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Effect of methyl jasmonate on physical, biochemical and yield behavior of strawberry cv. Nabila

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Abstract
This experiment was conducted at Bihar Agricultural University, Sabour, and Bhagalpur in the Department of Horticulture (Fruit & Fruit Tech.) to show the effect of methyl jasmonate on physical, biochemical and yield behavior of strawberry cv. Nabila. Highest fruit length (4.70 cm) was recorded in treatment T_3. There were no significant differences in fruit width, fruit weight, fruit volume were observed among the treatments of different doses of MeJA. The treatment of MeJA in regards to total soluble solids content in fruits was found significant with highest (11.77 ^oB) in 0.6 mM MeJA treated fruits. However, a non-significant difference in acidity of treated fruits was observed. The total sugar content of fruits had the highest (7.78%) and ascorbic acid content (78.65 mg/100g) under fruits treated with 0.6 mM MeJA. In case of phenolic content, the 0.9 mM MeJA treatment showed the highest (79.39 mg/100g). The effect of chemicals on the antioxidant and anthocyanin content of the fruits was found to be non-significant. Antioxidant content ranged between (25.94 µmol TE/g to 19.42 µmol TE/g). The anthocyanin content ranges from (32.35 mg/100g to 36.70 mg/100g). Maximum yield (18.33 fruits per plant) and was found in 0.6 mM MeJA treated plants.

Keywords: Strawberry, methyl jasmonate, physical parameters, biochemical parameters, yield

Introduction
The luscious, attractive and nutritious modern cultivated strawberry (*Fragaria x ananassa* Duch.) is one of the most important soft fruits in the world. It is a crop of octaploid in nature (2n = 8x = 56), belonging to the family Rosaceae, Being a rich source of vitamins and minerals, like vitamin A (60 IU/100 g of edible portion) and vitamin C (30-120 mg/100g of edible portion), they are mainly consumed as fresh fruits, and are also commonly utilized for flavouring in manufacturing and processing industries for different processed products and beauty products as well. The red colour of the fruits is due to the presence of anthocyanin pigment (Sharma, 2002 and Chadha, 2001) [14, 4]. It contains few aromatic compounds in different concentrations which are responsible for the aroma and flavour of the fruits, *viz*., ethyl hexanoate, methyl hexanoate, ethyl propionate, ethyl butanoate, methyl butanoate, furanone and linalool.

As to this, several low-cost strategies have been developed throughout the world to enhance its quality and productivity. Among them the use of different plant growth regulators is a common practice in different crops in order to get better yield and quality from the crop. Several PGRs have been exogenously applied in different experiments and have been found to be one of the most effective for enhancing the production and quality of the crop produce. The use of PGRs showed higher yield with improved fruit quality in different crops, such as Apple (Turk and Stopar, 2010) [16], Citrus (Gonzales and Borroto, 1987) [5], Mango (Wahdan *et al*., 2011) [17], etc. The exogenous application of PGRs has also been found to stimulate the growth, flowering and fruiting of different fruit crops (Al-Duljaili *et al*., 1987; Randhawa *et al*., 1959) [1, 12] which resulted from the improvement of endogenous level of phytohormone (Al-Duljaili *et al*., 1987) [1] and Mineral nutrients (Bist, 1990) [3].

Some reports also suggest that methyl jasmonate plays an important role in defense mechanism of plants and diverse developmental pathways such as seed germination, root growth, etc. However, literatures on the exact response of methyl jasmonate on strawberry plants and fruits are very less to improve the yield and quality. Therefore, more experiments are required on this field to study its function on various parameters of the plant, especially under sub-tropical condition.

Materials and Methods

Physical parameters

Fruit Length

The length of fruits was calculated with the help of digital Vernier caliper. It was recorded from the base of the fruit stalk to the end and expressed in centimeter (cm).

Fruit width

The width of fruits was also calculated with the help of digital Vernier caliper at the point where it was observed maximum and expressed in centimeter (cm).

Fruit weight

The weight of fruits from each tagged plant was taken using the digital weighing balance and the mean was expressed as weight of fruit in gram.

Fruit volume

The volume of fruits was calculated by water displacement method where the volume of the water displaced was measured, as given by Gustafson (1926) [7], and expressed in milliliter.

Biochemical parameters

Total soluble solids (TSS)

Total soluble solids of the fruits were measured at room temperature with the help of hand refractometer according to the method described by Rangana (2010) [13] and were expressed in degree brix (°B). To measure the value, some amount of juice was extracted from the fruits and 2-3 drops were taken in the clean glass on the prism base of the refractometer. The refractometer was then turned on and the reading displayed on the screen of the digital refractometer was then taken.

Titratable acidity (TA)

The titratable acidity was determined by using the titration method (Rangana, 2010) [13]. Two grams of fruit sample was weighed and were crushed. The crushed samples were then transferred to volumetric flask where they were further diluted with 50 ml of distilled water. They were then mixed and 10 ml of it was taken separately where 2 drops of phenolphthalein indicator was then added and were titrated against 0.1N NaOH solution. The titrated value was used for calculating the titratable acidity value as per cent by using the given formula:

$$\text{TA (\%)} = \frac{\text{Titre value x Normality of alkali x Volume made up x Equivalent weight of acid x 100}}{\text{Volume of sample taken for estimation x Weight of sample taken x 100}}$$

TSS: Acid ratio

The ratio was calculated by dividing the TSS value with the titratable acidity from each replication.

Ascorbic acid content

Ascorbic acid content in fruit was determined by using 2, 6-dichlorophenol indophenol dye method (Jones and Hughes, 1983) [8].

Reagents used

1. **Dye Solution:** 50 mg of 2, 6-dichlorophenol indophenol dye and 42 mg of sodium bicarbonate were mixed in 100 ml of hot water. The volume was then made up to 200 ml.
2. **3% Metaphosphoric acid solution:** 30 grams of MPA was dissolved in 1 litre of distilled water.
3. **Standard ascorbic acid solution:** 100 mg of ascorbic acid was taken and dissolved in 100 ml of 3% MPA solution. 10 ml of it was taken out and is again made upto 100 ml with 3% MPA solution. 10 ml of this standard ascorbic acid solution is taken and is titrated against the dye solution.

$$\text{Dye Factor} = \frac{1}{\text{Titre value}}$$

Procedure

Two grams of the sample was weighed from the fruit and crushed with 3% MPA solution. The volume is then made up to 50 ml with the same 3% MPA solution in a volumetric flask. It was then kept for 15 minutes after which charcoal powder was added in each sample solution and is mixed. They were then filtered with the help of filter paper and funnel where the filtrate needs to be colourless. If the filtrate is not colourless, more charcoal powder is then added to which they were filtered again. 10 ml of the filtrate was taken and is then titrated against the dye solution. The ascorbic acid content is then calculated by using the formula:

$$\text{Ascorbic acid content (mg/100g)} = \frac{\text{Titre value x Dye factor x Volume made up x 100}}{\text{Aliquot of extract x Volume of sample taken}}$$

Total anthocyanin content

Reagent used

Ethanolic HCl Solution: 95% ethanol and 1.5 N HCl were mixed in the ratio of 85:15.

Procedure

Two grams of the sample was weighed from the fruits and are crushed alongwith ethanolic HCl in mortar with pestle. They are then transferred to volumetric flask in which the volume was then made up to 20 ml with ethanolic HCl. The flasks were covered with aluminium foil and are kept in the refrigerator overnight at 4 °C. They are then filtered through filter paper with funnel where the optical density (O.D) of the filtrate is recorded at 535 nm wavelength using ethanolic HCl as blank. The total anthocyanin content is then calculated by using the formula:

Total anthocyanin content (mg/100g) = $\frac{Total\ O.D/100g}{98.2}$ as 98.2 is the E value for 1% solution at 535 nm.

Where,

$$\text{Total O.D/100 g} = \frac{\text{O.D x Volume made up x 100}}{\text{Weight of sample}}$$

Total antioxidant capacity

Reagents used

1. **Neocuproine solution:** 0.1562 gram of neocuproine was

dissolved in 100 ml of 96% of ethanol.
2. **Copper chloride solution:** 0.1705 gram of copper chloride was dissolved in 100 ml of distilled water.
3. **Ammonium acetate solution:** 7.708 grams of ammonium acetate was dissolved in 100 ml of distilled water.
4. 80% ethanol.

Procedure
1gram of fruit sample was weighed from the fruits and is crushed in 10 ml 80% ethanol. The sample homogenates were then centrifuged at 10,000 rpm for 20 minutes at 4 ºC temperature. 1 ml each of neocuproine, copper chloride, ammonium acetate solution and distilled water were added in a small test tube to which 100 µl each of the supernatant were then added in the test tubes. In case of blank, 100 µl distilled water is added instead of the supernatant sample. After the samples are mixed, their absorbances are measured in a spectrophotometer at 450 nm wavelength. The total antioxidant capacity is then calculated using the formula:

$$\text{Total antioxidant capacity} = \frac{\text{O.D x 4.1 x Volume made up x 1000 x 100}}{\text{Weight of sample taken x 1.67 x 10000 x 0.1}}$$

Total phenolic content
The total phenolic content in fruits was determined using the method suggested by Singleton and Rossi (1965) [15].

Reagents used
1. 80% ethanol.
2. Folin - ciocalteau reagent.
3. 20% sodium carbonate solution.

Procedure
2 grams of fruit sample was weighed from the fruits and are crushed in 10 ml 80% ethanol. The sample homogenates were then centrifuged at 10,000 rpm for 20 minutes at 4 ºC temperature. 2.8 ml of distilled water, 200 µl of supernatant sample and 0.5 ml of 2N Folin-ciocalteau reagent were taken and mixed in a small test tube. After keeping them for 3 minutes, 2 ml of 20% Sodium carbonate was added in test tube. The prepared samples were then kept for some time till it become blue black in colour. In case of blank, 200 µl is added instead of the supernatant sample. The absorbance is then taken for each sample in a spectrophotometer at 760 nm wavelength. Gallic acid was used to produce standard calibration curve. The total phenolic content is then calculated using the formula:

$$\text{Total phenol content (mg GAE/100g)} = \frac{\text{O.D X Volume made up with 80\% ethanol x 100}}{\text{Aliquot taken x Weight of sample x 1000}}$$

Total sugar content
The sugar content was measured using a method developed by Lane and Eynone (1923) [9].

Reagents used
1. Fehling's solution A
2. Fehling's solution B
3. Methylene blue indicator
4. 45% Neutral lead acetate solution: - 250 gram of neutral lead acetate was dissolved in distilled water and the volume was made up to 500 ml.
5. 22% Potassium oxalate solution: - 110 gram of potassium oxalate was dissolved in distilled water and the volume was made up to 500 ml.

Procedure
10 grams of the fruit sample was weighed from the fruits and were crushed with distilled water. They are then transferred to volumetric flask in which the volume was then made up to 100 ml. To it, 2 ml of lead acetate solution was added, followed by 1.9 ml of potassium oxalate solution. The volume is then made up to 250 ml with distilled water. The solution is then filtered through filter paper with the help of funnel. 50 ml of the filtrate is then taken in 100 ml volumetric flask to which 5 ml of concentrated HCl is added to the filtrate. They are then kept for 24 hours. After 24 hours, 2 drops of phenolphthalein indicator were added, followed by 40% NaOH until pink colour appears. Then, 0.1N HCl was added to it drop by drop as late as pink colour disappears. The volume was then made up to 100 ml with distilled water. They are then kept in burette for titration. In a beaker, 5 ml each of Fehling's solution A and B were mixed to which the volume was made up to 50 ml with distilled water. 2 drops of methylene blue were added. The mixture is then heated to which titration is done when it starts to boil. The reading is then taken when the brick red colour appears and stays on it even when methylene blue is added. The total sugar content is then calculated using the formula:

$$\text{Total sugar content (\%)} = \frac{\text{Factor x Dilution x 100 x 100}}{\text{50 x Titre value x Weight of sample}}$$

Yield
Number of fruits per plant
It was recorded by counting the number of fruits produced by the individual plant throughout the fruiting period in each treatment.

Results and Discussion
Physical parameters
The maximum fruit length (4.70 cm) was recorded with spray of MeJA at the concentration of 0.6 mM MeJA and ranges from 4.0 to 4.7 cm showing significant result in the fruit length. The treatment for fruit width, fruit weight and fruit volume were found to be non-significant. The possible causes for increased fruit size, weight and volume could be attributed to enhanced fruit growth due to rapid cell enlargement and cell division and decreased intercellular space. This can also be attributed by a rise in photosynthetic processes and the production of further carbohydrates by significant varieties of plants and leaves. All these data for physical parameters has shown that the untreated fruits were found to be the most inferior as compared to the MeJA treated fruits. Amongst the treated fruits, treatment with 0.6 mM MeJA was found to be the best dosage for treatment of fruits as it showed the best

result. Similar finding in terms of fruit volume was also reported by Martines-Espla *et al.* (2014) [11] where 0.5 mM MeJA was the best dosage among the different concentrations on Plum cvs. Black Splendour and Royal Rosa.

Biochemical parameters

The treatment of MeJA in regarding to total soluble solids content in fruits was found significant with 11.77 ^{o}B the highest in 0.6 mM MeJA treated fruits. The depletion of TSS in untreated fruits could be the result of a higher fruit metabolism and senescence processes. By contrast, lower concentrations of respiration in treated fruits could have led to the accumulation of higher amounts of carbohydrates in tissues. However, a non-significant difference in acidity of differently treated fruits was observed where the untreated fruits were found to be the most acidic (0.98%) while the 0.3 mM MeJA treated fruits were the least acidic (0.88%). The total sugar content of fruits had a significant change in different treatment dosage as the fruits treated with 0.6 mM MeJA had the highest sugar content with 7.78%.

A significant effect of MeJA treatment was found in the fruits for ascorbic acid and phenolic content. The fruits treated with 0.6 mM MeJA had the highest ascorbic acid content (78.65 mg/100g) as compared to other treatments. This finding is in favour with Lolaei *et al.* (2013) [10] reported that the MeJA treatment with 0.5 mM and 1 mM showed higher ascorbic acid content than fruits treated with other concentrations or untreated fruits in strawberry cvs. Selva and Queen Elisa. In case of phenolic content, the 0.9 mM MeJA treatment showed the most beneficial response as the fruits had the highest phenolic content (79.39 mg/100g). In contrary to the present investigation, Gonzalez-Aguilar *et al.* (2004) [6] reported that total phenols were not affected by the MeJA treatment in guava fruits.

The effect of chemicals on the antioxidant and anthocyanin content of the fruits was found to be non-significant. However, the fruits treated with 0.9 mM MeJA had the antioxidant content with 25.94 µmol TE/g. The anthocyanin content ranges from 32.35 mg/100g to 36.70 mg/100g with a general mean value of 35.11 mg/100g. Ayala-Zavala *et al.* (2005) [2] also reported the same on strawberry crop.

Yield parameters

The present investigation showed the impact of different concentrations of MeJA in the yield parameters of strawberry cv. Nabila. The data obtained has revealed that the treatment is significant in improving the fruit yield where maximum yield (18.33 fruits per plant) and was found in 0.6 mM MeJA treated plants. On the other hand, the minimum (12.65 fruits per plant) was obtained in control. Similar findings as to increase of fruit yield in MeJA treatment was also observed by Garcia-Pastor *et al.* (2019) [18] in pomegranate and Lolaei *et al.* (2013) [10] in strawberry. The increase in weight of fruits per plant was due to increase in number of fruits ripened and harvested.

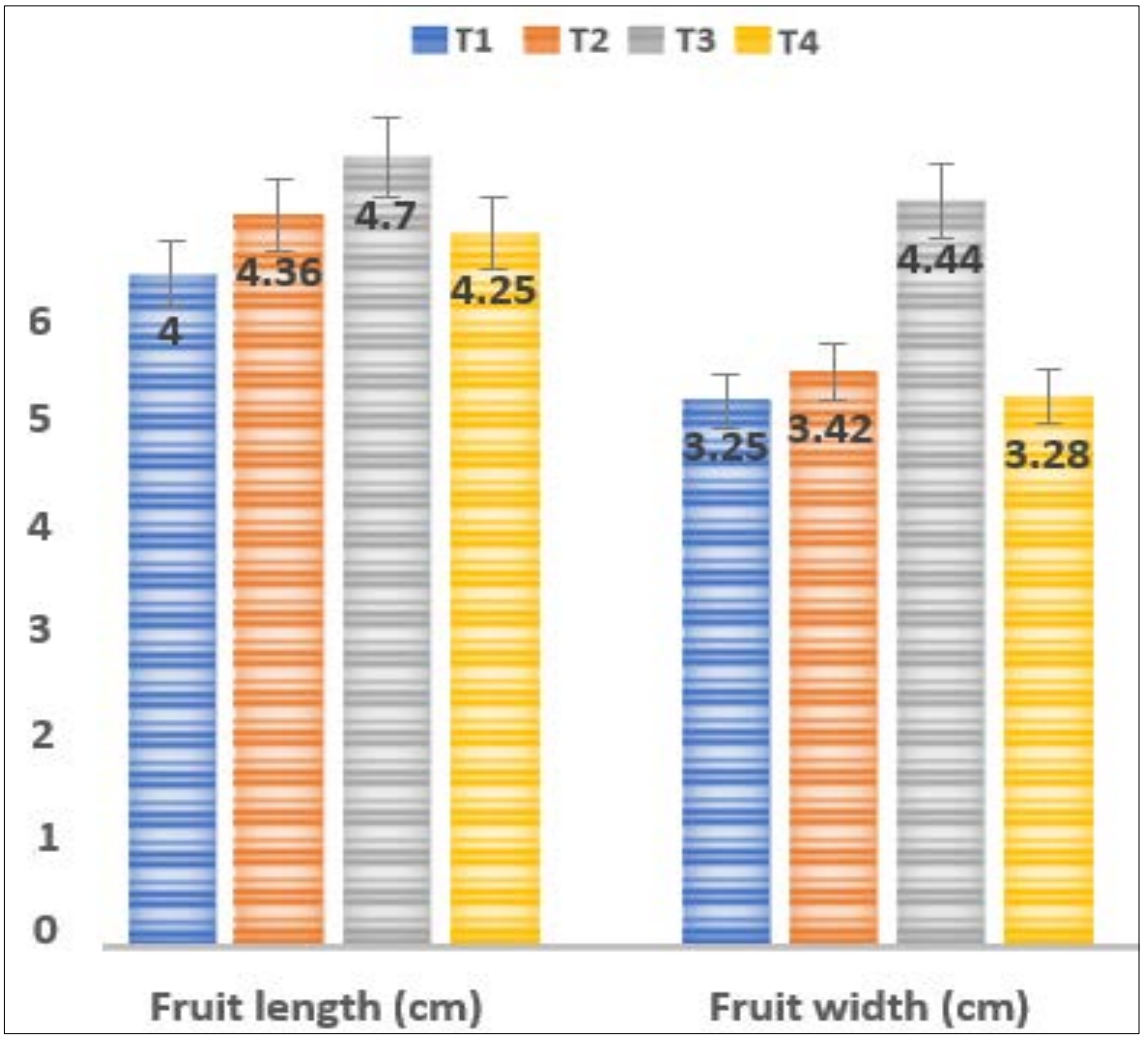

Fig 1a, b: Physical parameters of strawberry fruits

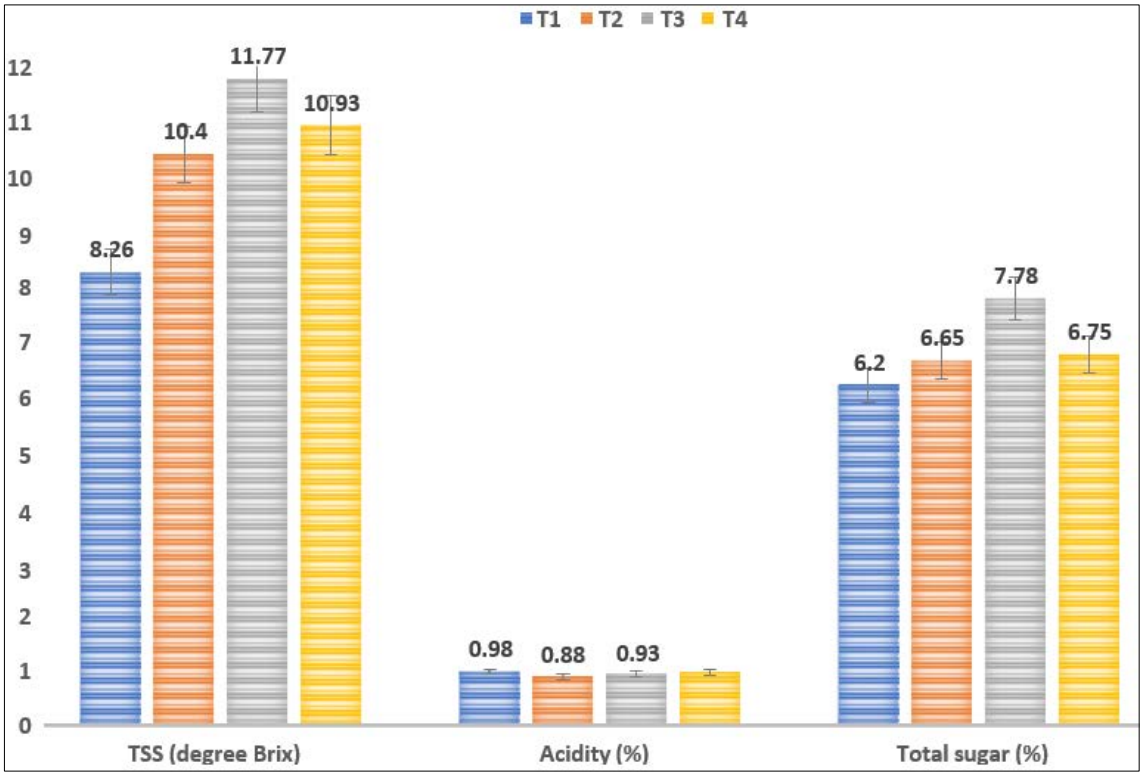

Fig 2: Effect of methyl jasmonate on biochemical parameters

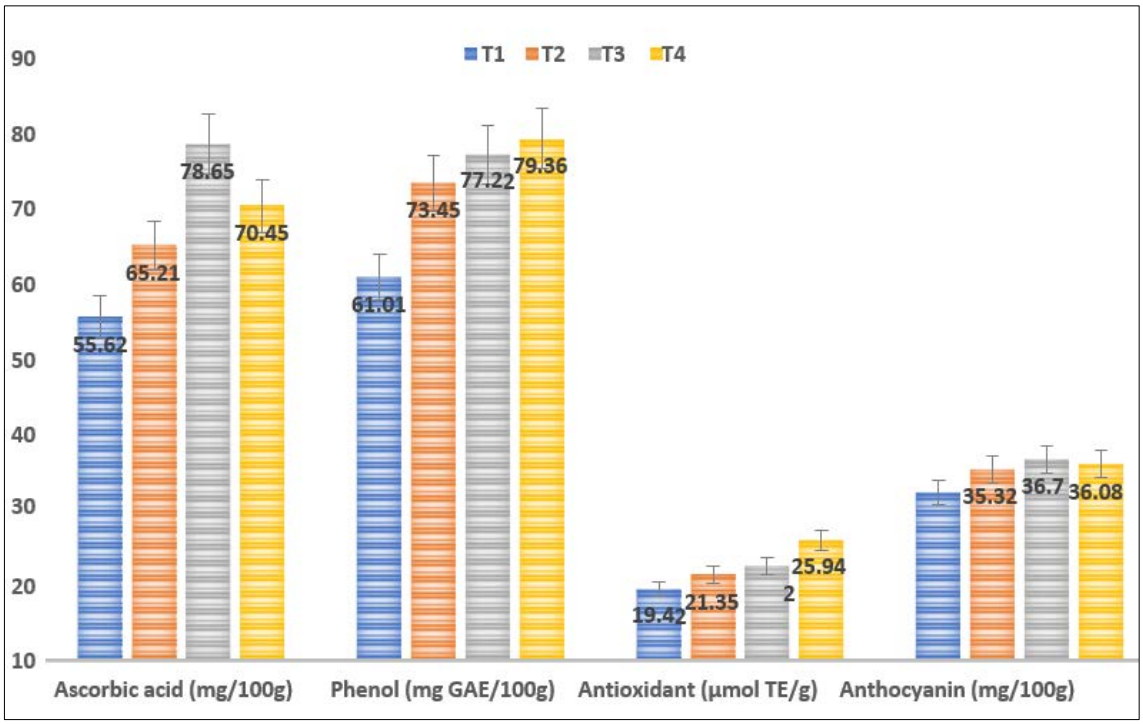

Fig 3: Effect of methyl jasmonate on biochemical parameters

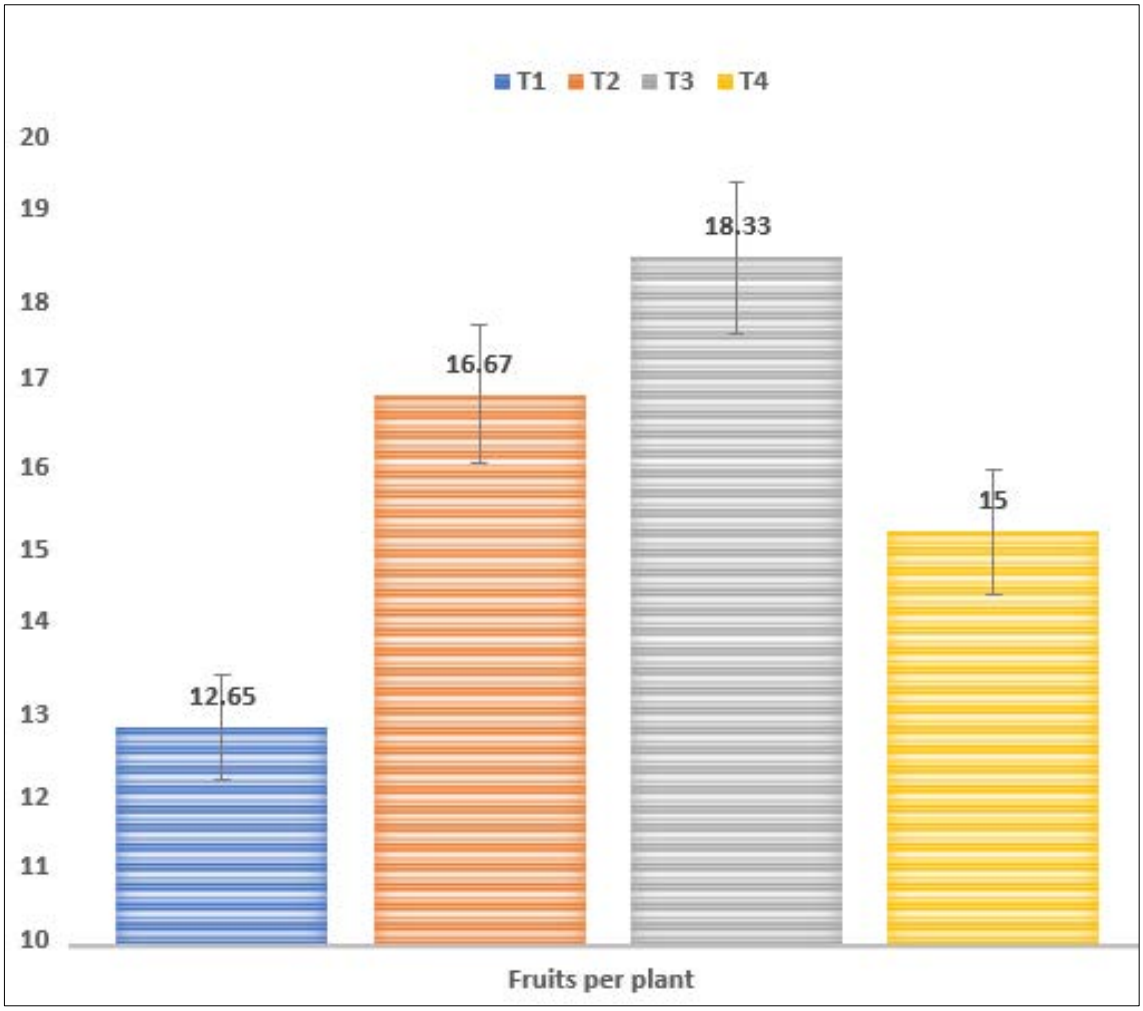

Fig 4: Effect of methyl jasmonate on yield (numbers) parameters

Conclusion

Positive response on physical, biochemical and yield parameters were observed by the application of methyl jasmonate two times, first at the time of before initiation of flowers and second after fifteen days of first spray. The treatment of MeJA at the rate of 0.6 mM concentration was found the best result in all aspect.

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