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Effect of exogenous application of salicylic acid and triacontanol on growth characters and yield of strawberry

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Abstract
The present investigation was carried on entitled "Effect of exogenous application of salicylic acid and triacontanol on growth characters and yield of strawberry (Fragaria x ananassa Duch.) cv. Camarosa ". Treatments contained three levels of salicylic acid (0, 1 and 2 mM) and three levels of triacontanol (0, 5 and 10 µM), constituting 9 treatment combinations. The experiment was laid out in Randomised Complete Block Design (RCBD) with factorial arrangements and replicated thrice. Results revealed that the exogenous usage of salicylic acid and triacontanol had significant impact on different growth characters, fruiting and yield of strawberry as compared to control, although the interaction effect of these two growth regulators was non-significant for all the studied parameters. Among the salicylic acid and triacontanol treatments, the salicylic acid @ 2 mM and triacontanol @10 µM show maximum growth in vegetative characters of strawberry. Both the growth regulators significantly increased floral characters and fruiting characters.

Keywords: Salicylic acid, strawberry, triacontanol, vegetative growth and yield

Introduction
The genus, *Fragaria,* of the family Rosaceae (sub-family *Rosoideae*) includes 20 wild species, three naturally occurring hybrid species and the modern cultivated strawberry (*F.* × *ananassa* Duch.), originated in North America, South America, Asia and Europe [17]. Modern cultivated strawberry (*Fragaria* × *ananassa* Duch.) is an octaploid (2n=8x=56 chromosomes) hybrid species [12]. The main stem of the strawberry plant is much shortened called 'crown'. The leaves and flowering stems emerges from woody crown in a spiral arrangement. Each leaf has three or more leaflets depending on the variety, and the leaflets are oval to oblong with coarsely toothed edge. The strawberry inflorescence is a modified stem called cyme; terminates by a primary blossom; typically followed by two secondary, four tertiary and depending on the variety and time of season, eight quaternary flowers [13]. Flower induction in strawberry is controlled primarily by the interaction of photoperiod, temperature, and genotype [7]. An individual flower typically has 10 green sepals, five white petals, 20 to 30 stamens and 60 to 600 pistils. The greatest number of pistils occurs on the primary flower and decreases successively down the inflorescence. Each pistil contains a single ovary that develops into an achene [7]. The achenes (the true fruits of strawberry) together with receptacle form an aggregate fruit, which is referred to as a berry (the eterio of achenes), but it is not a true berry in the botanical sense [11].

Strawberry is important in all climates with mild winters. Camarosa is an early short-day cultivar with a vigorous growing habit. Produces large to very large firm fruit, has a good appearance, good flavor and is widely adapted producing fruit over an extended period at low latitudes consistently throughout the growing season [2].

In Kashmir valley, strawberry crop deserves special attention not only because of quick and high remunerative returns per unit area, but also due to growing interest of the farmers as the crop is ready for harvesting early in the season. In field conditions, strawberry crop often face adverse weather conditions like occurrence of temperature fluctuations, frost, heavy rains and hails [12, 25]. Under such conditions, exogenous application of growth regulators can alter these levels and cause change in the synthesis of various compounds in the plant, enhancing or modifying an effect on growth and development of plant. In recent decades, naturally occurring phyto-chemicals such as salicylic acid and triacontanol have been recognized as plant growth regulators because of their regulatory activities in the plants.

Salicylic acid (SA) naturally occurs in plants and various aspects of plant life cycle can be affected by this phytohormone. Salicylic acid (SA), a ubiquitous plant phenolic compound, regulates a number of processes in plants. Salicylic acid is a natural phenolic compound presented in many plants and is an important component in the signal transduction pathway. Salicylic acid (SA), an endogenous plant growth regulator has been found to generate a wide range of metabolic and physiological responses in plants there by affecting their growth and development [14].

Exogenous application of triacontanol has found beneficial effect on chlorophyll contents, photosynthetic rate and chlorophyll fluorescence [32, 23]. Various studies indicate strong evidences that the application of triacontanol applied either to the root medium or to leaves enhanced the growth and yield of crops, including agronomic and horticultural crops as well as medicinal and aromatic crop plants under normal and adverse conditions [26].

Application of Salicylic Acid and Triacontanol on Growth, Yield of Strawberry (*Fragaria* × *ananassa* Duch.) cv. Camarosa" laid the role of in the growth of different characters, flowering, fruiting and yield of strawberry.

Materials and Methods

This study was performed at the experimental field, Division of Fruit Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir during 2015 to study effect of effect of exogenous application of salicylic acid and triacontanol on growth characters and yield of strawberry (*Fragaria* x *ananassa* Duch.) cv. Camarosa". The experimental site is situated at an elevation of 1685 meters above mean sea level between 34º 9'0" N latitude, 74º 52'55" E longitude. Different treatments of salicylic acid (0, 1 and 2 mM) and triacontanol (0, 5 and 10 µM) were applied. Two sprays were done; 1st at 3-4 leaf stage during spring growth & 2nd at 15 days after 1st application. The different characters were measured and were randomly selected from each treatment for recording data on plant height, plant spread and number of leaves per plant while 20 leaves were taken into account for leaf area measurements.The height of plants was measured from the marked point at ground level to the leaf apex with the help of measuring scale and expressed in cm. Plant spread was measured with the help of measuring scale from North to South and East to West and the average of both the direction's measurements was expressed as plant spread in cm. The number of leaves produced by randomly selected plants was counted and expressed as average number leaf per plant. The leaf area of 20 fully developed leaves was measured using Leaf Area Meter (Systronics) and values were expressed in cm [2]. The time period between the date of start of flowering to the end of the flowering of 50 per cent plants (n=10) under each treatment was recorded and the average values were expressed in days. Total number of flowers borne on randomly selected plants in each treatment was noted during whole flowering period and expressed as number of flowers per plant. Total number of flowers per plant was counted and number of berry harvested from the sampled plants was recorded and thus, fruit set percent was worked out using formula [43]. Time (days) taken from anthesis of flowers to the development of desired color of the fruits (i.e. harvest maturity) were recorded on 20 fruits and the average days taken to fruit maturity was expressed in days. The total number of fruits from ten randomly selected plants in each treatment were counted at each picking and average number of fruit per plant were worked out. Total fruits harvested from ten randomly selected plants in each treatment from all pickings were weighed with electronic balance and the average fruit yield per plant was expressed in gram (g). The total yield of berries under each treatment was recorded from ten randomly marked plants in each treatment and yield was calculated on hectare basis and expressed in kg ha^{-1}.

Experimental design and statistical analysis

The data collected on various parameters during an experiment were subjected to statistical analysis in Randomised Complete Block Design following 2 × 3 Factorial arrangements as per standard procedure [29].

Result and Discussion

Data on different growth characters (plant height, plant spread, number of leaves per plant, and leaf area per plant) of strawberry cv. Camarosa as influenced by salicylic acid and triacontanol treatments were recorded.

In present study, application of salicylic acid influenced vegetative growth of strawberry cv. Camarosa. The average plant height was recorded and was increased significantly due to the salicylic acid treatment as compared to control. Salicylic acid at 2 mM resulted in maximum plant height; but it was statistically at par with 1 mM salicylic acid. Salicylic acid at 2 mM also showed maximum plant spread; exhibiting significantly higher values over control. The number of leaves per plant was significantly increased due to salicylic acid treatment over control. An increase in leaf area of strawberry during the study period was observed due to salicylic acid treatment, but significant results over control were noted. These results are similar to as reported in strawberry [21, 9]. It has been suggested that the growth-promoting effects of salicylic acid could be related to changes in the hormonal status [37, 1] or by role in photosynthesis, transpiration, and stomatal conductance [8, 41]. Salicylic acid promotes some physiological processes and inhibits others depending on its concentration, plant species, developmental stages and environmental conditions [36, 14] Salicylic acid has been found to generate a wide range of metabolic and physiological responses in plants thereby affecting their growth and development, although the effect of salicylic acid on growth depends on the plant species, developmental stage, and the salicylic acid concentrations [15]. In Basil and Marjoram low concentration of salicylic acid increased photosynthetic activity, which enhanced their plant height, number of internodes, number of branches and leaves as well as fresh and dry weights [10].

Application of triacontanol greatly influenced vegetative growth of strawberry cv. Camarosa. Significantly maximum plant height was noted due to triacontanol application as compared to control. Triacontanol at 10µM recorded highest plant height, maximum plant spread, maximum leaf number per plant was counted and an increase in leaf area of strawberry during the study period was observed compared to control. Triacontanol induced improvement in growth of strawberry as observed in the present study might be due to increasing the level of RuBisCO[5], regulation of many genes [40] related to photosynthesis, modulation in activities of antioxidants [31] by triggering secondary messenger for activity of those enzymes involved in carbohydrate metabolism and several physiological responses and biochemical processes [34]. Increased vegetative growth in strawberry recorded [24] when prayed 1.25, 2.50 or 5ppm triacontanol. Salicylic acid application significantly increased the duration of flowering, number of flowers per plant, percent fruit set and number of fruits per plant in strawberry cv. Camarosa however non-significant difference in days taken to fruit maturity was observed as compared to control. Maximum duration of flowering (36.79 days) was noted with salicylic acid @ 2 mM but it was statistically at pat with salicylic acid @ 1 mM. Maximum number of flowers per plant (18.36) was recorded with 2 mM salicylic acid followed by 1 mM salicylic acid (17.49) and lowest number of flowers per plant (16.58) was recorded in control. Although, the exact mechanism of flowering inducing property of salicylic acid is yet to be explored, Oota [28] hypothesized that O-hydroxyl of salicylic acid confers the metal chelating property that favours induction of flowering. Beneficial effects of salicylic acid on flowering in present study might be attributed to vigorous plants as reflected on improving vegetative growth and followed by active translocation of the photosynthesis products from source to flower production. Also, salicylic acid ameliorates the adverse effect of stressful environment [14] that can be related to increased photosynthetic efficiency by stabilization of chlorophyll, higher production and translocation of photosynthetic reserves for enhanced flowering. The present study is also in consonance with the reports of Qureshi [33] and Kazemi [21]. Increased number of flowers per inflorescence in strawberry due to salicylic acid application under stress. Highest fruit set was noted with the application of salicylic acid @ 2 mM, but statistically at par with salicylic acid @1 mM while lowest fruit set (%) in control[3]. These results are in close agreement with the findings of Qureshi [33] who demonstrated that the salicylic acid application increased fruit set in strawberry. It is probable that aqueous spraying with salicylic acid may have interfered with the biosynthesis/action of ethylene, which in turn reduced flower/fruitlet abscission [27]. Progressive increase in salicylic acid concentration significantly enhanced number of fruits per plant as compared to control and recorded maximum number of fruits per plant (14.47), followed by 1 mM salicylic acid (13.50 and minimum number of fruits per plant (12.57) was observed in control. These results are also corroborated with as in strawberry [19]. Higher number of fruits per plant in present study is the cumulative effects of enhanced duration of flowering, increased number of flowers per plant and higher fruit set percentage due to salicylic acid application. Moreover, salicylic acid increased fruit retention in mango might be due to the better photosynthetic activity leading to proper supply of carbohydrates to sink [39].

Triacontanol application significantly increased duration of flowering and number of flowers per plant in strawberry cv. Camarosa compared to control and best results were obtained with the application of triacontanol @ 10 µM. Beneficial effects of triacontanol at flowering in strawberry might be attributed to vigorous plants as reflected on improving vegetative growth and followed by active translocation of the photosynthesis products from source to flowering organs. These results are in conformity with the findings of Kumar [24] and Sharma [38] in olive. Fruit set showed significant increase when the concentration of triacontanol was raised from control to 10µM. It was found that highest fruit set per cent of (81.92%) was noticed when plants were applied with triacontanol at 10 µM followed by 5 µM triacontanol which recorded fruit set (79.63%). Control treatment recorded lowest fruit set (76.63%). Enhanced fruit set percent due to triacontanol might be attributed to the inhibition of pre-abscission pectinase and cellulase activities [16]. These findings were in agreement as reported in mango [30], in 'New Castle' apricot [20], and in olive [38]. Triacontanol significantly delayed fruit maturation compared to the control. Triacontanol @ 10 µM took maximum days (25.48) for fruit maturity although; it was statistically at par with 5 µM triacontanol (24.98 days). Lowest number of days (23.39) to fruit maturity were recorded in control. These results were also corroborated as in mandarin[6]. Exogenous application of triacontanol significantly increased with each increased level of triacontanol. Maximum number of fruits per plant (14.62) were recorded with 10µM triacontanol followed by 5µM triacontanol which recorded 13.44 number of fruits per plant whereas the lowest number of fruits per plant (12.47) was recorded when no triacontanol was sprayed. These results were in accordance with findings as in mandarin[6] and in tomato [22].

Application of salicylic acid significantly enhanced fruit yield per plant as well as per ha compared to control and the best results were obtained with 2 mM salicylic acid, exhibiting highest yield (161.85 g per plant and 11989 kg per ha) with the application of 2 mM salicylic acid, followed by 1 mM salicylic acid (150.68 g per plant and 11162 kg per ha). The lowest yield (135.68 g per plant and 10151 kg per ha) was noted in control. In present study, salicylic acid treatments have attributed to prolonged flowering duration, exhibiting more number of flowers per plant and higher fruit set percent, resulted in more number of fruits per plant with higher weight of individual fruits than those in control, and ultimately realized the enhanced fruit yield. Similar results have also been observed who also reported increased fruit yield in strawberry due to salicylic acid application [19, 15].

Application of triacontanol significantly enhanced fruit yield per plant as well as per ha compared to control. Highest fruit yield (166.29 g per plant and 11989 kg ha) was recorded with the application of 10 µM triacontanol, followed by 5 µM triacontanol (148.18 g per plant and 11162 kg per ha) and lowest fruit yield (133.75 g per plant and 10051 kg per ha) was noted in control. In present study, triacontanol treatments has attributed to prolonged flowering duration, exhibiting more number of flowers per plant and higher fruit set percent, resulted in more number of fruits per plant with higher weight of individual fruits than those in control, and ultimately realized the enhanced fruit yield. These results are also in accordance as in plum [4], in Guava [18], in strawberry [24] and in mandarin [6] who reported yield enhancement due to triacontanol application [42]. Foliar applied triacontanol at different growth stages enhance the translocation of assimilates resulting in increased yield and yield attributes, however optimal concentrations of triacontanol and plant age are among the important factors that control the growth and final yield of various plant species [35].

Conclusion

In this study, we determined that both the growth regulators were found effective for increase in vegetative growth characters (plant height, plant spread, number of leaves per plants and leaf area), duration of flowering, number of flowers (as well as fruits) per plant and higher fruit yield.

Table 1: Effect of salicylic acid and triacontanol on an average plant height (cm), Plant Spread, Number of Leaves per Plant and Leaf area of strawberry cv. Camarosa

S↓ C→	Plant Height				Plant Spread				Number of Leaves Per Plant				Leaf Area			
	C_0	C_1	C_2	Mean	C_0	C_1	C_2	Mean	C_0	C_1	C_2	Mean	C_0	C_1	C_2	Mean
S_0	17.249	17.607	19.726	18.194	15.54	16.64	17.56	16.61	8.93	9.82	10.28	9.68	90.25	93.41	96.69	93.45
S_1	18.800	19.823	21.299	19.974	17.14	17.60	18.81	17.85	9.68	10.63	11.10	10.47	93.32	99.37	98.92	97.20
S_2	19.721	21.416	22.212	21.117	17.35	18.72	19.92	18.66	10.16	10.68	11.57	10.80	95.27	102.56	102.90	100.11
Mean	18.590	19.615	21.079		16.71	17.65	18.76		9.59	10.37	10.98		92.95	98.31	99.50	
CD at 5%																
Triacontanol (C)	0.429				0.358				0.288				2.53			
Salicylic acid (S)	0.429				0.358				0.288				2.53			
C × S	NS				NS				NS				NS			

S= Salicylic acid; S_0 -Control (water spray), S_1 - Salicylic acid (1 mM) and S_2 - Salicylic acid (2 mM). C = Triacontanol; C_0 - Control (water spray), C_1 - Triacontanol (5 µM) and C_2 - Triacontanol (10 µM). *Base period: Month in which growth regulators were applied

Table 2: Effect of salicylic acid and triacontanol on duration of flowering and number of flowers per plant of strawberry cv. Camarosa

S↓ C→	Duration of flowering (Days)				Number of flowers per plant			
	C_0	C_1	C_2	Mean	C_0	C_1	C_2	Mean
S_0	31.63	35.63	36.70	34.66	14.83	16.63	18.27	16.58
S_1	33.90	36.70	38.03	36.21	16.37	17.20	18.90	17.49
S_2	34.80	36.00	39.57	36.79	16.83	18.73	19.50	18.36
Mean	33.44	36.11	38.10		16.01	17.52	18.89	
CD at 5%								
Triacontanol (C):	1.65				0.82			
Salicylic acid (S):	1.65				0.82			
C × S :	NS				NS			

S= Salicylic acid; S0 -Control (water spray), S1 - Salicylic acid (1 mM) and S2 - Salicylic acid (2 mM). C = Triacontanol; C0 - Control (water spray), C1 - Triacontanol (5 µM) and C2 - Triacontanol (10 µM).

Table 3: Effect of salicylic acid and triacontanol on fruit set (%), days taken to fruit maturity and number of fruits per plant of strawberry cv. Camarosa

S↓ C→	Fruit set (%)				Days taken to fruit maturity				Number of fruits per plant			
	C_0	C_1	C_2	Mean	C_0	C_1	C_2	Mean	C_0	C_1	C_2	Mean
S_0	74.43	76.97	78.97	76.79	23.00	24.58	25.08	24.22	11.37	12.73	13.60	12.57
S_1	77.63	79.53	83.20	80.12	24.67	25.43	24.88	24.99	12.63	13.03	14.83	13.50
S_2	77.83	82.40	83.60	81.28	22.50	24.92	26.46	24.63	13.40	14.57	15.43	14.47
Mean	76.63	79.63	81.92		23.39	24.98	25.48		12.47	13.44	14.62	
CD at 5%												
Triacontanol (C):	2.14				1.40				0.53			
Salicylic acid (S):	2.14				NS				0.53			
C × S :	NS				NS				NS			

S= Salicylic acid; S_0 -Control (water spray), S_1 - Salicylic acid (1 mM) and S_2 - Salicylic acid (2 mM). C = Triacontanol; C_0 - Control (water spray), C_1 - Triacontanol (5 µM) and C_2 - Triacontanol (10 µM).

Table 4: Effect of salicylic acid and triacontanol on fruit yield per plant (g) and fruit yield per hectare (kg) of strawberry cv. Camarosa

S↓ C→	Fruit yield per plant (g)				Fruit yield per hectare (kg)			
	C_0	C_1	C_2	Mean	C_0	C_1	C_2	Mean
S_0	124.72	131.17	151.16	135.68	9238.5	9716.3	11197	10051
S_1	133.48	148.27	170.29	150.68	9887.6	10983	12614	11162
S_2	143.04	165.10	177.42	161.85	10596	12229	13142	11989
Mean	133.75	148.18	166.29		9907.3	10976	12318	
CD at 5%								
Triacontanol (C):	5.78				427.91			
Salicylic acid (S):	5.78				427.91			
C × S :	NS				NS			

S= Salicylic acid; S_0 -Control (water spray), S_1 - Salicylic acid (1 mM) and S_2 - Salicylic acid (2 mM). C = Triacontanol; C_0 - Control (water spray), C_1 - Triacontanol (5 µM) and C_2 - Triacontanol (10 µM).

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