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Application of Pyraclostrobin Enhances Growth, Quality, and Yield of Tomato

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ABSTRACT

Tomato is an important vegetable crop extensively cultivated not only in India but throughout the world for its fruit which is a rich source of important nutrients such as vitamin C, vitamin E, lycopene, and various minerals. Strobilurin compounds are commonly used by farmers as a fungicide against early blight and late blight disease of tomato. Several reports have shown that strobilurins, including pyraclostrobin, are capable of increasing growth and yield when applied to healthy plants. The present study was intended to check the effects of sequential applications of pyraclostrobin on tomato plants. Seeds of the tomato cultivar "Heemshikhar" were sown in a small plot and after 25 days, the plants were transferred to a one-acre field. Four sequential sprays of pyraclostrobin were applied to the tomato plants at 15 days interval. The first pyraclostrobin application commenced at 50 days after sowing. Parameters such as vegetative, reproductive, and fruit quality, as well as yield were compared at various intervals among control and treated plants. Plants that were given the cumulative pyraclostrobin application showed a significant increase in the height of the plant, leaf number, and flower number. The significantly higher content of lycopene and protein as well as the significantly lower content of carbohydrates in pyraclostrobin treated plants signified the beneficial effect of pyraclostrobin application on fruit quality. The application of this fungicide was also found to increase the size, weight, and yield of tomato.

Keywords: Lycopene; Protein; Strobilurin; Tomato

1. Introduction

Tomato (*Solanum lycopersicum* L.) belongs to *Solanaceae*, the nightshade family. It bears a cluster of berries classified as a vegetable. Tomato is one of the most frequently consumed vegetables in the world and is an important source of dietary antioxidants such as Lycopene, flavonoids, Vitamin C, and Vitamin E [1-3].

Agriculture is one of the most important professions and it contributes to more than 60% of the Indian economy. Among the various vegetable crops, tomato

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is the foremost one cultivated in the western provinces of India. The main season for planting the tomato in Gujarat is October-November. Fruits are picked after two months and this harvesting process lasts for the next two months.

Strobilurin fungicides have been reported, in the last couple of decades, to have plant growth-promoting effects in addition to their fungicidal activity. Pyraclostrobin is a member of the strobilurin group of fungicides. Azoxystrobin, Picoxystrobin, Kresoxy-methyl, Trifloxystrobin, and Metominostrobin are relative compounds of the same family [4].

Pyraclostrobin, a member of the strobilurin family, has shown some positive effects on plant growth [5]. Pyraclostrobin has been reported to stimulate nitric oxide, an important secondary messenger. It also has been reported to alleviate oxidative stress [6] and to retard senescence in plants [7]. Several studies have shown that the application of pyraclostrobin affects the phytohormonal level in plants including increasing the endogenous levels of abscisic acid (ABA) and IAA [6]. Application of pyraclostrobin increased stalk yield and green biomass index and ultimately the yield of sugarcane crop [8]. Studies on the effects of various strobilurins have confined their candidature to the category of plant growth-promoting substances apart from their fungicidal activity. The present study was framed to elucidate the effects of sequential application of pyraclostrobin on the growth of tomato plant and fruit quality. It was hypothesized that the application of pyraclostrobin enhances vegetative and reproductive growth, yield, and fruit quality. The whole study was conducted in the field of Bakrol village of Anand district. The average rainfall in Anand district is 882 mm per year [9] and the soil type is sandy loam. Heemshikhar is the most popular cultivar grown in this area. It has a prostrate habitat and lasts for about four months. The total production of tomato recorded in Anand district was 114120 metric tons in an area of 3600 hectares in the year 2016-17. Application of compounds like pyraclostrobin may help increase the yield and quality of tomato and thus, help improve the economy and income of farmers.

2. Materials and Methods

2.1 Field experiment

The field experiment was carried out in the year 2016-17. Randomized Block Design was used for the experiment. Four rows in terms of replicates were assigned to each treatment.

2.1.1 Plant material and pyraclostrobin application

Seeds of the tomato cultivar "Heemshikhar'' were purchased from the seed and agrochemical market of Anand City, India, and sown in a small plot during the first week of November in 2016. Plants were regularly monitored and provided with fertilizers and water. After 25 days of growth, they were transplanted to a one-acre field (N22.563855, E72.899390) in Anand district of Gujarat, India in December 2016. Distances between rows and between two plants in each row were spaced at 150 cm and 50 cm, respectively. Control and treated rows were separated by keeping one buffer row in between them. Cabrio top (0.3% w/v) (BASF) containing 5% pyraclostrobin, was applied to the canopy of tomato plants using a sprayer (Capacity: 16 Liter, Pressure: 0.2-0.4 Mega Pascal), 50 to 60 litres of cabrio top solution was applied per acre of the field. The application of pyraclostrobin commenced on 50 DAS (Days After Sowing) (Table 1).

Table 1. Pyraclostrobin sequential application schedule.

Treatment	1st spray (50DAS)	2nd spray (65DAS)	3rd spray (80DAS)	4th spray (95DAS)
Control	×	×	×	×
T1	Yes	×	×	×
T2	Yes	Yes	×	×
T3	Yes	Yes	Yes	×
T4	Yes	Yes	Yes	Yes

Note: Symbol '' ×'' shows that plants of particular rows were not given application of pyraclostrobin, DAS=Day after sowing; T1, T2, T3, and T4 are the different sets of plant acquiring one application, two applications, three applications, and four applications, respectively; control set was not given application of pyraclostrobin.

2.1.2 Chemicals, equipment, and instruments used in the experiment

Common chemicals such as acids, solvents, and chemicals for preparation of buffer were purchased from SRL Pvt. Ltd. India. Fine chemicals used for standard graph preparation were purchased from Sigma Aldrich, USA. In the experiment, water bath (Cole-parmer, USA), cooling centrifuge (Eppendorf 5430 R; Eppendorf 5415 R), and spectrophotometer (Shimadzu UV 1800) were used. Consumable items such as pipette tips and centrifuge tubes were purchased from Tarson, India.

2.1.3 Common farmer practices and fertilizers application

Watering, removal of weeds, and other common farming practices were followed in similar ways for all the sets of plants (Control and Treated). Fertilizers were provided regularly according to the need, equally to all sets of plants.

2.1.4 Plant growth parameters

The effects of sequential application of pyraclostrobin on vegetative growth of tomato were checked by selecting 25 random plants from each set and then the plant height and the total number of leaves were counted on the 15^{th} day after first and second sprays of pyraclostrobin. For the third and fourth sprays, 10 plants were selected for measuring plant growth parameters. On the 15^{th} day after each pyraclostrobin application, the number of floral branches and the number of flowers per floral branch were determined in each set to validate the effect of pyraclostrobin application on flowering and ultimately on the yield.

2.1.5 Nutrient content of fruit

2.1.5.1 Carbohydrate estimation

Fruit samples were collected in triplicate from the field and brought to the laboratory for analysis. The fruit was cut into small pieces and weighed 100 mg each in a boiling tube. Samples were hydrolyzed by adding 2.5 M HCl in each tube followed by keeping the tubes in a boiling water-bath for 3 hours. The mixture was then cooled and neutralized with sodium carbonate until the effervescences ceased at which point the volume was made up to 100 ml using distilled water. The mixture was transferred to a 50 ml polypropylene conical bottom centrifuge tube (Tarson, India) and centrifuged at 5000 rpm for 10 min at room temperature (Eppendorf centrifuge 5430R). From the clear supernatant, 0.5 ml and 1.0 ml aliquots were collected for analysis and the carbohydrate content in various fruit samples was determined using the Anthrone method [9].

2.1.5.2 Lycopene estimation

Extraction: Fruits of similar ripening stage were collected in triplicate from the field. The fruit was first cut into small pieces and weighed 2 g each followed by homogenization in hexane and acetone (60:40) using a pre-cooled mortar and pestle. Tubes were briefly centrifuged (Eppendorf centrifuge 5430R) and the upper organic phase was transferred to fresh capped tubes on ice. The remaining aqueous phase was extracted again with 5 ml of the same solvent repeatedly and the organic phase was transferred to the same tube until the aqueous layer became colorless.

Assay: 1 ml of the organic extract was taken in the tube separately and absorbance was measured at 502 nm against hexane and acetone (60:40) solvent as a blank and the amount of lycopene was calculated using the following equation [11].

$$\text{Lycopene } (\mu g/ml) = 3.12 \times OD_{502}$$

2.1.5.3 Protein estimation

Extraction of protein: The fruit sample was collected in triplicate for each set and brought to the laboratory for assay. The fruit was cut into small pieces and 1 g was weighed and homogenized in 2 ml of phosphate buffer (pH 7.0) in a mortar and pestle. Homogenate was transferred to the microcentrifuge tubes and subjected to centrifuge at 10000 rpm for 10 min at 4 ºC (Eppendorf centrifuge 5415 R). The supernatant was transferred to a fresh tube for further use. The amount of protein in the sample was determined following the Folin-Lowry method [12].

2.1.5.4 Ascorbic acid estimation

Extraction: Four replicate fruit samples were collected, in triplicate for each set, cut into small pieces, weighed 1 g, and homogenized in 20 ml of 4% TCA using a mortar and pestle. Homogenate was transferred into conical bottom 50 ml polypropylene centrifuge tube and subjected to centrifuge at 5000 rpm for 5 min at room temperature (Eppendorf centrifuge 5430R). The supernatant was collected in a fresh tube and a pinch of charcoal was added, followed by a 5 to 10 min incubation. Following this, it was subjected to centrifuge at 5000 rpm for 20 min at room temperature (Eppendorf centrifuge 5430R). The above step was repeated until complete removal of charcoal. The sample was stored at 4 ºC for further use.

Assay: 0.1 ml of the extract was taken into a test tube, the final volume was adjusted to 2 ml with 4% TCA and 0.5 ml of DNPH (2, 4-dinitrophenyl hydrazine) (Sigma-Aldrich, USA) in each tube. Then, 25 µl of 10% Thiourea (Sigma- Aldrich, USA) was added to this preparation followed by incubation at 37 ºC for 3 hours. After incubation, 2.5 ml of 35% cold H_2SO_4 was added, followed by 20 min of incubation at room temperature. Blank tube was prepared by adding H_2SO_4 before DNPH and Thiourea followed by incubation at room temperature for 30 min. The standard curve was prepared using 20 mg% ascorbic acid (Sigma- Aldrich, USA) in 4% TCA. Absorbance was measured (Shimadzu UV-1800) against blank and the amount of ascorbic acid in the sample was calculated using a standard curve [13].

2.1.6 Measurement of fruit size and weight

Fifteen fruits from each set were collected at similar ripening stage from the field after 15 days of the 4th spray of pyraclostrobin for measurement of size and weight of fruit. Fruit size was measured using Vernier calipers. Weight of the fruits was also measured and compared in all 5 sets. Fruit samples were also transversely cut for macroscopic observation.

2.1.7 Yield assessment

The yield of tomato for each set was also determined after 15 days of the 4th application of pyraclostrobin. Total expected yield per acre (MT/acre) was calculated by calculating the average yield per plant multiplied by the total number of plants in the one-acre field.

2.2 Statistical analysis

Statistical analysis was performed using IBM© SPSS Statistics software Version 22. Variables of the first pyraclostrobin application were subjected to an independent sample t-test whereas for the second and subsequent applications, variables were tested using one-way ANOVA with Tukey-B test and homogeneity groups were generated assigning characters of the alphabet in which different letters indicate the significant difference among sets.

3. Results and Discussion

3.1 Plant growth parameters

3.1.1 First application of pyraclostrobin

Independent sample t- test analysis indicated a significant increase in plant height, leaf number, and floral branches number. Plant height was significantly higher (significant at 0.01) as compared to control. The number of leaves was three folds higher (significant at $p = 0.05$) than the control (Fig. 1). Floral branches were also found to be significantly increased (significant at $p = 0.05$) as compared to control plants (Fig. 1). A significant difference was not found for the number of flowers per floral branch between control and treated (T1) sets.

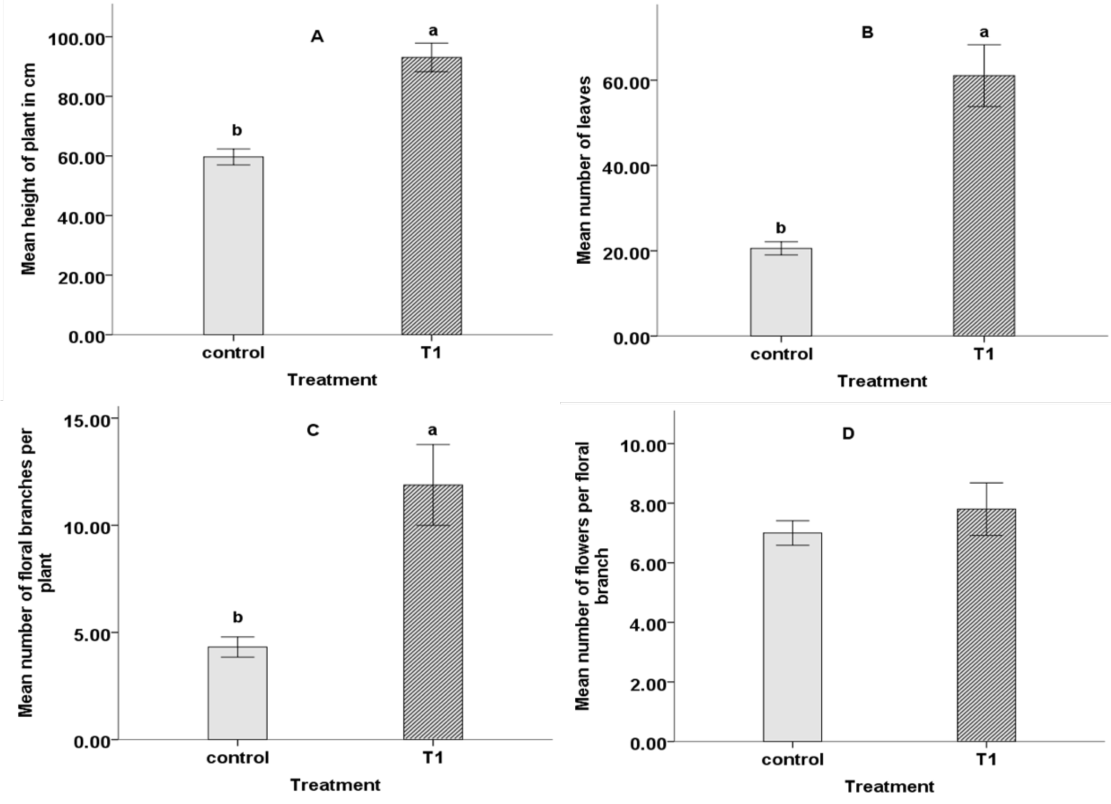

Fig. 1. Morphological parameters after the first application of Pyraclostrobin. A) Height of the plant in cm. B) Number of leaves C) Number of floral branches per plant D) Number of flowers per floral branch. Bars with different letter are significantly different at $p = 0.05$; cm=centimetre.

3.1.2 Second application of pyraclostrobin

After the second application of pyraclostrobin, growth parameters were compared among three sets of plants. Tukey-b test inferred that height of the plant, leaf number, and the number of floral branches were significantly (significant at $p = 0.05$) greater in both treated (T1 and T2) plants than in the control plants (Fig. 2). Both the treated (T1 and T2) plants produced a significantly greater number of flowers per floral branch as well (Fig. 2).

3.1.3 Third application of pyraclostrobin

On the 15th day after the third application of pyraclostrobin, height of the plant, leaf number, number of floral branches, and number of flowers per floral branch were significantly (significant at $p = 0.05$) greater in all treated plants compared to control plants (Fig. 3). Interestingly, the height of plants of T1, T2, and T3 were respectively 1.3-, 1.6-, and 2-fold greater than the control.

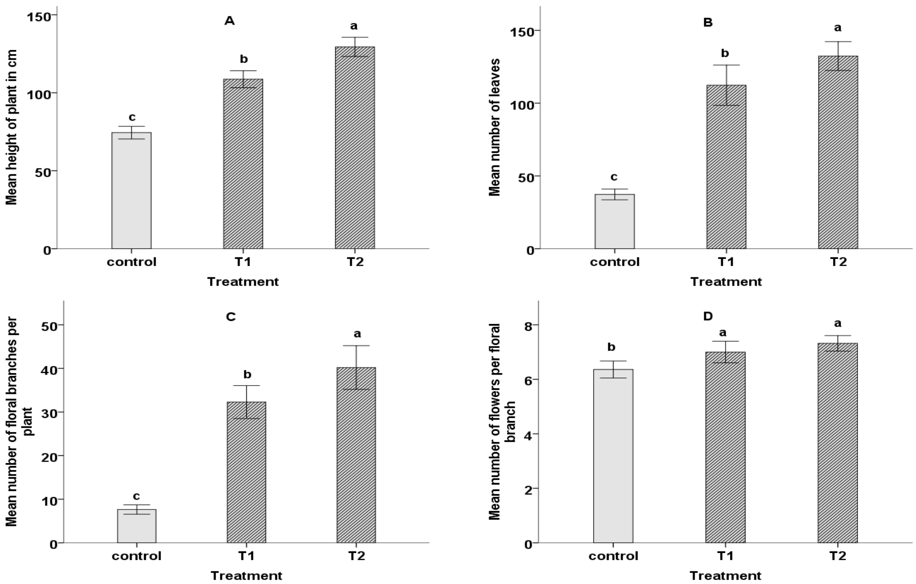

Fig. 2. Morphological parameters after the second application of Pyraclostrobin. A) Height of the plant in cm. B) Number of leaves C) Number of floral branches per plant D) Number of flowers per floral branch. Bars with different letter are significantly different at $p = 0.05$; cm=centimetre.

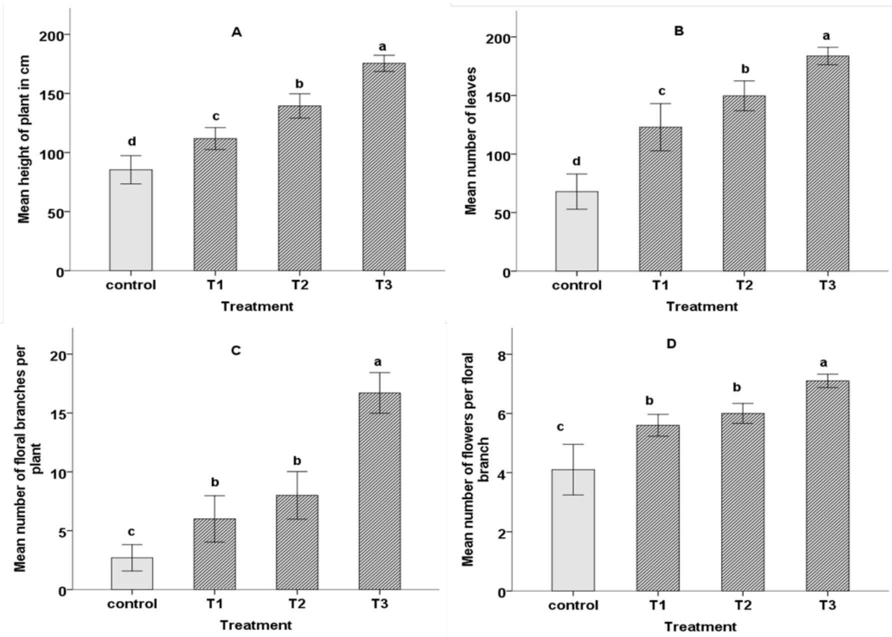

Fig. 3. Morphological parameters after the third application of Pyraclostrobin. A) Height of the plant in cm. B) Number of leaves C) Number of floral branches per plant D) Number of flowers per floral branch. Bars with different letter are significantly different at $p = 0.05$; cm=centimetre.

Also, the number of leaves was 1.8-, 2.2-, and 2.7-fold greater, the number of floral branches was 2.2-, 3-, and 6-fold greater, and the number of flowers per branch was 1.4-, 1.5-, and 1.7-fold greater, in the T1, T2, and T3 plants respectively, than control.

3.1.4 Fourth application of pyraclostrobin

Similar to the third application, after the fourth application, growth parameters indicated positive effects on vegetative growth and the reproductive organ, the flower. An increase in the number of leaves and height of plants as a result of the pyraclostrobin application suggests that the application of pyraclostrobin boosts the vegetative growth of tomato plants. An increase in vegetative growth might be due to an increase in nutrient uptake, and photosynthesis rate. This assertion can be supported by previous studies which show a similar positive effect of pyraclostrobin application in other crops [14-15]. The increase in the number of floral branches and number of flowers per branch indicates that more fruits will set in the treated plants, being highest in the T4 plants and gradually less in T3, T2, T1, and control (Fig. 4).

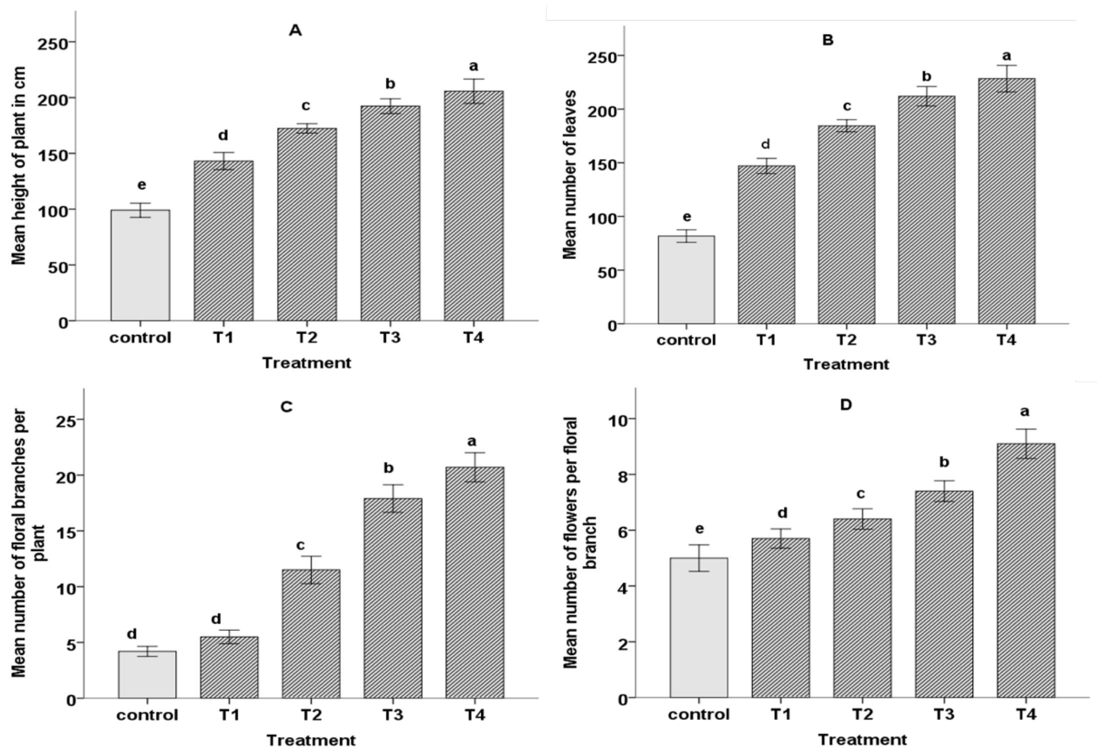

Fig. 4. Morphological parameters after the fourth application of Pyraclostrobin. A) Height of the plant in cm. B) Number of leaves C) Number of floral branches per plant D) Number of flowers per floral branch. Bars with different letter are significantly different at $p = 0.05$; cm=centimetre.

3.2 The dietary content of fruit

Dietary content parameters such as lycopene and protein content were significantly (significant at $p = 0.05$) greater in the T3 fruit than in fruit of control plants (Figs. 5 A, C, D). T1 showed the highest content of ascorbic acid among all the treatments. Interestingly, the carbohydrate content in fruits of all the groups (T1, T2, T3 and T4) was significantly (significant at $p = 0.05$) lower than the fruits of control plants. The lowest carbohydrate content was observed in the fruits of T3 plants (Fig. 5B). Reduced levels of carbohydrates in produce lowers the risk of obesity and liver dysfunction [16]. Such fruits having lower carbohydrate content make them a healthier choice. Pyraclostrobin has been reported to increase nitrogen fixation which is an ATP consuming process in the cell. ATP is generated at the cost of glucose, a carbohydrate in the process of glycolysis [17]. A decrease in carbohydrate content in fruits of pyraclostrobin treated tomato plants might be a result of increased consumption of carbohydrates in the root to account for increased nitrogen assimilation. Carbohydrates produced in the leaves are transported to roots for the nitrogen assimilation process. Increased nitrogen assimilation may result in increased synthesis of proteins. Also, one notable study performed on corn demonstrated that pyraclostrobin decreased starch by 1 to 2 g kg^{-1}, and decreased extractable starch by 3 to 4 g kg^{-1} compared to control [18].

Fruit containing increased levels of antioxidants such as ascorbic acid [19] and lycopene [20] is always preferred by consumers, as lycopene reduces the risk of certain types of cancer and aging effects [21] and ascorbic acid (vitamin C) helps in growth, development, and building body tissues. Lycopene also imparts the red color to fruits, which may be important to attract consumers. An increase in lycopene content in the tomato fruits of T3 plants might be due to increased expression of Stay green protein SlSGR1 which regulates chlorophyll degradation and lycopene synthesis in tomato fruit and leaves [21]. Higher protein content in tomatoes can be a priority choice for the preparation of tomato products as it is beneficial for health [22].

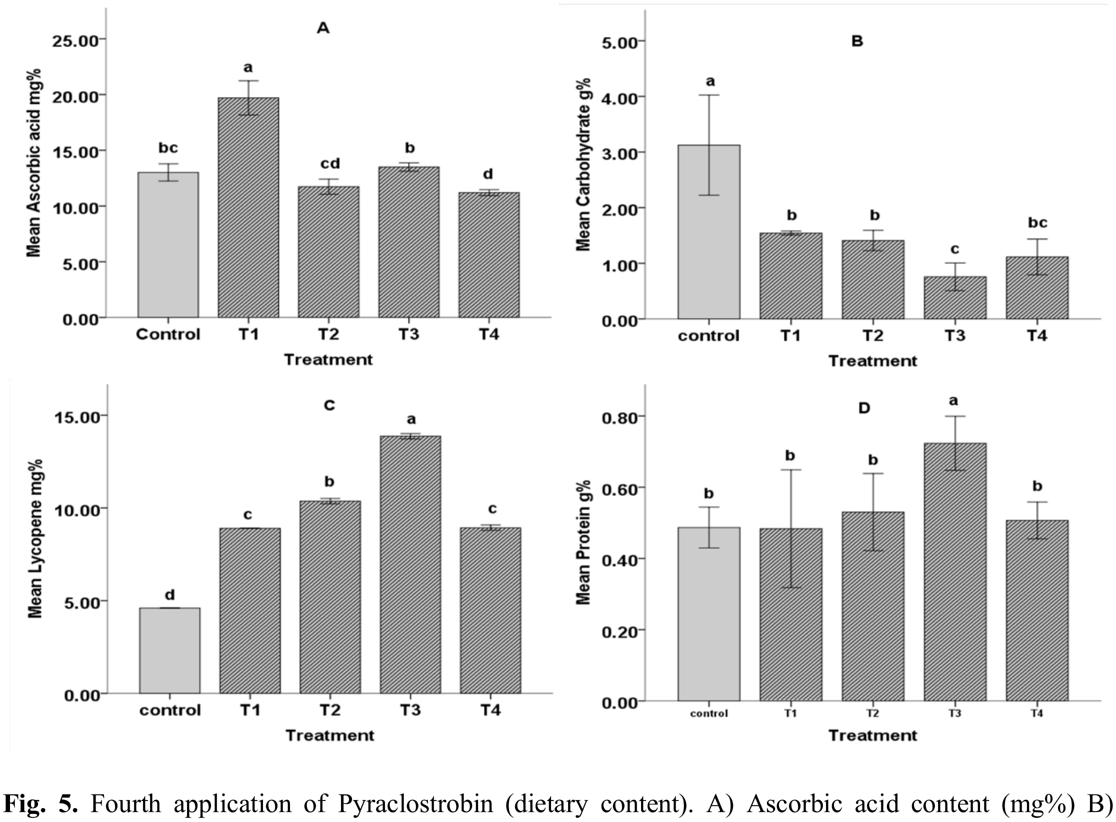

Fig. 5. Fourth application of Pyraclostrobin (dietary content). A) Ascorbic acid content (mg%) B) Carbohydrate (g%) C) Lycopene (mg%) D) Protein (g%). Bars with different letter are significantly different at p=0.05; g=gram, mg=milligram.

3.3 Fruit size and weight

One of the most significant changes was observed in the weight and size of treated fruits. All the treated plants except T1 produced significantly larger (significant at p=0.05) sized fruits as compared to the control (Figs. 6 A, B). Similarly, the weights of the fruits of all treated plants were found to be greater than the control. Among all treated plants, T4 plants had the fruit with the largest size and weight. Transversely cut fruits of plants showed that the highest number of locules was 4, also occurring in the T4 plants, whereas only 3 locules were present in the T1, T2, and T3 plants. Control plants produced fruits having two locules (Fig. 7).

Fig. 7. Fruit macroscopic structure. Labels above the each cut fruit shows the treatment given to that plant; Transversely cut fruit shows the comparative difference in size and number of locules in the fruit.

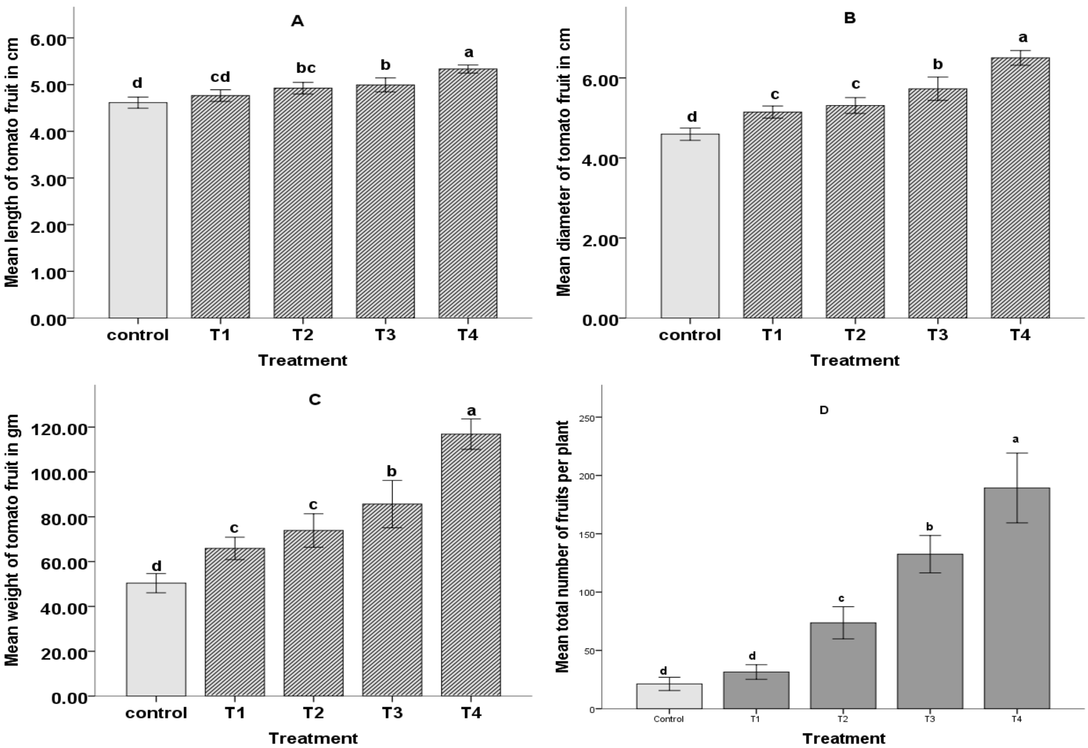

Fig. 6. Fourth application of Pyraclostrobin (Fruit size, weight and yield). A) Length of fruit (cm) B) Diameter of fruit (cm) C) Weight of fruit (g) D) Total number of fruits per plant, Bars with a different letter are significantly different at p = 0.05; cm=centimetre, g=gram.

3.4 Yield

Strobilurin has previously been reported to increase yield and size of seeds in soybean [23] and other crops. T4 plants produced about 7 times more fruit than the control plants. Moreover, T2 and T3 plants also produced a significantly greater number of fruits per plant than the controls. Depending upon the season and cultural practices, the average yield of Heemshikhar tomatoes is 25-30 MT/acre [24]. Application of four sequential sprays is expected to produce 57 MT/acre which is about double the usual yield (Table 2).

Table 2. Yield of tomato (MT/acre).

Treatment	Number of fruits per plant	Avg. weight of fruit (g)	Tomato yield per plant (g)	Yield MT/acre
C	21.30	32.29	687.77	4.63
T1	31.50	35.60	1121.40	7.56
T2	73.70	37.92	2794.70	18.85
T3	132.5	41.27	5468.27	36.88
T4	189.30	45.00	8518.50	57.45

Note: MT indicate Metric ton, equal to 1000 kg: g for gram: Avg for Average.

4. Conclusion

Tomato is considered one of the major vegetable crops in India as well as in the world. Its products are in great demand. Though the cultivation of tomato is possible in greenhouses, field production is much greater than greenhouse production in India. But the major problem of field production is the risk of various fungal diseases and poor farmer practices that ultimately lead to reduced yield.

Strobilurins are a class of compounds that can prevent various fungal and bacterial diseases. They are well known for their various positive effects on healthy plants. A similar effort was made here to study the positive effects of one of the strobilurin compounds, pyraclostrobin, on the tomato plant. The sequential application of pyraclostrobin showed a cumulative positive effect on the tomato plants. Plants receiving multiple applications of pyraclostrobin showed more vigorous growth and produced more flowers and floral branches.

Though T4 plants showed the foremost vegetative growth among all sets and also produced the largest sized fruits having the highest weight, T3 plants were better in producing higher quality fruits. Lycopene and protein content were found highest in fruits of T3 plants. Moreover, fruits of T3 plants had lower carbohydrate content which again makes it better in quality.

It can be concluded from these findings that three sequential applications of Pyraclostrobin at intervals of 15 days commencing on 50 DAS is an optimal schedule for the production of good quality fruits and better yield.

These findings validate the hypothesis that the sequential application of pyraclostrobin helps to bring vigorous vegetative growth of the tomato plant, in a dose dependent manner. Moreover, sequential applications of this compound also make the fruits better in quality.

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