CHAPTER - III

MATERIALS AND METHODS

3.1 SELECTION OF FRUITS FOR THE EXPERIMENT

The fruits were harvested in morning of the day for the purpose of the experiments, when the fruits had attained the full maturity. Fruits were hand picked to avoid any mechanical injury and carried to the experimental site in cartons. Only the firm and well developed fruits of uniform size and maturity, free from pests and diseases, injuries, bruises and blemishes were selected for the experiment. The fruits were washed with tap water twice and then graded by density gradation method to select fruits of uniform maturity. Fruits were then dipped in a bucket full of water. Only water sinkers were chosen used for this trial. Each fruit was then rubbed gently with tissue paper to remove excess water on the peel and placed in shade for uniform drying.

3.2 EXPERIMENTAL DESIGN

The present investigation was carried out in the year 2010 & 2011 at SHIATS, Allahabad. The detached fruits of Amrapali variety of fruits were selected for the present study. The fruits were given various post-harvest treatments with hot water, GA3, frutox wax emulsion, ALPF and LDPF in isolation and in different combinations as given in Table.3.1. After the application of post-harvest treatments, the mango fruit were kept at room temperature (34°C).

The experiment was laid out in completely randomized design. There were altogether twenty-four treatments with three replications. Three fruits per treatment were used.
### Table-3.1 Treatment details

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Particular</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Control</td>
</tr>
<tr>
<td>T2</td>
<td>Gibberellic acid</td>
</tr>
<tr>
<td>T3</td>
<td>Hot water treatment</td>
</tr>
<tr>
<td>T4</td>
<td>Frutox wax emulsion</td>
</tr>
<tr>
<td>T5</td>
<td>Gibberellic acid+ Hot water treatment</td>
</tr>
<tr>
<td>T6</td>
<td>Gibberellic acid+ Frutox wax emulsion</td>
</tr>
<tr>
<td>T7</td>
<td>Hot water treatment+ Frutox wax emulsion</td>
</tr>
<tr>
<td>T8</td>
<td>Gibberellic acid+ Frutox wax emulsion+ Hot water treatment</td>
</tr>
<tr>
<td>T9</td>
<td>Low Density Polythene Film</td>
</tr>
<tr>
<td>T10</td>
<td>Gibberellic acid+ Low Density Polythene Film</td>
</tr>
<tr>
<td>T11</td>
<td>Hot water treatment+ Low Density Polythene Film</td>
</tr>
<tr>
<td>T12</td>
<td>Frutox wax emulsion+ Low Density Polythene Film</td>
</tr>
<tr>
<td>T13</td>
<td>Gibberellic acid+ Hot water treatment+ Low Density Polythene Film</td>
</tr>
<tr>
<td>T14</td>
<td>Gibberellic acid+ Frutox wax emulsion+ Low Density Polythene Film</td>
</tr>
<tr>
<td>T15</td>
<td>Hot water treatment+ Frutox wax emulsion+ Low Density Polythene Film</td>
</tr>
<tr>
<td>T16</td>
<td>Gibberellic acid+ Hot water treatment+ Frutox wax emulsion+ Low Density Polythene Film</td>
</tr>
<tr>
<td>T17</td>
<td>Aluminium laminted polythene film</td>
</tr>
<tr>
<td>T18</td>
<td>Gibberellic acid+ Aluminium laminted polythene film</td>
</tr>
<tr>
<td>T19</td>
<td>Hot water treatment+ Aluminium laminted polythene film</td>
</tr>
<tr>
<td>T20</td>
<td>Frutox wax emulsion+ Aluminium laminted polythene film</td>
</tr>
<tr>
<td>T21</td>
<td>Gibberellic acid+ Hot water treatment+ Aluminium laminted polythene film</td>
</tr>
<tr>
<td>T22</td>
<td>Hot water treatment+ Frutox wax emulsion+ Aluminium laminted polythene film</td>
</tr>
<tr>
<td>T23</td>
<td>Gibberellic acid+ Frutox wax emulsion+ Aluminium laminted polythene film</td>
</tr>
<tr>
<td>T24</td>
<td>Gibberellic acid+ Hot water treatment+ Frutox wax emulsion+ Aluminium laminted polythene film</td>
</tr>
</tbody>
</table>
Table-3.2 Layout plan for the experimentation
3.3 PREPARATION AND APPLICATION OF GA3, SOLUTION

GA3 (Gibberellic acid) was used in the study. The stock solution was prepared first by dissolving 1g of the material (GA3) in 50 ppm of absolute alcohol. The stock solution was diluted and made to the required volume by adding 20 litres of distilled water to make 50 ppm solution. Ten liters of such solution, was taken for dipping fruits for five minutes. After taking out from GA3 solution, treated fruits were spread evenly over paper sheets and then exposed under an electric fan.

3.4 HOT WATER TREATMENT

The hot water treatment of mango fruits cv. Amrapali was given in an aluminium container of 50 litre capacity. The container then filled with clean water leaving 2" space from the brim. The container was then placed on stove with mild flame. As the temperature of water reached 52°C mango fruits were wrapped in muslin cloth and dipped in water for five minutes and then spread thinly on newspaper and then placed under electric fan for drying. Temperature of water was measured from time to time with the help of thermometer, so that temperature may not rise or fall 52°C during the period of dipping. At the time of initial dipping of fruits, the temperature of water was raised 2°C above the required temperature i.e. 540C, so that with the dipping of fruits the temperature of water may not fall below 520C.

3.5 PREPARATION AND APPLICATION OF WAX EMULSION

Frutox waxol, 0-12 (12 per cent strength) was obtained from M/S Indian chemical specialists, 131; New Ramdhani Path, Nagpur.

To prepare six per cent wax emulsion, Frutox wax emulsion was emulsified by adding one part hot distilled water and one part waxol and was stirred to make six per cent emulsion. Fruits to be treated with waxol emulsion (6 per cent) were dipped for fifty seconds, then taken out and allowed to dry.

3.6 PROCEDURE FOR WRAPPING OF FRUITS

Polyethylene bags (13 cm X 18 cm size) made of low density polyethylene film (400 gauge) i.e. LDPF and Aluminum laminated polyethylene film (260 gauge) i.e. ALPF with pin holes were prepared and used for this experiment. The individual fruit was kept in each bag.
After keeping the fruits the bags were properly sealed with bag sealing machine; and kept separately as per the required treatment.

**Tagging**- The bamboo baskets containing fruits of each of the twenty four treatments in two lots were tagged with paper tags of 3 cm X 5 cm size. The tag carried full information regarding the treatment symbol and other information in the shortest possible way.

**3.7 OBSERVATIONS RECORDED**

Altogether 24 treatments were studied in three replications. Mango fruits under different treatments were kept separately and randomly following the random table. Observations were recorded at 4th, 7th, 10th and 13th day of storage.

The parameters and methods followed for recording various observations were as under.

**3.7.1 PHYSICAL CHARACTERS**

Physical characters of the fruits i.e. loss in weight, volume, specific gravity, size, weight of pulp and rind, pulp, peel ratio, pulp, stone ratio and all the physical parameters were recorded at regular intervals to record the impact of different treatments on physical characters of mango fruits during storage.

**3.7.1.1 PHYSIOLOGICAL LOSS IN WEIGHT (PLW)**

To find out the cumulative physiological loss in weight, freshly harvested fruits from each treatment were weighed before subjecting them to different treatments. Same fruits were then weighed at alternate day. The loss in weight was derived by subtracting the weight of fruit on the date from the original fresh weight. The percentage loss in weight was calculated by the formula.

\[
\text{Physiological loss in weight (PLW) = \frac{\text{Loss in weight}}{\text{Original fresh weight}}} \times 100
\]

**3.7.1.2 LOSS IN VOLUME**
All randomly marked fruit per treatment was taken out and the volume recorded before subjecting to treatment. Another observation was taken on the last date of storage. The volume of the fruit was recorded by water displacement method, using calibrated measuring cylinder (Gustafson, 1926). The percentage of loss in volume was calculated by using the formula.

\[
\text{Loss in volume} = \frac{\text{Percentage of loss in volume}}{\text{Original volume}} \times 100
\]

3.7.1.3 SPECIFIC GRAVITY

The specific gravity of mango fruits was determined by dividing the weight of the fruit in air by the volume of the fruits as obtained by water displacement method (Gustafson, 1926).

\[
\text{Sp. gravity} = \frac{\text{Weight of the fruits}}{\text{Volume of the fruit}}
\]

3.7.1.4 CHANGE IN SIZE (LENGTH AND DIAMETER) OF FRUITS

All the randomly marked fruits per treatment, replication wise were taken out with the help of slide calipers. Their length and diameter were measured on first day and last day of the investigation and average size in terms of length and diameter was calculated. For calculating the change in size of fruits, following formula was adopted.

\[
\text{Percentage of change in size} = \frac{\text{Change in size (Length and diameter)}}{\text{Original size (Length and diameter)}} \times 100
\]

3.7.1.5 WEIGHT OF PULP, STONE AND PEEL
Mango fruits were randomly taken out. They were peeled off and crushed with the help of mortar and pestle. The average weight of pulp, peel and stone were recorded on the first and final day of observation with the help of chemical balance. The final weight of pulp, peel and stone were recorded separately under each treatment.

3.7.1.6 PULP/PEEL RATIO

The pulp/peel ratio of the fruit at the end of storage was determined by dividing the weight.

3.7.1.7 STONE/PULP RATIO

The Stone/pulp ratio of the fruit at the end of storage was determined by dividing the weight of stone by the weight of pulp.

3.7.1.8 RESPIRATION RATE

Respiration rate of the fruits was measured by gas flow method (Mayer et al, 1955) after acclimatizing the fruits. In this method CO2 free air was drawn into the respiration chamber and then affluent gas was bubbled for a definite period through absorption tower having known volume of standard solution of barium hydroxide. This barium hydroxide solution was titrated against 0.1 N HCl solutions. Blank was also run side by side with the sample and the quantity of CO2 produced by the sample was calculated and expressed as mg of CO2 per kg of fruits per hour (mg CO2/kg/hour).
3.7.1.9 TOTAL DAYS TAKEN FOR COMPLETE RIPENING OF FRUITS

Total days taken for the complete ripening of fruits under different treatments (economic life) was determined by counting the number of days on the date on which cumulative loss in weight of fruit due to the spoilage of fruits in a particular treatment exceeded 15 per cent from the date of harvest of the fruits. Number of days so obtained indicated the complete ripening (economic life) of the fruit under that treatment. It was decided on the basis of water dip, organoleptic tests and pressure methods (Singh, 1996).

3.7.1.10 DAYS TAKEN FOR SENESCENCE

Senescence or the last stage of fruit development (ripening), when anabolic biochemical process gave way to catabolic processes leading to death of the tissues, was recorded at regular intervals, till the process was completed under different treatments. It was judged with the help of organoleptic tests and general appearance of the fruits.

3.7.2 CHEMICAL CHARACTERS

The initial observations were recorded on the first day of the experiment and then at each alternate day of the storage.

3.7.2.1 TOTAL SOLUBLE SOLIDS (TSS)

The total soluble solids (TSS) of fruits (°Brix) was recorded by the help of hand refractometer and the value were corrected to 20° C and expressed in percentage. The original data of percentage were transformed in to degree using the formula given by C.I Bliss (1937) as Angle = Arc Sin \( \sqrt{\frac{\text{PERCENTAGE}}{180}} \) for the purpose of analysis.

3.7.2.2 TITRABLE ACIDITY

The acidity of mango fruits was estimated by titrating a known volume of juice contained from the fruits from each replication of all the treatments against standard sodium hydroxide solution (0.1 N) using phenolphthalein as an indicator. The acidity of fruits so obtained was expressed as grams of anhydrous citric acid per 100 g of pulp. The formula used was.

\[ 0.1 \text{ N NaOH consumed} \]
Acidity percent = \[ \frac{\text{Volume-of juice (ml.)}}{\text{X} \times 64 \times X \times 100} \]

3.7.2.3 pH VALUE

pH value in fruit juice was measured directly with the help of systronic pH meter.

3.7.2.4 ASCORBIC ACID

Ascorbic acid or vitamin C was estimated by titrating a known volume of juice diluted in 3 per cent solution of metaphosphoric acid against the dye 2-4 dichlorophenol indophenol. The vitamin C on test so found was expressed as milligrams of ascorbic acid per 100 gram of the pulp.

3.7.2.5 REDUCING SUGAR

Reducing sugar in the fruit juice was estimated by Lane and Eynon (1923) Hopper titration method as explained below.

5 ml each of Fehling solution A and B were taken in conical flask and 10 ml of distilled water was added. The juice was titrated against boiling solution of Fehling using methylene blue as indicator. The appearance of brick red colour determined the end point. The reducing sugar in g/100g of pulp in terms of glucose was worked out.

3.7.2.6 TOTAL SUGAR

Ten milliliter of fruit juice was hydrolyzed by adding 3 ml of concentrated HCl. It was left for 24 hours. After that it was neutralized by adding sodium hydroxide 4N solution. For complete neutralization blue and red litmus papers were used. This solution was then titrated against Fehling A and B as in the case of reducing sugar and the percentage of total sugar in g/100g of fruit pulp was worked out considering Fehling A and B = 0.05 g of glucose.

3.7.2.7 NON-REDUCING SUGAR

The non-reducing sugar was calculated by deducting the reducing sugar from total sugar and subsequently multiplying with the necessary factor (0.95). The amount of non-reducing sugar estimated, was expressed in g/100 g of juice.
3.7.3 ORGANOLEPTIC EVALUATION

Organoleptic studies were conducted by score card system with a panel of three judges. The observations were recorded when 75 per cent fruits ripened. Following marks were allotted to each characteristic for their scoring.
Table 3.3- Organoleptic qualities and their maximum marks

<table>
<thead>
<tr>
<th>SI. No.</th>
<th>Quality</th>
<th>Maximum marks allotted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Texture and firmness of fruit</td>
<td>20 marks</td>
</tr>
<tr>
<td>2.</td>
<td>Taste of fruit</td>
<td>20 marks</td>
</tr>
<tr>
<td>3.</td>
<td>Flavour of fruit</td>
<td>20 marks</td>
</tr>
<tr>
<td>4.</td>
<td>Shape, colour and shine of fruit</td>
<td>20 marks</td>
</tr>
<tr>
<td>5.</td>
<td>General appearance of fruit (blemishes, injury, spot etc.)</td>
<td>20 marks</td>
</tr>
</tbody>
</table>

Table 3.4 The rating of the organoleptic score

<table>
<thead>
<tr>
<th>SI. No.</th>
<th>Quality</th>
<th>Maximum marks allotted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Excellent quality</td>
<td>90-100</td>
</tr>
<tr>
<td>2.</td>
<td>Good quality</td>
<td>80-89</td>
</tr>
<tr>
<td>3.</td>
<td>Fair quality</td>
<td>70-79</td>
</tr>
<tr>
<td>4.</td>
<td>Poor quality</td>
<td>60-70</td>
</tr>
<tr>
<td>5.</td>
<td>Very poor quality</td>
<td>Less than 60</td>
</tr>
</tbody>
</table>
3.7.3.1 TEXTURE AND FIRMNESS OF FRUIT

Fruits with good texture, firmness and fresh in appearance were categorized for high texture marking. The lower marks were allotted to fruits having variation from this natural texture and uneven maturity.

3.7.3.2 TASTE OF THE FRUIT

The scoring for the taste was done on the basis of sweetness, acidity and tastefulness. Sugar/acid blend of the fruit was considered as most important factor and maximum marks were allotted on desirable blending of sweetness and acidity. Fruits showing deviation from natural taste and freshness obtained lower marks.

3.7.3.3 FLAVOUR OF THE FRUIT

Higher marks were allotted to the fruits with excellent and natural flavour of mango. Over ripened or less ripened fruits with poor flavour fetched lower marks.

3.7.3.4 SHAPE, COLOUR AND SHINE OF FRUITS

Shape, colour, shine i.e. the external appearance of fruits plays very important role in marketing of fruits. Fruits with elongated uniform shape and glossy skin with uniform greenish yellow colour gained better marks. Amrapali fruits are known for its deep orange coloured pulp, which indicates possesses 2.5 to 3.0 times more B carotene content. Fruits with deep orange coloured pulp were preferred over light yellow or deep yellow coloured pulp and fetched better marks.

3.7.3.5 GENERAL APPEARANCE OF FRUIT

The mango fruits without any external deformities, rots, blemishes, shrivelling and having smoother skin were awarded better marks and those with variation from the normal characteristics were awarded poor marks during the organoleptic evaluation of fruits.

3.7.4 LOSS DUE TO SPOILAGE OF FRUITS

The spoiled fruits on alternate day of observations were separated out replication wise from all the treatments. The fruits so obtained were weighed separately and thus the percentage of weight loss due to spoiled fruits on each date was calculated by using the following formula.
Weight of spoiled fruits

\[
\text{Percentage weight loss} = \frac{\text{Weight of spoiled fruits}}{\text{Original weight of fruit}} \times 100
\]

3.7.5 ASSESSMENT OF THE EXTENT OF FRUIT ROT

The number of fruits rotten during the whole storage period was counted in each treatment replication wise. The percentage of rot was worked out by the formula.

\[
\text{Percentage of fruit rot} = \frac{\text{Number of rotten fruits}}{\text{Total number of fruits stored}} \times 100
\]

3.8 STATISTICAL ANALYSIS

Data recorded during the course of investigation were subjected to analysis of variance by completely randomized design and the significance of the treatments was tested by error mean square by Fisher "F" test of probability levels at 0.05. After the individual analysis, the data were subjected to pooled analysis where ever necessary.

3.9 ECONOMICS OF STORAGE

The unit cost for each of the treatments was calculated separately and the cost so evolved was recorded. The net profit for the same in each treatment was calculated on the particular day, when the fruits under best treatment retained their "good" scoring in overall organoleptic evaluation and maintained the economic level of spoilage. Economics was calculated on the basis of market rate prevailed at that period.