Influence of foliar application of bio-stimulants on growth, yield and chemical composition of tomato


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Key words: Fruit quality, growth, plant extracts, Salicylic acid, yield.

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Abstract

The experiment was carried out 2018 at the University of Agriculture Peshawar-Pakistan to investigate the influence of different plant extracts and salicylic acid as a foliar spray on growth, yield and fruit quality of tomato (Cv. Rio Grande). The design of the experiment was a Randomized Complete Block Design (RCBD) with split plot arrangement with three replicates were used. Different levels of plant extracts i.e (control, moringa 6% neem 10% and garlic 4%) and Salicylic acid concentrations i.e (Control, 2, 4 and 6mM) were applied for foliar application. Results indicated that foliar application of plant extracts (moringa 6%) gave Maximum chlorophyll content (0.0511 mg cm\(^{-2}\)), plant height (90.44 cm), single fruit weight (57.79 g), fruit volume (55.68 cm\(^3\)), fruit firmness (3.63 kg cm\(^{-2}\)), titratable acidity (0.40 %) and ascorbic acid (11.76 mg 100gm\(^{-1}\)) content, while maximum pH was recorded in control treatment. Whereas salicylic acid concentration (6mM) also effected all the attributes as compared to all treatment. maximum chlorophyll content (0.0522 mg cm\(^{-2}\)), plant height (88.38 cm), single fruit weight (54.91 g), fruit volume (51.91cm\(^3\)), fruit firmness (3.28 kg cm\(^{-2}\)), titratable acidity (0.37 kg %), and ascorbic acid (10.87 mg 100g\(^{-1}\)) content with minimum fruit juice pH (4.19) was recorded.

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Introduction

Tomato is cultivated throughout the world (Fanasca, 2007). It contains high levels of antioxidant active compounds such as vitamin C, carotenoids (Tommonaro et al., 2012).

Foliar feeding of vegetable plants can effectively supplement soil fertilization. It has been found that elements foliar application is in a same level and even more influential compared to soil application.

It was suggested that foliar feeding could be applied successfully to compensate shortage of those elements.

Salicylic acid (SA) treatments were generally effective on vegetative growth, photosynthetic pigments, minerals, yield of tomato fruit quality (Kazemi, 2014). SA is a natural and safe chemical to maintain postharvest quality of horticultural crops and nowadays its application is adopting, although chemical treatments are banning in many countries (Supapvanich and Promyou, 2013). SA is an active element of aspirin, and it regulates a number of processes in plants (Kumar et al., 2013). SA inhibit grey mould growth, significantly decrease weight loss, increase storage life, maintain total soluble solids, titratable acidity, antioxidant, ascorbic acid and pH value in Kiwi fruits (Fatemi et al., 2013). Foliar SA application increase plant growth, chlorophyll content in leaves, early yield and total yield and soluble solids, although it had no effect on pH, ascorbic acid and titratable acidity of tomato (Yildirim and Dursun, 2008).

Moringa, Neem, Garlic leaf extract was sprayed onto leaves of tomato, melon and maize, sorghum, coffee, tea, onions, bell pepper, soya beans, chili and was shown to increase yields of these crops (Fuglie, 2000).

The objective of the current study is to test effect Moringa, Neem, Garlic leaf extract with Salicylic acid to increase the growth, yield, fruit quality characteristics

Materials and methods

Experimental site

An experiment to study “Influence of foliar application of bio-stimulants on growth, yield and chemical composition of tomato” was conducted in Horticulture Research Farm and Post-harvest Laboratory, The University of Agriculture Peshawar during 2018.

Design of Experiment

The research work was conducted in Randomized Complete Block Design (RCBD) with two factorial split plot arrangement. Salicylic acid concentrations along with control were allotted to main plot with four levels (0, 2, 4, and 6 mM) while, different plant extracts along with control were allotted to sub plot with four levels (0, moringa 6%, neem 10% and garlic 4%) having three replications were used. Salicylic acid and different plant extracts was applied in liquid form as foliar spray to the tomato plants.

Treatments

The total numbers of treatments were 48.

Plant extracts preparation

Leaf samples of Moringa and Neem were collected from Horticulture Research Farm, Malakandhair and Garlic cloves were collected from local market and brought to the laboratory. All the samples were washed thoroughly with tape water by putting it in net basket. Then these samples were mixed with water in such a way that samples: water (1: 5) and then were ground with small grinder. The grinded sample was filtered through masculine cloth to remove the impurities. The filtered solution was taken as standard solution. From the standard solution, the recommended solutions were prepared for each sample i.e. Moringa (6%), Neem (10%) and Garlic (4%) (Price, 2007).

Nursery raising and cultural practices

The seeds (Cv. Rio Grande) were sown in the last week of January. The field was prepared one week before transplantation of the crop. All the stone, stub, root or any other material which may result in barrier
to the crop were removed. N-P-K was applied as a basal dose at 112-80-40 kg ha⁻¹ respectively. Tomato seedlings transplanted when they reached 2-4 leaves stage and were transplanted early in morning.

**Stage of foliar application**

The crop was sprayed fifteen (15) days after transplantation.

**Variables studied**

Chlorophyll content (mg cm⁻²): It was measured from randomly selected leaves of desirable plant with the help of spad meter and then the mean was calculated.

Plant height (cm): From each treatment five plants were randomly selected and through measuring tape their height was measured from bottom to the top of the plant. Their mean was computed.

Fruit weight (g): Mean was computed by weighing the randomly picked fruit from plants of each treatment of all replications and calculated by following formula.

Fruit volume (cm³): The fruit volume was measured from randomly five fruit by water displacement method. 500ml beaker was taken and placed in a collector then the beaker was filled with water and the fruit was putted in the beaker and then the drained water was measured in graduated cylinder. The fruit volume was measured form five randomly taken fruits and then the average was taken.

Fruit firmness (kg cm⁻²): Penetrometer Model-Wanger FT-327 having the capacity of 28 lbs was used to find the fruit firmness. The prob of the instrument was penetrated into the pulp of tomato and reading was recorded.

Percent Titratable Acidity: The percent titratable acidity determined by standard method AOAC (2000) and calculated by following formula.

\[ \text{Percent titratable acidity} = \frac{N \times T \times F \times 100}{D \times S} \times 100 \]

Where

\[ N = \text{Normality of NaOH} \]
\[ T = \text{ml of 0.1 N NaOH used} \]
\[ F = \text{Constant acid factor 0.0067 (citric acid)} \]
\[ D = \text{ml of sample taken of tomato juice.} \]
\[ S = \text{ml of diluted sample taken for titration.} \]

Ascorbic acid content (mg 100g⁻¹): “Dye method” was used to find ascorbic acid content. Fruit juice was extracted from the selected fruit samples at the rate of 10 ml and poured into the volumetric flask, then oxalic acid was supplemented to move up the volume to 100 ml. By this 10% solution was made. After that 10 ml solution was picked and titrated with Dye. The observance of pink color indicates the completion of reaction. Furthermore, the content of Vitamin C will be calculated through the given formula:

\[ \text{Ascorbic acid content (mg/100g)} = \frac{F \times T \times 10}{D \times S} \times 100 \]

Where

\[ T = \text{Amount of Dye solution consumed from burette (ml)} \]
\[ F = \text{Constant Factor of Dye} \]
\[ S = \text{Fruit juice (g) taken for dilution} \]
\[ D = \text{Amount of diluted sample used for titration (ml)} \]

Fruit juice pH: pH was measured from the juice of the fruit with the help of pH meter by dipping the probe of the meter into the juice until the reading stop and then the reading was noted.

**Statistical analysis**

All the data collected was analyzed by using Randomized Complete Block Design. In case the data was found significant. Least significant difference was applied for mean comparison. A statistical package (8.1) was used for analyzing the data (Jan et al., 2009). The foliar application of plant extracts salicylic acid significant effected the growth, yield and fruit quality of tomato.

**Results**

The foliar application of different plant extracts and salicylic acid concentrations significantly affected the growth, yield and fruit quality of tomato.
Table 1. Influence of different plant extracts and salicylic acid on plant height and physico-chemical component of tomato.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant extracts (%)</th>
<th>Plant height (cm)</th>
<th>Chlorophyll content (mg cm$^{-2}$)</th>
<th>Fruit weight (g)</th>
<th>Fruit volume (cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_0$</td>
<td>68.61C</td>
<td>0.0406b</td>
<td>43.40C</td>
<td>43.67C</td>
<td></td>
</tr>
<tr>
<td>$E_1$</td>
<td>90.44A</td>
<td>0.0511a</td>
<td>57.79A</td>
<td>55.68A</td>
<td></td>
</tr>
<tr>
<td>$E_2$</td>
<td>78.72B</td>
<td>0.0434b</td>
<td>48.98B</td>
<td>47.76B</td>
<td></td>
</tr>
<tr>
<td>$E_3$</td>
<td>77.88BC</td>
<td>0.0429b</td>
<td>46.39BC</td>
<td>45.52BC</td>
<td></td>
</tr>
</tbody>
</table>

Salicylic acid (mM)

| $S_0 = 0$ | 70.06c | 0.0539c | 42.34c | 44.50c |
| $S_1 = 2$ | 74.73bc| 0.0429bc| 48.92b | 47.92b |
| $S_2 = 4$ | 82.48ab| 0.0470ab| 50.39b | 48.30b |
| $S_3 = 6$ | 88.38a | 0.0522a | 54.91a | 51.91a |

LSD at 1%

| 9.9698 | 6.331  | 4.6016 | 2.4726 |

LSD 5%

| 9.1308 | 8.752  | 5.7656 | 3.4023 |

$E_0 = \) Control, $E_1 = \) Moringa (6%), $E_2 = \) Neem (10%), $E_3 = \) Garlic (4%), $S_0 = 0, S_1 = 2 \) mM, $S_2 = 4 \) mM, $S_3 = 6 \) mM.

The maximum plant height 90.44 (cm) was recorded when the foliar spray of plant excrete (6% moringa) was applied. While the minimum plant height (68.61 cm) was noted in control. In case of Salicylic acid (6mM) the maximum plant height (88.38 cm) was recorded, while minimum plant height (70.06 cm) was noted from control.

In case of plant extracts, highest chlorophyll content (0.0511 mg cm$^{-2}$) was recorded when plants sprayed with 6% moringa leaf extract while lowest chlorophyll content (0.0406 mg cm$^{-2}$) of tomato leaves was recorded in control treatment.

Likewise, the maximum fruit weight (57.79 g) was observed with foliar spray of 6% moringa leaf extract while the minimum tomato fruit weight (43.40 g) was found in control treatment. In case of Salicylic acid, the maximum fruit weight (54.91 g) was recorded in plants treated with 6mM salicylic acid as a result of foliar spray, while least fruit weight (42.34 g) was recorded in control.

Table 2. Influence of different plant extracts and salicylic acid in physico-chemical component tomato.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant extracts (%)</th>
<th>Fruit firmness (kg cm$^{-2}$)</th>
<th>Fruit juice pH</th>
<th>%Titratable Acidity</th>
<th>Vit.C (mg 100$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_0$</td>
<td>2.04c</td>
<td>5.49a</td>
<td>0.25c</td>
<td>6.83c</td>
<td></td>
</tr>
<tr>
<td>$E_1$</td>
<td>3.63a</td>
<td>4.06c</td>
<td>0.40a</td>
<td>11.76a</td>
<td></td>
</tr>
<tr>
<td>$E_2$</td>
<td>2.88b</td>
<td>4.72bc</td>
<td>0.30b</td>
<td>9.59b</td>
<td></td>
</tr>
<tr>
<td>$E_3$</td>
<td>2.41bc</td>
<td>5.26ab</td>
<td>0.28bc</td>
<td>7.03c</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>0.5233</td>
<td>0.6778</td>
<td>0.0458</td>
<td>1.1331</td>
<td></td>
</tr>
</tbody>
</table>

Salicylic acid (mM)

| $S_0 = 0$ | 2.34c | 5.59a | 0.24e | 6.43c |
| $S_1 = 2$ | 2.51bc| 4.94ab| 0.29bc| 8.57b |
| $S_2 = 4$ | 2.83ab| 4.81bc| 0.33ab| 9.34ab|
| $S_3 = 6$ | 3.28a | 4.19c | 0.37a | 10.87a|
| LSD       | 0.4645| 0.6472| 0.0699| 2.0943|

$E_0 = \) Control, $E_1 = \) Moringa (6%), $E_2 = \) Neem (10%), $E_3 = \) Garlic (4%), $S_0 = 0, S_1 = 2 mM, S_2 = 4 mM, S_3 = 6 mM.
Similarly, moringa leaf extracts also effected the fruit volume. The maximum fruit volume (55.68 cm$^3$) was recorded for the plants sprayed with 6% moringa leaf extract, while the minimum fruit volume (43.67 cm$^3$) was recorded in control plants. In case of Salicylic acid, highest fruit volume (51.91 cm$^3$) was noted in plant treated with 6mM salicylic acid as a foliar spray, while minimum fruit volume (44.50 cm$^3$) was recorded in control.

The foliar application of different plant extracts and salicylic acid concentrations significantly affected fruit quality in Table 2. In case of moringa leaf extracts spray, the maximum fruit firmness (3.63 kg cm$^{-2}$) was recorded for the plants sprayed with 6% moringa leaf extract, while minimum fruit firmness (2.04 kg cm$^{-2}$) was recorded in control plants. Whereas, Salicylic acid showed maximum fruit firmness (3.28 kg cm$^{-2}$) in treated plants with 6mM salicylic acid while minimum fruit firmness (2.34 kg cm$^{-2}$) was recorded in control. The minimum fruit juice pH (4.06) was recorded for the plants sprayed with 6% moringa leaf extract, while the maximum fruit juice pH (5.49) was recorded in control plants. In case of Salicylic acid, minimum fruit juice pH (4.19) was recorded in plants treated with 6mM salicylic acid while maximum (5.59) was recorded in control.

In case of moringa leaf extracts, the maximum titratable acidity (0.40 %) was recorded for the plants sprayed with 6% moringa leaf extract, while the minimum titratable acidity (0.25 %) was recorded in control plants. Whereas, Salicylic acid showed maximum titratable acidity (0.37 %) was recorded in plants treated with 6mM salicylic acid while minimum titratable acidity (0.24 %) was recorded in control.

Similarly, the maximum ascorbic acid content (11.76 mg 100g$^{-1}$) was recorded for the plants sprayed with 6% moringa leaf extract, while the minimum ascorbic acid content (6.83 mg 100g$^{-1}$) was recorded in control plants. While Salicylic acid, the maximum ascorbic acid (10.87 mg 100g$^{-1}$) content was recorded in plants treated with 6mM salicylic acid while minimum ascorbic acid (6.43 mg 100g$^{-1}$) content was recorded in control.

**Discussion**

**Chlorophyll content**

This increased in chlorophyll content might be due to the fact that aqueous solution of moringa leaf extract is a rich source of magnesium which is the central atom in the structure of chlorophyll (Khan et al., 2008). Rehman and Basra (2010) reported that application of moringa leaf extract prevent the premature leaf senescence and also promote the leaf area with more chlorophyll. Gunes et al. (2007) and Yildirim et al. (2006) reported that chlorophyll content enhanced through the application of salicylic acid as a foliar spray in maize and cucumber respectively.

**Plant height**

The analysis of variance indicated that plant height of tomato plant was significantly affected by different plant extracts and salicylic acid concentrations. The increment in plant height might be due to the presence of macro and micro nutrients in moringa leaf extract that positively increased vegetative and reproductive growth of plant. Similar results were also observed by Kato et al., (2002).

Salicylic acid is required for auxin synthesis which is synthesized in the meristematic tissue of the plant that is responsible for vegetative growth, hence maximum plant height was due to the application of salicylic acid and its effect on auxin.

**Fruit weight (g)**

Yasmeen (2011) found that spraying wheat, peas and tomato with Moringa leaf extract at 3.5% increased fruit weight. Similarly Hafez and El-Metwally (2007) reported that maximum fruit weight of kinnow was found by foliar application of moringa leaf extract. Yildirim et al. (2006) stated that SA enhances the carbohydrate production and speed up the breakdown of synthesized carbohydrate in both source and sink tissue of the treated plant which
results in maximum fruit weight. Salicylic acid increases the membrane permeability which enhances the uptake of mineral nutrients which promote the growth and yield as well as fruit weight (Javaheri et al., 2012).

Fruit volume (cm³)
The increased in fruit volume might be due to the high content of cytokinin in moringa leaf extract which plays good role in enhancing cell division and expansion which ultimately leads to fruit with greater volume (Sheren et al., 2015). So, the increase in fruit volume in case of salicylic acid might be due to an increase in photosynthetic activity and the supply of more assimilates to the developing fruits which results in increased fruit volume. Similar results are reported by Hubbard et al. (1989) and Marcelis (1993) who stated that the fruit cell size increases with the higher level of assimilate and steady supply of carbohydrate which leads to increased fruit diameter.

Fruit firmness (kg cm⁻²)
This increased in the current findings of fruit firmness might be due to the high calcium content present in the aqueous solution of moringa leaf extract (Mishra et al., 2013). Since calcium play a leading role in the structure of cell wall contributing in the firmness of fruit (Burns and Pressey, 1987). Maximum firmness at higher concentration salicylic acid foliar spray intends to increase the shelf life of peach early grand variety with 1.5 mM (Tareen et al., 2012). Application of repeated foliar spray of salicylic acid showed their indirect role through firmer fruit in prolonging the shelf life of the peach cultivar.

Percent Titratable Acidity
The increased in the total acidity of tomato fruit juice might be due to the fact that moringa leaf extract is a good source of certain acids like ascorbic acid, which is directly involved in the rising of total acidity at final harvest, moreover certain nutrients such as potassium also directly involved in the acidity of fruit juice. Higher the potassium, higher will be the juice acidity and lower the potassium, lower will be the juice acidity (Zekri and Obreza, 2009). Foliar application of salicylic acid increased the titratable acidity with the passage of time that acidity decrease due to conversion of acids into sugar as a result of respiration (Han et al., 1997).

Ascorbic acid content (mg 100g⁻¹)
The increased in the amount of ascorbic acid in tomato fruit might be due to the high amount of ascorbate in moringa leaf extract. The exogenous application of ascorbate in the solution form of moringa leaf extract trigger the production of internal ascorbic acid, which leads to the high quantity of ascorbic acid in the fruits of treated plants (Mengel, 1997). Nasira et al. (2016) also found that foliar application of moringa leaf aqueous extract increased vitamin C of ‘Kinnow’ mandarin. Javaheri et al. (2012) reported maximum vitamin C in tomato crop treated with salicylic acid foliar spray as compared to control plants. Cara novel orange was found with maximum ascorbic acid content when treated with salicylic acid (Reuhua et al., 2008).

Fruit juice pH
The decreased in the juice pH of tomato fruit might be due to the high content of potassium. Ashraf et al. (2016), who reported that foliar application of moringa leaf extract on ‘Kinnow’ mandarine decreased the acidity of fruit juice. Hafeznia et al. (2014) reported that the combine effect of salicylic acid and methyl jasmonate (0.5+ 0.5 mM) significantly decrease the fruit juice pH in tomato crop. Yildirim (2007) reported that foliar application of these substances decreased the pH in various crop species.

Conclusion
The results showed that the application of Moringa leaf extract (6 %) with salicylic acid concentrations significantly effected all the growth, yield and quality attributes. The Moringa leaf extract (6 %) enhanced chlorophyll content, plant height, fruit weight, fruit volume, fruit firmness, Titratable acidity and ascorbic acid content. Application of salicylic acid at 6mM improved the growth, yield and quality attributes of
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