SHORT COMMUNICATION

Copper level and Fatty acid Composition among Healthy and Cracked fruits of Pomegranate (*Punica granatum* L.) cv. Bhagwa

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Abstract

Punica granatum L. (Pomegranate) is commercially valuable fruit belonging to Punicaceae family. Copper is essential micronutrient that plant require for various processes. Copper level and fatty acid composition was studied in order to find variation among healthy and cracked fruit of *Punica granatum L*. Fatty acid composition of pomegranate leaves was determined using hyphenated GC-MS technique while copper concentration was found using Atomic Absorption Spectrophotometer. Variation among fatty acids was found in healthy and cracked fruit. Predominant fatty acid in healthy fruit was Linolenic acid (40.95%) while Palmitic acid (41.38%) was in highest proportion in cracked fruit. Stearic acid was found absent in cracked fruits. Findings from AAS showed that excess copper (1.77ppm) was found in leaves of plant bearing cracked fruit. Variation in fatty acid and absence of stearic acid at early developing stage can be used for possible cracking detection in pomegranate fruit also, high copper level increase cracking in pomegranate, so pesticides and fungicide containing copper should be used in adequate amounts in order to reduce cracking in pomegranate fruits.

Keywords: *Punica granatum* L., Fattyacids, Copper, Cracking, GC-MS

Highlights

- Pomegranate varieties differ in fatty acid composition.
- Fatty acid composition and copper level were identified in pomegranate bearing healthy and cracked fruits.
- Predominant fatty acid in healthy fruit is Linolenic acid (40.95%) while Palmitic acid (41.38%) is in highest proportion in cracked fruit.
- Excess copper was found inleaves of plant bearing cracked fruit.

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Introduction

Punica granatum L. is a commercially valuable fruit belonging to the Punicaceae family, also known as pomegranate. The plant is grown especially in the Mediterranean region, cultivated extensively in countries like India, Afghanistan, Iran, Saudi Arabia and Pakistan (Bakeer, 2012). Fruit cracking is the top problem that negatively affects pomegranate production and quality (Singh *et al*., 2020). Splitting of pomegranate is a trouble around its producing regions over the world but its strength of hassle depends on weather, vareity, heredity, fruit growth and other practices. (Saad *et al*., 1988).

Copper is an important element for various metabolic processes. As it is a micronutrient, less copper is needed and becomes toxic at a higher level. (Delas, 1963; Alva and Chen, 1995). According to (Karataglis and Babalonas, 1985), with increased Cu concentration, plant height, biomass of shoots and roots, output of flowers and fruits all are reduced. Fatty acids and lipids are important as well as fundamental constituents in every plant cell; they serve as mediators for signal transduction and provide energy and structural integrity for various metabolic functions. Fatty acids and lipids can function as extracellular and intracellular signals, respectively. Additionally, acylic and cyclic products made during the metabolism of fatty acids can serve as helpful, significant chemical signals (Lim *et al*., 2017). Common fatty acids present in plant lipids include palmitic acid, stearic acid, oleic acid, linolenic acid and linoleic acid (Moire *et al*., 2004). Among these, fatty acids that carry double bonds between two unsaturated carbons such as oleic acid, linolenic acid and linoleic acid play vital roles in plant protections (Walley *et al*. 2013). Due to elevated levels of linolenic and linoleic acid, high resistance was obtained for *Colletotrichum gloeosporioides* in avocado and *Pseudomonas syringae* in tomato (Madi *et al*., 2003; Yaeno, Matsuda *et al*., 2004). Linoleic Acid has several therapeutic applications like anti-inflammatory, acne reductive, skin-brightening, and moisture-retentive properties (Walley *et al*.,2013; Adiguzel *et al*., 2006; Moire *et al*., 2004; Ozen *et al*., 2004).

Current developments in the fields of horticulture and biological sciences are opening up powerful avenues for accurately identifying genetic and biochemical variations among plant species.

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Since the 1990s, phylogenetic relationships have been analyzed using techniques like FAME (fatty acid methyl esters) and random amplified polymorphic DNA (RAPD) (Adiguzel *et al*., 2006; Harris ,1995; Ozen *et al*., 2004; Williams *et al*., 1993).

The study aims to find variation among fatty acid and copper levels in two conditioned pomegranate using the hyphenated technique GC-MS and AAS.

Materials And Methods

Sample collection for *Punica granatum L.* leaves

In end of January 2021, leaves of *Punica granatum L*. were gathered from the village of Lohariya mota in Anjar town of Kachchh district .Location coordinates lies at 69°86'E longitude and 23°09'N latitude. The sampling site experiences an arid climate with an average of 412 mm of annual rainfall. The soil had 1.68% organic carbon and were non saline and slightly alkaline, containing pH 7.8 and EC value 1.07dSm^{-1}. Two distinct orchards were chosen, one with plants that produced healthy fruit and another with plants that had nearly all of their fruits cracked. Location of both the orchards lies at 1.5 km apart. Both the orchards contained two-and-a-half-year-old plants with drip irrigation systems. Leaves from all round the plants from both varieties were collected, washed with water, air dried using lab sample grinder.

Determination of copper (Cu^{2+}) using AAS

Sample preparation for AAS was carried out according to methodology given by (Pequerul et al. 1993). 10mL nitric acid was added to 1g Leaves powder in dry flask and stirred well, then 8mL hydrogen peroxide was added carefully in the fume hood, heated on hot plate till the evolution of brown fumes, solution was cooled and diluted with distill water up to 50 mL. This solution were analyzed for Cu element using Absorption Spectrometer Shimadzu AA-7000 and a calibration curve was obtained using Merck Copper (Cu) solution.

Extraction, Isolation and Identification of Fatty Acids from the leaves of *Punica granatum L.*

50g of dried leaves powder of both varieties of *Punica granatum L*. were soaked overnight in petroleum ether and continously extracted for 8 hours using soxhlet extraction appartus. After extraction procedure solvent was distilled off in reduced pressure. The crude obtained was subjected to saponification using 20% KOH in methanol, and excess methanol was distilled. The saponified crude obtained was diluted with 100 ml water and obtained aqueous phase was extracted with diethyl ether (50 mL). The dried fatty acids obtained were further used.

Fatty acid methylation was performed using 1g fatty acid mixture and heated with 100mL mixture of methanol: Benzene:Con.H_2SO_4 for eight hours at 80 to 90˚C over water bath under long air-condenser stream. After cooling the reaction mixture, 25 mL solution was extracted with n-hexane. Methyl esters of fatty acids was dried over mixture containing 1:3 sodium bicarbonate and anhydrous sodium sulphate .The dried mixture of esters of fatty acid were stored and utilized for further analysis.

Identification of Fatty acids from *Punica granatum L.* leaves using GC-MS

Methyl esters of fatty acid were taken in minimum quantity and dissolved in minimum amount of chloroform. GC–MS analysis were carried out on a gas chromatography shimadzu QP 2010 GC coupled with mass spectrometer with electron impact ionization with energy (70 eV). Capillary column of (30 × 0.53 ID × 3 µm) was used. For the analysis, a split mode injection of 2 µL sample was performed using helium gas as carrier gas, flowing at a rate of 1.4mL/min with ion source temperature of 230˚C and injector temperature of 250˚C. Mass spectra with fragments from 60 to 1000Da and scan interval of 0.5s were obtained at 70eV. GC-MS ran for 26 minutes in total .The obtained peaks in the spectrum were interpreted. The component's names, molecular weight and structures were determined by comparing the obtained unknown peaks with wiley library. Each fatty acid's percentage were determined by comparing total area and area occupied by fatty acids .

Results And Discussion

Copper (Cu^{2+}) levels in leaves of *Punica granatum L.* bearing healthy and cracked fruit

Cu levels in leaves of plant containing Healthy and cracked fruit is shown in (Table 1). Elemental variation for various plant depend on water, fertilizers used and soil content, in addition to that absorbility of plant to uptake various elements. Therefore nature of plant is responsible for variation in concentration of different elements.(Rajurkar and Damame, 1997).Results indicated Excess of Copper concentration in cracked fruit (1.77ppm) in comparision to healthy fruit(1.61ppm) (Table 1).

According to (Bergmann, 1992), the formation of the chloroplast involves copper, where copper play crucial role for the production of protiens ,carbohydrates and nitrate reduction It play major role in demonstrating chlorophyll and shielding it from premature destruction within plant cells which has an impact on the photosynthesis process and synthesis of several enzymes including tyrosinase and cytochrome oxidase. (Al-Naimi,1999;Emadi,1991).According to (Hussein and Hasan, 2020) spraying of fluoratone and copper at concentration of 0.5g L^{-1} +1gL^{-1} reduced cracking to great extent.

GC-MS of Fatty acids methyl esters obtained from the leaves of *Punica granatum L.*

Figs 1 and 2 depicts the results of gas chromatography combined with mass spectrometry analysis of fatty acids in leaves of plant bearing healthy and cracked fruit .The healthy and cracked fruit were variated among presence and absence of four main fatty acid and its relative proportions.

Table 1: Concentration of Cu^{2+} (ppm) in leaves of pomegranate plant

No.	*Name*	*Copper (Cu^{2+}) concentration (ppm)*
1	Leaves of plant that produces healthy fruit	1.61
2	Leaves of plant that produces cracked fruit	1.77

Table 2 : Fatty Acids in leaves of plant producing cracked and healthy fruit

Fatty Acids							
Leaves of plant bearing Healthy fruit				*Leaves of plant bearing cracked fruit*			
Peak No.	*Retension Time*	*Name*	*Percentage (%)*	*Peak No.*	*Retension Time*	*Name*	*Percentage (%)*
1	13.939	Palmitic acid	32.52	1	13.937	Palmitic acid	41.38
2	15.010	Linoleic acid	16.65	2	15.010	Linoleic acid	27.20
3	15.038	Linolenic acid	40.95	3	15.037	Linolenic acid	31.42
4	15.233	Stearic acid	9.87	-	-	-	-

(-) = Absence of Fatty Acid

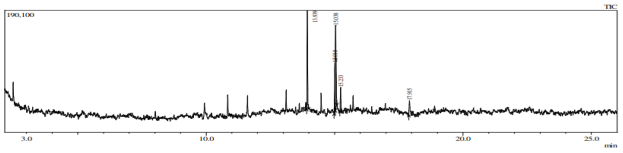

Fig. 1: TIC chromatogram of fatty acids in leaves of plant producing healthy fruit

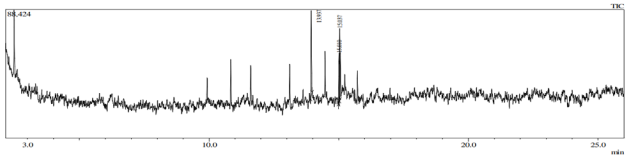

Fig. 2: TIC chromatogram of fatty acids in leaves of plant producing cracked fruit

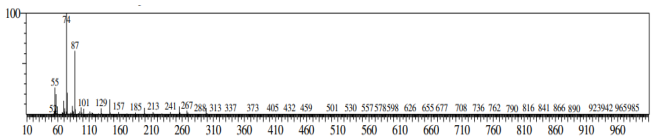

Fig. 3: Mass spectrum of Stearic acid found in healthy fruit

Results of fatty acid analysis revealed that leaves of both variety contained three common compounds. In healthy fruit, linolenic acid was predominant fatty acid (40.95%) followed by Palmitic acid(32.52%),Linoleic acid(16.65%) and stearic acid (9.65%) (Table 2) (Fig. 3). In cracked fruit the predominant fatty acid were Palmitic acid (41.38%) followed by linolenic acid(31.42%),Linoleic acid(27.42%) (Table 2). All the obtained fatty acid have medicinal properties. Linolenic acid methyl ester and palmitic acid methyl ester composed almost 70% of total fatty acid. Solvent peaks are excluded and fatty acid peaks are only shown in the chromatogram. According to (Ando *et al*., 1998; Darmstadt *et al*., 2002; Letawe *et al*.,1998).Anti-inflammatory, acne-reducing, skin-lightening and moisture retaining qualities are all carried by linoleic acid.

Table 2 : Fatty acids present in leaves of *Punica granatum* L. producing cracked and healthy fruit.

In both variety (Healthy and cracked fruit) composition of fatty acids in leaves were same but with different proportion except absence of stearic acid in leaves of plant bearing the cracked fruit. Despite reports of caprylic acid being the predominant fatty acid in sweet Egyptian pomegranate ,but we could not confirm its presence (El-Nemr *et al*., 1990). Fatty acid analysis can be one of the useful parameter to identify healthy and cracked fruit as linolenic acid is predominant in healthy fruit while palmitic acid is predominant in cracked it.

Conclusion

From the current study it can be concluded that high amount of copper in comparison with healthy fruit was found in cracked fruit .Excess copper levels harm the plant growth, So fertilizers and fungicides containing copper should be used in adequate amount as excess may lead to cracking of pomegranate fruit. Plant-bearing healthy fruit contained four different fatty acids while three fatty acids were present in cracked fruit. The predominant fatty acid in healthy fruit was linolenic acid while palmitic acid was in the highest proportion in cracked fruit, stearic acid was found absent in cracked fruit. Fatty acid analysis can be one of the useful parameters to identify healthy and cracked fruit as linolenic acid is predominant in healthy fruit while palmitic acid is predominant in cracked fruit.Moreover as palmitic acid,linolenic acid and stearic acid have many applications in the field of cosmetics and medicine, so these fatty acids can be used in various fields.

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Author Contribution

Author Pooja Mesurani was involved in planning and author Vijay Ram supervised the work. Pooja Mesurani did experimental work ,carried out analysis and author Somiya ajani and Author Pooja Mesurani drafted manuscript.All the three authors discussed results, reviewed it and approved the manuscript with mutual consent.

Conflict Of Interest

None

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