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Effect of plant bio stimulant application on plant health, growth, fruit yield and quality of apple (*Malus x domestica*) under temperate conditions of Jammu and Kashmir, India

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Abstract
A research trial was conducted to study the efficacy of commercial product G5 granules and liquid (a plant bio stimulant consisting of sea weed extract, amino acids, herbal extract, humic acid and anti-fungal substances) on plant health, growth, fruit yield and quality attributes of apple *cv.* Red Delicious under temperate conditions of Jammu and Kashmir during the year 2022-23. The treatments comprised: T_1 (soil application of G5 granules @500 g/tree as basal dose at pre-flowering stage), T_2 (T_1 + G5 liquid @250 ml/100 l of water as foliar spray at petal fall stage), T_3 (T_2 + G5 liquid @250 ml/100 l of water as foliar spray at walnut size stage), T_4 (T_3+G5 liquid @250 ml/100 l of water as foliar spray at fruit development stage) and T_5 (control) in addition to standard recommendations. Results of study showed that the highest growth in terms of shoot length was obtained in T_4 followed by T_3, T_2 and T_1 treatments. The highest fruit set was observed in treatment T_2 that was statistically at par with T_3 and T_4. T_4 recorded the highest fruit retention, fruit yield and the lowest fruit drop. The lowest growth, fruit set, fruit retention, fruit yield and the highest fruit drop was observed in control. All treatments were found to be highly superior to control in terms of fruit weight, length, breadth and firmness with T_3 and T_4 showing marked increase in fruit colour as compared to other treatments. As regards chemical fruit quality attributes, again T_4 recorded the highest TSS, reducing sugars and total sugars while the values of these attributes were the lowest in control and *vice versa* for acidity. There was no apparent phyto-toxic effect of any of the treatments on tree/fruit health. Thus, treatment T_4 (soil application of G5 granules as basal dose @500 g/tree at pre-flowering stage + G5 liquid foliar sprays @250ml/100 l of water one each at petal fall, walnut size and fruit development stage) was found to be the best among all treatments in enhancing growth, fruit yield and quality attributes of apple.

Keywords: Apple, foliar spray, g5 bio stimulant, soil application, quality, yield

Introduction
Apple (*Malus x domestica* Borkh.) is one of the major fruit crops popular in temperate regions of the world because of its high acreage, production, economic returns and nutritional value. In India, Jammu and Kashmir state is the largest producer of apple fruit accounting for 75% of country's total apple production. Apple alone occupies 51% of the total area under all temperate fruit crops grown in the state and is a major contributor to the state economy. During the year 2021-22, area under apple cultivation in Jammu and Kashmir was 168.57 thousand ha with the production of 1898.59 thousand tonnes of fruit (Anonymous 2022) [2]. Although average productivity of commercially important apple cultivars in Jammu and Kashmir is highest in the country (10-12 tonnes/ha), it compares poorly to the yield levels (20-30 tonnes/ha) in horticulturally advanced countries of the world. Lack of proper nutrition has been cited as one of the main causes of low productivity amongst several other factors like alternate bearing, defective training and pruning, use of seedling rootstock of unknown performance, poor water management, inadequate suitable pollinizers and ineffective control of pests and diseases. Further, the horticulture sector today is faced with the dual challenge of boosting production of

quality fruits to meet the demand of increasing population on one hand and curtailing excessive use of agrochemicals to maintain quality of environment, on the other. Use of bio stimulants at large scale is a viable option to address to these issues and can be considered as an alternative technology to organic management. Certain bio stimulants have the potential to increase the amount of nutrients taken up by plants (Halpern *et al.* 2015) [23]. Reducing the amount of mineral fertilizers introduced into the soil limits environmental degradation.

Plant bio stimulants such as seaweed extracts, herbal extracts, amino acids, humic and fulvic acids, protein hydrolysates *etc.* (Battacharyya *et al.* 2015, Canellas *et al.* 2015, Colla *et al.* 2017) [8, 12, 13], have recently gained popularity as promising production tools that can enhance the efficiency of horticultural inputs (irrigation water and fertilizers), crop tolerance towards abiotic stresses (drought, salinity, extreme temperature and radiation) and the quality of produce with minimum impact on environment. Several reviews on bio stimulants (Calvo *et al.* 2014, Du Jardin 2015, Rouphael and Colla 2020, Tandon and Dubey 2015) [11, 17, 39, 45, 13] have been published in recent years mainly concerning agronomical aspects, but the research pertaining to their contribution to growth, yield and quality of fruit crops remains extremely limited. Therefore, the present studies were undertaken to evaluate the efficacy of G5 bio stimulant on growth and performance of apple in temperate Jammu and Kashmir.

Materials and Methods

An experiment was conducted during the year 2022-23 at the research farm of Regional Horticultural Research Sub-Station Bhaderwah of Sher-e-Kashmir University of Agricultural Sciences and Technology-Jammu, India on 14 years old Red Delicious apple plants of uniform size and vigour raised on seedling rootstock planted at a distance of 5m x 5m. The study area lies between geographical co-ordinates of $32^0$56’59”-$32^0$57’12” N and $75^0$43’31”-$75^0$43’39” E. Soils of experimental site were sandy clay loam in texture with pH 5.42, EC value of 0.08 dS/m having organic carbon content of 20.0 mg kg^{-1}. G5 (a commercial bio stimulant formulation used in the current studies) is a crop supplement of plant origin containing sea weed extract, amino acids, herbal extract, humic acid and anti-fungal substances that has been reported to improve nutrient use efficiency and plant defence mechanism resulting in better crop performance.

The experimental design in this study was randomised block design (RBD) with four replicates for each treatment. The treatments consisted of T_1 (soil application of G5 granules @500 g/tree as basal dose at pre-flowering stage), T_2 (T_1+ G5 liquid @250 ml/100 l of water as foliar spray at petal fall stage), T_3 (T_2+ G5 liquid @250 ml/100 l of water as foliar spray at walnut size stage), T_4 (T_3+G5 liquid @250 ml/100 l of water as foliar spray at fruit development stage) and T_5 (control). All treatments including control received uniform doses of fertilisers, plant protection measures and other inter-cultural operations as per standard recommendations.

Ten shoots randomly selected around the periphery of plant were tagged. Their extension growth was measured in centimetres before pruning. Fruit set was recorded three weeks after petal fall and per cent fruit set was calculated by following formula (Westwood 1993) [50]:

Fruit set (%) = (Number of fruit set/ Number of flowers cluster) x100

Total numbers of fruits retained on the tagged branches were counted at harvesting and the percentage of fruit retained was calculated on the basis of total number of fruits at the time of fruit set by following formula:

Fruit retention (%) = (Number of fruits at harvest/ Number of fruit set) x100

Percent fruit drop was calculated by subtracting per cent fruit retention from 100 and average was worked out. Fruit yield was recorded by the removal of crop load during harvesting at commercial maturity (mid-August) and expressed as kg/tree. Weight of fruit was recorded on a top pan balance. The unit sample consisted of ten fruits and the results were expressed as g/fruit. Fruit length and breadth were recorded with the help of Vernier caliper. Fruit firmness was measured with the help of Magness and Taylor pressure tester and expressed as kg/cm^2. Visible fruit skin colour was used to calculate percentage of fruit colour. Total soluble solids (TSS) were determined using a hand refractometer. Percentage of titratable acidity in fruit juice was determined according to AOAC (1995) [3]. Total sugars in the fruit pulp were determined by phenol sulphuric method (Dubois *et al.* 1956) [17, 16]. Observations on different phyto-toxicity indicators (leaf injury on tips/surface, wilting, yellowing, vein clearing, necrosis, epinasty, hyponasty and stunting) were recorded 0, l, 3, 5, 7, 10 and 15 days after each treatment on 0-10 point scale with 0 indicating no adverse effect while 10 indicating 91-100% crop being adversely effected, using the ratings as given in table 1.

Table 1: Phyto-toxicity ratings used in present studies

Injury (%)	Rating	Injury (%)	Rating
0	No phyto-toxicity	51-60	6
1-10	1	61-70	7
11-20	2	71-80	8
21-30	3	81-90	9
31-40	4	91-100	10
41-50	5		

Data on both the years were pooled and the mean values are presented in tables 2 to 4. MS Excel 2007 was used for data processing. Analysis of variance (ANOVA) was performed using the software package Fast Statistics v2.0.4 Build 0627. Means were differentiated using CD values at 1% level of significance ($p \leq 0.01$).

Results and Discussion

Growth, yield and fruiting characteristics

Data presented in table 2 showed that the highest shoot extension growth (61.77 cm) was obtained in T_4 treatment followed by T_3, T_2, T_1 treatments with the lowest (49.73 cm) being in T_5 (control). All treatments differed significantly from each other with respect to this parameter. The observed increase in vegetative growth might be due to the amino acids and hormones like auxins and cytokinins present in G5 formulation. Amino acids are the N containing components of proteins and required for initial growth of deciduous plants in spring during cell division (Bi *et al.* 2003) [10]. Auxin IAA and cytokinins are known to play an important role in cell division (Moore 1979) [36]. The highest fruit set (25.81%) was observed in treatment T_2 followed by T_1 (22.44%) and the lowest (18.31%) in control (T_5). Treatments T_2, T_3, T_4 were statistically at par with each other. Humic acid has been reported to increase floral stimulus due to its florigenic activity (Bhatt and Singh 2022; Ngullie *et* *al.* 2014) [9, 38]. Cytokinins are involved in induction of flowering, fruiting and senesence (Kumar *et al.* 2013, Kunikowska *et al.* 2013, Schmülling 2002) [28, 29, 40] while amino acid sprays enhance pollen tube ovule penetration and delay ovule senesence increasing fruit set and yield (Arabloo *et al.* 2017) [4]. The highest fruit retention (60.39%) was also observed in treatment T_4 followed by T_3, T_2 and T_1; treatments T_1 and T_2 being statistically at par with each other. However, fruit drop was recorded highest (49.59%) in control (T_5) followed by T_1 (48.31%), T_2 (47.41%), T_3 (44.93%) and T_4 (39.61%), wherein treatments T_1 and T_2 were statistically at par with each other. NAA, an auxin has been successfully used as an effective treatment in reducing pre harvest fruit drop (Iqbal *et al.* 2009, Maurya and Singh 1981) [25, 35]. Among treatments, T_4 recorded the highest fruit yield (32.53 kg/ tree) whereas the control had the lowest one (24.53 kg/tree). Treatments T_2, T_4 and T_5 differed significantly from each other; while T_1, T_2 and T_3 were statistically at par with each other. Thus, shoot extension growth, fruit set, fruit retention, fruit drop and fruit yield were significantly influenced by G5 application in all combinations except for T_1 in case of fruit retention and fruit drop; and T_1, T_3 in case of fruit yield where the effect was non-significant. Treatment effect was more pronounced in dual (soil + foliar) application of G5 as compared to soil application alone that showed increasing trend with the increase in frequency of its application indicating their positive effect.

The results obtained are a manifestation of varied effects of different constituent components of G5 (the bio stimulant under study) as supported by the results of a number of studies. Humic substances have been shown to increase the number of fruits and flowers and improve fruit quality (Arancon *et al.* 2006) [5] both as soil as well as foliar application. When applied to soil, it improves soil structure and micronutrients solubility, alters root morphology, increases root function with regards to ATPase activity and increases the action of nitrate assimilation enzymes (Halpern *et al.* 2015) [23]. Seaweed extracts contain plant hormones such as auxins, cytokinin, abscisic acid and also amino acids (Khan *et al.* 2009, Lötze and Hoffman 2016) [31, 33] besides other constituents like nutrients, sterols and betaines known to act on soil as well as on plant (Halpern *et al.* 2015, Lötze and Hoffman 2016) [23, 33] to promote plant growth, chlorophyll levels, flowering, yield and seed germination. Higher fruit yield in G5 treated plants was due to relatively higher fruit set, fruit retention, size and weight of fruit recorded. Thanaa *et al.* (2016) [46] ascribed increased yield of Anna apples by bio stimulant application to their positive effect on endogenous levels of growth promoters and nutrients. Bio stimulants can modulate plant molecular and physiological processes that boost the plant growth, productivity and quality (Soppelsa *et al.* 2018) [42]. Increased yield by amino acid and bio stimulant application were also reported by Fayek *et al.* (2011) [21] in Le Conte pear, El-Shenawi *et al.* (2008) [19] in Grandnain banana and Aisha *et al.* (2006) [1] in Flame Seedless grapes. Jat *et al.* (2024) [26] obtained significant effect of spraying Anna apple plants with nutrients and organic formulations on growth, flowering, yield and quality while De Sousa *et al.* (2018) [15] reported Gala apple trees treated with seaweed extract showing increase in fruit set, number, weight and length of fruits. Yang *et al.* (2023) [51] found significant increase in net photosynthetic rate, chlorophyll content and Rubisco activity in Fuji apple leaves following seaweed application as also reported by Spinelli *et al.* (2009) [44]. Seaweed spray stimulated shoot and leaf growth, improved flower quality and prolonged blooming time in different apple cultivars (Basak 2008) [7].

Table 2: Effect of G5 application on yield and fruiting characteristics of apple cv. Red Delicious (values are means of four replicates)

Treatments	Shoot extension growth (cm)	Fruit set (%)	Fruit retention (%)	Fruit drop (%)	Fruit yield (kg/tree)
T_1- G5 granules @500 g/tree	53. 43	22.44	51.69	48.31	26.15
T_2-T_1+G5 liquid @250 ml/100 l	56.11	25.81	52.59	47.41	27.59
T_3-T_2+G5 liquid @250 ml/100 l	58.90	24.96	55.07	44.93	26.41
T_4-T_2+G5 liquid @250 ml/100 l	61.77	24.88	60.39	39.61	32.53
T_5-Control	49.73	18.31	50.44	49.59	24.53
CD $_{0.01}$	1.66	1.97	1.40	1.39	2.89

Physical fruit quality characteristics

Data presented in table 3 showed that treatment T_4 recorded the highest fruit weight (154.57 g), length (7.58 cm), breadth (7.62 cm) and firmness (7.45 kg/cm^2) followed by T_3, T_2, T_1 and T_5 (control). All treatments were found to be statistically superior to control with respect to these parameters except for T_1 in case of fruit firmness which was observed to be non-significant. Treatments T_1 and T_2 were statistically at par with each other in case of fruit length and breadth. Treatments T_2, T_3 and T_3, T_4 were statistically at par with each other in case of fruit firmness. Increase in fruit weight and diameter following bio stimulant application may be attributed to enhanced uptake of mineral nutrients by plant (Jat *et al.* 2024) [26]. Also, the possible hormone like activity of humic acid could be responsible for increase in fruit weight and diameter (Nardi *et al.* 2021) [37]. Increase in fruit weight with the application of amino acids with/ without seaweed was reported by Khan *et al.* (2012) [30] in Perlette grapes, Koksal *et al.* (1999) [32] in Williams pear and Sharaf *et al.* (2011) [41] in Washington Navel orange. Jat *et al.* (2024) [26] attributed higher fruit diameter, weight, firmness and yield to positive interaction of organic substances with micronutrients resulting in increase in cell size and intercellular space (Baker and Davis 1951) [6] in Anna apple plants under semi-arid conditions of Haryana, India. El-Boray *et al.* (2016) [18] reported significant increase in peach fruit firmness following application of bio stimulants. Similar results were found in apple (Thanaa *et al.* 2016) [46] and pear (Fayek *et al.* 2011) [21]. Fruit colour, to some extent was found to be influenced by G5 treatment that ranged from 90-95% in T_1, T_2, T_5 and from 95-100% in T_3 and T_4 treatments. Similar results were reported by Frioni *et al.* (2018) [22] in grapes. Malaguti *et al.* (2002) [34] found that foliar application of seaweed extract improved the intensity and distribution of color of fruit peel in Mondial Gala apple. Changes in fruit color depend upon the degradation of the chlorophyll content and accumulation of the coloring pigments. Increase in red color of apple fruits might be due to the modulation of metabolism of plant endogenous growth regulators obtained with the application of bio stimulant substances (Wally *et al.* 2013) [49] leading to an induction of anthocyanin biosynthesis and accumulation of the same in fruit skin before harvest. Seaweed extract has been reported to induce anthocyanin biosynthesis in fruit peel (Soppelsa *et al.* 2018) [42]. Anthocyanin showed positive correlation with TSS and negative with titratable acidity (Jat *et al.* 2024) [26]. Seaweed spray increased size and colour of fruits in Jonagold Decosta apple (Basak 2008) [7]. Similarly, Hermans *et al.* (2006) [24] reported an increase in anthocyanin content of fruits following application of humic acid.

Table 3: Effect of G5 application on physical fruit quality characteristics of apple *cv.* Red Delicious (values are means of four replicates)

Treatments	Fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	Fruit firmness (kg/cm^2)	Colour Index (%)
T_1- G5 granules @500 g/tree	141.27	7.24	7.29	7.30	90-95
T_2-T_1+G5 liquid @250 ml/100 l	145.31	7.34	7.37	7.38	90-95
T_3-T_2+G5 liquid @250 ml/100 l	149.24	7.46	7.51	7.42	95-100
T_4-T_2+G5 liquid @250 ml/100 l	154.57	7.58	7.62	7.45	95-100
T_5-Control	132.26	7.09	7.15	7.26	90-95
CD $_{0.01}$	2.13	0.10	0.08	0.05	-

Chemical fruit quality characteristics

Scrutiny of data given in table 4 revealed that T_4 had the highest values of TSS (13.43^{0}B), reducing sugar (6.41%), total sugars (7.35%) and the lowest acidity (0.19%) among the treatments which differed significantly from control (T_5). Treatments T_1, T_2, T_3 in case of TSS; T_1, T_2, T_3, T_4 in case of acidity; T_2, T_3 in case of reducing sugars and T_1, T_2 in case of total sugars were found to be statistically at par with each other.

Thanaa *et al.* (2016) [46] observed that amino acids enhanced the synthesis of different proteins, acids and sugars, which increased TSS content in fruits. El-Boray *et al.* (2016) [18] reported that foliar application of bio stimulants increased TSS, total sugars and reduced acidity due to enhanced photosynthesis of plants, increased leaf area and potassium content which resulted in improved fruit quality in peach. Exogenous application of amino acids improved total soluble solids, total sugars and decreased acidity in peach (Kaur *et al.* 2019) [27]. Similarly, Fathy *et al.* (2010) [20] reported that spraying of bio stimulants reduced acidity in peach. The enhanced photosynthesis, metabolism and overall health of plants might have resulted in increasing the total sugars contents of peach fruits under different treatments. Jat *et al.* (2024) [26] found negative correlation of titratable acidity with total sugars in Anna apple cultivar. Also, total sugars showed an increase after the application of humic acid (Hermans *et al.* 2006) [24].

Table 4: Effect of G5 application on chemical fruit quality characteristics of apple cv. Red Delicious (values are means of four replicates)

Treatments	TSS (^{0}B)	Acidity (%)	Reducing sugars (%)	Total sugars (%)
T_1- G5 Granules @500 g/tree	12.46	0.21	6.06	6.97
T_2-T_1+G5 liquid @250 ml/100 l	12.53	0.20	6.18	7.01
T_3-T_2+G5 liquid @250 ml/100 l	12.61	0.20	6.19	7.12
T_4-T_2+G5 liquid @250 ml/100 l	13.43	0.19	6.41	7.35
T_5-Control	12.31	0.23	5.96	6.92
CD $_{0.01}$	0.53	0.03	0.10	0.08

Phyto-toxicity studies

None of the phyto-toxicity symptoms *viz.* leaf injury on tips/surface, wilting, yellowing, vein clearing, necrosis, epinasty, hyponasty and stunting was observed with any of the treatments under study, which indicated that the use of G5 bio stimulant formulation is safe for apple fruit/plant in the doses used in the present studies. Bio stimulants as natural substances can enhance plant resilience against different abiotic stressors (Zhang and Schmidt 2000, Zhang *et al.* 2003) [52, 53] and improve nutrient uptake from soil (Verkleij 1992, Turan and Köse 2004) [48, 47] and assimilation (De Pascale *et al.* 2017, Souri and Hatamian 2019) [14, 43] and enhance antioxidant properties (Verkleij 1992) [48]. Also, the antifungal component of G5 formulation might have prevented the spread of any disease resulting in better general health of plant and fruits.

On the basis of results obtained in the present studies, it could be concluded that application of plant bio stimulant G5 at different stages of apple plant growth influenced the vegetative growth, fruit yield, fruiting and fruit quality characteristics of Red Delicious apple without any apparent phyto-toxicity. Treatment effect was more pronounced in combined (soil + foliar) application of bio stimulant compared to soil application alone. Soil application of G5 granules as basal dose @500 g/tree at pre-flowering stage + G5 liquid foliar sprays @250 ml/100 l of water one each at petal fall, walnut size and fruit development stage was found to be the best treatment that resulted in most significant increase in shoot extension growth, fruit set, fruit retention, fruit yield, fruit weight, fruit length, fruit breadth, fruit firmness, fruit colour, TSS, reducing sugars and total sugars with lowest fruit drop and acidity when compared to the untreated control under temperate conditions of Jammu and Kashmir.

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