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Effect of Foliar Application of Vasicine on Gas Exchange Parameters, Proline Content and SOD Activity in Tomato (*Solanum lycopersicum* L.)

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ABSTRACT: Vasicine is a new biostimulant extracted from *Adhatoda* plant has influence many physiological and biochemical processes in plant. Because of highly consumable vegetable, increased production of tomato without usage any chemical compounds is a big challenge in agriculture. It may be possible only by using plant extracts called as biostimulants evidenced with physiological approaches. The present study was conducted to assess the impact of vasicine on gas exchange parameters, proline content, SOD activity and fruit yield in tomato under pot culture condition. The vasicine treatments include soil application (4 kg acre^{-1}), foliar application (2 mL litre^{-1}), combined application (soil + foliar) and control. The highest photosynthetic rate of 34.24 µmol m^{-2} s^{-1} and transpiration rate of 19.18 mmol m^{-2} s^{-1} were registered by combined application of vasicine. Foliar application of vasicine showed least leaf temperature of 33.05ºC and control recorded highest (35.10ºC). Combined application recorded highest proline content (39.75 mg g^{-1}) and foliar application registered highest superoxide dismutase (SOD) activity (311.58 units mg protein^{-1}) and lowest was recorded by unsprayed control. Combined application of vasicine recorded highest fruit yield of 26.23 t ha^{-1} which is on par with foliar spray of vasicine (25.68 t ha^{-1}).

Keywords: Vasicine, tomato, photosynthetic rate, leaf temperature, proline, SOD, fruit yield

INTRODUCTION

Tomato (*Solanum lycopersicum* L) is an important and most consumed vegetable worldwide. India is the second largest producer of tomato accounting 11.10% of the world's production. The popularity of tomato is rising among the consumers because of its vitamins, minerals and good anti-oxidants (Erica *et al.,* 2010). Bhowmik *et al.* (2012) reported that the tomato can make people healthier and decrease the risk of cancer, osteoporosis and cardiovascular disease. Around 12% of the world population suffer by hunger and live without quality and nutritional food (Magdoff and Tokar, 2009). To achieve nutritional security of people, quantity and quality of the produce like tomato may be increased. There are number of previous studies are reported to increase the crop production by using chemicals, nutrients and plant growth regulators. Foliar application of plant growth regulators (Salicylic acid and gibberellic acid) and nutrient (Boric acid) increased the plant height, tiller numbers and grain yield with higher BC ratio in crops (Ashwini *et al.,* 2021). However, there was no report available for the yield increment by using biostimulants in tomato.

Since, the tomato is an unavoidable vegetable in the daily food, there is an essential requirement to increase the production and quality of tomato by using any biostimulants. Biostimulants are plant derived additive products used in crop production to enhance productivity and quality of the produce. They also enhanced the activity of soil microbes, growth hormones and photosynthetic enzynes in plants (Giannattasio *et al.*, 2013). Santos *et al.* (2019) reported that a biostimulant consisted auxin (IBA), cytokinin (Kinetin) and gibberellic acid which are major growth regulators responsible for plant growth, yield and quality. They promote root growth, water and nutrients uptake and helping the restoration of plant hormonal balance. Generally, most of the biostimulants act as plant growth regulators.

Natural products are the most important source for the discovery of new and potential biostimulants. *Adhatoda vasica* (L.) is known as Malabar nut shrub growing throughout India. An active principle compound present in the quinazoline alkaloids is vasicine which extracted from the leaves of *Adhatoda*. Claeson *et al.* (2000) reported that the *Adhatoda* plant contains alkaloids such as vasicine, vasicinone, vasicol, adhatodinine and vasicinol. Vasicine regulates vitamin metabolism in plant through glucuronidation pathway was reported by Dan *et al.* (2019). Vasicine obtained from *Adhatoda* *vasica* leaves has antimicrobial and antioxidant property (Duraipandiyan *et al.,* 2015).

Santos *et al.* (2019) registered that the exogenous application of biostimulant recorded higher net photosynthetic rate, transpiration rate and stomatal conductance compared to control. The use of biostimulants is an innovative solution to address the novel challenge to improve the sustainability of agricultural systems and reduce the use of chemical fertilizers (Di Stasio *et al.,* 2018). Sivakumar and Jeyakumar (2020) reported that the vasicine is a biostimulant showed some positive action on crop growth and yield.

Hence, the present study is undertaken to investigate the impact of a new biostimulant - vasicine on gas exchange parameters, proline and SOD activity with fruit yield. The study on impact of vasicine on gas exchange parameters of plant is meager, since it is the first study undertaken.

MATERIAL AND METHODS

The experiment was carried out in the pot culture under glass house condition at Tamil Nadu Agricultural University, Coimbatore during 2017-2019. Red sandy soil was used for pot culture experiment. Soil mixture was prepared by using red soil, vermicompost and sand in the ratio of 3:2:1. Transplanting was carried out with 25^{th} days old seedlings in pots.

Basal application was done before transplanting as per the treatment schedule. Irrigation was given once in five days and plant protection measures were carried out as per the recommendations of TNAU Crop Production Guide. The experiment was laid out in completely randomized block design with three replications.

The treatments like (T1 - Control) recommended fertilizer (RF) (150:100:50 kg of N, P and K ha^{-1}) and foliar spray of triacontanol (1 ppm) at 15 days after transplanting (DAT), (T2) RF + soil application of vasicine @ 4 kg $acre^{-1}$ (50% as basal & 50% at 15 DAT), (T3) RF + foliar application of vasicine @ 2 ml $litre^{-1}$ at 15 and 30 DAT, (T4) RF + soil application of vasicine @ 4 kg $acre^{-1}$ (50% as basal & 50% at 15 DAT) + foliar application of vasicine @ 2 mL $litre^{-1}$ at 15 and 30 DAT were used for this experiment. Vasicine was obtained in the name of VIGO from Bio products Division, Research and Development Centre, Coromandel International Limited, Tamil Nadu, India.

Measurement of gas exchange parameters was performed by using Portable Photosynthesis System (PPS) (Model LC*pro*-SD., ADC BioScientific Ltd., Hoddesdon, UK) equipped with a halogen lamp (6400-02B LED) positioned on the cuvette. Third leaf from top was used for the measurements with replicated thrice. Leaf was inserted in 3 cm^2 leaf chamber and PPFD at 1500 μmol photons m^{-2} s^{-1} and relative humidity of 50-55% were set. The readings were taken between 9 am to 11.30 am and the value is expressed as respective units. The gas exchange parameters *viz.,* photosynthetic rate (μmol m^{-2} s^{-1}), transpiration rate (mmol m^{-2} s^{-1}), stomatal conductance (cm s^{-1}) and leaf temperature (ºC) were measured.

The proline content was estimated by using the protocol of Bates *et al.* (1973) and the amount of proline in the sample is expressed as mg g^{-1}. 0.5 g of leaf sample was homogenized with 10 mL of 3% sulphosalicylic acid and centrifuged at 3000 rpm for 10 minutes.

Two mL of the supernatant was taken and 2 mL of glacial acetic acid, 2 mL of ortho phosphoric acid and 2 mL of acid ninhydrin mixture were added. The contents were allowed to react at 100ºC for 1 hour under water bath and then incubated on ice for 10 minutes to terminate the reaction. The reaction mixture was mixed vigorously with 4 mL toluene for 15 to 20 seconds. The chromophore containing toluene was aspired from the aqueous phase and the optical density was read at 520 nm. The proline content in the sample was calculated by using pure proline as standard.

The superoxide dismutase (SOD) activity was estimated by following the protocol given by Beau-Champ and Fridovich (1971) with the reaction mixture consisting 50 mM HEPES-KOH, 0.1 mM EDTA, 50 mM Na_2CO_3, 12 mM methionine, NBT 75 M and 1 M riboflavin. 500 mg of sample was macerated with 10 mL HEPES-KOH buffer and centrifuged at 15000 ppm for 15 min at 4°C. 1 mL of the enzyme extract was taken and mixed with 3 mL of reaction mixture. One unit SOD activity was defined as the amount of enzyme required to result a 50 per cent inhibition of the rate of NBT reduction at 560 nm. The result is expressed as enzyme units mg $protein^{-1}$ g^{-1}.

The total weight of fruits harvested from each plant from all picking was added, and yield per plant was worked out, and estimated fruit yield was obtained and expressed as t ha^{-1}. The data on various parameters were analyzed statistically as per the procedure suggested by Gomez and Gomez (1984). Wherever the treatment differences were found significant, critical differences were worked out at five per cent probability level and the values were furnished.

RESULTS AND DISCUSSION

The role of tomato fruits in health and disease risk reduction extends beyond antioxidant function to include other protective mechanisms such as antithrombotic and anti-inflammatory functions (Freeman and Reimers, 2011). Hence, yield enhancement in tomato by using non-chemical bioproducts is essentially required. The results gathered in the present study showed that the application of biostimulant - vasicine increased the gas exchange parameters, proline content, SOD activity and fruit yield in tomato.

The data on gas exchange parameters showed significant differences among the treatments at flowering and fruiting stages. However, photosynthetic and transpiration rate showed significant difference between all the treatments at fruiting stage compared to flowering stage (Table 1). Among the treatments, higher photosynthetic rate of 34.24 µmol m^{-2} s^{-1} was registered in combined application which is on par with foliar application (32.62 µmol m^{-2} s^{-1}) at flowering stage. Compare to soil application (27.15), foliar application alone registered highest photosynthetic rate of 30.55 µmol m^{-2} s^{-1} and the lowest photosynthetic rate of 23.41 µmol m^{-2} s^{-1} was observed in unsprayed control during fruiting stage. Combined application of vasicine enhanced the photosynthetic rate in tomato up to 31.3% and 41.1% during flowering and fruiting stages respectively compared to control. The increment of photosynthetic rate by the application of vasicine might be due to its involvement action in stomatal movement through hormonal metabolism. Santos *et al.* (2019) reported that the biostimulant composed kinetin which restores plant hormonal balance. The cytokinin hormone involved in the stomatal opening which enhances the photosynthetic rate in crop plants. Cytokinin and auxin-induced opening of stomata involves a decrease of H_2O_2 levels of guard cells in *Vicia faba* (Song *et al.*, 2006). Application of bio-stimulant increased the intercellular CO_2 concentration and net photosynthesis in maize (Anjum *et al.*, 2011). The present study is agreed with earlier findings.

The transpiration rate showed similar trend of photosynthetic rate. Exogenous application of vasicine increased the transpiration rate compared to unsprayed control in tomato (Table 1). Among the two stages, the highest transpiration rate was observed in flowering stage compared to fruiting stage. The highest transpiration rate of 19.18 mmol m^{-2} s^{-1} was recorded in combined application which is on par with foliar application of vasicine (18.57 mmol m^{-2} s^{-1}) followed by soil application (16.98 mmol m^{-2} s^{-1}) and lowest by unsprayed control (15.62 mmol m^{-2} s^{-1}) at flowering stage. However, all the treatments showed significant difference between each other at fruiting stage. Maintenance of transpiration rate is unavoidable phenomena for the continuation of photosynthesis in crop plants.

Under water is not a limiting factor, the enhancement of transpiration rate is totally advantage for the yield increment. In the present study, transpiration rate is increased by the application of vasicine in tomato up to 22.8% and 33.6% at flowering and fruiting stages respectively compared to control. Foliar application is more advantage than soil application might be due to the direct contact of leaf lamina. The increment of transpiration rate by vasicine might be due to involvement in the stomatal opening. The vasicine might contain cytokinin like compounds which promotes stomatal opening. Kaluzewicz *et al.* (2017) found that the application of biostimulants increased the stomatal conductance and transpiration in broccoli.

Stomatal conductance exhibited significant differences between unsprayed control, soil application and other treatments. However, there was no significant difference was observed between foliar application and combined application (Soil + Foliar) of vasicine (Table 1). Combined application of vasicine recorded the highest stomatal conductance of 1.23 and 1.20 cm s^{-1} are on par with foliar application (1.19 and 1.11 cm s^{-1}) followed by soil application of vasicine (1.03 and 0.99 cm s^{-1}) at flowering and fruiting stages respectively. Compared to control, an increment of 33.7% was observed in stomatal conductance by the application of vasicine at flowering stage. Stomatal conductance represents the conductive nature of the stomata for CO_2 and water vapour under given environment condition. Stomatal conductance is the main parameter to control net photosynthesis of plant and these two traits are with linear and exponential relations between them. Less stomatal conductance decreases CO_2 supply to the mesophyll cells resulting in lower net photosynthetic rate (Laxman *et al.*, 2016). In the present study, combined and foliar application of vasicine increased

Table 1: Impact of vasicine on gas exchange parameters in tomato.

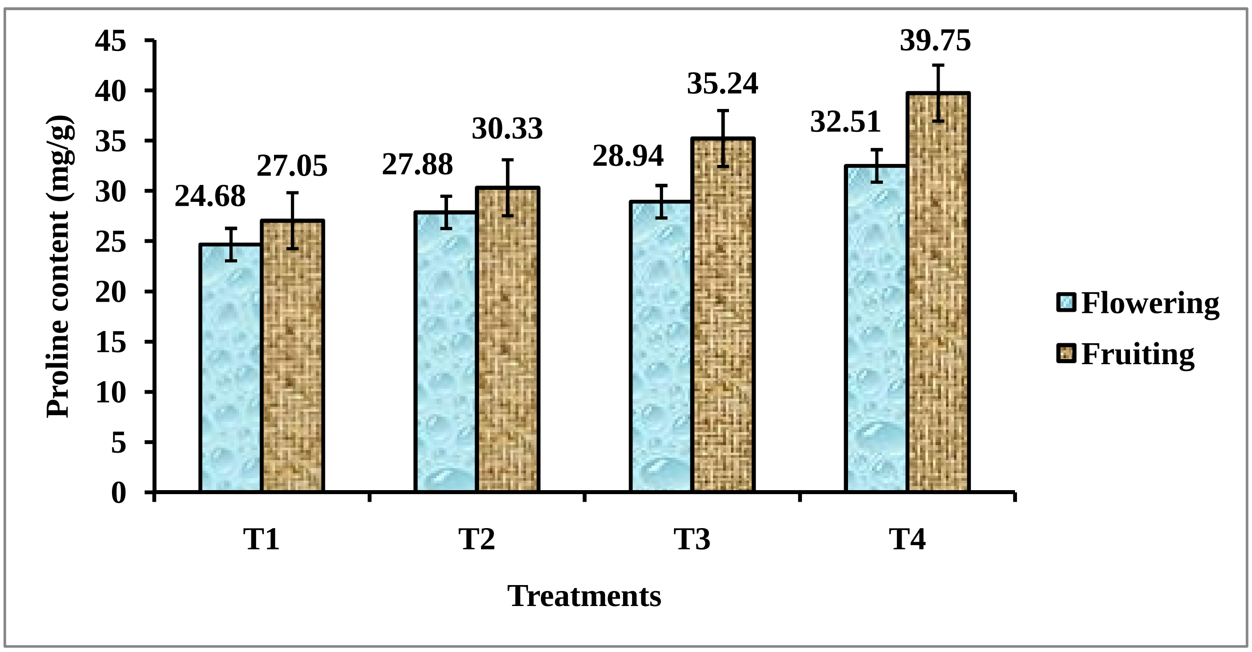
Treatments	Photosynthetic rate (µmol m^{-2} s^{-1})		Stomatal conductance (cm s^{-1})		Transpiration rate (mmol m^{-2} s^{-1})		Leaf temperature (°C)	
	Flowering	Fruiting	Flowering	Fruiting	Flowering	Fruiting	Flowering	Fruiting
T_1: Control	26.07 **C**	23.41 **D**	0.92 **C**	0.87 **C**	15.62 **C**	14.24 **D**	34.82 **C**	35.10 **C**
T_2: Vasicine - Soil	29.82 **B**	27.15 **C**	1.03 **B**	0.99 **B**	16.98 **B**	16.10 **C**	33.83 **B**	33.97 **B**
T_3: Vasicine - Foliar	32.62 **A**	30.55 **B**	1.19 **A**	1.11 **A**	18.57 **A**	17.38 **B**	33.05 **A**	33.25 **A**
T_4: Vasicine - Soil + Foliar	34.24 **A**	33.10 **A**	1.23 **A**	1.20 **A**	19.18 **A**	19.03 **A**	33.16 **A**	33.12 **A**
SEd	0.96	1.03	0.04	0.05	0.42	0.38	0.32	0.29
CD (P=0.05)	2.22	2.08	0.09	0.10	0.97	0.77	0.71	0.60

the stomatal conductance in tomato. However, soil application showed less impact on stomatal conductance compared to foliar.

The increased stomatal conductance by the application of vasicine might be due to the support of stomatal opening. The use of biostimulant provided an increase in stomatal conductance by 27% compared to control was observed in pepper. The results may be related to the composition of the biostimulant, such as phytohormones and organic substances that stimulate cell elongation and hormonal balance (Melo *et al.*, 2020). Mudo *et al.* (2020) reported that the foliar spray of biostimulant showed positive result with increased the stomatal conductance in mango. These findings are corroborated with the present study.

Similar trend was observed in leaf temperature as in the case of stomatal conductance. The data indicated that the exogenous application of vasicine decreased the leaf temperature compared to control (Table 1). Combined application of vasicine and foliar application recorded the lowest leaf temperature compared to other two treatments. Among the treatments, the lowest leaf temperature of 33.12ºC was recorded by combined application which is on par value with foliar application (33.25ºC) at fruiting stage. Unsprayed control showed higher leaf temperature of 35.1ºC followed by soil application (33.97ºC). Application of vasicine decreased the leaf temperature in tomato up to 1.98ºC compared to unsprayed control. However the decrement is higher in fruiting stage compared to flowering stage (1.66ºC). Leaf temperature depends on external weather conditions and it decides the enzymatic reaction of plant metabolism. Leaf temperature is negative correlation with the stomatal conductance. Higher stomatal conductance increases the loss of water through transpiration leading to lesser leaf temperature which helps the plants to tolerate the excessive heat load. Exogenous application of vasicine decreased the leaf temperature from 35.1ºC to 33.12ºC. The decreased leaf temperature by vasicine treatment might be due to the enhancement of stomatal conductance and transpiration rate.

Shukla *et al.* (2018) reported that the biostimulant extracted from seaweed treatment significantly increased the stomatal conductance of soybean plants up to 46% compared to control plants. Seaweed extract reducing the leaf temperature with improved stomatal conductance in soybean was reported by Martynenko *et al.* (2016). The data showed clear increment of proline content by the application of vasicine in tomato (Fig. 1). The plant had higher proline content at fruiting stage (39.75 mg g^{-1}) than flowering stage (32.51 mg g^{-1}). Among the treatments, combined application of vasicine registered highest proline content followed by foliar application (35.24 mg g^{-1}), soil application (30.33 mg g^{-1}) and the lowest was recorded in unsprayed control (27.05 mg g^{-1}). Vasicine showed a positive influence on proline content in tomato with an increment of 31.7% compared to control at flowering stage. The effect of vasicine on proline content is on par values by soil and foliar application at flowering stage and significantly differed at fruiting stage (Fig. 1).



Treatments: T_1 = Control, T_2 = Vasicine (Soil), T_3 = Vasicine (Foliar), T_4 = Vasicine (Soil + Foliar).

Fig. 1. Impact of vasicine on proline content in tomato.

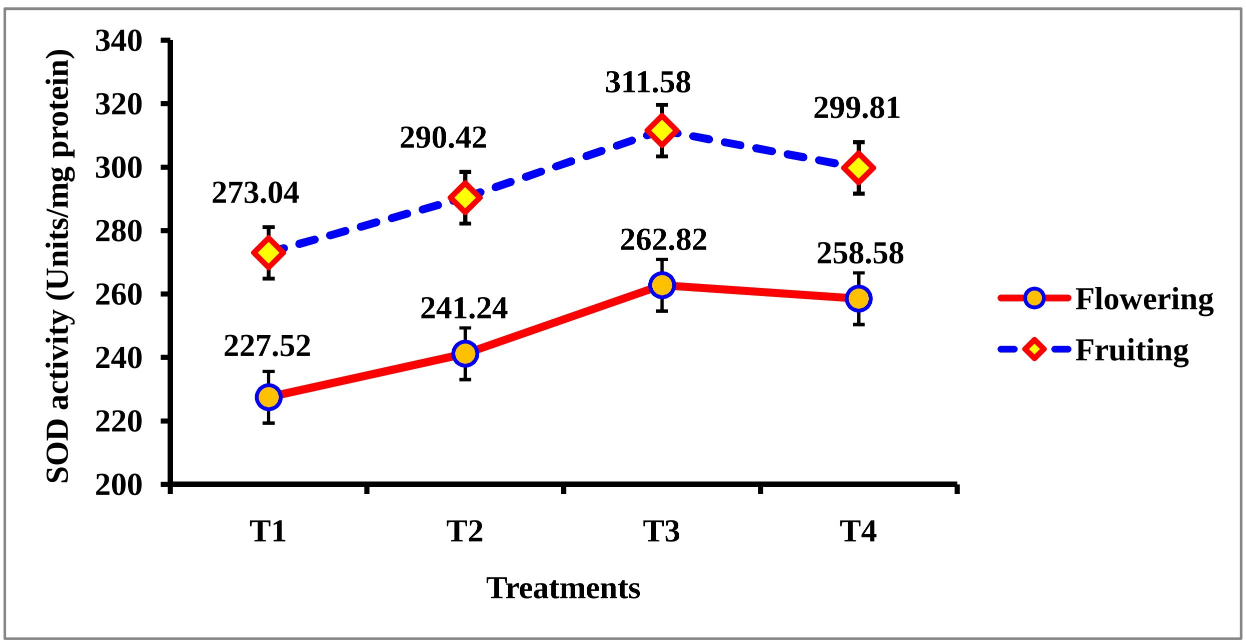
Proline is a multifunctional amino acid which protects the plant from any adverse conditions. Even in normal conditions, proline used for protein synthesis and a component of metabolic signaling network controlling mitochondrial functions. Hence, increased proline content under normal conditions enhances protein synthesis and decides the productivity of crop plants.

In the present study, application of vasicine increased the proline content in tomato might be due to its association with increased expression of proline synthesis genes. The increment is higher in fruiting stage than flowering stage might be due to the continuous action of vasicine in plant. Application of vasicine with soil and foliar increased protein content might be due to its positive action on denovo synthesis of amino acids was reported by Sivakumar and Jeyakumar (2020). Foliar application of biostimulant derived from algae extract increased the proline content in *Solanum* sp. (Leguizamon *et al.*, 2016).

Elansary *et al.* (2016) reported that the treatment with seaweed extract increased the proline content in *Spiraea* and *Pittosporum*. Many scientists reported that the application of biostimulant and plant extracts increased the proline content in crop plants (Van Oosten *et al.*, 2017). Ali *et al.* (2021) reported that seaweed extracts are highly organic and ideally suitable for organic farming and environmentally sensitive crop production. Application of seaweed extract increased the proline content by the way it gives varies stress tolerance to the crop plants. The present study agreed with the earlier investigations.

SOD activity exhibited different pattern of enhancement by the application of vasicine (Fig. 2). The treatments attained statistical significance at all the two stages studied. Regarding treatments, foliar applied plants showed higher enzyme activity of 262.82 and 311.58 at flowering and fruiting stages respectively than the plants imposed with combined application (258.58 and 299.81). Treatment with vasicine showed positive impact on increment of SOD activity in tomato compared to untreated one. The highest SOD activity of 311.58 enzyme units mg protein^{-1} was recorded by foliar application of vasicine followed by combined application (299.81), soil application (290.42) and lowest was observed in unsprayed control (273.04). Superoxide Dismutase is a key enzyme to nullify the effect of super oxide which is produced by Haber-Weiss reaction.

Sharma *et al.* (2012) opined that the primary anti-oxidative defense consists of several enzymatic systems, the most important being superoxide dismutase (SOD). Interestingly, foliar application of vasicine increased the SOD activity without any adverse conditions. In the present study, foliar applied plant had higher SOD activity than combined (soil + foliar) and the reason is unclear. The increased SOD activity by the application of vasicine might be due to its phenolic hydroxyl group, which is responsible for the enhancement of anti-oxidant activity. However, soil applied biostimulants increased the SOD activity in soybean was reported by Vasconcelos *et al.* (2009).



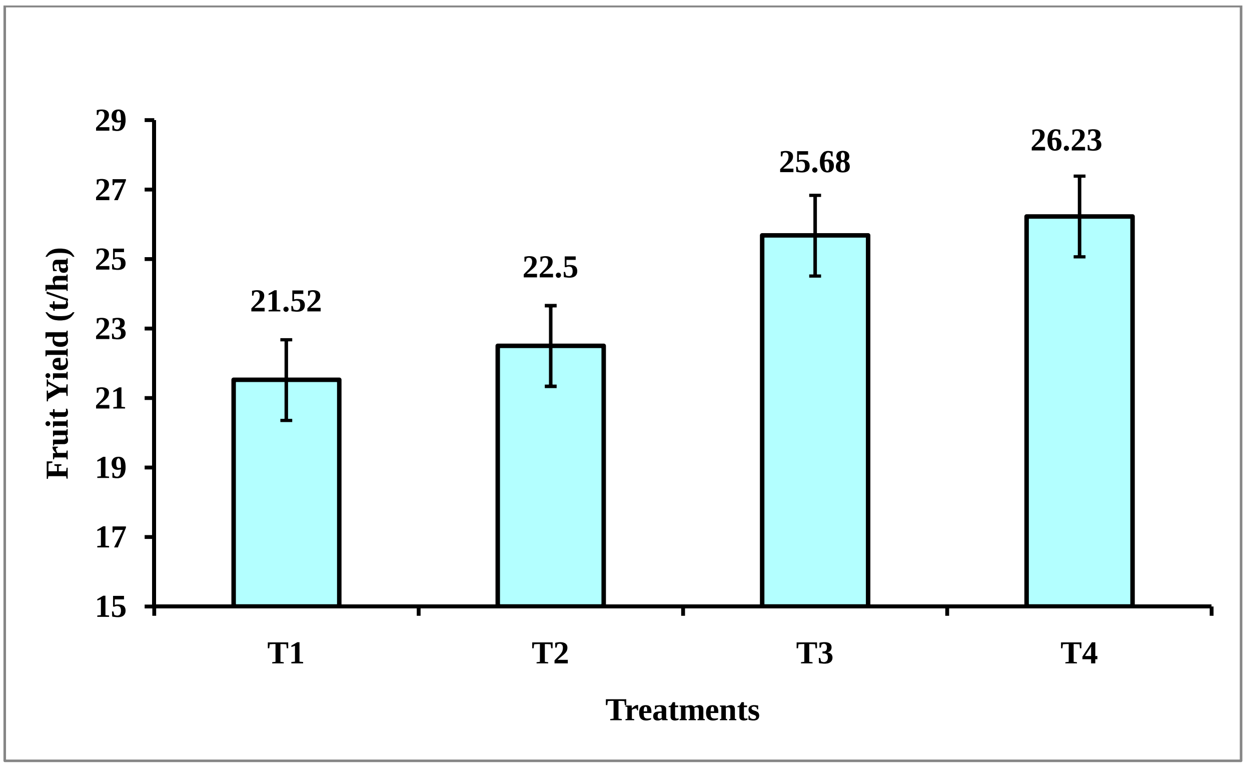
Treatments: T_1 = Control, T_2 = Vasicine (Soil), T_3 = Vasicine (Foliar), T_4 = Vasicine (Soil + Foliar).

Fig. 2. Impact of vasicine on SOD activity in tomato.

Francesca *et al.* (2020) reported that the foliar application of plant based biostimulant increased the antioxidants contents compared to non-treated plants both in leaves and fruits.

Application of vasicine increased the fruit yield was observed in tomato (Fig. 3). Combined application of vasicine recorded the highest fruit yield of 26.23 t ha^{-1} which is on par with foliar spray (25.68 t ha^{-1}) in tomato. Soil application of vasicine recorded 22.50 t ha^{-1} and lowest fruit yield of 21.52 t ha^{-1} u was registered in unsprayed control. Combined application of vasicine through soil and foliar increased tomato fruit yield up to 21.88% compared to control. As a challenge given prior in this research, a non-chemical plant derived biostimulant vasicine increased a distinguished yield in tomato. Combined application and foliar spray showed on par values compared to soil application might be due to foliar spray during flowering directly contributed to the yield rather than soil application contributed mainly to vegetative growth. The yield increment by the application of vasicine might be due to the enhancement of photosynthetic rate, proline content and SOD activity, and decreased leaf temperature which are direct contributors to the photosynthesis and yield. The popularity of biostimulants in agriculture/horticulture is associated with the prospect of achieving higher yields. Paradikovic *et al.* (2011) reported that the application of biostimulants could be considered as a good production strategy for obtaining higher yield in vegetables with lower impact on the environment. Foliar and soil application of seaweed extracts, Superzyme gold, Root master and Sea bomb increased the crop yield and yield attributes in cow pea (Leelavathi *et al.*, 2021).

Vasicine is a new biostimulant which showed positive impact on growth and yield was reported by Sivakumar and Jeyakumar (2020) in tomato. They also registered that the yield increment by the application of vasicine might be due to the enhancement of chlorophyll and protein. The present investigation agreed with the earlier studies.



Treatments: T_1 = Control, T_2 = Vasicine (Soil), T_3 = Vasicine (Foliar), T_4 = Vasicine (Soil + Foliar).

Fig. 3. Impact of vasicine on fruit yield in tomato.

CONCLUSIONS

The present study concluded that the combined application of vasicine in the form of vigo through soil (4 kg $acre^{-1}$) and foliar spray (2 mL L^{-1}) along with a recommended dose of fertilizers enhanced the photosynthetic rate, transpiration rate, proline content, SOD activity and decreased leaf temperature with increased fruit yield compared to control in tomato. In comparison, foliar application of vasicine @ 2 mL L^{-1} twice at 15 and 30 days after transplanting is on par vales with the combined application. Hence, combined/foliar application of vasicine may be a viable and effective technology to increase fruit yield in tomato with environment safe.

FUTURE SCOPE

The biostimulant - Vasicine present in the leaf extract of *Adhatoda* plant can be assessed for almost all vegetable crops to increase the production without affecting the quality of the produce. There is big scope to use vasicine in agricultural crops and also assess the quality of the produce to ensure the non-chemical nature of the produce. This research may be given ways for the extraction of many biostimulants from various plants and assess the utility in agriculture and horticultural crops.

Conflict of Interest. The author declares that there is no conflict of interest about the manuscript

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