Original Article
DOI: https://doi.org/10.70749/ijbr.v3i2.689

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

The Influence of Gibberellic Acid (GA_3) Application and Partial Coating on Pre and Postharvest Performance of Grapefruit

Sara Nisar[1], Nadia Samad[2], Sidra Mukhtar[2], Imran Qazi[3], Qazi Shoaib Ali[1], Ayesha Zahir[4], Nagina Zeb[2], Hajra Shah[2], Muhammad Sohaib Khattak[1], Shehla Gul Khan[1]

[1]Department of Horticulture, University of Agriculture, Peshawar, KP, Pakistan.
[2]Agricultural Research Institute, Tarnab, Peshawar, KP, Pakistan.
[3]Pakistan Agricultural Research Council, Adaptive Research cum Demonstration Institute, Wana South Waziristan, KP, Pakistan.
[4]National Integrated Development Association, (NIDA) Pakistan.

ARTICLE INFO

Keywords
Grapefruit, Gibberellic Acid (GA_3), Yield, Fruit Quality, Partial Coating, Shelf Life.

Corresponding Author: Imran Qazi, Pakistan Agricultural Research Council, Adaptive Research cum Demonstration Institute, Wana South Wazristan, KP, Pakistan.
Email: hortus.qazi@gmail.com

Declaration
Authors' Contribution: All authors equally contributed to the study and approved the final manuscript. *Detail is given at the end.
Conflict of Interest: No conflict of interest.
Funding: No funding received by the authors.

Article History
Received: 03-01-2025
Revised: 13-02-2025
Accepted: 17-02-2025

ABSTRACT

Gibberellic acid (GA3) a widely used plant growth regulator is involved in regulating the processes of growth and development such as, vegetative growth, bud dormancy, fruit set, increasing yield, fruit quality, fruit ripening and optimization of fruit storage. This study was conducted at Horticulture Research Farm, and Postharvest Laboratory, The University of Agriculture Peshawar, during winter (2018-19). Pre-harvest research trial was planned as Randomized Complete Block Design (RCBD) having one factor. GA3 foliar spray of different concentrations, i.e 0, 15, 30 and 45 ppm were applied on grapefruits. Two factorial experiment by using Completely Randomized Design (CRD) was carried out in laboratory. Grapefruits were partially coated with coconut oil and stored for 60 days. Data was taken at 20 days interval. Results showed that GA3 concentrations significantly influenced all the quality attributes. Fruits treated with 45 ppm GA3 showed maximum days to maturity, number of fruits tree^{-1}, yield tree^{-1} (kg), with less total soluble solids (^{o}Brix), maximum ascorbic acid (mg.100g^{-1}), less reducing sugars (%), maximum non-reducing sugars (%) and least weight loss (%). All the quality attributes were significantly influenced by various intervals of storage, which showed maximum total soluble solids (^{o}Brix), with less ascorbic acid (mg.100g^{-1}), maximum reducing-sugars (%), less non-reducing-sugars (%) and maximum weight loss (%) were recorded in fruits stored for 60 days. The interactive effect of storage duration and GA3 concentrations exhibited non-significant effect on all attributes except weight loss and ascorbic acid. It is inferred that grapefruits can be sprayed with 45 ppm GA3 at physiological maturity stage and coated with coconut oil to better retain the quality attributes and increase the storability of fruits.

INTRODUCTION

Grapefruit (*Citrus* × *paradisi*) an important member of the genus citrus, belongs to the family Rutaceae with semi-sweet to sour or slightly bitter fruits. (Sean *et al.*, 2003). It thrives well in warm and subtropical climate. The citrus fruits are highly valued and widely cultivated in the world. Citrus is ranked second in world and 1st in Pakistan with area of approximately (206,569 ha) and production (2.36 million tons) among the fruits respectively. Citrus constitutes about 30% of the total fruit production in our country. In Pakistan, 95% of citrus production is in Punjab because of the well-suited temperature and environmental conditions of the region. In KP it is mostly cultivated in the areas of Peshawar, Mardan, Swat, Swabi, Nowshera, Hazara.

The grapefruit is highly cherished for its nutrional and therapeutic values. It contains higher amount of nutrients, vitamin C, carotenoids, antioxidants and fiber. It also has other compounds like eriocitrin, hesperidin, nidymin, nairutin, naringin, neohespiridin, neoeriocitrin, and poncirin. It plays a crucial role in lowering glucose and cholesterol levels in body. It uplifts and energizes the body functions and nourishes the skin, also supports immune system of the body by increasing body's natural defense. Its fragrance helps in reducing depression from our mind. Moreover, grapefruit helps in reducing body weight, aids in preventing arthritis, cancer and is also used as an antiseptic. Also, it is commonly used for common cold and fever treatment (Box, 1993).

The yield and quality of grapefruits is affected by both pre and postharvest problems. Citrus fruits are susceptible to temperature changes, pathogenic attacks, pre-harvest fruit drop, various diseases and problems from blooming till harvesting stage, which greatly affect its yield, production and quality, causing the larger yield gap. Fruit drop, postharvest pathogenic attacks and microbial attacks reasonably deteriorate fruit quality and also decrease its market value. (Tripathi and Dubey, 2003). The excessive supply during peak production is another cause of lower prices and less income. Therefore, storage is required to extend the availability and to fetch good prices. Storage losses increase when fruits are kept in undesirable conditions that change the metabolism pathway (Holland *et al*., 2005) making undesirable physiological changes, which damages 10-30% of the total yield of crop (Kader, 2002). A substitute for storage is picking the fruits early or late in season to outstretch marketing season. However, it may adversely affect fruit quality and storability (Holland *et al*., 2002).

Thus, the use of plant growth regulators (PGR's) is an effective alternative to increase the crop production and extend storage life. Plant growth regulators are enormously used in horticulture to improve growth and yield of plants by enhancing fruit set, increasing fruit number and size of fruits. The use of gibberellins is gaining importance, as they play a role to balance the improper source–sink relationships and other hormonal imbalances in plants that may occur during growth (Shah *et al*., 2006). Chemically, gibberellins are a group of plant hormones, actually acids found in most plant tissues. They play a role inside the plant body in such a way that activates several enzymes which play a significant role for growth and the development of the plants and other processes, such as germination, seed set, stimulate cell and stem elongation, leaf area expansion, flowering, affecting flower morphology, earliness, fruit set, ion transport and osmoregulation, fruit development, rind firmness, color development, delay fruit ripening, improve fruit quality at harvest, extend shelf life, reduce disease incidence, improve fruit size, yield, and market outturns. (Hedden *et al*., 2015; Azuma *et al*., 1997; Yang *et al*., 1992).

Use of GA_3 to improve crop productivity or to control postharvest quality has been evaluated in many fruits and vegetables (Aharoni *et al*., 1993; Yu *et al*., 2006; Singh *et al*., 1995). The preliminary application of gibberellic acid from "color break", delays senescence related processes as reduction in peel firmness and maintains the chemical composition of flavedo, peel color and peel oil (Greany *et al*., 1991). GA treated fruits are also less susceptible to the late season abscission, postharvest decay and have desirable flavor, rind size, quality, peel properties for at least three months longer than the untreated fruits, and are been in use to control and retard the postharvest changes in fruits, vegetables, and flowers (Aharoni *et al*., 1993; Yu *et al*., 2006). The effects of GA are dependent on both the timing of application and the concentration of GA applied.

On the other hand to reduce moisture loss and avoid fruit puffiness, fruits are washed and waxed/oil coated as soon as possible after harvest for better marketability. Oil coating acts as a semipermeable physical barrier against O_2, CO_2, solute and moisture movement, reducing the water loss and rate of respiration. Referring to some previous studies of (Shein *et al*., 2008; Bayindirli *et al*., 1995; Sadasivan *et al*., 1974), fruit coating is considered as cheaper alternative method to maintain shelf life of citrus species. The non-chemical measures of disease control are generally preferred in postharvest disease suppression (Al-Obeed and Harhash, 2006). It is documented that surface coating can alleviate fruit weight loss upto 50%, depending on the type of coating and its concentration. Furthermore, it can retain the freshness of the product and enhance their appearance (Baldwin, 1994). A primary disadvantage of (full) coatings is the development of off-flavor, which can be unfavorably affected by the coating for reasons regarding permeation of gases between the peel and external environment (Hagenmaier and Baker, 1993). In anaerobic conditions, excessive build-up of volatile compounds such as acetaldehyde and ethanol in citrus fruits can result in the production of off-flavor (Chen and Nussinovitch, 2000, 2001; Shaw *et al*., 1991). Thereby better option for coating is doing partial coating of fruits. Coating the fruits provide more resistance to pathogens and increasing their storage and marketing (Petracek *et al*., 1998; Park, 1999).

So, it is concluded from the above studies that for good pre and postharvest quality of grapefruit gibberellic acid foliar spray and oil coating are reasonable alternatives and a better approach to keep the nutrients in fruits at optimal level and also maintaining their freshness as compared to the other expensive methods used.

MATERIALS AND METHODS

Experimental Site

An experiment entitled "The influence of gibberellic acid (GA_3) application and partial coating on pre and postharvest performance of grapefruit" was performed at Horticulture Research Farm, and Postharvest Laboratory, The University of Agriculture Peshawar, Khyber Pakhtunkhwa in winter (Late Sep 2018 - Feb 2019).

Pre-Harvest Experiment

Pre harvest research trial was planned as Randomized Complete Block Design (RCBD) having one factor. Total 12 grapefruit trees of variety "Reed" were selected. First each tree was sprayed with distilled water to check the amount of spray required. The gibberellic acid was applied on trees as a foliar spray having concentrations of 0, 15, 30, 45 ppm at physiological maturity stage.

Gibberellic Acid Spray Preparation Procedure: For making GA_3 solution, 15, 30 and 45 mg GA_3 were diluted in water in separate beakers and then raised the volumes upto 1 liter respectively. But according to the requirement of individual tree, 2 liters solution was made for each tree, so all the three concentrations were taken in double amount and diluted in 2 liters of water respectively. The solution was applied through a manual spray. Treatments were repeated thrice. The treated fruits harvested from the Horticulture Research Farm at edible maturity stage were brought to Postharvest Laboratory and were analyzed for different qualitative parameters.

Storage Experiment: Two factorial experiment by using Completely Randomized Design (CRD) was carried out in laboratory. The treated fruits with GA_3 levels (0, 15, 30 and 45 ppm) were kept for 60 days of storage with data recording interval of 20 days. The partial coating with "coconut oil" was done on equatorial region of the fruits. All the treated fruits were stored at of 16 ± 1°C temperature with 56-58% humidity level.

The studied pre and postharvest quality attributes included days to maturity, number of fruits tree^{-1}, yield tree^{-1} (kg), total soluble solids (TSS), ascorbic acid (mg/100g), reducing sugars (%), non-reducing sugars (%) and weight loss (%).

Days to Maturity: Days to maturity were calculated for fruits from all the trees and average was taken for the replicates from each treatment.

Fruit Number Tree^{-1}: Number of fruits tree^{-1} were counted and average was taken for the replicates of each treatment.

Yield Tree^{-1} (kg): Yield tree^{-1} (kg) was calculated by weighing the fruits at the time of harvesting from each treatment and average weight was taken for respective replications.

Total Soluble Solids (°Brix): Total soluble solids were determined through hand refractometer (Model ATAGO, Japan). Through squeezer, fruit juice was extracted and was passed from the mesh. All the seeds were removed with the help of mesh. Then the juice drops were poured on the hand refractometer and the data was noted for each treatment per replicate and then average was taken.

Ascorbic Acid (mg/100g): Ascorbic acid was determined in fruit juice by using the Dye method described by (Rangana, 1977).

Reducing Sugars (%): Reducing sugars were determined through following reagents.

Fehling-A Solution: It was prepared from copper sulphate. 34.65 gm of copper sulphate was mixed in 500 ml distilled water to make Fehling-A solution.

Fehling-B Solution: 173 gm sodium potassium nitrate, and 50 gm of sodium hydroxide were added in beaker and then diluted with distilled water to make 500 ml volume.

Methylene Blue Indicator: 1 gm methylene blue indicator was added in 100 ml distilled water to make indicator.

Procedure: Grapefruit juice was prepared. After juice preparation 10 ml juice was added in a graduated cylinder. Volume of solution in graduated cylinder was upraised up to 100 ml through distilled water. Then the solution was poured into the burette. Then in a flask a 5 ml of Fehling-A and B solution along with 10 ml distilled water were mixed. The solution was boiled. Little drops from the burette were added in the flask solution until the red-brick color appeared. 1-2 drops of methylene-blue as indicator were added to the flask until the appearance of red-brick color.

Calculation: 5 ml of Fehling-A and 5 ml of Fehling-B = X ml of 10% syrup-solution = 0.05 g of reducing-sugars.

100 ml of 10% sample solution contains = $\frac{0.05 \times 100}{Xml}$

= Y gm of reducing sugars

% of reducing-sugars = $\frac{Y \times 100}{10}$

Non-Reducing sugars (%): Non-Reducing sugars concentrations were calculated by using the Lane and Eynon procedure, as described in AOAC (2012), method No. 920.184. Following reagents were used to determine the non-reducing sugars.

Fehling-A solution: 34.65 gm of copper sulphate was dissolved in 500 ml distilled water to make Fehling-A solution.

Fehling-B solution: 173 gm sodium potassium titrate, and 50 gm of sodium hydroxide were added in beaker and then dissolved with distilled water to make 500 ml volume.

Indicator: Methylene-blue indicator was used as indicator.

Procedure: Grapefruit juice was prepared and 10 ml sample was transferred to a graduated cylinder. Volume of sample was upraised up to 100 ml by addition of distill water. 20 ml of sample was added in a flask with 10 ml of 1 normal hydrochloric acid (HCL) solution. This solution was heated for about 5-10 minutes, when cooled then 10 ml of 1N NaOH was poured and 250 ml of distilled water was added so that the volume reached the mark and then 5 ml of Fehling-A and B solutions were added in 10 ml distilled water and boiled. The solution was titrated against sample solution in burette until its boiling and red-brick color appearance. Indicator methylene-blue was added to test the sample solutions and red color presence.

Calculation: X ml of syrup solution contains = 0.05 g of reducing-sugars

250 ml of syrup solution contains = $\frac{250 \times 0.05}{Xml}$

= Y gm of reducing-sugars

250 ml of syrup solution was made from 20 ml of 10% sample solution

So, 20 ml of 10% sample solution contains Y gm of reducing-sugars

100 ml of 10% solution contains = $\frac{Y \times 100}{20}$ = P gm reducing-sugars

10 ml of sample solution contains P gm of reducing-sugars

100 ml sample solution contains = $\frac{P \times 100}{10}$ = Q gm of total reducing-sugars

Q gm of total reducing-sugars = Inverted-sugars + Free reducing-sugars

Non-reducing-sugars = total reducing-sugars – Free reducing-sugars

Weight loss (%): Following formula was used to determine weight loss.

$$WL\ (\%) = \frac{\text{Fresh weight- After interval weight}}{\text{Fresh fruits weight}} \times 100$$

Statistical Analysis

The recorded data were analyzed by analysis of variance (ANOVA) method for finding the difference among different treatments and their interaction. LSD test was applied for significant results at 1% probability level (Steel *et al*., 1997) of the studied attributes. (Statistix 8.1) software was used for the determination of ANOVA and LSD.

RESULTS

Mean data regarding days to maturity of fruits, number of fruits tree^{-1} and yield tree^{-1} (kg) are represented in Table 1. Different concentrations of GA_3 significantly influenced days to maturity of fruits, number of fruits tree^{-1} and yield tree^{-1} (kg).

Maximum days to maturity of fruits (81.66), number of fruits tree^{-1} (159.33), yield tree^{-1} (51.25 kg) were noted in fruits applied with 45 ppm GA_3. On the contrary minimum days to maturity (54.33), number of fruits tree^{-1} (105.33), and yield tree^{-1} (27.17 kg) were recorded for untreated fruits.

Mean data regarding total soluble solids (°Brix), ascorbic acid (mg.100g^{-1}), reducing sugars (%), non-reducing sugars (%) and weight loss (%) are represented in Table 2. Different concentrations of GA_3 and storage duration notably influenced total soluble solids (°Brix), ascorbic acid (mg.100g^{-1}), reducing sugars (%), non-reducing sugars (%) and weight loss (%). While the interactive effect of GA_3 levels and storage duration exhibited non-significant effect on all of the postharvest quality attributes except ascorbic acid and weight loss.

Regarding GA_3 concentrations, maximum total soluble solids (11.36°Brix) were recorded in untreated fruits while minimum total soluble solids (10.56°Brix) were noted in 45 ppm GA_3 treated fruits. In case of storage duration, total soluble solids were maximum (11.47°Brix) in fruits kept for 60 days, as compared to minimum (10.48°Brix) in freshly harvested fruits. The maximum ascorbic acid (45.47 mg.100g^{-1}) was recorded in fruits treated with 45 ppm GA_3 while minimum ascorbic acid (40.55 mg.100g^{-1}) was noted in untreated fruits. Ascorbic acid was maximum (47.70 mg.100g^{-1}) in freshly harvested fruits while minimum ascorbic acid (38.60 mg.100g^{-1}) was recorded in fruits stored for 60 days. The maximum reducing sugars (6.66%) were observed in fruits at control while minimum reducing sugars (5.69%) were noted in 45 ppm GA_3 treated fruits. Reducing sugars were maximum (6.80%) in fruits stored for 60 days while minimum reducing sugars (5.65%) were recorded in freshly picked fruits. The maximum non-reducing sugars (12.35%) were recorded in fruits sprayed with 45 ppm GA_3 while minimum non-reducing sugars (11.81%) were observed in unsprayed fruits. Non-reducing sugars were maximum (13.72%) in freshly harvested fruits while minimum non-reducing sugars (10.51%) were calculated in fruits kept for 60 days. The maximum weight loss (9.07%) was recorded in untreated fruits while minimum weight loss (5.06%) was recorded in fruits sprayed with 45 ppm GA_3. In case of storage duration, weight loss was maximum (14.87%) in fruits stored for 60 days, as compared to no weight loss (0.00%) in freshly harvested fruits.

Table 1

Days to maturity, number of fruits tree^{-1} and yield tree^{-1} (kg) as affected by various concentrations of GA_3.

GA_3 conc. (ppm)	Days to maturity	Number of fruits tree^{-1}	Yield tree^{-1}(kg)
Control	54.33 D	105.33 D	27.17 D
15	60.00 C	121.33 C	34.14 C
30	70.33 B	137.00 B	40.84 B
45	81.66 A	159.33 A	51.25 A
LSD	2.80	7.73	3.84

Means having different letters are significantly different from each other at $P \leq 0.01$.

Table 2

Total soluble solids (°Brix), ascorbic acid (mg.100g^{-1}), reducing sugars (%), non- reducing sugars (%) and weight loss (%) as affected by different concentrations of GA_3 and storage duration.

GA_3 conc. (ppm)	Total soluble solids (°Brix)	Ascorbic acid (mg/100g)	Reducing sugars (%)	Non-Reducing sugars (%)	Weight loss (%)
Control	11.36 A	40.55 D	6.66 A	11.81 C	9.07 A
15	11.11 AB	43.45 C	6.39 B	11.96 C	7.82 B
30	10.85 B	44.27 B	6.20 C	12.13 B	6.75 C
45	10.56 C	45.47 A	5.69 D	12.35 A	5.06 D
LSD	0.2767	0.0340	0.1190	0.1617	0.4779
Storage duration					
0	10.48 B	47.70 A	5.65 D	13.72 A	0.00D
20	10.74 B	45.05 B	6.10 C	12.51 B	4.24 C
40	11.20 A	42.39 C	6.38 B	11.51 C	9.59 B
60	11.47 A	38.60 D	6.80 A	10.51 D	14.87 A
LSD	0.2767	0.0340	0.1190	0.1617	0.4779

Interaction GA × SD	NS	**	NS	NS	**

Means having different letters are significantly different from each other at P≤ 0.01.
GA = Gibberellic Acid
SD = Storage Duration

DISCUSSIONS

GA_3 remarkably influenced days to maturity of grapefruit, because GA_3 triggers or increases the production of substances in the fruit peel that are then translocated into pulp tissue causing a delay in the ripening process. Gibberellins are a group of substances, known to delay maturation and senescence of the fruits. GA_3 not only affects the color development but also other aspects of ripening processes as well. GA_3 retards degradation of chlorophyll and fruit softening (Vendrell, 1970 and Khader, 1992). GA_3 acts on hormonal or enzymatic activities associated with ripening processes. It should be noted that GA_3 concentrations and their responses differ in different species and their stage of growth at which it is applied. Pre-harvest treatment of GA_3 reduces fruit drop which in turn leads to retention of greater number of fruits (Almeida *et al*., 2004). Gibberellic acid is responsible in modifying growth, number of fruits tree^{-1}, yield and yield related characters of the plants (Rafeekher *et al*., 2002). Cell division as well as cell elongation are stimulated by GA_3 treatments which improve the weight and yield of fruits. Also, it plays a role to control fruit set, reduce the prior to harvest fruit drop and increases fruit yield and storage life of fruits (Adlakha and Verma, 1964). (Khan *et al*., 2006) described the remarkable role of GA_3 to enhance fruit set which ultimately produces greater fruits plant^{-1}, improve fruit size and productivity.

GA_3 also affected all the postharvest attributes like total soluble solids (°Brix), ascorbic acid (mg.100g^{-1}), reducing sugars (%), non-reducing sugars (%) and weight loss (%). GA_3 slows down the breakdown of acids and ultimately their conversion to sugars so the TSS contents were found less than those in untreated fruits. So the consequences of this trail were matched with the discoveries of (Ahmed and Tingwa, 1995) in banana and (Murthy and Rao, 1982) in banana, who also found a decrease in total soluble solids with the GA_3 treatments. Increased ascorbic acid content with GA_3 application could be either due to biosynthesis of ascorbic acid or prevention of synthesized ascorbic acid from oxidizing through the activation of an enzyme, ascorbic acid oxidase by reducing the respiration rate in fruits. Producing fruits with higher vitamin C content and preserving vitamin C content during postharvest storage, is essential for maintaining their nutritional value. GA_3 treatments can also affect metabolism and redistribution of nitrogen to different plant organs and its supply for different biochemical processes (Miceli *et al*., 2019) thus, likely influencing ascorbic acid production. Generally, during the process of maturation, reducing sugars increase in the fruit (Ladaniya, 2008). Rapid increment in total and reducing sugars of control fruits is also due to the higher rate of ripening in which starch is converted into simpler sugars, whereas the slow rate of ripening in GA treated fruits is attributed to the inhibitory effects of this hormone on ripening (Golding *et al*., 2005). The retarded loss of non-reducing sugars in this experiment might be due to increased nutrient uptake, synthesis of metabolites and rapid translocation of photosynthates and minerals by the trees due to GA and consequently more synthesis of carbohydrates and other metabolites and their translocation to the fruits. (Sankar *et al*., 2013) also stated that application of PGRs and pruning increase non-reducing sugars in pomegranate fruits. The reduced weight loss observed in GA_3 applied fruits could be due to the maintenance of tissue strength because of the lower activity of enzymes responsible for disintegrating cellular structure. Surface coatings are widely applicable to reduce loss of water from fruits, puffiness of the fruit peel, delay ripening, and thus delay the deterioration of fruit quality. These coatings are considered as additional physical barrier against O_2, CO_2, dehydration and solutes movement. Hence, decline in the rates of the respiration, moisture loss and less oxidation occur (Baldwin *et al*., 1999) and (Park, 1999). The edible coatings also restrict the action of ethylene by partially inhibiting gaseous exchange occurring through fruit peel. This mechanism provides good protection and reducing postharvest losses in fruits.

Conclusion

According to the results observed in this experiment, the following conclusions are derived. Fruits sprayed with 45 ppm GA_3 and coconut oil coating enhanced all the qualitative attributes i.e days to maturity, number of fruits tree^{-1}, yield tree^{-1} (kg), with less total soluble solids (TSS) (°Brix), more ascorbic acid (mg.100g^{-1}), less reducing sugars (%), more non-reducing sugars (%) and minimum weight loss (%). GA_3 along with partial coating of oil enhances different qualitative features of grapefruit, and contribute to production and maintenance of good quality fruits and increasing their marketability. Keeping in view the results, GA_3 treatment on fruits seems to be suitable for delaying the harvest season of grapefruit and reducing postharvest losses, improving quality and increasing shelf-life of fruits. While partial coating with coconut oil retained good texture of the fruits by avoiding water loss which causes puffiness in fruits.

Authors Contribution

NS designed the Experiment, SN performed the experiment, SM and IQ analyzed the data and prepared the manuscript.

NS designed the experiment, SN performed the experiment and collected the data, SM and IQ, analyzed the data and prepared the manuscript, QSA guided the experiment, AZ, NZ, HS, MSK and SGK helped in conducting the experiment.

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