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Effect of Plant Biostimulants on Growth, Chlorophyll Content, Flower Drop and Fruit Set of Pomegranate Cv. Kandhari Kabuli

Aziz Rahman Abubakar, Naira Ashraf* and Moieza Ashraf

Department of Fruit Science, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh, India

Email: naira.ashraf@gmail.com

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Abstract

The experiment was laid out in the pomegranate experimental block of the Department of Fruit Science, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. The experiment consisted of 19 treatments with three replications. The pomegranate trees under investigation were subjected to different concentrations of biostimulants viz. Vipul (TRIA) (5 ml/l, 10 ml/l, 15 ml/l), Spic cytozyme (1 ml/l, 2 ml/l, 4 ml/l), Homobrassinolides (Godrej Double) (0.5 ml/l, 1 ml/l, 1.5 ml/l), Biozyme Crop Plus (1 ml/l, 2 ml/l, 3 ml/l) and Vipul + Homobrassinolides (0.5 + 5 ml/l, 1 + 5 ml/l, 1.5 + 5 ml/l, 5 + 0.5 ml/l, 5 + 1 ml/l, 5 + 1.5 ml/l). The results revealed that the highest tree growth was recorded in trees treated with Spic cytozyme at 4 ml/l. The highest total chlorophyll content was observed with the application of Vipul 15ml/l, whereas the highest fruit set and minimum flower drop was recorded with the application of Vipul + Homobrassinolides (1.5 + 5 ml/l).

Highlights

- An experiment was laid out on pomegranate cv.Kandhari Kabuli with varied concentrations of biostimulants.
- Results revealed that the highest tree growth was recorded in trees treated with Spic cytozyme @ 4 ml/l, where as highest total chlorophyll content was observed with Vipul @15ml/l, and highest fruit set and minimum flower drop was recorded with the application of Vipul and Homobrassinolides @1.5 and 5 ml/l respectively.

Keywords: Pomegranate, growth, fruit set and biostimulants

Pomegranate (*Punica granatum* L.) belongs to the family Punicaceae and is mainly grown in tropical and sub-tropical regions of the world. High quality fruits can be produced where there is cool winter and hot dry summer. Pomegranate flowers are classified into three types: male, hermaphroditic, and intermediate (Chaudhari and Desai, 1993) as observations of gradients of flower types in some pomegranate genotypes support this concept. However, generally flowering in pomegranate is characterized as having both hermaphroditic (bisexual) flowers and functionally male flowers on the same plant, a condition referred to as andromonoecy (Wetzstein, 2011). A positive relation was found between the percentage of bisexual flowers and bearing capacity (Chaudhari and Desai, 1993). Manipulating the relative ratio of flower types to environmental conditions can be very advantageous. Male flowers drop and generally fail to set; thus, fruits develop exclusively from bisexual flowers. In Himachal Pradesh, pomegranate is mainly cultivated under rainfed conditions and flowering occurs in April-May, when high temperature is an issue. Application of plant growth regulators can influence the sex expression and distribution of flower types in pomegranate (Chaudhari and Desai, 1993). Gibberellic acid induced more male flowers and reduced hermaphrodite flowers, whereas ethrel and maleic hydrazide induced more hermaphrodite and fewer male flowers. Optimizing cultural conditions may be a means to promote the development of greater numbers of bisexual flowers with high vigor to obtain maximum fruit set and yield (Wetzstein, 2011).

Plant growth and development, as well as the responses to environmental factors, are highly regulated by complex and coordinated action of the endogenous hormones and plant biostimulants. They have the potential of increasing plant productivity and quality through influence on various metabolic processes. They have the potential of increasing plant productivity and quality through influence on various metabolic processes. Vipul, Spic cytozyme, Biozyme Crop plus and Godrej Double are few such chemicals available in the market. Vipul is a commercial formulation of triacontanol (TRIA) which is a long chain 30 carbon primary alcohol and occurs in nature as a natural constituent of bee wax and plant waxes. Biozyme Crop Plus is a commercial formulation of seaweed extract (*Ascophyllum nodosum*), enzymes and hydrolyzed proteins whereas, Spic cytozyme contain gibberellic acid, auxins, cytokinins, seaweed extract (*Ascophyllum nodosum*), hydrolysed proteins and trace elements. Godrej Double is a commercial formulation having homobrassinolides, belongs to brassinosteroids group of plant hormones. Brassinosteroids are relatively new endogenous phytohormones (Swamy and Rao, 2010) which was first isolated from pollen grains of *Brassica napus*, participate with other plant hormones in the regulation of numerous aspects of plant development, including shoot and root growth, vascular differentiation, fertility, and seed germination (Clouse and Sasse, 1998; Nomura *et al*., 2007). The present study was therefore, carried out to find the suitability of these chemicals on growth and production of pomegranate.

Materials and methods

The experiment was conducted in the experimental orchard of the Department of Fruit Science, Dr. Y .S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. The experimental orchard is situated between 310 N latitude and 770 E longitude at an altitude of 1276 m above mean sea level. Treatment plots were arranged in a randomized complete block design with 3 replications. The experiment consisted of 19 treatments with three replications laid out in Randomized Block Design. The biostimulants applied were Vipul (5 ml/l, 10 ml/l, 15 ml/l), Spic cytozyme (1 ml/l, 2 ml/l, 4 ml/l), Homobrassinolides (0.5 ml/l, 1 ml/l, 1.5 ml/l), Biozyme Crop plus (1 ml/l, 2 ml/l, 3 ml/l), Vipul + Homobrassinolides (0.5 + 5 ml/l, 1 + 5 ml/l, 1.5 + 5 ml/l, 5 + 0.5 ml/l, 5 + 1 ml/l, 5 + 1.5 ml/l) and control (water spray). Seven years old pomegranate trees of cv. Kandhari Kabuli with uniform vigour and size, planted at a spacing of 3m x 3m were selected for the study. The biostimulants were applied 45 days after bud burst and repeated 10 days after fruit set. The solutions of biostimulants were prepared by dissolving them in water directly and sprayed with the help of foot sprayer. Spraying (four liters/tree) was done in a clear and calm day during the morning hours to obtain better effect till the leaves / twigs were wet and droplets of solutions started trickling down. Before spraying, 0.5ml of wetting agent (Indtron-AE) per litre of solution was added as surfactant to reduce surface tension and to facilitate the absorption of solution. The observations in respect of annual shoot growth was recorded by selecting ten shoots from the current season's growth of each tree on east, west, south and north sides of the tree periphery. The length of these shoots was measured with a measuring tape after the cessation of growth and expressed in centimeters. Trunk girth was recorded at 15 cm above ground level with the help of Digital Vernier calliper once before the start of the experiment and again after the completion of the experiment. The average increase in trunk girth was expressed in centimeter. The canopy volume was worked out as per the procedure given by Westwood (1993) and expressed in cubic meter. For recording the average leaf area, thirty fully expanded and mature leaves were collected at random from the periphery of each tree in the month of July. The leaf area of each replication was measured with the help of LI-COR 3100 Leaf Area Meter. The average leaf area was calculated by dividing the total leaf area with the number of leaves and expressed in square centimeter. The total chlorophyll content was determined by taking the O.D. values of the leaf extracts in Spectronic-20 at 645 and 663 nm wavelengths against a dimethyl sulphoxide as blank and the results thus obtained were expressed as mg of total chlorophyll per gram of fresh weight. The flower drop was recorded by selecting four branches of one meter length on east, west, south and north sides of the periphery of each tree and the number of flowers were counted on 1st of May and again at the time of final fruit set on 15th of

May and expressed in percentage. The fruit set was determined by counting the number of fruits at final fruit set on 15th of May and expressed in percentage. The data recorded was analyzed using the statistical procedure as described by Gomez and Gomez (1984).

Results and discussion

Tree growth and vigour

The biostimulants had a marked influence on annual shoot growth, trunk girth and canopy volume (Table 1). The maximum shoot growth (26.33 cm), trunk girth (0.93 cm) and canopy volume (5.45 m^3), leaf area (12.48 cm^2) were recorded in treatment T6 (Spic cytozyme 4ml/l) which was significantly superior to all other treatments. The increase in growth with the use of Spic cytozyme is in accordance with the work of Mandal and Kumar (1989) in guava, biozyme crop plus and protozyme in apple (Sharma, 1990), actiwave (seaweed extract) in strawberry (Spinelli *et al*., 2010).The effect of Spic cytozyme might be due to the increase in photosynthesis as a result of increased chlorophyll content and uptake of nutrients. Spic cytozyme possesses cytokinins, auxins and gibberellins like activity, individually or collectively may have influenced different growth characters through increase in cell division, enlargement and mineral absorption (Taiz and Zeiger, 2006). The minimum values for shoot growth (15.88 cm), trunk girth (0.44 cm), canopy volume (2.58 m^3) and leaf area (9.20 cm2) were observed in treatment T19 (control).

The chlorophyll content values were observed to range from 2.09 to 3.23 mg/g (Table 2). The highest chlorophyll (3.23 mg/g) was recorded in treatment T3 (Vipul 15 ml/l) which was statistically at par with treatment T6 (3.15 mg/g), T12 (2.95 mg/g), T2 (2.89 mg/g) and T15 (2.86 mg/g). These results are in consonance with those obtained by Sharma (1990) in apple with the application of Vipul. Effect of Vipul might be due to increase in photosynthesis, nutrient uptake (Ries *et al*., 1993), iron (Hinkle and Eisenmenger, 1958) and Mg (Blunden *et al.*, 1996) which are essential elements for chlorophyll biosynthesis. Biostimulants might have increased the uptake of these elements along with water and thereby, increased the chlorophyll content. The minimum chlorophyll (2.09 mg/g) was observed in treatment T19 (control) and was statistically at par with T16 (2.17 mg/g), T7 (2.19 mg/g), T8 (2.21 mg/g), T9 (2.27 mg/g), T4 (2.31 mg/g), T10 (2.32 mg/g), T5 (2.32 mg/g) and T13 (2.43 mg/g). Treatment T3 and T6 resulted in 1.5 fold increase in chlorophyll content when compared to the control.

Table 1: Effect of plant biostimulants on growth and vigour of pomegranate

Treatment	Concentration (ml/l)	Annual shoot growth (cm)	Increase in trunk girth (cm)	Canopy volume (m^3)	Average leaf area (cm^2)
T_1 Vipul(TRIA*)	5	17.04	0.54	9.61	3.44
T_2 Vipul	10	18.29	0.57	10.06	3.48
T_3 Vipul	15	20.96	0.67	10.21	3.69
T_4 Cytozyme	1	18.46	0.49	9.69	3.45
T_5 Cytozyme	2	20.25	0.53	10.42	3.70
T_6 Cytozyme	4	26.33	0.93	12.48	5.45
T_7 HBRs**	0.5	16.25	0.45	9.34	2.53
T_8 HBRs	1	16.42	0.46	9.35	2.72
T_9 HBRs	1.5	16.48	0.47	9.49	2.88
T_{10} Biozyme	1	17.71	0.48	9.39	3.31
T_{11} Biozyme	2	18.33	0.59	9.68	3.42
T_{12} Biozyme	3	20.46	0.59	10.32	3.81
T_{13} Vipul+HBRs	0.5 +5	20.33	0.55	10.20	3.49
T_{14} Vipul+HBRs	1 +5	20.44	0.59	10.32	3.63
T_{15} Vipul+HBRs	1.5 +5	21.25	0.67	10.64	4.21
T_{16} Vipul+HBRs	5 +0.5	18.17	0.56	9.96	3.39
T_{17} Vipul+HBRs	5 +1	18.58	0.56	10.08	3.48
T_{18} Vipul+HBRs	5 +1.5	19.08	0.59	10.38	3.55
T_{19} Control	Water Spray	15.88	0.44	9.20	2.58
$CD_{0.05}$		0.75	0.05	0.48	0.68

Chlorophyll content

Table 2: Effect of plant biostimulants on total chlorophyll content, flower drop and fruit set of pomegranate

Treatment	Concentration (ml/l)	Total chlorophyll (mg/g fresh weight)	Flower drop (%)	Fruit set (%)
T_1 Vipul(TRIA*)	5	68.23 (55.69)	31.77 (34.31)	2.50
T_2 Vipul	10	67.22 (55.08)	32.78 (34.93)	2.89
T_3 Vipul	15	65.18 (53.84)	34.82 (36.17)	3.23
T_4 Cytozyme	1	65.63 (54.11)	34.37 (35.89)	2.31
T_5 Cytozyme	2	65.16 (53.83)	34.84 (36.18)	2.32
T_6 Cytozyme	4	61.40 (51.59)	38.60 (38.41)	3.15
T_7 HBRs**	0.5	68.97 (56.15)	31.03 (33.85)	2.19
T_8 HBRs	1	66.76 (54.79)	33.24 (35.21)	2.21
T_9 HBRs	1.5	65.26 (53.89)	34.74 (36.11)	2.27
T_{10} Biozyme	1	65.15 (53.82)	34.85 (36.18)	2.32
T_{11} Biozyme	2	64.44 (53.40)	35.56 (36.61)	2.60
T_{12} Biozyme	3	62.33 (52.14)	37.67 (37.86)	2.95
T_{13} Vipul+HBRs	0.5 +5	61.33 (51.55)	38.67 (38.45)	2.43
T_{14} Vipul+HBRs	1 +5	59.00 (50.19)	41.00 (39.81)	2.58
T_{15} Vipul+HBRs	1.5 +5	57.23 (49.16)	42.77 (40.84)	2.86
T_{16} Vipul+HBRs	5 +0.5	67.41 (55.19)	32.59 (34.81)	2.17
T_{17} Vipul+HBRs	5 +1	65.93 (54.29)	34.07 (35.71)	2.55
T_{18} Vipul+HBRs	5 +1.5	64.25 (53.28)	35.75 (36.72)	2.65
T_{19} Control	Water Spray	72.21 (58.19)	27.79 (31.81)	2.09
$CD_{0.05}$		1.16	1.16	0.40

Figures in parenthesis are arcsine transformed values.

Flower drop and fruit set

All the treatments of biostimulants influenced the flower drop and fruit set in comparison to control (Table 2). The minimum flower drop (57.23 %) and maximum fruit set (42.77 %) were recorded in treatment T15 (Vipul + HBRs 1.5 + 5ml/l) which was significantly superior to all other treatments and was closely followed by treatment T14 (59.00%, 41.00 %) respectively. The results confirm the findings of Sharma (1990) who recorded increase in fruit set with Biozyme crop plus and Protozyme in Red Delicious apple. Reduced flower drop and increase in fruit set may be due to delay in abscission (the effect of cytokinins and auxins) through preservation of loss of pectin material in middle lamella (Kachave and Bhosale, 2007), influenced sex expression (Chaudhari and Desai, 1993) and promotion of tube growth may have lead to better fruit set and thus prevented flower drop. Similarly, the results with Vipul application is in agreement with those of Chandel (1985) who recorded increase in fruit set in Santa Rosa plum with triacontanol (Vipul), and with application of Miraculan improved fruit set in apricot (Sharma, 1991) with the reason that it may have increased carbohydrates levels and C/N ratio (Sharma, 1990). Moreover, enhanced fruit set and reduced flower drop due to HBRs is in line with the application of GA3 + brassinosteroids + BA in Thompson Seedless grapes (Warusavitharana *et al.,* 2008). HBRs are known to facilitate pollen tube growth (Mussig, 2005), retard abscission, enhance resistance to water, nutrient stress (Fujioka, 1997), enhanced photosynthesis and mobilization of metabolites to the flowers (Bhatia and Kaur, 1997) which resulted in less flower drop and more fruit set. Homobrassinolides is the sixth group of growth promoting hormones with broad spectrum of physiomorphological responses that influence the levels of nucleic acids, increase nitrogen fixation and enhance soluble protein content, photosynthesis, cell division and cell expansion (Fujioka, 1997). The maximum flower drop (72.21 %) and minimum fruit set (27.79 %) were observed in treatment T19 (control) which was significantly lower than all other treatments. The results further show that the mean value of flower drop obtained from all the concentrations of Vipul + HBRs was least followed by Biozyme Crop plus, Spic cytozyme, Vipul and HBRs.

Conclusion

From this study, it is evident that the application of plant biostimulants significantly improved plant growth, flowering, fruit set, and reduced flower drop. The highest tree growth was recorded in trees treated with Cytozyme at 4 ml/l. The highest total chlorophyll content was observed with the application of Vipul 15ml/l, whereas the highest fruit set and minimum flower drop was recorded with the application of Vipul + Homobrassinolides (1.5 + 5 ml/l).

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