methyl gallate | Ph. | 5.592 | 274.81 | 15 | 449.32 | 24.53 | 713.97 | 38.97 |
| 5 | Coffeic acid | Ph. | 6.050 | 234.06 | 18 | 14.00 | 1.08 | 6.56 | 0.50 |
| 6 | Syringic acid | Fl. | 6.583 | 253.65 | 17.2 | 70.14 | 4.76 | 151.47 | 10.27 |
| 7 | Pyro catechol | Ph. | 6.794 | 277.97 | 40 | 3.00 | 0.43 | 9.23 | 1.33 |
| 8 | Rutin | Fl. | 7.984 | 223.90 | 26 | 22.47 | 2.61 | 40.03 | 4.65 |
| 9 | Ellagic acid | Ph. | 8.847 | 647.33 | 120 | 1305.00 | 241.92 | 1151.37 | 213.44 |
| 10 | Coumaric acid | Ph. | 9.168 | 633.94 | 20 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11 | Vanillin | Ph. | 9.781 | 294.73 | 12.9 | 72.35 | 3.17 | 46.15 | 2.02 |
| 12 | Ferulic acid | Ph. | 10.255 | 292.74 | 20 | 85.03 | 5.81 | 47.32 | 3.23 |
| 13 | Naringenin | Fl. | 10.488 | 248.79 | 30 | 0.00 | 0.00 | 0.00 | 0.00 |
| 14 | Daidzein | Fl. | 12.276 | 566.52 | 35 | 861.21 | 53.21 | 0.00 | 0.00 |
| 15 | Querectin | Fl. | 12.761 | 290.40 | 40 | 3477.36 | 478.97 | 5007.76 | 689.76 |
| 16 | Cinnamic acid | Ph. | 14.072 | 542.85 | 10 | 283.13 | 5.22 | 1023.38 | 18.85 |
| 17 | Apigenin | Fl. | 14.547 | 656.85 | 50 | 4584.70 | 348.99 | 6968.91 | 530.48 |
| 18 | Kaempferol | Ph. | 15.043 | 257.92 | 20 | 0.00 | 0.00 | 0.00 | 0.00 |
| 19 | Hesperetin | Fl. | 15.614 | 343.09 | 20 | 3775.66 | 220.09 | 4862.18 | 283.43 |

- Ph. (Phenolic compound), Fl. (Flavonoids)

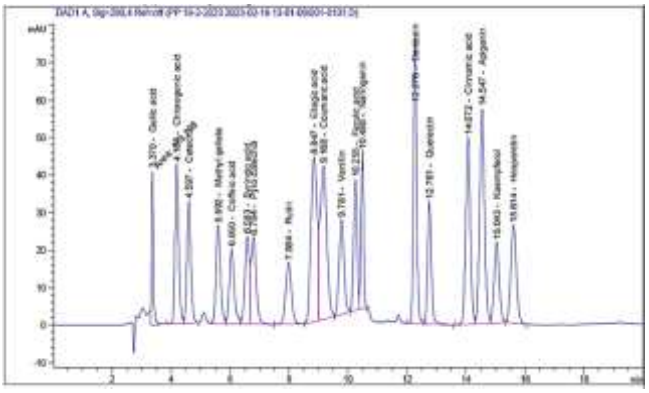

**Fig. 5:** HPLC Chromatogram of standards; Peaks appeared with its retention time sequentially of each compound.

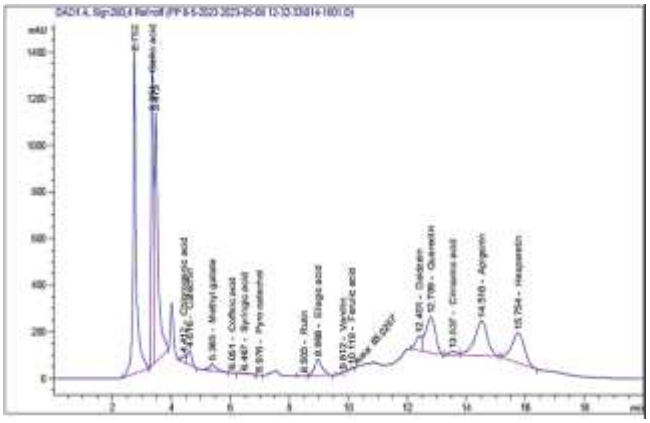

**Fig. 6:** HPLC chromatogram of ethanolic extract of unirradiated pomegranate peel

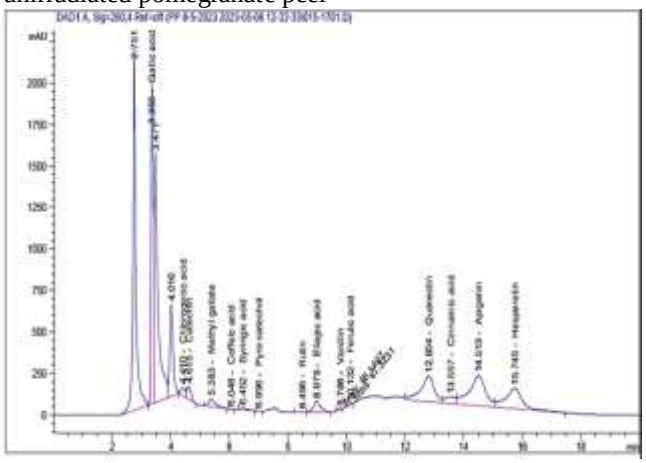

**Fig.7:** HPLC chromatogram of ethanolic extract of irradiated pomegranate peel with 10 kGy.

## 4 Discussion

The effectiveness of plant extracts in controlling stored insect pests has been documented by numerous researchers [33,34,35,36]. In the current study, the ethanolic extract of pomegranate peel was found to significantly reduce the population of *Sitophilus oryzae* adults, demonstrating its effectiveness. This is consistent with the findings of Gandhi et al. [37] for *Tribolium castaneum*, they found that *Punica granatum* was highly insecticidal and protective of seeds. Belmain et al. [38] found that adding 5% w/w dry leaf powder of *Cassia sophera* to cowpea or wheat increased adult mortality of *Callosobruchus maculates* and *Rhyzopertha dominica*. Saljoqi et al. [39] found that a 10% concentration of ethanol extract from banana fruit was effective in repelling and killing *S. oryzae*.

In many African and Asian countries, mixing plant parts with grains is a traditional method to control stored grain pests [40,41]. Plant factors typically exhibit antifeedant, repellent, and growth-regulating effects. In such cases, the insect becomes lethargic, refuses to feed, and does not bore into the seeds. If the plant components are toxic, the insect may only scarify the seeds before dying. However, if the insect is not killed, it may experience a debilitating effect, which could prolong its life cycle. Ultimately, this leads to reduced damage to stored grains [42]. Effects of Plant biochemical components on the developmental growth may lead to minimal damage for stored grains [43]. Rate of mortality increases and support grain protection by increasing the plant extract concentration, When *Rhyzopertha dominica* was treated with pomegranate leaf powder**,** Gandhi and Pillai [44] found that there was a significant death rate and that the rate of development was reduced when they tested *P. granatum* and *Murraya koenigii* on *R. dominica***.**

Plant extracts can penetrate the insect cuticle because they are very lipophilic [45]. The active component of neem, azadirachtin, has various effects on insect pests. It can sterilize them, disrupt the moulting of larvae and nymphs, prevent mating and sexual communication, block the synthesis of chitin, impair fitness, and reproductive activity. Additionally, it alters insect development by inhibiting the release of allatotropins and prothoracicotropic hormones [46]. Although it does not have contact toxicity (as noted by Islam and Talukder [47] and Morgan [48], rotenone is an inhibitor of cellular respiration that exerts toxic effects on nerve and muscle cells. Meanwhile, pyrethrins block voltage-gated sodium channels in nerve axons, and some plant derivatives can inhibit moulting processes, leading to a significant reduction in insect populations [49]; interfere with normal growth processes [50,51].

This is the first report on the effect of gamma radiation on the insecticidal efficacy of *P. granatum* peel extract for the control of *S. oryzae* in stored wheat. The peel of a pomegranate was exposed to gamma radiation at a level of 10 KGy. This dose was determined based on the guidelines set by the FAO/IAEA/WHO Joint Expert Committee on the Wholesomeness of Food Irradiation (JECFI), which states that food irradiation up to 10 KGy is safe and does not require any toxicological testing. According to the World Health Organization (WHO) [52], irradiating food up to 10 KGy did not cause any significant nutritional or microbiological issues. According to the Manual of Good Practice in Food Irradiation, using medium doses (1-10 KGy) is recommended for reducing microbiological contamination in spices and dried food ingredients [53]. In Indonesia, the FDA allows for a maximum absorbed dose of 10 KGy to reduce pathogenic microbes in dried vegetables, seasonings, dry herbs, and herbal teas [54]. There was no observable physical difference between the irradiated and control (0 KGy) samples.

Results of feeding toxicity show that unirradiated and irradiated extract caused different mortalities but the irradiated extract was the highest effect. Additionally, repellent effect increased with increasing the irradiated extract concentration. This may be because the increase of total phenolic content and antioxidants in the irradiated extract, according to the results of the analyses performed on the irradiated and unirradiated extract. Our results

indicated that treatment of wheat grains with irradiated peel extract caused higher toxicity to *S. oryzae* adults than unirradiated peel extract. The obtained results were conformity with the finding of Mali et al. [55], they recorded increasing in total phenolic content that may break the complex components to increase extract toxicity for insect. Kumari et al. [56] achieved close results, where they demonstrated increasing the content of gallic acid and total phenolics because of irradiation that may change the extract toxicity. Results were similar with Hamouda et al. [12] they stated exhibited antifeeding effects of *P. granatum* against *T. castaneum* larvae by utilizing several extracts such as ethanol, methanol and aqueous fruit peel.

When identify the chemical compositions of *P. granatum* by GC/MS, numerous effective components were determined such as tannins, polyphenols and secondary metabolites, flavonoids , phenolics, alkaloids and steroids Mohammad et al. [57] and Tripathi et al. [58]. Biochemical components of *P. granatum* recorded significant effects against *R. dominica, S. oryzae, and T. castaneum* Liu et al. [59]; Nararak et al. [60]. Leaf powder of *P. granatum* recorded significant insecticidal effect on *T. castaneum* that may because of the contents of tannins Hamouda et al. [12]. Nararak et al. [60] and Liao et al. [61] recorded two bioactive components such as Caryophyllene and caryophyllene oxide which inhibit the carboxylesterase, glutathione S-transferase and acetylcholinesterase activities; they also play as repellent activity against *T. castaneum*.

After analyzing both extracts of pomegranate peel using HPLC, the findings indicated that the irradiated extract had a higher concentration of bioactive compounds compared to the non-irradiated extract. This could be attributed to the gamma irradiation process which may have led to the breakdown of the chemical bonds of polyphenols, thus freeing soluble phenols of lower molecular weight [25]. Several studies have shown that gamma irradiation has a positive effect on the phytochemical content, which is in line with our research. When *Ziziphus mauritiana* leaves were exposed to gamma radiation doses of up to 12.5 KGy, there was an increase in the content of phenolic compounds, flavonoids, saponins, and tannins. This is likely due to the release of active compounds from the more complex structures that are degraded by gamma rays, as demonstrated by Khattak and Rahman [62]. Studies have shown that a 10 KGy dose can boost the phenolic content of persimmon and mulberry leaf extracts, as well as increase the isoflavone content of Serbian soybean. Mugwort extract also benefits from this dose, as it enhances the total phenolic and flavonoid content. These findings were documented by Cho et al. [63,64]; Popovic et al. [65] and Hwang et al. [66].

## 5 Conclusions

After processing pomegranate fruits, their peels are usually discarded as waste. However, this waste contributes to severe environmental pollution as it gradually ferments and releases odors due to microbial contamination. The study proved that it is possible to use these peel extract for controlling the rice weevil, because of a high concentration of the total phenolic content in it. Gamma radiation (10 KGy) helps in increasing the proportion of phenols from the peel extract.

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