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RESEARCH

Influence of Gibberellic Acid and Mulch Materials on Yield and Biochemical Attributes of Strawberry (*Fragaria × ananassa*) cv. Festival

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Abstract

One of the most important factors in strawberry (*Fragaria × ananassa*) cv. Festival production for achieving higher yields is the availability of strawberries with better quality. The experiment was carried out during the period from November 2020 to March 2021 to find out the responses of gibberellic acid (GA_3) and mulch materials on biochemical and quality attributes related to strawberry yield characteristics. The experiment consisted of two treatments including different mulch materials: black polythene, white polythene, sawdust, and control (no mulch); and GA_3 (0 and 200 mg/L) were studied. Various quality parameters, namely total soluble solids (TSS), pH, titratable acidity, ascorbic acid, total sugar, phenolic, and anthocyanin content of the strawberries were determined. The results observed in the GA_3-treated plants showed more significant results than the plants not treated with GA_3. Among the mulch materials, sawdust performed better than the other mulch materials in terms of yield, biochemical, and quality attributes. The highest fruit set percentage (89) and fruit yield (18.70 t/ha) were obtained from the GA_3 and sawdust mulch treatments. GA_3 and sawdust mulch performed best in terms of quality parameters including TSS, pH, ascorbic acid, total sugar, phenolic, and anthocyanin content. Accordingly, sawdust and GA_3 are more responsive to strawberry plant quality parameters. These findings provide practical insights for sustainable strawberry cultivation practices, contributing to increased fruit availability and improved nutritional value for consumers.

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Statement of Sustainability: This study emphasized an integrated approach to achieve sufficient yield and maintain good quality by using GA_3 and various mulch materials associated with sustainable agricultural practices. With the combined application of GA_3 and different mulch materials, the proposed integrated strategy aims to create a circular model where sustainable production and good quality can be maintained to increase nutrient content and storage capacity. This study aims to contribute to a more sustainable and environmentally conscious future by promoting the efficient use of GA_3 and mulch materials, thereby minimizing production shortages and nutrient deficiencies.

1. Introduction

The strawberry (*Fragaria × ananassa* Duch.) is one of the world's most attractive, nutritious, sweet and refreshing fruits. It is a member of the rose family (Rosaceae). In tropical and subtropical regions, this crop behaves as an annual, but in temperate regions, it behaves as a perennial (Yadav et al., 2020). Due to its wide range of environmental adaptations, high degree of heterozygosity, and genotypic diversity, it is the most widely cultivated fruit crop in the world. It is currently grown in approximately 75 countries around the world (Vishal et al., 2016). Strawberries are a good source of natural antioxidants (Huang et al., 2012). One of the tastiest fruits in the world, it has high levels of vitamins A (60 UI/100g edible part), C (30–120 mg/100g edible part), 5.0% total sugars, 0.90–1.85% acids, fiber and pectin (0.55%), minerals and water (90%) (Kher et al., 2010). Strawberry has become an important table fruit for millions of people around the world due to its high vitamin and mineral content and delicate flavor (Sahana et al., 2020). However, the quality and storage of strawberry fruits are the main problems in gaining popularity and importance in production. The availability of high-quality fruit is one of the most important elements in strawberry production to achieve high yields. Strawberry has become an important table fruit for millions of people around the world due to its high vitamin and mineral content and delicate flavor (Lakshmikanth et al., 2020).

Plant Growth Regulators (PGRs), also known as phytohormones, are organic compounds that are naturally synthesized in higher plants and affect growth or other physiological activities. Fruit development and quality are determined by a variety of factors closely related to nutrient uptake by the plant and PGRs. The use of plant growth regulators has resulted in some excellent successes in terms of growth, production, and quality in numerous fruit crops (Suman et al., 2017). Gibberellic acid promotes early flowering, cell elongation, extended flowering time, and yield. The use of gibberellic acid has been shown to improve leaf size and petiole length (Vishal et al., 2016). Several researchers have investigated the effect of growth regulators on strawberry fruit yield and quality measures such as total soluble solids, ascorbic acid, acidity, and sugar content. Vishal et al. (2017) reported the beneficial effects of exogenously applied growth regulators on total vegetative growth, flowering, fruit set (%), yield, and physicochemical quality parameters of strawberry. Sharma and Singh (2009) showed that the addition of GA_3 to the leaves of several horticultural crops increased yield and quality. It has been observed that strawberry fruit quality varies due to some factors, including environmental factors, genotype selection, and cultural practices used (Pincemail et al., 2012). Mulching is the most important cultural technique as it plays an important role in maintaining soil moisture, weed control, and regulating the soil hydrothermal regime, in addition to keeping the fragile fruit clean and tidy as strawberry is a low-growing perennial plant (Tarara, 2000). Strawberries are particularly sensitive to different mulch materials and climatic conditions, according to research on the effects of different mulch materials on fruit quality (Shylla and Sharma, 2010). Mulch improves the thermal state of the soil in the morning and protects the soil from absorbing heat during the day (Arun, 2016). Mulching also promotes strawberry plant growth, fruit set (%), fruit yield, and quality (Singh and Asrey, 2005). Plant debris is broken down by enzymes that are activated when organic mulches are added to the soil (Palei et al., 2016). Mulches increase crop quality and yield and have a significant impact on harvest duration due to improved nutrient availability, weed control, and frost protection (Soliman et al., 2015), and it reduces the incidence of disease, and unclean plants, and improves soil and moisture conservation (Li et al., 2021).

Various studies on the influence of GA_3 and mulch materials on the morphological, physiological and yield attributes of strawberry have been reported in the context of strawberry production (Bisht et al., 2018; Kumar et al., 2014). Thus, the current study was conducted to evaluate the influence of GA_3 and different mulch materials on the yield and biochemical and qualitative attributes of strawberries, which are directly or indirectly related to the shelf life and storability of strawberries.

2. Materials and Methods

2.1. Plant Materials and Environmental Conditions

The experiment was conducted at the horticulture farm of Sher-e-Bangla Agricultural University in Dhaka, Bangladesh from November 2020 to March 2021 under natural lighting (250-260 μmol/m^2/s). Dhaka is located at 23°42′37′′ N (latitude), 90°24′26′′ E (longitude) and has an average elevation of 4 m (13.12 ft), according to the National Mapping Organization of Bangladesh. In the current investigation, gibberellic acid (0 and 200 mg/L GA_3) was applied with various mulch materials, including no mulch, sawdust, and white and black polyethylene. The experiment used a randomized complete block design with three replications. Festival strawberries were planted in 60 × 30 cm raised beds under a net house. During the experiment, the average temperature was 243 °C during the day and 132°C at night, with relative humidity ranging from 65 to 80%. All essential cultural practices and phytosanitary measures were applied to all plots during the experiment. In each replication, growth, yield, and physicochemical characteristics were assessed on randomly selected plants.

2.2. Fruit Set Percentage and Fruit Yield (t/ha) of Strawberry

Fruit set efficiency of each treatment was assessed by marking flowers at full bloom stage and calculated using the formula:

$$\text{Fruit set (\%)} = (\text{Number of Flowers Set / Total Number of Flowers Marked}) \times 100$$

The weight of fruit from each plot was measured using a balance and converted to hectare, and fruit yield per hectare was expressed in t/ha.

2.3. Measurements of Biochemical Parameters

The TSS content of strawberries was determined using a digital refractometer (MA871; Romania). A drop of strawberry juice was deposited on the refractometer prism using a dropper. Total soluble solids were measured with a refractometer. A digital pH meter (HI 2211; Romania) was used to determine the pH of the fruit juices of each treated strawberry. To measure the titratable acidity, a 5 g sample was crushed in a mortar and pestle. After filtering and adding distilled water, the total volume was 100 mL. In this, 10 mL of the stock solution, and 2 drops of phenolphthalein were then added to a conical flask. The solution was titrated with 0.1N NaOH. The total acid content was calculated as maleic acid equivalents based on triplicate analyses. The ascorbic acid content of strawberries was determined by the oxidation-reduction titration method (Tee et al., 1988). The fruit was mixed and then filtered through Whatman No. 1 filter paper. A volume of 100 mL of oxalic acid (5%) was prepared. Titration was carried out using the 2, 6-dichlorophenol indophenol dye solution. The mean observations revealed the amount of dye required to oxidize an unknown concentration of a given amount of L-ascorbic acid solution using L-ascorbic acid as the known sample. Each titration used 5 mL of the solution, and the last titration point was detected by the pink color that lasted for 10 seconds. As a result, a burette reading was recorded.

The phenol-sulfuric acid method was used to quantify reducing sugars (Dubois et al., 1956). The extract was filtered with deionized water after homogenization of 0.2 g of fresh fruit. To 2 mL of solution, 0.4 mL of 5% phenol was added. The liquid was then immediately diluted with 2 cc of 98% sulfuric acid. To allow for color development, the test tubes were kept at room temperature for 10 minutes before being immersed in a 30 °C water bath for 20 minutes. Absorbance was measured at 540 nm using a spectrophotometer. The same procedure was used to prepare a blank solution, but this time distilled water was used instead of fruit extract. Sugar reduction was expressed as mg/g fresh weight (FW). 1.0 g of fruit pulp was mixed with 1 mL of 85% ethanol + 15% HCl 1.5N. After extraction, 1 mL of the sample solution was taken and diluted to 10 mL. Absorbance was measured at a wavelength of 535 nm. The concentration of anthocyanins was calculated as follows:

$$\text{Anthocyanin} = (\text{A535 nm} \times \text{Volume of Extraction Solution} \times 100)/\text{ Sample Weight in g} \times 98.2$$

The same procedure as described above is used to prepare reference solutions, except that the fruit extract is replaced by distilled water (Lapornik et al., 2005).

The phenolic content was determined by Singleton et al. (1999). Fresh fruits (250 mg) were homogenized in 85% methanol. The supernatant was obtained after centrifugation of the extract at 3000 rpm for 15 min at 10 °C. 2 mL of the supernatant was mixed with 2 mL of Folin and Ciocalteu reagent. A 7.5% sodium carbonate solution (2 mL) was added to each test tube and the absorbance was measured at a wavelength of 725 nm against a reagent blank after 30-45 minutes. To quantify the concentration of total phenolics in the unknown sample, a standard curve was generated using gallic acid.

2.4. Analyses of Statistics

Trials were conducted in a randomized complete block design (RCBD) with three replications for each treatment and five plants in each replication. Statistical analyses were performed using IBM SPSS Statistics 21 (IBM Corp, Armonk, NY, USA). Means among treatments were considered statistically significant at $p < 0.05$. The mean and standard error of each outcome were determined using the replicated data. Microsoft Excel 2016 (Microsoft Corp., USA) was used to generate the graphs. The effects of GA_3, mulch materials and their interactions on several growth, yield and quality parameters were studied using analysis of variance (ANOVA).

3. Results and Discussion

3.1. Fruit Set (%) of Strawberry

All GA_3 treated plants showed maximum fruit set (%) than the non-GA_3-treated plants. Fruit set (%) of strawberry at different growth stages varied significantly due to different mulch materials (Figure 1). The maximum fruit set (89.14%) was recorded under sawdust mulch with GA_3 treated plants. The minimum fruit set (61.95%) was recorded under black polythene and no GA_3 treatment, followed by the control with no GA_3 treatment (66.56%). Exogenous GA_3 treatment had an indirect effect on auxin metabolism resulting in higher fruit set (%) and hence increased fruit yield (Singh et al., 2022). Saima et al. (2014) found a significant increase in flower number and fruit set in strawberry plants after application of gibberellic acid. GA_3 plays a regulatory role in the mobilization of metabolites from the source (leaves) to the sink (developing fruits) (Iqbal et al., 2011). Excess biomass in GA_3-treated plants may be able to produce more metabolites through photosynthetic activity, which ultimately sinks into the developing fruits. In the absence of GA_3, all flowers in control plants could not be transformed into fruits due to lack of pollination. As a result, the number of fruits and the percentage of fruit set were the lowest. The maximum fruit set (%) under sawdust mulch was observed by Ikeh et al. (2019). The probable cause is the control of weed growth, resulting in less conflict between the crop and weeds for nutrients and moisture, which may have improved nutrient use efficiency and large plant reproduction. This observation is consistent with the recent report by Ikeh and Akpan (2018) that organic mulch material, especially at higher dosages, inhibits weed seed germination. The low weed density and biomass observed in the mulched plots compared to the control plots could be because organic and inorganic mulch materials were able to shade and smother some weeds. This is consistent with the findings of Ikeorgu et al. (2006) who reported that covering soils with cover crops, straw, grasses, or even synthetic materials could effectively suppress weed growth and prevent them from receiving sunlight.

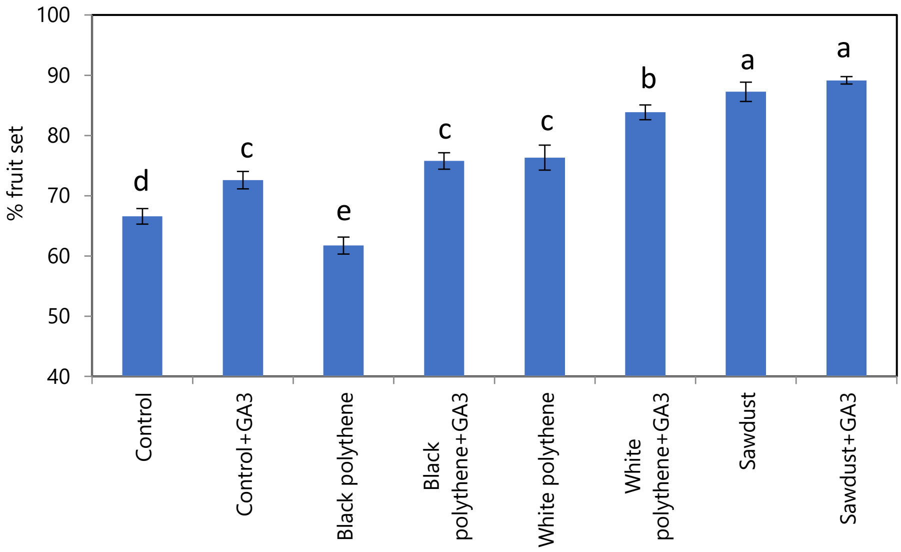

Figure 1. Effect of GA_3 and mulch materials on fruit set (%) of strawberry.

3.2. Fruit Yield of Strawberry

A significant effect was found for fruit yield/ha of strawberry as influenced by GA_3 and non-GA_3 (Figure 2). The results showed that the fruit yield of all GA_3 treated plants was comparatively high than the non-GA_3-treated plants. Fruit yield/ha of strawberries under different types of mulch materials was statistically different (Figure 2). The maximum fruit yield (17.95 t/ha) was found in GA_3 with sawdust mulch treatment plants followed by sawdust mulch treated plants (17.55 t/ha). The minimum fruit yield (6.07 t/ha) was found under black polythene with no GA_3 sprayed plants. Kumar et al. (2022) found that GA_3 gave the best results in terms of strawberry plant growth, yield, and fruit quality compared to other treatments. The increase in yield could be attributed to the influence of GA_3 on growth and development after cell division and elongation, as well as an indirect effect on auxin metabolism, which resulted in a greater number of marketable fruits and thus increased fruit yield. Kumar et al. (2014) also reported an increase in fruit size, weight, yield, and water-soluble dry matter due to GA treatments. Application of GA_3 could increase carbohydrate content and dry matter content due to higher photosynthesis rate and increase cell division and elongation, resulting in larger fruits (Saima et al., 2014). According to Paunovic et al. (2020) and Iwuagwu et al. (2020), mulching improved soil temperature, reduced weed infestation, and increased soil moisture, all of which improved plant development and fruit yield. Mulch could improve the photosynthetic capacity of the leaves in addition to the role of polyethylene in enhancing root growth, as well as the absorption of water and nutrients, respectively, thus improving the metabolic activities within the plant during the period of growth and the reproductive process. The plant had a large number of shoots per plant and a wide leaf area with high leaf chlorophyll content, which induced more photosynthetic rates as mentioned earlier (Ike et al., 2019). This resulted in a high glucose yield, which caused more cell division and enlargement, resulting in more vegetative vigorous growth, which resulted in higher total yield.

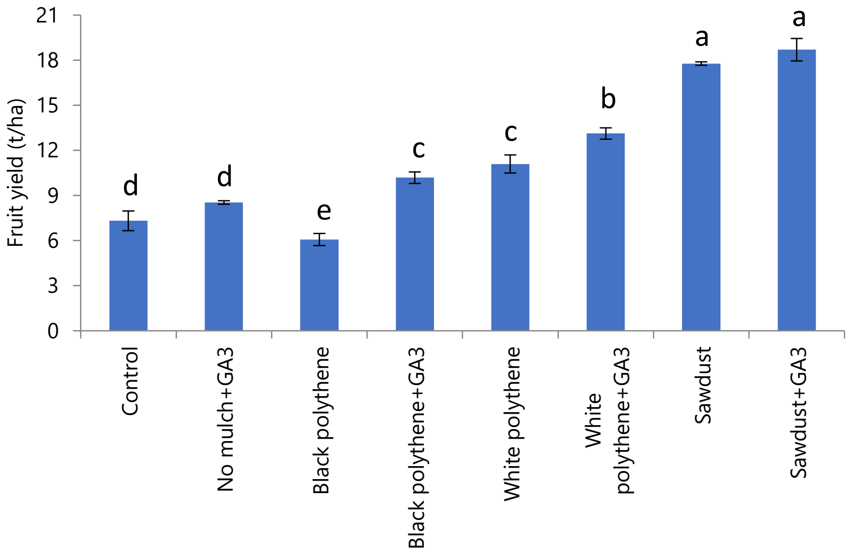

Figure 2. Effect of GA_3 and mulch materials on fruit yield (ton/ha) of strawberry.

3.3. TSS, pH, and Ascorbic Acid of Strawberry

A significant effect was observed for the TSS of strawberry as influenced by GA_3 and non-GA_3 (Table 1). All GA_3 sprayed plants showed increased TSS compared to non-GA_3 sprayed plants. The highest TSS of strawberry (8.90%) was found in GA_3-treated plants with sawdust mulch followed by GA_3 without mulch (8.06%) plants and the lowest TSS (6.30%) was observed in black polythene treated plants with no GA_3 followed by black polythene treated with GA_3 plants (6.53%). The highest TSS was found in sawdust mulch (8.45%) (Table 2). Palei et al. (2016) and Khunte et al. (2014) recorded higher TSS of strawberry fruits with the application of GA_3 compared to untreated plants. This could be because of the treatment on physiological sugar accumulation and changes in metabolism, resulting in increased retention of TSS and total sugars. The enzyme invertase breaks down sucrose into fructose and glucose, thereby increasing the amount of reducing sugars (Liu et al., 2014). Strik and Davis (2021) found that sawdust mulch resulted in higher TSS than other mulches. A weed-free environment, better moisture retention, ideal soil or canopy temperature, and maximum nutrient uptake under sawdust mulch treatment are associated with improved fruit quality (Strik et al., 2017). Significant variation in strawberry pH was observed under different types of mulch materials. The highest pH (3.35) was recorded under sawdust mulch with GA_3 treated plants. The lowest pH value (3.08) was recorded under the plants with black polyethylene mulch (Table 1). Sadek et al. (2021) and Kumar et al. (2012) found the highest pH value in the study of strawberries with sawdust mulch. Available phosphorus, nitrogen, exchangeable potassium, calcium concentrations and electrical conductivity were significantly increased in the soil under sawdust treatment and contributed to soil acidification (Sas-Paszt et al., 2014).

Table 1. Effect of GA_3 and mulch materials on total soluble solids, pH and titratable acidity of strawberry.

Treatments	Total Soluble Solids (TSS: %)	pH	Titrable Acidity (%)
No mulch	7.94 a	3.29 a	0.38 b
Black Polythene	6.41 c	3.12 b	0.46 a
White polythene	7.23 b	3.15 b	0.40 b
Sawdust	8.45 a	3.31 a	0.36 b
No mulch+GA_3	8.06 ab	3.25 b	0.40 abc
Black polythene+GA_3	6.53 c	3.17 c	0.48 a
White polythene+GA_3	7.70 b	3.12 cd	0.41 abc
Sawdust+GA_3	8.90 a	3.35 a	0.37 bc
CV%	7.08	1.15	7.23
$LSD_{0.05}$	0.931	0.064	0.079

At a 5% level of LSD, means a column that has the same letter(s) don't differ significantly from one another.

Among the mulch materials, black polyethylene showed the highest titratable acidity. The combination of black polyethylene with GA_3 gave higher results in terms of titratable acidity, while the lowest titratable acidity (0.35%) was recorded under the sawdust mulch treated with no GA_3, which was statistically similar to the control plants (0.36%) (Table 1). Higher moisture and nutrient availability, higher root activities including higher water and nutrient uptake, high photosynthetic and other enzymatic activities, and accumulation of more carbohydrates in the presence of optimum soil moisture appear to increase total acidity in fruits, as well as their involvement at the metabolic level in regulating vital physiological and biochemical processes. This is in line with the findings of Sener and Turemis (2017). This agrees with the findings of Sharma and Sharma (2003). Heat loss from the soil through evaporation, heat transfer from the mulch itself as radiation and conduction, and absorption of solar radiation all contributed to the varying thermal regimes of the soil and altered microsite conditions (Phet and Mpuat, 2016).

3.4. Ascorbic Acid, Total Sugar, Phenol, and Anthocyanin Content

All GA_3 sprayed plants showed increased ascorbic acid compared to non-GA_3 treated plants. The ascorbic acid content of strawberry (50.56 mg/100g) was maximum in GA_3 treated plants with sawdust mulch, followed by GA_3 with white polythene, while the lowest ascorbic acid (26.88 mg/100g) was observed in non-GA_3-treated-white-polythene-plants (Table 2). Bhople et al. (2019) observed that the application of GA_3 had a significant effect on the ascorbic acid content of fruits. This was due to a positive effect on sink strength (reproductive growth) as indicated by higher TSS and juice mass (%) in fruits of auxin-treated trees compared to control and other growth regulators such as GA_3 (Khunte et al., 2014). Helaly et al. (2017) indicated that plants (black currant) grown on sawdust mulch increased the vitamin C content in berries. According to their findings, the increase in vitamin C concentration under sawdust mulch may be the result of the mulch's ability to stimulate plant development and metabolism, which improves the chemical composition. The increased soil moisture retention, weed-free conditions, and maximum nutrient uptake in sawdust mulch-treated soils are associated with good and higher fruit quality (Ochmian et al., 2008).

The increased total sugar content of strawberries was observed under GA_3 treated plants compared to non-GA_3-treated plants. The maximum content of total sugar of strawberry (6.84%) was found in GA_3 treated plants with sawdust mulch followed by plants treated with GA_3 and white polythene (6.78%). The minimum total sugar content (5.51%) was observed under black polythene with non-GA_3 plants followed by the control (no GA_3 and mulch) (Table 2). According to Al-Atrushy (2016) and Thakur et al. (2015), the use of gibberellic acid significantly increased the total sugar content in strawberry and grape juice. The significant increase in sugar content could be attributed to carbohydrate accumulation in strawberry fruit as a result of increased supply/absorption of GA_3 (Lal and Das, 2017). According to Kher et al. (2010), the use of sawdust mulch resulted in the highest levels of TSS, total sugars, and crude protein. Higher fruit quality is associated with weed-free conditions, increased moisture conservation, a favorable soil hydrothermal cycle, and maximum nutrient uptake, all of which may result in a positive response in terms of growth, flowering, and quality traits under the sawdust mulch treatment (Singh et al., 2010). All GA_3-sprayed plants showed increased phenolic content compared to non-GA_3-treated plants. The highest phenolic content of strawberry (6.41 mg/g) was recorded in the combined treatment of GA_3 and sawdust-treated plants, while the lowest phenolic content (4.87 mg/g) was observed in GA_3 and white polyethylene-treated plants, followed by control plants (no GA_3 and mulch) (Table 2). Pre-harvest application of gibberellin on 'El-Bayadi' table grapes confirmed better yield, high quality with balanced sugar/acid ratio, higher accumulation of anthocyanins and phenolic content in berries, and improved economic factor of grapes by increasing color intensity and uniformity (Gundogdu et al., 2021; Alrashdi et al., 2017). According to Ye et al. (2017), morphological characteristics, antioxidant enzyme activities, photosynthetic pigment content, and bioactive content of *Anoectochilus roxburghii* differed according to treatment with different colored polyethylene mulch. Different light quality affects qualitative characteristics of lettuce such as vitamin C, carotenoids, and phenolics, and sawdust mulch treatments were shown to have higher levels of these characteristics compared to other mulches (Jahan et al., 2018).

A significant influence was found for the anthocyanin content of strawberry as influenced by GA_3 and non-GA_3 (Table 2). The results showed that all GA_3 sprayed plants showed increased anthocyanin content compared to non-GA_3 treated plants. The maximum anthocyanin content in strawberries (30.37 mg/100g) was observed in GA_3-treated plants with white polyethylene, followed by GA_3 and sawdust mulch. The lowest anthocyanin content (23.84 mg/100g) was observed in plants treated with black polythene without GA_3. Among the mulch materials, sawdust mulch had the highest anthocyanin content (30.15 mg/100g). Quintero et al. (2013) and Tas et al. (2021) observed increased anthocyanin concentration in strawberries after GA_3 spraying. The higher anthocyanin levels observed in this study could be attributed to increased photosynthesis and either direct or indirect involvement of GA_3 in the synthesis of anthocyanin pigment or its precursor, or by participating in the movement of its precursor under the influence of plant growth regulators (Fleishon et al., 2011). According to Kaur and Mirza (2018), strawberries grown with sawdust mulch and GA_3 have higher anthocyanin content than strawberries grown with black polyethylene and no mulch, because these strawberries are surrounded by optimal air and soil temperatures and receive more short-wave light reflection involvement in the movement of its precursor under the influence of plant growth regulators (Todic et al., 2008).

Table 2. Effect of GA_3 and mulch materials on ascorbic acid, total sugar, phenolic and anthocyanin content of strawberry.

Treatments	Ascorbic Acid (mg/100g)	Total Sugar (%)	Phenolic Content (mg/g)	Anthocyanin Content (mg/100g)
No mulch	32.42 b	5.96 c	5.21 b	25.43 b
Black Polythene	41.25 a	5.90 c	5.76 a	25.15 b
White polythene	36.15 b	6.30 b	5.02 c	30.05 a
Sawdust	40.85 a	6.62 a	5.81 a	30.15 a
No mulch+GA_3	37.33 b	6.28 b	5.45 cd	26.32 b
Black polythene+GA_3	44.80 a	6.28 b	5.82 b	26.45 b
White polythene+GA_3	45.43 a	6.78 a	5.17 e	30.37 a
Sawdust+GA_3	50.56 a	6.84 a	6.41 a	30.23 a
CV%	9.30	1.42	2.72	2.63
$LSD_{0.05}$	6.133	0.154	0.260	1.273

At a 5% level of LSD, means in a column that have the same letter(s) don't differ significantly from one another.

4. Conclusion

In strawberry production, organic mulch (sawdust) showed better results in yield and biochemical characteristics than synthetic (black and white plastic) and no mulch. GA_3-treated plants also had higher quality characteristics than non-GA_3-treated plants. The results of the study suggest that the combination of sawdust mulch with GA_3 in strawberry production can improve the quality attributes of the fruit.

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