Pak. J. Agri. Sci., Vol. 53(4), 911-916; 2016
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/16.3682
http://www.pakjas.com.pk

FLOWERING AND FRUITING RESPONSES OF STRAWBERRY TO GROWTH HORMONE AND CHILLING GROWN UNDER TUNNEL CONDITIONS

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Strawberry (*Fragaria ananasa)* is a berry fruit belonging to the family Rosaceae and requires certain amount of cold temperature to initiate flowering. Present study was conducted with the objective to study flowering and fruiting responses of strawberry to growth hormone and chilling under tunnel conditions. The study comprised of six different treatments as; control, GA_3 (gibberellic acid) 200 ppm, GA_3 400 ppm, chilling at 4°C, chilling at 4°C + GA_3 200 ppm and chilling at 4°C + GA_3 400 ppm. Treatments were applied at vegetative stage before flowering. It was inferred from the study that strawberry plants responded much better to application of GA_3 at 400 ppm for their growth, flower stimulation, their number, quality features while chilling at 4°C proved better for maximum fruit production and better quality under tunnel condition.

Key words: Strawberry, gibberellic acid, chilling, flowering, fruiting, tunnel conditions

INTRODUCTION

Strawberry (*Fragaria ananasa*) a soft fruit that belongs to family Rosaceae is stimulated by cold weather. Strawberry is native to North America. Its name has been derived from farmers mulching called "Straw", earlier the strawberries were just planted for medicinal purpose but later on Carolus Linneaus for the first time introduced strawberry as edible fruit (Lieten *et al.*, 1995). Strawberry is herbaceous perennial which is propagated by means of seed, short rhizomes and leafless stolons. The beautiful red colored fleshy fruit is full of achenes on its surface (Anderson, 2006). Strawberry is rich source of vitamins mainly vitamin C, fiber, potassium and phytochemical especially ellagic acid and anthocyanins which act as anti-inflammatory and antioxidant agents (Hannum, 2004).

Chilling is required for breaking the dormancy of strawberry (Lieten *et al.*, 1995), and to accelerate flowering ability (Sung and Amasino, 2004). There are several models that have been developed for measuring winter chill, e.g. the Chilling Hours Model (Luedeling *et al.*, 2010), the Utah Model and the Dynamic Model (Fishman *et al.*, 1987). These models differ from each other greatly in their sensitivity to climate change (Luedeling *et al.*, 2009), which makes the choice of the model a crucial determinant of forecasting the extent of climate change and effects on winter chill. A certain quantity of chilling has been shown to improve flower bud formation of the short-day strawberry plant (Taylor, 2002; Calis *et al.*, 2015; Nezhadahmadi *et al.*, 2015).

Vegetative and reproductive developments of strawberry under the control of genes are extremely vulnerable to environmental factors (Battey *et al.*, 1998). GA_3 appliance improved length of petiole and area of leaf in strawberry plants when used in different concentrations (Paroussi *et al.*, 2002). The application of GA_3 (50 ppm) to the latent buds could achieve 97% break of their onset of dormancy (Ahmed and Ragab, 2005). A treatment of 10 ppm GA_3 applied to fruit frost protected plants during the 2nd week of January (Ozguven and Yilmaz, 2002). Ozguven and Yilmaz (2002) also reported that GA_3 applied at 5, 10 and 20 ppm had no effect on the average fruit weight and total soluble solid content in cv. 'Camarosa'.

Many studies have shown that runner quality is a major factor that affects both productivity and fruit quality (Durner *et al.*, 2000). High vitamin C quantity has been observed in strawberry fruit plant treated with GA_3 during pre-flowering stage (Sharma and Singh, 2009). Parallel results have been reported when GA_3 was sprayed at pre-flowering stage, caused high vitamin C contents and total soluble solids and also enhanced firmness in capsicum (Ouzounidou *et al.*, 2010) and sweet cherry (Kappel and Macdonald, 2007). Gibberellic acid application also reported to boost the fruit firmness (Usenik *et al.*, 2005). Hence present study was designed to investigate the effect of chilling and growth hormone on the reproductive growth of strawberry plants and the role of GA_3 in fulfilling chilling requirement to stimulate flowering and fruiting.

MATERIAL AND METHODS

Chemicals**:** Gibberellic acid, sodium hydroxide, hydrochloric acid and L-ascorbic acid were purchased from Sigma-Aldrich, Germany Milli-Q water with a resistivity of 18 MΩ cm was used to prepare all solutions. All of these chemicals were of analytical grade.

Growth conditions and plant materials**:** Present study was conducted in climatic conditions of Rawalpindi, Pakistan under plastic tunnel conditions. Runners of strawberry cv. "Chandler" were acquired from Swat, Pakistan. The runners were kept in cold chamber (Mack Pharmatech PVT Limited) at 4°C for about fifteen days to meet the chilling needs of strawberry plants. The runners were planted under field conditions in tunnels (well drained sandy loam soil, 22-25°C temperature with 65-75% humidity. Plantation was done on ridges by keeping plant to plant distance of 8 cm and row to row distance of 30 cm, irrigation was given soon after planting and then applied on weekly basis and as per plant requirement.

A foliar spray of GA_3 was applied before flowering after 45 days of plantation the concentration of GA_3 in combination with different chilling temperatures used were as follows: T_1:control, T_2:GA_3 200 ppm, T_3:GA_3 400 ppm, T_4:chilling 4°C, T_5: chilling 4°C + GA_3 200 ppm and T_6: chilling 4°C + GA_3 400 ppm.

Parameters evaluated**:** Following reproductive and quality parameters were studied; days to flower initiation, numbers of flowers, number of fruit set/plant, percentage fruit set, fruit weight (g), fruit size (cm) and firmness (pounds). Total soluble solids (TSS °Brix), L-ascorbic acid contents (mg/ml) and titratable acidity was determined by standard procedures (AOAC, 1990).

Statistical analysis**:** The experiment was laid down by using linear model randomized complete block design (RCBD). The collected observations were analyzed using Statistix 8.1. The means were compared by using Least Significant Difference (LSD) at 5% probability level (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

The data relating to the role of chilling and growth hormone affecting vegetative and reproductive growth of strawberry are presented in Table 1. The data was collected in the morning.

Number of days required to open first flower**:** It was observed (Table 1) that the lowest days (72.66) were taken by the plants treated with T_5 to start flower production followed by T_3 (74.33), T_4 (75.00), T_6 (75.33) the other treatments (T_2) stimulated flower after 80.66 days and maximum days (90.33) were taken by T_1 to flower induction. Gibberellic acid functions to induce early flowering in all horticultural plants including strawberry. Sharma and Singh (2009) suggested that GA_3 positively influenced initiation, duration, number of flowers, harvesting season and yield of strawberry plant. Early flowering has also been reported in other horticulture crops when sprayed with GA_3 before flowering. Moreover, number of fruits per plants and fruit size was also increased (Ouzounidou *et al.*, 2010). Paroussi *et al.* (2002) reported that flowering was boosted by 200 mg L^{-1} GA_3 treatment. GA_3 application caused noticeable inflorescence abnormalities especially in cv. Seascape, irrespective of photoperiod (Paroussi *et al.*, 2002) and the possible reason of inducing early flowering is due to the stimulation of Floragin hormone by gibberellic acid.

Number of flowers**:** The highest number of flowers per plant (25.33) was counted in treatment T_3 (400 ppm GA_3). Non-significant (p>0.05) data was shown by the rest of the treatments with the contribution of T4 (24.88) T_1 (23.66), T_5 (21.33), T_2 (20.11) and minimum number of flowers (18.77) were counted for T_6. Our results revealed that gibberellic acid and chilling help to produce more number of flowers as compared to control, similar results were stated by the previous work of many researchers that GA_3 application improved buds and open flowers thus, promoting earliness without effecting marketable yield (El-Shabasi *et al.*, 2009; Paroussi *et al.*, 2002). GA_3 at 10 ppm or ethrel at 250 ppm improved the number of flowers and total monthly yield (El-Shabasi *et al.*, 2009) (Table 1).

Table 1. Effect of chilling and growth hormone of strawberry on reproductive parameters of strawberry.

Treatment	Number of days required to open first flower	Average number of flowers	Flower diameter	No. of fruit set	Fruit set (%)	Total No. of fruits
T_1: Control	90.33$\pm$0.66^{A}	23.6$\pm$1.71^{AB}	3.32+0.10^{AB}	12.50$\pm$1.07^{AB}	90.70$\pm$6.68^{AB}	26.20$\pm$1.25^{ABC}
T_2: GA_3 200 ppm	80.66$\pm$2.40^{B}	20.10$\pm$0.40^{BC}	2.64+0.20^{C}	7.40$\pm$1.06^{B}	83.70$\pm$0.84^{AB}	29.16$\pm$1.06^{AB}
T_3: GA_3 400 ppm	74.33$\pm$0.28^{C}	25.30$\pm$0.88^{A}	2.81+0.15^{BC}	9.70$\pm$0.48^{B}	81.40$\pm$1.89^{B}	23.70$\pm$0.21^{BC}
T_4: Chilling 4°C	75.00$\pm$0.57^{C}	24.80$\pm$0.44^{AC}	3.34+0.42^{A}	15.40$\pm$0.78^{A}	93.70$\pm$2.99^{A}	31.50$\pm$0.76^{A}
T_5: Chilling 4°C (GA_3 200 ppm)	72.60$\pm$4.45^{C}	21.30$\pm$2.41^{ABC}	2.63+0.20^{C}	8.80$\pm$1.87^{B}	86.10$\pm$2.99^{AB}	20.80$\pm$1.89^{C}
T_6: Chilling 4°C (GA_3 400 ppm)	75.30$\pm$1.76^{C}	18.70$\pm$0.70^{C}	2.39+0.32^{C}	9.30$\pm$0.29^{B}	67.20$\pm$2.80^{C}	14.20$\pm$0.19^{D}

Means not sharing a letter differ significantly at P<0.05, ± = standard error.

***Flower diameter*:** The data related to flower diameter (cm) is presented in Table 1 which shows that maximum flower diameter (3.34 cm) was observed in T_4 followed by T_1 (3.22 cm). The rest of the treatments helped to produce flower diameter of 2.81 cm at T_3, 2.64 cm at T_2, 2.63 cm at T_5 and the lowest 2.39 cm was produced at T_6. Chilling temperatures were just as effective on reducing the time to first flower as previously reported (Risser and Robert, 1993). Chilling initiates the production of gibberellic acid and is also involved in breakdown of carbohydrates and other food reserves and their translocation to production sites that is why it help to produce larger fruit diameter in comparison with the rest of treatments and control.

***Number of fruit set*:** Number of fruit set effected by chilling and exogenous application of gibberellic acid (Table 1) explained that maximum fruit set was found in T_4 (15.4 fruits per plant) chilling treatment while fruit number of 12.55, 9.77, 9.33, 8.88 and 7.44 were produced by T_1, T_3, T_6, T_5 and T_2, respectively. Chilling produced the highest fruit set in strawberry as it stimulates the production of endogenous gibberellins production which help to boost fruit set number as mentioned in previous literature. Gibberellins enhanced number of flower and flower bud per plant and also the total marketable yield (Kasim *et al.*, 2007; Sharma and Singh, 2009). Gibberellic acid significantly influences the initiation and duration of flowering and harvesting season, numbers of flowers per plant, fruit set and numbers of fruits per plant. It also increased the total yield and decreased the average fruit size (Sharma and Singh, 2009). Pre harvest foliar application of GA_3 at 50 ppm showed major increment in early yield (as compared to other treatments) of Globe artichoke whereas, no such increase was observed in late yield, this increase in early yield was due to early flowering thus no positive results were observed in total yield (Kasim *et al*., 2007).

***Fruit set percentage*:** The data pertaining to percentage fruit set is presented in Table 1. It describes that the highest 93.78 fruit percentage was noted in T_4 followed by T_1(control) with the fruit set of 90.73%. The rest of the treatments contributed to 81.41% at T_3, 86.18% at T_5, 83.78% atT_2 and lowest of 67.24% at T_6, respectively. The research findings proved that chilling contributed to the highest fruit set, the fruits produced were fresh and of good size and weight. GA_3 also contributed to good fruit set percentage but most of the fruits were albino and were not of marketable quality. The reason was that most of the biosynthate produced were used for runner production which resulted in poor fruit quality. These findings were in line with the work of Sharma and Singh (2009), who stated that gibberellic acid significantly increased the total yield and decreased the average fruit size.

***Total number of fruits*:** The results obtained on total number of fruits presented in Table 1 proved that maximum number of total fruits (31.55) was obtained from treatment T_4 followed by three T_2 (29.16), T_1 (26.22), T_3 (23.78), T_5 (20.83) and T_6 (14.22) fruits, respectively. Chilling produced maximum number of fruit (put in fruit number) alone by stimulating the production of endogenous GA_3 as compared to other treatments. Similar results were found by Sharma and Singh (2009) who reported that gibberellic acid increased the number of fruits comparable results in other fruits like capsicum. Foliar application of GA_3 notably accelerated the time of flowering by 8.3% as compared to control in *Capsicum annum* and also increased number of fruits per plant (Ouzounidou *et al.*, 2010). Single spray of gibberellic acid also induce fruit setting in sweet oranges, whereas did not affect final yield. More numbers of fruits were also reported while fruit size remains unaltered (Agusti *et al.*, 1982).

***Fruit size (cm)*:** The fruit size is presented in Figure 1a explains that maximum fruit size (20.66 cm) was observed in T_4 followed by T_1 (18.77 cm) T_5 (18.46 cm), T_6 (16.40), T_3 (15.78) and minimum of 15.40 cm by T_2 respectively. Chilling contributed to the maximum fruit size by transferring all the biosynthates to the fruits while the treatments with gibberellic acid used them for runner production as a result fruit quality decreased in term of fruit size and weight. Our findings are in accordance with the work of (Paroussi *et al.*, 2002) who reported that gibberellic acid enhanced the percentage of aborted flowers and malformed fruits, resulting in a major reduction in total marketable yield (Agusti *et al*., 1982; Sharma and Singh, 2009). Gibberellic acid in late season on kiwi fruit canes increase in pedicel length but reduced berry weight and fruit diameter (Burge *et al.*, 1990). This might be due to sink of GA_3 treated plant's reserves towards early flower production and fruit setting and runner production.

***Fruit weight (g)*:** Fresh Fruit weight as affected by different treatments with chilling and exogenous application of gibberellic acid is presented in Figure 1b. It revealed that heaviest fruits (12.67 g) were obtained with T_4 chilling treatment alone followed by T_1 (control) with fruit weight of 10.66 which was then followed by T_2 (9.24 g), T_5 (7.38 g), T_3 (7.28 g) and the lightest (4.41 g) by T_6, respectively. Our findings proved the positive increasing effect of chilling on increasing fruit weight as compared to the rest of the treatments and the reason was the efficient supply of food reserves from source to sink. Our results further found the negative effect of gibberellins on fruit weight as described by other scientists (Sharma and Singh, 2009), tomatoes, kiwifruit (Burge *et al*., 1990) and globe artichoke (Kasim *et al*., 2007). GA_3 application reduced the time needed for inflorescence emergence, induced flowering and maximized the number of flower buds and open flowers in most growing conditions. GA_3 at (50 mg/l) did not affect total marketable yield, while at 200 mg/l, combined with long photoperiod, enhanced the percentage of aborted flowers plus malformed fruits, resulting in a significant reduction in total marketable yield (Paroussi *et al*., 2002).

***Fruit Firmness (pound)*:** The data related to fruit firmness is presented in Figure 2a which explains that the firmest fruits (0.82 pounds) were produced by T_3 followed by T_6 (0.79 pounds) and the other treatments contributed to the fruit firmness were as T_5 (0.75 pounds), T_4 (0.62 pounds), T_2 (0.60 pounds) and the lowest was noted in T_1 (0.56 pounds). Our research study found that gibberellic acid helped to maintain fruit firmness than chilling and control and this is because it regulates the activities of cell wall hydraulic enzymes which are responsible for fruit softening (Kondo and Danjo, 2001). These research findings are in agreement with the work of Canli and Orhan (2009), Cline and Trought (2007), Ozkaya and Dundar (2008). Application of 10, 20 and 30 ppm foliar sprays of gibberellic acid on sweet cherry can reduce the loss of fruit firmness (Ozkaya and Dundar, 2008). Sweet cherry trees were treated with gibberellic acid at the concentrations of 0, 15 and 25 ppm to investigate that which concentration is optimum for fruit quality. The results indicated that when gibberellic acid is sprayed at optimum concentration of 25 ppm at two different locations, caused 38% and 25% more fruit firmness than control (Canli and Orhan, 2009). The applications on sweet cherries revealed that foliar spray of gibberellic acid either applied repeatedly or singly, increased fruit cracking and fruit firmness but delayed the color development of fruits (Cline and Trought, 2007).

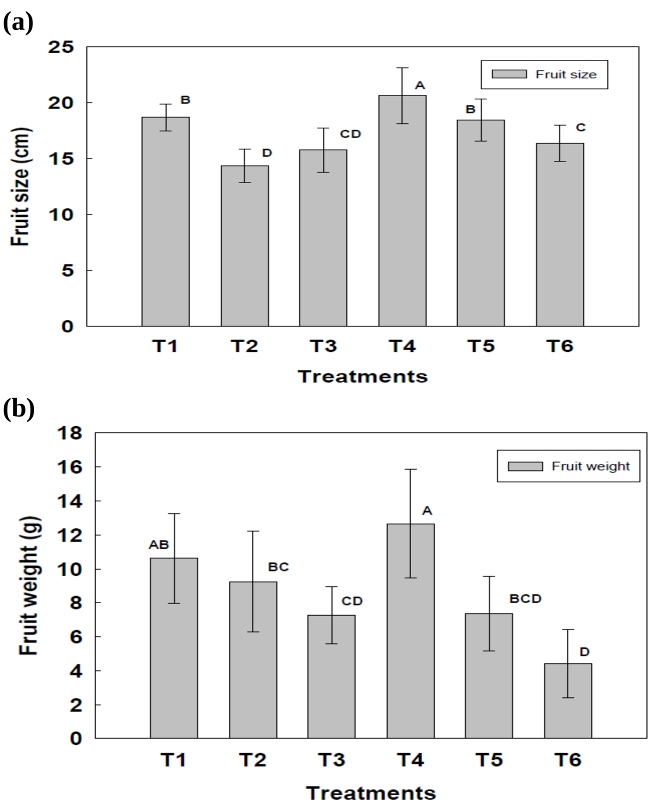

Figure 1. Effect of chilling and growth hormone on strawberry (a) fruit size (b) fruit weight (under tunnel conditions).

***Total soluble solids (TSS °Brix)*:** The statistical data presented in Figure 2b describes that maximum total soluble solids were produced by treatment with T_3 (10.80 °Brix) followed by T_2 (9.90 °Brix) statistically at par. The rest of the treatments contributed TSS as T_5 (8.66 °Brix), T_6 (7.96 °Brix), T_1 (6.56 °Brix) control, T_4 (6.50 °Brix), respectively. Gibberellic acid increase TSS in strawberry as compared with chilling and control treatments. These findings are similar with the work of Kappel and McDonald (2007) that GA_3 treated sweet cherries showed more TSS level as compared to control and chilling treatments and the maximum total soluble solids (TTS) was obtained with GA_3 application (Singh and Singh, 2006) and also the fruit were larger, firmer and had more soluble solids as compared to untreated plants (Facteau *et al.*, 1985).

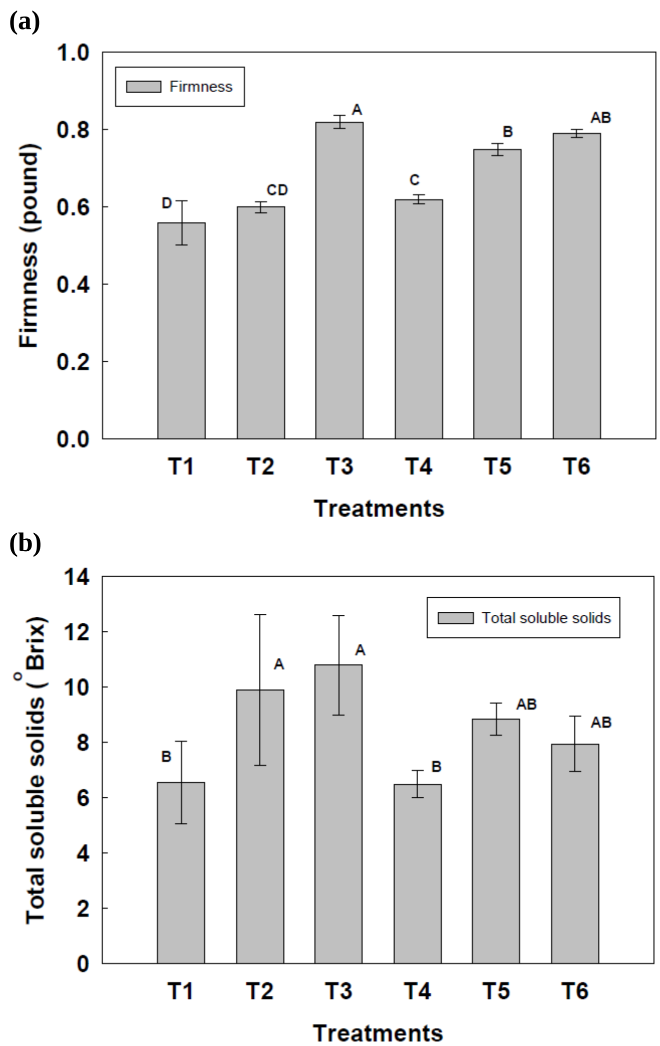

Figure 2. Effect of chilling and growth hormone on strawberry (a) firmness and (b) total soluble solids (under tunnel conditions).

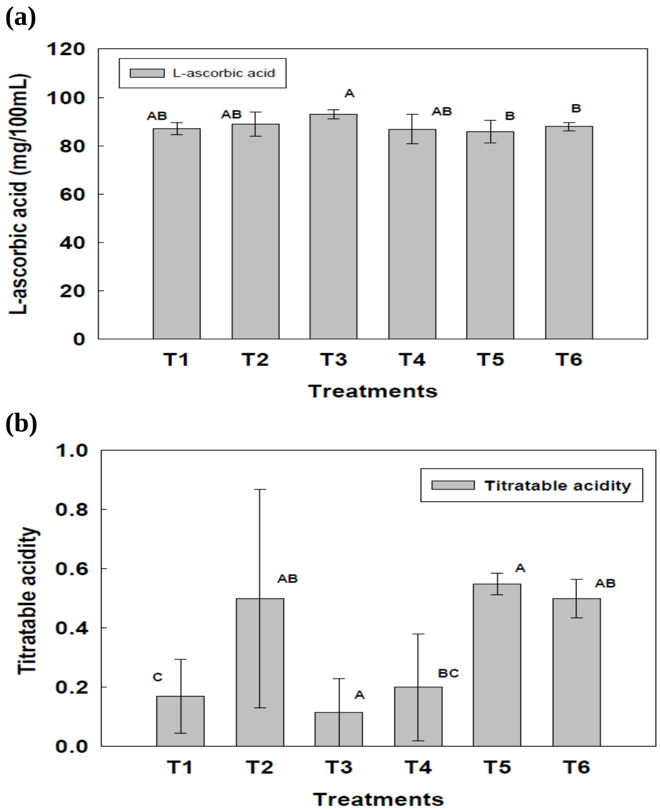

Figure 3. Effect of chilling and growth hormone on strawberry (a) ascorbic acid contents and (b) titratable acidity (under tunnel conditions).

***L-ascorbic acid (mg/100ml of juice)*:** L-ascorbic acid of 93.03 mg/100ml was produced by T_3 followed by T_2 (89.90 mg/100ml) and the rest of the treatments contributed as T_6 (88.56 mg/100ml) T_4 (86.90 mg/100ml), T_1 (87.00 mg/100ml) and 85.80 mg/100ml by T_5 (Fig. 3a). Strawberries are the vital source of ascorbic acid contents for the human diet (Food and Nutrition Board, 1989) and they contain more ascorbic acid as compared to oranges (Ayub *et al.,* 2010). Vitamin C is a main quality parameter which is most susceptible to degradation because of oxidation compared to the rest of nutrients during food processing and storage our results revealed the increasing effect of GA_3 application on vitamin C and these findings are in parallel with that of other scientists including Sharma and Singh (2009) reported high ascorbic acid quantity in strawberry fruit plant treated with GA_3 during pre-flowering stage, Kappel and Macdonald (2007) suggested that GA_3 at 90 ppm contributed to increase the production of quality parameters like ascorbic acid and acidity of strawberry and Ouzounidou *et al*. (2010) also observed similar results by working on capsicum.

***Titratable acidity*:** The sugars and acids are used as vital measure of taste attribute for strawberries (Wozniak *et al.,* 1997) which attracts the consumers. As described in (Fig. 3b) the most significant results were observed in treatment with T_3 (0.69) followed by T_5 (0.55), T_2 (0.00), T_6 (0.50), T_4 (0.20) and the lowest of 0.17 by T_1 (control). Titratable acidity is associated with the levels of organic acids present in the fruits as these are significant parameters for maintaining fruit quality. In strawberries citric acid is the chief acid adding to 90% of entire organic acid content. Our work revealed that gibbellins had positive effect on elevation of titratable acidity which is undesirable with respect to consumption. These results coincide with findings of Ouzounidou *et al.* (2010) who worked on effect of gibberellic acid in capsicum plant and found non-significant results regarding titratable acidity. El-Otmani and Coggins Jr (1991) also confirmed our results regarding effect of gibberellic acid and similar results were reported by Kappel and Macdonald (2007), they intimated that yield was not affected by GA_3 spray whereas early GA_3 spray produces firmer fruits. Total soluble solids in GA_3 treated strawberry plants were higher than control. Moreover, by delaying the time of spray titratable acidity was increased in gibberellic acid treated fruits.

It is concluded from the above mentioned results that gibberellic acid though induced early flowering but decreased marketable yield by producing malformed fruits either alone or in combination with chilling while chilling alone produced maximum quality yield by increasing fruit size weight and average number of fruits. Gibberellic acid concentration of 400 ppm improved quality parameters in term of TSS, firmness, titratable acidity and ascorbic acid so are recommended for processed fruit products.

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