



## Effect of Bio-stimulants on Improving Floral Characteristics, Yield and Quality of Apple cv. Red Delicious

Mohd Zubair<sup>1\*</sup>, F. A. Bandy<sup>2</sup>, Umar I. Waida<sup>2</sup>, Jahangir A. Baba<sup>1</sup>, S. S. Hussain<sup>1</sup>  
and Munib-u-Rehman<sup>1</sup>

<sup>1</sup>Krishi Vigyan Kendra, Extension Training Centre SKUAST-Kashmir, Malangpora (Pulwama) J & K, 192301, India.

<sup>2</sup>Division of Fruit Science, SKUAST-K, India.

### Authors' contributions

This work was carried out in collaboration between all authors. Author MZ designed the study, wrote the protocol, first draft and executed research. Author FAB directed and managed the analyses of the study. Authors UIW, JAB, SSH and MUR managed the literature searches and performed the statistical analysis. All authors wrote the manuscript and approved the final manuscript.

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### ABSTRACT

The Effect of bio-stimulants on improving floral characteristics, yield and quality of apple cv. Red Delicious was studied in the Division of Fruit Science, SKUAST-Kashmir, Shalimar, Srinagar during the year 2013 and 2014. Twenty five year old apple trees of cv. Red Delicious were selected at the Sher-e-Kashmir university of Agricultural Sciences and Technology, Shalimar, Kashmir. The soluble boron of solubor (0.1%) and bio-stimulants of Biozyme (1.5 ml/l) and triacontanol (10 ppm) and their combinations were sprayed at three timings: (i) At pink bud stage (ii) three weeks after fruit set of apple (iii) two months after second spray. Two months after second spray, solubor was replaced with 0.5% CaCl<sub>2</sub>. The results revealed that combination of solubor + biozyme + triacontanol and solubor + biozyme was more effective to improve floral and yield characteristics with fruit set (74.71 and 69.50%) and yield (97.75 and 92.70 kg/trees) Fruits were harvested and analysed for their

\*Corresponding author: E-mail: takzubair@gmail.com;

physico-chemical characteristics. Foliar application of solubor + biozyme + triacontanol and biozyme + triacontanol improved fruit color, size, weight, volume, firmness and TSS, sugars while acidity declined in all treatments at various stages.

**Keywords:** Apple; bio-stimulants; solubor; CaCl<sub>2</sub>

## 1. INTRODUCTION

The cultivated apple, *Malus x domestica* Borkh., belongs to the Pomoideae subfamily of the Rosaceae [1]. Apple is a rich source of carbohydrates, proteins; minerals like iron and potassium etc. and contain vitamin-C and beta carotene [2]. Apple is good in normalizing the intestines, for treatment of anaemia. Apple is believed to reduce the incidence of dental caries, helps to control obesity and supply extra energy for heavy exercise. Apples are the fourth most widely produced fruit in the world after bananas, oranges and grapes with references like [3] and [4]. In India, apple is grown in the states of Jammu and Kashmir, Himachal Pradesh, Uttarakhand and North Eastern states. However, Jammu and Kashmir enjoys a vital place for the production of world class apples due to its agro-climatic condition. The major apple producing countries in the world are China, Russia, India, Ukraine, Poland, USA, Iran, France, Romania etc. Fruit culture is an important industry in the economy of the Jammu and Kashmir state and it occupies about 173.63 thousand hectares with an annual production of 1500.25 thousand MT [5]. In India, amongst the temperate fruits, apple accounts for the highest production. But average productivity (5.8 t/ ha) is low as compared to USA (26 t/ha) [6]. It accounts for about 10 per cent of the total fruit production of the country. This is indicative of a great scope available for us to improve the productivity and production of apple in Kashmir. Red Delicious is one of the most famous and most widely grown apple varieties in Kashmir. Apple tree, like other plants, need different nutrients in varying quantities to achieve proper growth and fruiting. The decreasing trend in apple productivity during the last decade due to changing climate scenario has caused a serious concern to the fruit growers and planners of the country. Several factors like inadequate pollinizer proportion, reduction in natural population of pollinating agents, occurrence of spring frosts, hails and gales, nutrient deficiencies, droughts etc. are the factors leading to poor fruit setting in Delicious apple [7]. Among these the main important cause of reduction in apple production and quality are deficiency of nutrients. Due to Ca and B deficiency in apple orchards fruits remain small,

malformed and suffer from various disorders. Apples low in Ca ripens earlier and loses more weight during storage while as B deficiency causes pitting, skin discoloration, cracking and corking. Besides, nutrients all plants require endogenous PBR's for normal growth and development. Application of seaweed extract as an organic bio stimulant is fast becoming an accepted practice in horticulture. Many physiological responses shown by crop plants are reported due to cytokines, Biozyme is a known example. Triacontanol also plays an active role in the up-regulation of many biochemical and physiological processes in plants. Application of N in the form of urea tends to increase tree N storage and regulate N distribution, results in healthy spurs and better flowering. Thus for increasing the apple production and productivity, it seems to be desirable to have some of technological intervention in the package of practice for apple growers so that we can be able to boost up the productivity and encourage the farmer to grow and earn more.

Keeping the above points in view, purposes of this study were to evaluate the effect of bio-stimulants and their combinations on increasing the yield and quality of apple cv. Red Delicious.

## 2. MATERIALS AND METHODS

The present experiment was carried out at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar, Srinagar, Jammu and Kashmir during the year 2013 and 2014. The experiment was conducted on twenty five years old trees of uniform size and vigor. The Kashmir represents the climatic conditions prevailing in the temperate zone of the Jammu and Kashmir state. The maximum and minimum temperature of valley during the crop season ranged between 23°C and 29.9°C and -5.8°C to 12°C, respectively with relative humidity of 43.90 per cent and 650-800 mm rainfall mostly which was received from December to April.

The bio-stimulants were applied at three different timings to see the effect of chemicals on fruit set, quality of apple Table 1.

The three timings are:

- 1) At pink-bud stage of apple
- 2) Three weeks after fruit set of apple
- 3) Two months after second spray

## 2.1 Experimental Design

Randomized Block Design With a randomized block design, the experimenter divides subjects into subgroups called blocks, such that the variability within blocks is less than the variability between blocks. Then, subjects within each block are randomly assigned to treatment conditions.

The various floral and yield characteristics and physio-chemical observations of fruit were recorded during the study, like

Bloom (flower) index =

$$\frac{\text{Flower buds}}{\text{Flower buds + Vegetative buds}} \times 100$$

$$\text{Fruit set (\%)} = \frac{\text{Number of fruit set}}{\text{Number of flowers}} \times 100$$

Fruit retention (%) =

$$\frac{\text{Number of fruits}}{\text{Total number of flowers}} \times 100$$

$$\text{Titrate acidity (\%)} = \frac{0.0064 \times \frac{N}{10} \text{ NaoH used}}{\text{Volume of the fruit taken}} \times 100 \quad [8]$$

$$\text{Specific gravity value} = \frac{\text{Weight of the fruit}}{\text{Volume of the fruit}}$$

Fruit volume was measured by water displacement method and expressed as cubic centimeters. The volume of fruit samples was calculated with the help of measuring cylinder and recorded for

$$V = b - a$$

Where,

$$\begin{aligned} V &= \text{Volume of all the fruits} \\ b &= \text{Final volume of water} \\ a &= \text{Initial volume of water} \end{aligned}$$

Average volume

$$A = b - a/n$$

Where,

$$\begin{aligned} A &= \text{Average volume of fruit samples} \\ b &= \text{Final volume of water} \\ a &= \text{Initial volume of water} \\ n &= \text{Number of fruits present in the samples} \end{aligned} \quad [9].$$

### 2.1.1 TSS: Acid ratio

Total soluble solids/ acid ratio was calculated by dividing TSS with that of the corresponding titratable acidity.

### 2.1.2 Sugars

10 ml of the juice was taken in 100 ml beaker and 5 mg of lead acetate was added to solution. The solution was kept for half an hour and filtered through Whatman's filter No.1. Then 1 gm of potassium oxalate was added to remove the excess lead and the volume was made 100 ml with distilled water. This aliquot was used for determining the reducing sugars and total sugars.

### 2.1.3 Reducing sugars (%)

The solutions Fehling A and B(5 ml volume of each) were taken in burette and titrated against Fehling solution (A and B) mixed over a hot plate. Four drops of methylene blue indicator were added to the solution in the flask before titrating. Titration was then started and continued till the end point indicating the appearance of permanent brick red color. The volume of aliquot used was noted as 'A'. The percentage of reducing sugars was calculated by using the following formula [10]

$$\begin{aligned} \text{RS (\%)} &= \text{Titrate value} \times \frac{\text{Stock solution}}{\text{Weight of sample} \times \text{solution used}} \times 100 \\ &= 0.05 \times \frac{100}{10 \times 'A'} \times 100 \end{aligned}$$

### 2.1.4 Total sugars (%)

Total sugars were estimated by hydrolysing the non-reducing sugars first. 25 ml of above aliquot was taken into 100 ml measuring flask and 25 ml of distilled water and hydrolysis was done by the addition of 5 ml of HCl (60 per cent volume) and then it was warmed complete hydrolysis. When non-reducing sugars were hydrolysed, the extract was cooked to room temperature and the pH of this was made neutral or slightly alkaline with the help of N/10. pH was tested with the help of red litmus paper [10].

$$\begin{aligned} \text{Total sugars (\%)} &= 0.05 \times \frac{\text{Stock solution}}{\text{Weight of sample}} \times \frac{\text{Second stock solution}}{\text{Volume of aliquot taken} \times \text{Volume of aliquot used}} \times 100 \\ &= 0.05 \times \frac{100}{10} \times \frac{100}{25 \times 'A'} \times 100 \end{aligned}$$

### 3. RESULTS AND DISCUSSION

#### 3.1 Effect of Chemicals on Floral and Yield Characteristics

The present studies indicated that the foliar application of solobur and bio-stimulants definitely alter the floral and yield characteristics of apple orchard cv. Red Delicious. All the treatments of bio-stimulant and their combinations influenced the floral and yield characteristics. The highest (74.71%) fruit set and lowest drop (11.19%) were observed in solubor + biozyme + triacontanol followed by solubor + biozyme (65.25, 11.34%) and solubor + triacontanol (53.42, 12.31%) of fruit set and fruit drop. The possible reason for higher fruit set is boron and bio-stimulants. Boron is important in pollen germination and pollen tube growth, which is likely to increase fruit set. Therefore, boron fertilization may increase yield. Although the mechanism of boron translocation within the plants has not been well understood. The results confirm the findings of [11] and [12]. Reduced flower drop and increase in fruit set may be due to delay in abscission (the effect of cytokinins and auxin) through prevention of pectin material in middle lamella [13]. Similarly, the results with vipul (triacontanol) application is in agreement with [14] who recorded increase in fruit set in Santa Rosa plum with traiconatnol (vipul) and with application of miraculan improved fruit set in apricot [15] with the reason it may have increased carbohydrates level and C/N ratio [16]. The another possible reasons may be the presence of minerals and some growth regulators, proteins, carbohydrates and vitamins such as thiamine, riboflavin, vit-B<sub>12</sub> and folic acid in biozyme which may have positive effect on increasing fruit set and decreasing fruit drop. This may be due to the beneficial effect of such treatments on nutritional status of the trees which reflected on increasing fruit set and fruit retention. Maximum fruits were retained by solubor + biozyme + triacontanol treatment (61.72%). The results obtained may confirm the previous work done by [17] and [18] who reported that yield in mango and orange were increased by algae and yeast extracts. It could be noticed from Table 2 that all treatments

significantly increased yield (kg/tree) than the control in both the seasons under study.

#### 3.2 Effect of Chemicals on Physical Changes of Fruit

Consumers highly appreciate red intensively coloured apple fruits and the market usually rewards those growers able to alter them. Red color is based on the amount of anthocyanin that depends on the degradation of chlorophyll. The red color formation was significantly increased by the combination of solubor + biozyme + triacontanol (4 scores). Effect of triacontanol might be due to increase in photosynthesis, nutrient uptake [19], iron [20] and Mg [21] which are essential elements for chlorophyll biosynthesis and biozyme contain different acids (Fulvic and alginic acid) and more than 5000 enzymes [22]. More over seaweed extracts contain natural plant growth regulators which control growth and structural development of plants [22]. The results are in consonance with those obtained by [16] and [23].

Fruit size is one of the most important quality parameter, larger fruit have proportionally more flesh and strongly preferred by most consumers, as they have greater visual appeal and have better flavour [24]. Fruit length, breadth and weight with the application of chemicals was found to be altered.

Highest fruit length (8.31 cm), fruit breadth (8.97 cm), fruit weight (266.37g) and fruit volume (255.44 cc) was measured from solubor + biozyme + triacontanol and followed by biozyme + triacontanol. Increased fruit size at harvest may be due to enhanced cell enlargement by growth regulators during developmental stages. The major plant growth regulators present in biozyme are auxins, cytokinins, indoles and hormones. These PGRs are present in biozyme in very small quantities, generally measured in parts per million. The cytokinins in biozyme are a major factor applied to apple and peach trees in promoting the growth of fruiting spurs and reduce premature dropping of fruit and improve the quality of the fruit [25]. Same results were obtained by [23] and [26] who described that application of biozyme increased fruit size in

apple trees. Similarly, triacontanol is a growth regulator which is a long 30 carbon primary alcohol and occurs in nature as a natural consistent of bee wax and plant waxes [27].

Foliar applications of chemicals improved the firmness of Red Delicious apple. Maximum firmness (10.01 kg/cm<sup>2</sup>) was obtained with solubor + biozyme + triacontanol followed by solubor + biozyme (9.68 kg/cm<sup>2</sup>) and biozyme + triacontanol (9.49 kg/cm<sup>2</sup>). The flesh firmness degree has been regarded as a good index for judging the maturation of fruits. After two months of second spray, solubor was replaced with calcium chloride. The calcium sprays improved the flesh firmness. Calcium is an important nutrient involved in the structure of cell walls and cell membranes [28]. [29] Noticed that fruits having higher concentration of calcium compounds in pre-harvest spray retained higher

firmness. [30] also reported that pre-harvest calcium sprays significantly increased the calcium content in the peel (24-25%) as well as in flesh (11-12%) of the peach fruits after harvest. [31] observed that formulations containing calcium chloride were the most suitable for increasing calcium content.

The presence of GA<sub>3</sub> in biozyme may increase fruit firmness degree in fruits. Concurring with findings from other studies, [32] observed that GA<sub>3</sub> treated fruits were consistently more firmer than untreated fruits. The relative small increase in calcium content in the skin tissue with only CaCl<sub>2</sub> application can be attributed to low absorption and incorporation of calcium content in the cell wall [33] The boron might promote calcium incorporation into the cell wall [34]. These results are in agreement with findings of [35].

**Table 1. List of treatments applied during the study**

Treatment	Common name	Concentration
T <sub>1</sub>	Solubor	0.1 per cent
T <sub>2</sub>	Biozyme	1.5 ml/lt
T <sub>3</sub>	Triacontanol	10 ppm
T <sub>4</sub> (T <sub>1</sub> + T <sub>2</sub> )	Solubor + Biozyme	0.1 per cent + 1.5 ml/lt
T <sub>5</sub> (T <sub>1</sub> +T <sub>3</sub> )	Solubor + Triacontanol	0.1 per cent + 10 ppm
T <sub>6</sub> (T <sub>2</sub> + T <sub>3</sub> )	Biozyme + Triacontanol	1.5 ml/lt +10 ppm
T <sub>7</sub> (T <sub>1</sub> +T <sub>2</sub> +T <sub>3</sub> )	Solubor+Biozyme+ Triacontanol	0.1 % +1.5 ml/lt+10 ppm
T <sub>8</sub>	Control	Water spray

Note: Two months after 2<sup>nd</sup> spray, solubor was replaced with 0.5 per cent CaCl<sub>2</sub>

**Table 2. Effect of solobur & bio-stimulants on floral and yield characteristics of apple fruit cv. "Red Delicious"**

Treatment	Concentration	Bloom (flower) index (%)	Fruit set (%)	Fruit drop (%)	Fruit retention (%)	Fruit yield (%)	
		Pooled	Pooled	Pooled	Pooled	Pooled	
T <sub>1</sub>	Solubor*	0.1%	29.84	47.92	15.34	32.58	76.50
T <sub>2</sub>	Biozyme	1.5 ml/lt	29.72	46.83	15.34	27.79	72.13
T <sub>3</sub>	Triacontanol	10 ppm	29.81	44.35	18.89	29.44	66.50
T <sub>4</sub>	Solubor* + Biozyme	0.1% + 1.5 ml/lt	43.62	65.25	11.34	54.07	92.67
T <sub>5</sub>	Solubor* + Triacontanol	0.1% + 10 ppm	40.29	53.42	12.31	47.08	87.55
T <sub>6</sub>	Biozyme + Triacontanol	1.5 ml/lt + 10 ppm	30.30	50.42	12.99	38.21	82.28
T <sub>7</sub>	Solubor* + Biozyme + Triacontanol	0.1% + 1.5 ml/lt + 10 ppm	51.65	74.71	11.19	61.72	97.75
T <sub>8</sub>	Control	Water	29.04	43.13	25.09	19.26	60.88
C.D (p < 0.05)			0.89	0.89	0.85	0.68	0.59

\*Two months after 2<sup>nd</sup> spray, solubor was replaced with 0.5% CaCl<sub>2</sub>

### 3.3 Effect of Chemicals on Bio-chemical Characteristics of Fruit

The results obtained clearly indicate that solubor, biozyme, triacontanol and their combinations were most effective in influencing the bio-chemical characteristics of fruit. Maximum TSS (15.74%) was noted in solubor + biozyme + triacontanol treatment followed by biozyme + triacontanol (14.99%). Possible reasons may be assigned to the faster hydrolysis or degradation of starch into simple sugars by the  $\alpha$ -amylase activities [36]. The values of acidity with treatments were 0.09 to 0.11 and 0.09 to 0.10 per cent during 2012 and 2014 seasons comparing with 0.12 to 0.13 per cent in control. Results showed that there was a gradual decrease in titratable acidity during the consequent year in treated and untreated fruits. All the treatments recorded the lower values for titratable acidity as compared with control. These results are in line with those reported by [37] who reported that Anna apple fruit with calcium chloride spray plus dipping decrease the acidity during storage.

Effect of chemicals application on fruit of reducing sugars content and total sugars are presented in Table 5. Data showed that the treatments resulted in highest reducing sugars (8.85%) and total sugars (11.80%) in solubor + biozyme + triacontanol treatment followed by biozyme + triacontanol (8.43 and 11.24%) of

reducing sugars during 2013 and 2014, respectively.

Sugars in the pome fruits are sucrose, glucose and fructose. Glucose and fructose have together been determined as reducing sugars [38] Increase in TSS during maturation process may be due to conversion of starch and other polysaccharides into soluble solids. [39] also reported an increase in total soluble solids as well as total acidity due to boron applications. This can be attributed to transportation of higher amount of assimilates into fruit tissues.

[40] found that boron has a main role in many processes specially transport of sugars and carbohydrate metabolism. Biozyme and triacontanol are other important reasons to increase the TSS and sugars in apple. Beneficial effects of biozyme may be ascribed to the hormonal action of sea weed extract, which increase the endogenous hormonal action. A seaweed extract has been found to contain biologically active cytokinins, which promote cell division [41].

Increase in TSS, sugars and TSS: acid ratio may be related with enzymes which are present in seaweed extract (biozyme) that enhanced the synthesis of different proteins, acids and sugars [42]. Also observed that application of bio-stimulants improved the SSC: TA ratio in 'Roomy Red' and 'Thompson Seedless' grapes.

**Table 3. Effect of solobur & bio-stimulants on physical changes of apple fruit cv. "Red Delicious"**

Treatment	Concentration	Fruit color (0-5 scores)	Fruit length (cm)	Fruit breadth (cm)	Fruit weight (g)
		Pooled	Pooled	Pooled	Pooled
T <sub>1</sub> Solubor*	0.1%	2.65	7.45	8.68	197.14
T <sub>2</sub> Biozyme	1.5 ml/lit	2.96	7.39	8.35	215.36
T <sub>3</sub> Triacontanol	10 ppm	3.10	7.57	8.00	205.25
T <sub>4</sub> Solubor* + Biozyme	0.1 % + 1.5 ml/lit	3.41	8.04	8.28	228.05
T <sub>5</sub> Solubor* + Triacontanol	0.1 % + 10 ppm	3.42	8.07	8.02	226.90
T <sub>6</sub> Biozyme + Triacontanol	1.5 ml/lit + 10 ppm	3.59	8.16	8.71	235.19
T <sub>7</sub> Solubor* + Biozyme + Triacontanol	0.1% + 1.5 ml/lit + 10 ppm	4.00	8.31	8.97	252.85
T <sub>8</sub> Control	Water	2.06	6.08	6.95	170.78
C.D p < 0.05)		0.73	0.85	NS	0.76

\*Two months after 2<sup>nd</sup> spray, solubor was replaced with 0.5% CaCl<sub>2</sub>

**Table 4. Effect of solubur & bio-stimulants on physical changes of apple fruit cv. "Red Delicious"**

Treatment	Concentration	Fruit volume (cc)	Specific gravity	Fruit firmness (kg/cm <sup>2</sup> )	Fruit juice (%)
		Pooled	Pooled	Pooled	Pooled
T <sub>1</sub> Solubor*	0.1%	188.00	1.04	9.10	52.27
T <sub>2</sub> Biozyme	1.5 ml/lt	195.13	1.05	8.85	54.03
T <sub>3</sub> Triacantanol	10 ppm	188.50	1.05	8.95	53.36
T <sub>4</sub> Solubor* + Biozyme	0.1 % + 1.5 ml/lt	218.07	1.09	9.68	58.42
T <sub>5</sub> Solubor* + Triacantanol	0.1 % + 10 ppm	205.25	1.05	9.20	55.55
T <sub>6</sub> Biozyme + Triacantanol	1.5 ml/lt + 10 ppm	245.57	1.11	9.49	66.06
T <sub>7</sub> Solubor* + Biozyme + Triacantanol	0.1% + 1.5 ml/lt + 10 ppm	255.44	1.11	10.01	68.61
T <sub>8</sub> Control	Water	165.73	1.03	8.22	50.29
C.D (p < 0.05)		0.80	NS	0.81	0.54

\*Two months after 2<sup>nd</sup> spray, solubor was replaced with 0.5% CaCl<sub>2</sub>

**Table 5. Effect of solubur & bio-stimulants on bio-chemical characteristics of applefruit cv. "Red Delicious"**

Treatment	Concentration	TSS %		Acidity %	Sugars	
		TSS: Acid		TSS: Acid	Reducing %	Total %
		Pooled	Pooled		Pooled	Pooled
T <sub>1</sub> Solubor*	0.1%	13.99	0.10	133.66	7.87	10.49
T <sub>2</sub> Biozyme	1.5 ml/lt	14.08	0.10	140.75	7.86	10.49
T <sub>3</sub> Triacantanol	10 ppm	13.99	0.11	133.61	7.92	10.56
T <sub>4</sub> Solubor* + Biozyme	0.1 % + 1.5 ml/lt	14.83	0.10	148.26	8.34	11.12
T <sub>5</sub> Solubor*+Triacantanol	0.1 % + 10 ppm	14.66	0.10	155.08	8.25	10.99
T <sub>6</sub> Biozyme+ Triacantanol	1.5 ml/lt + 10 ppm	14.99	0.09	161.00	8.43	11.24
T <sub>7</sub> Solubor* + Biozyme + Triacantanol	0.1% + 1.5 ml/lt + 10 ppm	15.74	0.09	174.89	8.85	11.80
T <sub>8</sub> Control	Water	12.31	0.13	102.74	7.04	9.39
C.D p < 0.05)		NS	0.85	0.82	0.008	0.88

\*Two months after 2<sup>nd</sup> spray, solubor was replaced with 0.5% CaCl<sub>2</sub>

Enhanced level of chlorophyll level in the treated fruits ultimately resulted in increased rate of photosynthesis and accumulation of carbohydrates reserves in the apple. [18] also reported that foliar application of yeast extract and some micronutrients increased the reducing sugars percentage of 'Red Romy' grape vines. [23] observed that apple plants treated with biozyme fruit at the rate of 1.5 ml per litre of water resulted in higher TSS and total sugars content. Fruits were more coloured and matured earlier with this treatment. [43] reported that application of triacantanol (TRIA) 10 days before harvest to Santa Rosa plum significantly increased total soluble solids. Exogenous application of TRIA has been reported to

enhance some major processes such as chlorophyll fluorescence, photosynthesis, tree amino acids, reducing sugars, soluble solids, proteins and crop yield [44].

#### 4. CONCLUSION

While going through the results, it is revealed that the combinations of solubor, biozyme and triacantanol were best to improve the fruit set and yield of apple fruits. The highest fruit set and minimum fruit drop was recorded with the application of solubor + biozyme + triacantanol and solubor + biozyme. Good quality glossy fruits were produced by solubor + biozyme + triacantanol and biozyme + triacantanol

treatments and these treatments produced apple fruits of excellent acceptability with very much improved physico-chemical characteristics. Solubor + biozyme + triacontanol.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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