



## Response of okra (*Abelmoschus esculentus* L.) to soil and foliar applied L-tryptophan

Ayesha Mustafa<sup>1,3</sup>, Azhar Hussain<sup>2</sup>, Muhammad Naveed<sup>1</sup>, Allah Ditta<sup>4</sup>, Zill-e-Huma Nazli<sup>5</sup> and Annum Sattar<sup>1</sup>

<sup>1</sup>Institute of Soil & Environmental Sciences, University of Agriculture, 38040-Faisalabad-Pakistan

<sup>2</sup>Department of Soil Science, University College of Agri. & Environmental Sciences, The Islamia University of Bahawalpur

<sup>3</sup>Nuclear Institute of Agriculture and Biology (NIAB), 38000-Faisalabad-Pakistan

<sup>4</sup>Department of Environmental Sciences, PMAS Arid Agriculture University, Rawalpindi-Pakistan

<sup>5</sup>Govt. College for Women University, Faisalabad

### Abstract

*L-tryptophan, an auxin precursor, is crucial for the regulation and coordination of many metabolic and physiological processes of plants. It was hypothesized that exogenous application of L-tryptophan may help in reducing time to onset of flowering and floral bud drop. This pot experiment was conducted to evaluate the effect of soil and foliar applied L-tryptophan on the phenology, growth and gas exchange and yield of okra. L-tryptophan was delivered as soil application (0, 20, 40 and 80 mg kg<sup>-1</sup>) or foliar spray (0, 5, 10 and 20 mg L<sup>-1</sup>) each at seedling emergence, flower initiation and fruiting. Both soil and foliar applied L-tryptophan significantly improved all growth, physiological and yield parameters of okra. Soil and foliar application of L-tryptophan at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively were the most effective. Foliar application of L-tryptophan at 10 mg L<sup>-1</sup> increased the plant height, internodal distance and fruit yield per treatment up to 49, 79 and 96%, respectively, while its soil application at 40 mg kg<sup>-1</sup> increased these parameters by 58, 94 and 47%, respectively over control. Soil and foliar application at these levels also reduced time to onset of flowering (15 and 14%) and floral bud drop (92 and 85%). Both soil and foliar applied L-tryptophan improved the physiological parameters, chlorophyll content and photosynthetic rate. These findings may imply that both soil and foliar application of L-tryptophan (40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively) could be used to reduce early floral bud drop and to increase the growth and yield of okra.*

**Key words:** L-tryptophan, phytohormones, bud drop, early flowering, okra

### Introduction

Okra (*Abelmoschus esculentus* (L.) Moench), being one of the world's oldest vegetable crops, is cultivated around the Inter-Tropical and Mediterranean regions for its young fruits. It is rich in minerals such as Ca and vitamins. In Pakistan, okra is vernacularly known as "Bhindi" and is an important vegetable, grown from mid February to mid May. The total area under okra cultivation in Pakistan is about  $13.9 \times 10^3$  hectares with production of  $1.03 \times 10^5$  tons of green pods (Fruit, vegetable and condiments statistics of Pakistan 2011-12). Okra yield is low due to different reasons which include abscission of floral buds, flowers and fruits. It is a major yield limiting factor in agricultural crops (Marcelis *et al.*, 2004). To overcome this problem and to get high production, surplus of young fruits and flowers might be removed or chemical amendment like plant growth regulators might be used (Estronell *et al.*, 2013).

Plant growth regulators, small signaling molecules, are effective at very low concentration, biologically active and play very important role in controlling physiological processes of plant (Frankenberger and Arshad, 1995;

Ahmad *et al.*, 2007). To improve crop yield, use of plant growth regulators is becoming very common (Zahir *et al.*, 2000a) due to their positive impact on plant growth and development by regulating and balancing the endogenous hormone levels which affect physiological processes of plant body and ultimately the plant yield (Dawood and Sadak, 2008).

Exogenous application of hormones and their precursors helps to improve the plant growth and yield by altering the level of endogenous hormones (Frankenberger and Arshad, 1995; Zahir *et al.*, 2000a). Soil micro-biota is another potential source of phytohormones, however, exogenous application of their precursors has been found to increase microbial production of phytohormones (Zahir *et al.*, 2000b). Auxin is considered to be an important plant growth regulator (Etesami *et al.*, 2009; Anjum *et al.*, 2011) as it plays a vital role in cell division and cell elongation, formation of adventitious roots, (Ahmad *et al.*, 2008; Etesami *et al.*, 2009) and apical dominance (Abdoli *et al.*, 2013). Under normal growth conditions, auxins are produced in the reproductive organs and inhibit the

\*Email: ayesha.2099@gmail.com

formation of abscission zones and may prevent abscission in plants (Wien and Zhang, 1991; El-Saeid *et al.*, 2010).

L-tryptophan is biologically active precursor of auxin (Ahmad *et al.*, 1999; Akhtar *et al.*, 2007; Hussain *et al.*, 2011; Abbas *et al.*, 2013). Exogenous application of auxin could be helpful in triggering the transition of flowers to growing fruits because it has link with ovule development (Pandolfini *et al.*, 2007; Mignolli *et al.*, 2012). As compared to pure auxin, L-tryptophan has better effect on the seed germination, nutrient uptake, plant growth and yield (Rao *et al.*, 2012; Abbas *et al.*, 2013). It affects plant growth and yield either after its conversion to auxin metabolites through the rhizosphere microflora or after its direct uptake by the plants and then its conversion into auxin within plant tissues (Frankenberger and Arshad, 1995; Ahmad *et al.*, 2007, 2008; Abbas *et al.*, 2013). Its application has positively affected the growth and yield of radish (Frankenberger *et al.*, 1990), potato and tomato (Zahir *et al.*, 1997) and watermelon and muskmelon (Frankenberger and Arshad, 1991). For application of phytohormones and their precursors to plants, different application methods have been used. It has been found by many researchers that application of L-tryptophan both through soil and foliar showed positive effects on the growth of different agricultural crops (Zahir *et al.*, 2000a; Tallat *et al.*, 2005; Dahab and El-Aziz, 2006; Nahed *et al.*, 2009; Abbas *et al.*, 2013). Dipping of rice seedlings in L-tryptophan solution also positively affected the rice growth and yield (Zahir *et al.*, 1999).

As there is meager information regarding the optimum level of L-tryptophan application and also on the method of application, therefore, a pot experiment was conducted to find out the optimum levels of L-tryptophan applied through soil and foliar on the growth and yield of okra and to find out the best method of application soil or foliar.

## Materials and Methods

### Plant material and growth conditions

A pot experiment was conducted to evaluate the effect of soil and foliar applied L-tryptophan at four levels on the growth, fruit set and yield of okra (*Abelmoschus esculentus* L.) in wire house of Institute of Soil & Environmental Sciences (ISES), University of Agriculture, Faisalabad. Okra variety *Sabz Pari* was used as a test crop. Before the start of experiment, bulk surface soil sample (0-15 cm) was collected from the ISES Research Area, University of Agriculture, Faisalabad and pre soil analysis was performed. The soil was dried, ground and passed through 2 mm sieve before the analysis. The physico-chemical properties of soil used for filling of pots were, texture, loam; pH, 7.93; E<sub>Ce</sub>, 2.75 dS m<sup>-1</sup>; organic matter, 0.75%;

total nitrogen, 0.05% and available phosphorus, 9.3 mg kg<sup>-1</sup>. To each pot, 8 kg of soil was filled and pots were arranged according to the completely randomized design (CRD) with three replications. Six healthy seeds of okra (var. *Sabz Pari*) were sown per pot on February 20, 2014. After the plant establishment, only three plants were maintained in each pot by uprooting rest of them.

Fertilizers were applied at 50, 12.5 and 12.5 mg kg<sup>-1</sup> soil NPK, respectively, using urea, diammonium phosphate (DAP) and sulphate of potash (SOP), respectively, as sources. Half of nitrogen and whole of phosphorus and potash were applied as basal dose; remaining half of nitrogen was applied at flowering. Irrigation was applied as and when required.

### L-tryptophan treatment

Four levels of L-tryptophan were applied to soil (0, 20, 40 and 80 mg kg<sup>-1</sup>) and to the plants (0, 5, 10 and 20 mg L<sup>-1</sup>) at three different growth stages (seedling, flowering and fruiting) in triplicates. For foliar application, 0.1% Tween 20 was used as binding agent.

### Plant growth measurements

Crop was harvested after 90 days of sowing and the data regarding growth, physiological and yield parameters of okra were recorded during the growth period and after harvesting.

### Agronomic parameters

Plant height, internodal distance, root and fruit length were recorded using measuring tape. Number of leaves and branches plant<sup>-1</sup> were counted while the data regarding number of days taken to flowering and fruiting, number of floral buds dropped and number of flowers plant<sup>-1</sup> were recorded on daily basis. Root, shoot and fruit fresh and dry weight were recorded after harvesting of okra. The soil from each pot was washed away to collect root samples. Both shoot and root samples were thoroughly washed with distilled water and then blotted dry with tissue papers before sun drying. Sun dried plants were placed in an oven at 72°C for 72 h until constant weight was achieved.

### Gaseous exchange measurement

Data regarding gas exchange i.e. photosynthetic rate (A), transpiration rate (E), stomatal conductance (g<sub>s</sub>) and sub-stomatal CO<sub>2</sub> concentration were measured by using CIRAS-3 (PP System, Amesbury, MA, USA) with PLC 3 universal leaf cuvette. At flowering stage, both sides of first fully developed leaf of each plant were measured at the ambient light (10:15 to 11:30 hrs). Light emitting diodes (LED) with a photon flux of 1000 µmol m<sup>-2</sup> s<sup>-1</sup> was used to provide light to cuvette.



## Chlorophyll content measurement

At flowering stage, chlorophyll contents were measured from first fully developed leaf of each plant (10:15 to 11:30 hrs) by using chlorophyll meter (SPAD-502, Konica Minolta, Japan). Before measurement, leaves were kept under shade for 30 min.

## Statistical analysis

Data regarding growth, physiological and yield attributes were analyzed statistically using software "Statistix®" version 8.1 (Copy right 2005, Analytical Software, USA) for analysis of variance (ANOVA) and means were compared by using least significant difference (LSD) test (Steel *et al.*, 1997).

## Results

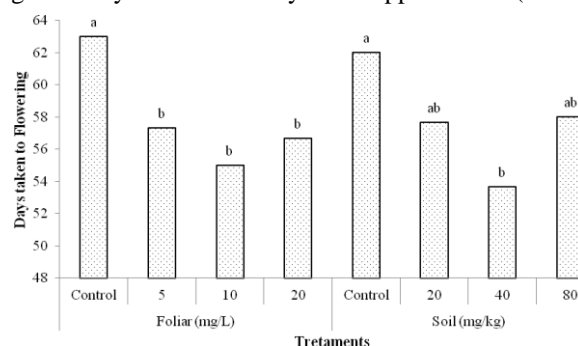
Results revealed that all growth, physiological and yield parameters of okra were significantly increased in response to both soil and foliar applied L-tryptophan.

## Agronomic parameters

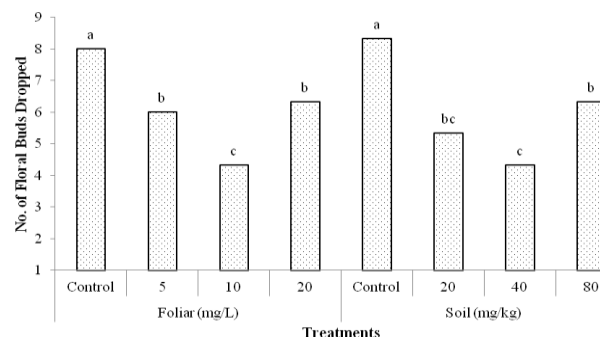
Both soil and foliar applied L-tryptophan have significant effect on the time to onset flowering. Figure 1 revealed that minimum number of days taken to flowering was observed where soil and foliar application of L-tryptophan was done at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively. Time to onset flowering was reduced up to 13.4 and 12.7%, respectively in comparison to control where flower formation took the maximum no. of days to flowering (62, 63). L-tryptophan induced early flowering and so the time taken to fruit formation was also significantly reduced. It was observed that with increasing concentration of soil and foliar applied L-tryptophan, floral bud drop was also significantly reduced (Figure 2) but soil and foliar application of L-tryptophan at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup> showed the most promising results as floral bud drop was reduced up to 50.0 and 45.8%, respectively in comparison with control.

Plant height was significantly increased by soil and foliar application of L-tryptophan at varying levels but the maximum increase in plant height (58.4 and 48.7% higher than control) was observed when soil and foliar application of L-tryptophan was done at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively (Table 1). Similarly, soil and foliar application of L-tryptophan at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively, was more effective in increasing root and fruit length (Table 2) and the maximum was observed with soil and foliar application of L-tryptophan at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively. Increase in root length was 97.3 and 74.1% while in fruit length was 27 and 76.6% higher than control. In the same way soil and foliar application of L-

tryptophan (at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively) showed better results in increasing number of flowers per plant, internodal distance, number of leaves and branches per plant in comparison to all other treatments. Shoot fresh and dry weight and root fresh and dry weight was also significantly increased by its application (Table 3).



**Figure 1: Effect of soil and foliar applied L-tryptophan on the days taken to flowering of okra**



**Figure 2: Effect of soil and foliar applied L-tryptophan on number of floral buds dropped per plant of okra**

Number of fruits per plant was significantly affected by different levels of soil and foliar applied L-tryptophan. The maximum number of fruits (84.8 and 78.4% higher than control) were obtained where L-tryptophan was applied at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup> through soil and foliar application, respectively, whereas, minimum number of fruits were recorded in control (Table 4). Significant effects of soil and foliar applied L-tryptophan concentrations were recorded on fruit fresh and dry weight. Maximum fruit yield was recorded where soil and foliar application of L-tryptophan was done at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively (Figure 3). Fruit yield was increased up to 46.6 and 94.6%, respectively, over control. Although its soil and foliar application at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively, increased the fruit diameter in comparison to control but this increase was non-significant.



**Table 1: Effect of soil and foliar applied L-tryptophan on growth parameters in okra**

Treatment	No. of days taken to fruiting	No. of flowers per pot	Plant height (cm)	Internodal distance (cm)
<b>Foliar application (mg L<sup>-1</sup>)</b>				
0	65.0 <sup>a</sup>	4.00 <sup>d</sup>	20.1 <sup>c</sup>	3.33 <sup>c</sup>
5	58.3 <sup>b</sup> (-12.06)	5.33 <sup>c</sup> (33)	23.5 <sup>b</sup> (16.9)	4.47 <sup>b</sup> (34.2)
10	56.0 <sup>b</sup> (-16.07)	8.33 <sup>a</sup> (108.2)	29.9 <sup>a</sup> (48.7)	5.97 <sup>a</sup> (79.3)
20	56.0 <sup>b</sup> (-14.03)	7.33 <sup>b</sup> (83)	23.9 <sup>b</sup> (18.9)	3.53 <sup>c</sup> (6)
<b>Soil application (mg kg<sup>-1</sup>)</b>				
0	64.0 <sup>a</sup>	5.00 <sup>d</sup>	21.4 <sup>d</sup>	2.80 <sup>c</sup>
20	58.7 <sup>ab</sup> (-9.08)	8.00 <sup>b</sup> (60)	28.6 <sup>b</sup> (35.0)	3.60 <sup>b</sup> (28.6)
40	55.0 <sup>b</sup> (-16.4)	9.67 <sup>a</sup> (93)	33.9 <sup>a</sup> (58.4)	5.43 <sup>a</sup> (93.9)
80	59.0 <sup>ab</sup> (-8.47)	6.33 <sup>c</sup> (26.6)	25.6 <sup>c</sup> (19.6)	3.17 <sup>c</sup> (13.2)

() = % increase from the control; (-) = % decrease from the control; \*Means followed by the same letters are not statistically different at  $P < 0.05$  according to the least significant difference (LSD) test

**Table 2: Effect of soil and foliar applied L-tryptophan on growth parameters in okra**

Treatment	No. of leaves per plant	No. of branches per plant	Root length (cm)	Fruit length (cm)
<b>Foliar application (mg L<sup>-1</sup>)</b>				
0	6.67 <sup>c</sup>	5.67 <sup>d</sup>	16.7 <sup>c</sup>	4.15 <sup>d</sup>
5	9.00 <sup>b</sup> (34.9)	7.33 <sup>c</sup> (29.3)	20.9 <sup>b</sup> (25.1)	4.82 <sup>c</sup> (16.1)
10	10.7 <sup>a</sup> (60)	10.33 <sup>a</sup> (82.2)	29.1 <sup>a</sup> (74.1)	7.33 <sup>a</sup> (76.6)
20	8.33 <sup>b</sup> (24.9)	8.67 <sup>b</sup> (52.9)	23.5 <sup>b</sup> (40.7)	5.93 <sup>b</sup> (42.9)
<b>Soil application (mg kg<sup>-1</sup>)</b>				
0	7.67 <sup>b</sup>	6.67 <sup>c</sup>	21.1 <sup>d</sup>	5.73 <sup>c</sup>
20	8.67 <sup>b</sup> (13)	7.00 <sup>c</sup> (4.95)	23.5 <sup>c</sup> (11.7)	6.82 <sup>ab</sup> (19)
40	12.0 <sup>a</sup> (56.4)	11.00 <sup>a</sup> (64.9)	41.6 <sup>a</sup> (97.3)	7.28 <sup>a</sup> (27)
80	9.00 <sup>b</sup> (17.3)	8.33 <sup>b</sup> (24.9)	26.0 <sup>b</sup> (23.5)	6.53 <sup>b</sup> (14)

() = % increase from the control; \*Means followed by the same letters are not statistically different at  $P < 0.05$  according to the least significant difference (LSD) test

**Table 3: Effect of soil and foliar applied L-tryptophan on growth parameters in okra**

Treatment	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
<b>Foliar application (mg L<sup>-1</sup>)</b>				
0	19.3 <sup>b</sup>	7.29 <sup>c</sup>	2.88 <sup>b</sup>	0.87 <sup>d</sup>
5	20.5 <sup>b</sup> (5.95)	9.32 <sup>b</sup> (27.8)	5.12 <sup>a</sup> (77.8)	1.50 <sup>b</sup> (72.4)
10	26.0 <sup>a</sup> (34.6)	10.8 <sup>a</sup> (47.9)	5.73 <sup>a</sup> (99)	1.71 <sup>a</sup> (96.5)
20	21.6 <sup>b</sup> (11.8)	8.32 <sup>b</sup> (14.1)	3.26 <sup>b</sup> (13.2)	1.23 <sup>c</sup> (41.4)
<b>Soil application (mg kg<sup>-1</sup>)</b>				
0	16.5 <sup>c</sup>	8.82 <sup>b</sup>	2.68 <sup>c</sup>	0.86 <sup>d</sup>
20	20.4 <sup>b</sup> (23.9)	6.74 <sup>a</sup> (52.4)	3.45 <sup>b</sup> (28.7)	1.21 <sup>c</sup> (40.7)
40	23.1 <sup>a</sup> (40)	10.1 <sup>a</sup> (74.9)	5.30 <sup>a</sup> (97.7)	1.69 <sup>a</sup> (96.5)
80	18.5 <sup>b</sup> (12.3)	5.78 <sup>b</sup> (16.6)	3.72 <sup>b</sup> (38.8)	1.29 <sup>b</sup> (50)

() = % increase from the control; \*Means followed by the same letters are not statistically different at  $P < 0.05$  according to the least significant difference (LSD) test

## Physiological parameters

It was revealed by the data (Table 5) that various levels of L-tryptophan application (both through soil and foliar) to okra significantly increased all measured physiological parameters. The maximum chlorophyll contents were

observed where L-tryptophan was applied at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup> through soil and foliar application which was

85.8 and 98.1%, respectively higher than control (Figure 4). Due to increase in chlorophyll content in response to L-tryptophan application at these levels, photosynthetic rate





**Table 4: Effect of soil and foliar applied L-tryptophan on yield parameters in okra**

Treatment	No. of fruits per pot	Fruit diameter(cm)	Fruit fresh weight (g)	Fruit dry weight (g)
<b>Foliar application (mg L<sup>-1</sup>)</b>				
0	4.67 <sup>c</sup>	2.31 <sup>a</sup>	5.76 <sup>c</sup>	4.99 <sup>c</sup>
5	5.00 <sup>c</sup> (7.07)	2.63 <sup>a</sup> (13.8)	7.59 <sup>b</sup> (31.8)	6.75 <sup>b</sup> (35.3)
10	8.33 <sup>a</sup> (78.4)	2.67 <sup>a</sup> (15.6)	11.3 <sup>a</sup> (95.5)	9.54 <sup>a</sup> (91.2)
20	6.67 <sup>b</sup> (62.8)	2.61 <sup>a</sup> (13.0)	7.14 <sup>b</sup> (23.9)	6.53 <sup>b</sup> (30.9)
<b>Soil application (mg kg<sup>-1</sup>)</b>				
0	4.33 <sup>c</sup>	2.52 <sup>a</sup>	6.23 <sup>c</sup>	4.99 <sup>c</sup>
20	6.67 <sup>b</sup> (54)	2.63 <sup>a</sup> (4.36)	7.57 <sup>c</sup> (21.5)	6.65 <sup>b</sup> (21.1)
40	8.00 <sup>a</sup> (84.8)	2.73 <sup>a</sup> (8.33)	9.14 <sup>a</sup> (46.7)	7.73 <sup>a</sup> (40.8)
80	5.00 <sup>c</sup> (15.5)	2.64 <sup>a</sup> (4.76)	7.29 <sup>b</sup> (17)	6.27 <sup>bc</sup> (14.2)

() = % increase from the control; \*Means followed by the same letters are not statistically different at  $P < 0.05$  according to the least significant difference (LSD) test

**Table 5: Effect of soil and foliar applied L-tryptophan on physiological parameters in okra**

Treatment	Photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	Transpiration rate ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )	Stomatal conductance ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )	Sub-stomatal CO <sub>2</sub> Conc. ( $\text{mmol mol}^{-1}$ )
<b>Foliar application (mg L<sup>-1</sup>)</b>				
0	8.30 <sup>d</sup>	4.83 <sup>d</sup>	263 <sup>d</sup>	221 <sup>c</sup>
5	11.6 <sup>c</sup> (40.1)	6.78 <sup>c</sup> (40.4)	348 <sup>c</sup> (31.9)	250 <sup>b</sup> (12.9)
10	16.3 <sup>a</sup> (96.7)	9.59 <sup>a</sup> (98.6)	516 <sup>a</sup> (95.7)	285 <sup>a</sup> (16.7)
20	14.4 <sup>b</sup> (73.5)	7.96 <sup>b</sup> (64.8)	382 <sup>b</sup> (45.1)	240 <sup>b</sup> (8.6)
<b>Soil application (mg kg<sup>-1</sup>)</b>				
0	13.9 <sup>d</sup>	5.35 <sup>d</sup>	264 <sup>c</sup>	202 <sup>b</sup>
20	18.5 <sup>b</sup> (33.3)	7.18 <sup>c</sup> (34.2)	319 <sup>b</sup> (20.7)	257 <sup>a</sup> (27.2)
40	22.1 <sup>a</sup> (59.0)	9.66 <sup>a</sup> (80.6)	510 <sup>a</sup> (93.2)	269 <sup>a</sup> (33.3)
80	16.5 <sup>c</sup> (18.9)	8.57 <sup>b</sup> (60.2)	424 <sup>b</sup> (60.5)	244 <sup>a</sup> (20.6)

() = % increase from the control; \*Means followed by the same letters are not statistically different at  $P < 0.05$  according to the least significant difference (LSD) test

was also increased. In case of stomatal conductance, soil and foliar application of L-tryptophan at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively, gave significantly better results and it was increased by 93.2 and 95.7%, respectively, in comparison to control. In the same way soil and foliar application of L-tryptophan (at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively) showed more promising results in increasing transpiration rate and sub-stomatal CO<sub>2</sub> concentration in comparison to all other treatments (Table 5).

## Discussion

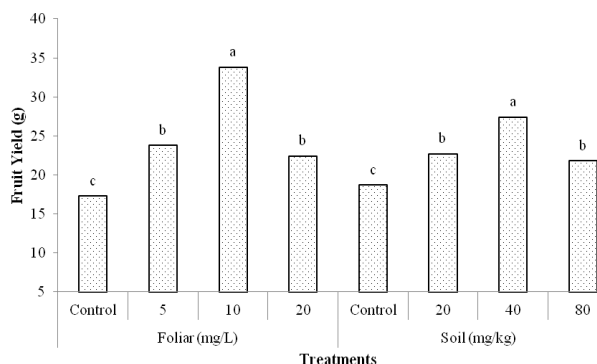
The results of present study clearly showed that both the soil and foliar application of L-tryptophan improved plant growth and yield of okra. L-tryptophan is considered biologically active precursor of auxin (Hussain *et al.*, 2011; Abbas *et al.*, 2013) and more beneficial as compared to pure auxin (Rao *et al.*, 2012) and affect different physiological processes of plant (Abbas *et al.*, 2013). Positive effects of L-tryptophan on the plant growth might be due to either direct uptake of L-tryptophan or auxin through the roots from the soil or its absorption through the

cuticle from the leaves (Ahmad *et al.*, 2008; Abbas *et al.*, 2013).

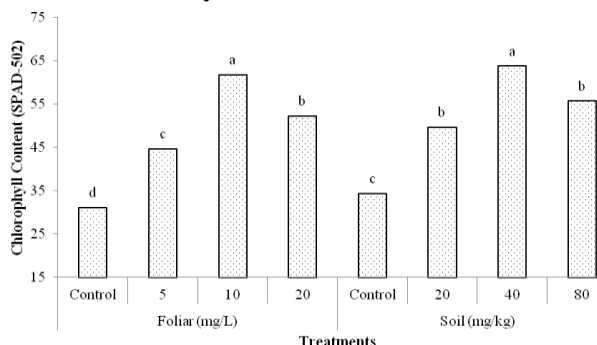
Addition of L-tryptophan increases the biosynthesis of auxin in soil (Nahed *et al.*, 2009; Rahmatzadeh *et al.*, 2012). Not only the plants, but also the microorganisms (bacteria and fungi) have the ability to synthesize auxin in their body (Etesami *et al.*, 2009). In plants, both root exudates and hydrolysis of proteins of dying cells release L-tryptophan in soil (Patten and Glick, 1996; Barazani and Friedman, 2000). In present study, it was observed that both soil and foliar application of L-tryptophan improved okra growth and physiological parameters (root/shoot biomass and reduced floral bud drop compared to un-amended control) (Tables 1-3). In response to L-tryptophan application, increase in total root system has commonly been observed in various crop plants (Barazani and Friedman, 2000). It affects root growth and development in several ways due to its conversion into auxin (Mantelin and Touraine, 2004). Microflora residing in rhizosphere uses L-tryptophan either from root exudates or exogenously applied for production of auxin which is responsible for



root structure (Ludwig-Müller, 2011). L-Tryptophan induced alterations in root structure might have increased its total biomass and length and resulted in improved nutrient and water uptake, which may have positive effect on plant growth as a whole. In control where no L-tryptophan was applied, yield was very less due to low levels of IAA (Table 4).



**Figure 3: Effect of soil and foliar applied L-tryptophan on fruit yield of okra**



**Figure 4: Effect of soil and foliar applied L-tryptophan on chlorophyll content of okra**

Our results strongly indicate that the improvement of plant growth and development is due to auxin production, particularly when L-tryptophan is applied (Tables 1-3). These findings are supported by the work of other researchers who determined the effect of L-tryptophan application on growth and development of various crops (Attoa *et al.*, 2002; Mirza *et al.*, 2007).

Moreover, auxin produced from L-tryptophan promoted root growth either by stimulating cell division or cell expansion or by influencing bacterial ACC deaminase activity. ACC deaminase hydrolyzes plant ACC, the immediate precursor of ethylene, and thereby prevents the production of plant growth-inhibiting levels of ethylene (Sun *et al.*, 2009). Exogenous IAA is known to increase transcription and activity of ACC synthase, which catalyzes

the production of ACC in plants (Patten and Glick, 2002). ACC production in plant stimulates ACC deaminase activity in bacteria (Li *et al.*, 2001).

Development of roots, root morphology and plant growth are controlled by the auxins which are an important class of hormones. Plant growth and development positively influenced by the exogenous application of L-tryptophan (Arshad and Frankenberger, 1997). Majority of plant growth promoting rhizobacteria have ability to produce ( $\geq 80\%$ ) phytohormones e.g. auxins, which directly influence plant growth (Bais *et al.*, 2006). Root exudates could supply the pool of precursors of different hormones for rhizosphere bacteria. Indole acetic acid production could not fulfill by the endogenous levels of tryptophan. As appreciable levels of indole acetic acid are not produced, so, an external source of tryptophan is supplied to the rhizosphere bacteria. Bacteria have high demand for tryptophan, as it is used to produce many essential compounds such as vitamins and proteins (Martens and Frankenberger, 1993).

In the present study, the soil and foliar applied L-tryptophan at 40 mg kg<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively, proved to be more effective in improving the growth, physiological and yield parameters of okra compared to the untreated control. These positive effects of L-tryptophan could be attributed to physiologically active levels of auxin at this substrate concentration. The mechanism of action of L-tryptophan on plant growth may be attributed to direct uptake of these compounds by plant roots, a change in the endogenous level or balance of naturally occurring hormones or allowing the modification of growth depending on age and physiological state of plant, state of nutrition and environmental conditions resulting in a beneficial rhizosphere for plant growth (Abeles *et al.*, 1992; Arshad and Frankenberger, 2002). In our study, improvement in the plant growth might be due to L-tryptophan dependent IAA biosynthesis, which might optimize the endogenous suboptimal plant hormone level, or improve mineral uptake by plant roots.

Fewer studies have described the role of stimulated IAA production by its precursor L-tryptophan in plant growth promotion by reducing time to onset flowering and floral bud drop. Under normal growth conditions, auxins are produced in the reproductive organs of plant and inhibit the formation of abscission zones. However, under stress, its efficiency is reduced due the production of ethylene which reduces its level in abscission organs. Ethylene produced acts in the pedicel base and cells are separated in the abscission zones (Wien and Zhang, 1991), in this way auxin prevent abscission of different plant parts in plants (El-Saeid *et al.*, 2010). In present study, it has been



elucidated that both soil and foliar applied L-tryptophan significantly reduced the early floral bud abscission and time to onset flowering (Figure 1 and 2). These results are in line with findings of Kumar *et al.* (2012). L-tryptophan has stimulatory effect on initiating photosynthetic pathway that leads to chlorophyll formation by supporting the function of succinyl CoA and glycine (Aziz *et al.*, 2009). It might be the reason that soil and foliar application of L-tryptophan increased the chlorophyll content. Similar results were obtained when L-tryptophan was applied to different crops (Dahab and El-Aziz, 2006; Nahed *et al.*, 2009; Rao *et al.*, 2012).

We provide the evidence that L-tryptophan dependent IAA biosynthesis affects growth and yield of okra plants. Based on our results we conclude that both soil and foliar application of L-tryptophan is effective to improve growth, yield and physiological parameters than control. Overall, this study implies that the soil application of L-tryptophan is an attractive approach for improving the growth and yield of plants. However, further field investigations are needed to confirm its potential on okra plants under natural soil environment.

## References

- Abbas, S.H., M. Sohail, M. Saleem, T. Mahmood, I. Aziz, M. Qamar, A. Majeed and M. Arif. 2013. Effect of L-tryptophan on plant weight and pod weight in chickpea under rainfed conditions. *Sciences and Technical Devison* 32(4): 277-280.
- Abdoli, M., M. Saeidi, S. Jalali-Honarmand and M. Azhand. 2013. The effect of foliar application of indol-3-acetic acid (IAA) and roles of ear photosynthesis on grain yield production of two wheat cultivars (*Triticum aestivum* L.) under post anthesis water deficit. *International Research Journal of Applied and Basic Sciences* 4(6): 1406-1413.
- Abeles, F.B., P.W. Morgan and M.E. Saltveit, Jr. 1992. Ethylene in plant biology, 2nd Ed. *San Diego: Academic Press*.
- Ahmad, M., M.A. Pervez, F.M. Tahir and Anwar-Ul-Haq. 1999. Effect of L-tryptophan on the growth and yield of potato cv. Pars 70. *International Journal of Agriculture and Biology* 1: 30-32.
- Ahmad, R., M. Khalid, M. Naveed, S.M. Shahzad, Z.A. Zahir and S.N. Khokhar. 2008. Comparative efficiency of auxin and its precursor applied through compost for improving growth and yield of maize. *Pakistan Journal of Botany* 40(4): 1703-1710.
- Ahmad, R., S.M. Shahzad, A. Khalid, M. Arshad and M. H. Mahmood. 2007. Growth and yield response of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) to nitrogen and L-tryptophan enriched compost. *Pakistan Journal of Botany* 39(2): 541-549.
- Akhtar, M.J., H.N. Asghar, M. Asif and Z.A. Zahir. 2007. Growth and yield of wheat as affected by compost enriched with chemical fertilizer, L-tryptophan and rhizobacteria. *Pakistan Journal of Agricultural Sciences* 44(1): 136-140.
- Anjum, M.A., Z.A. Zahir, M. Arshad and M. Ashraf. 2011. Isolation and screening of rhizobia for auxin biosynthesis and growth promotion of mung bean (*Vigna radiata* L.) seedlings under axenic conditions. *Soil & Environment* 30(1): 18-26.
- Arshad, M. and W.T. Frankenberger (Jr.). 1997. Plant growth regulating substances in the rhizosphere: microbial production and functions. *Advances in Agronomy* 62:45-151.
- Arshad, M., and W.T. Frankenberger, Jr. 2002. Ethylene: Agricultural sources and application. *Kluwer Academic/ Plenum Publisher, NY, USA*.
- Attoa, G.E., H.E. Wahba and A.A. Farhat. 2002. Effect of some amino acids and sulphur fertilization on growth and chemical composition of *Iberis amara* L. plant. *Egyptian Journal of Horticulture* 29: 17-37.
- Aziz, N.G.B., N.H. Mahgoub and A.A.M. Mazher. 2009. Physiological effect of phenylalanine and tryptophan on the growth and chemical constituents of *Antirrhium majus* plants. *Journal of Applied Sciences* 2: 399-407.
- Bais, H.P., T.L. Weir, L.G. Perry, S. Gilroy and J.M. Vivanco. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Reviews of Plant Biology* 57: 233-266.
- Barazani, O. and J. Friedman. 2000. Effect of exogenously applied L-tryptophan on allelochemical activity of plant growth promoting rhizobacteria (PGPR). *Journal of Chemical Ecology* 26(2): 343-349.
- Dahab, T.A.M.A. and N.G.A. El-Aziz. 2006. Physiological effect of diphenylamine and tryptophan on the growth and chemical constituents of *Philodendron erubescens* plants. *World Journal of Agriculture Sciences* 2(1): 75-81.
- Dawood, M.G. and M.S. Sadak. 2008. Physiological response of canola plants (*Brassica napus*) to tryptophan and benzylalanine. *Seria Agronomie* 50: 198-207.
- El-Saeid, H.M., S.D. Abou-Hussein and W.A. El-Tohamy. 2010. Growth characters, yield and endogenous hormones of cowpea plants in response to IAA application. *Research journal of Agriculture and Biological Sciences* 6(1): 27-31.
- Estronell, L.H., J. Agusti, P. Merelo, M. Talon and F.R. Tadeo. 2013. Elucidating mechanisms underlying organ abscission. *Plant Sciences* 199-200: 48-60.



- Etesami, H., H.A. Alikhani and A. Aliakbari. 2009. Evaluation of plant growth hormones production (IAA) ability by Iranian soils rhizobial strains and effects of superior strains application on wheat growth indexes. *World Applied Sciences* 6(11): 1576-1584.
- Frankenberger, W.T. Jr., A. Chang and M. Arshad. 1990. Response of *Raphanus sativus* to auxin precursor L-tryptophan applied to soil. *Plant and Soil* 129: 235-241.
- Frankenberger, W.T. Jr. and M. Arshad. 1991. Yield response of watermelon and muskmelon to L-tryptophan applied to soil. *Horticulture Sciences* 26(1): 35-37.
- Frankenberger, W.T. Jr. and M. Arshad. 1995. Phytohormones in soil: Microbial production and function. *Marcel Dekker Inc. NY. USA*. 503p.
- Fruit, Vegetable and Condiments Statistics of Pakistan 2011-12.2013. *Government of Pakistan. Ministry of National Food Security and Research, Islamabad*.
- Hussain, M.I., M.J. Akhtar, H.N. Asghar and M. Ahmad. 2011. Growth, nodulation and yield of mash bean (*Vigna mungo* L.) as affected by rhizobium inoculation and soil applied L-tryptophan. *Soil & Environment* 30(1): 13-17.
- Kumar, C.S., A. Singh, R.K. Sagar, M.P.S. Negi and J.N. Maurya. 2012. Impact of exogenous application of indole acetic acid on accumulation of heavy metal and antioxidants in wheat (*Triticum aestivum* L.) under sewage water irrigation. *Recent Research in Science and Technology* 4(7): 16-22.
- Li, J., D.H. Ovaskim, T.C. Charles and B.R. Glick. 2000. An ACC deaminase minus mutant of *Enterobacter cloacae* UW4 no longer promotes root elongation. *Current Microbiology* 41: 101-105.
- Ludwig-Müller, J.J. 2011. Auxin conjugates: Their role for plant development and in the evolution of land plants. *Journal of Experimental Botany* 62:1757-1773.
- Mantelin, S. and B. Touraine. 2004. Plant growth-promoting bacteria and nitrate availability: Impact of development and nitrate uptake. *Journal of Experimental Botany* 55: 27-34.
- Marcelis, L.F.M., E. Heuvelink, L.R. Baan Hofman-Eijer, J. Den bakker and L.B. Xue. 2004. Flower and fruit abortion in sweet pepper in relation to source and sink strength. *Journal of Experimental Botany* 55(406): 2261-2268.
- Martens, D.A. and W.T. Frankenberger (Jr.). 1993. Metabolism of tryptophan in soil. *Soil Biology and Biochemistry* 25: 1679-1687.
- Mignolli, F., L. Mariotti, L. Lombardi, M.L. Vidoz, N. ceccarelli and P. Picciarelli. 2012. Tomato fruit development in the auxin-resistant dgt mutant is induced by pollination but not by auxin treatment. *Journal of Plant Physiology* 169: 1165-1172.
- Mirza, B.S., M.S. Mirza, A. Bano and K.A. Malik. 2007. Co-inoculation of chickpea with Rhizobium isolates from roots and nodules and phytohormone-producing *Enterobacter* strains. *Australian Journal of Experimental Agriculture* 47: 1008-1015.
- Nahed, G., A. Aziz, Mona, H. Mahgoub, A.M. Mazher and Azza. 2009. Physiological effect of phenylalanine and tryptophan on the growth and chemical constituents of *Antirrhinum majus* plants. *Ozean Journal of Applied Sciences* 2(4): 399-407.
- Pandolfini, T., B. Molesini and A. Spena. 2007. Molecular dissection of the role of auxin in fruit initiation. *Trends in Plant Sciences* 12(8): 327-329.
- Patten, C.L. and B.R. Glick. 1996. Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology* 42: 207-220.
- Patten, C.L. and B.R. Glick. 2002. Regulation of indole acetic acid production in *Pseudomonas putida* GR12-2 by tryptophan and the stationary-phase sigma factor RpoS. *Canadian Journal of Microbiology* 48:635-642.
- Rahmatzadeh, S., J. Khara, and S.K. Kazemitabar. 2012. Effects of tryptophan on growth and some physiological parameters in mycorrhizal inoculated plants of *Catharanthus roseus* (L.) G. Don. *International Journal of Agriculture* 2(5): 564-572.
- Rao, S.R., A. Qayyum, A. Razzaq, M. Ahmad, I. Mahmood and A. Sher. 2012. Role of foliar application of salicylic acid and L-tryptophan in drought tolerance of maize. *Journal of Animal and Plant Sciences* 22(3): 768-772.
- Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Ed. *McGraw-Hill, Book International Co., Singapore*.
- Sun, Y., Z. Cheng and B.R. Glick. 2009. The presence of a 1-aminocyclopropane-1-carboxylate (ACC) deaminase deletion mutation alters the physiology of the endophytic plant growth promoting bacterium *Burkholderia phytofirmans* PsJN. *FEMS Microbiology Letters* 296: 131-136.
- Tallat, I.M., M.A.Bekheta and M.H. Mahgoub. 2005. Physiological response of periwinkle plants (*Catharanthus roseus* L.) to tryptophan and putrescine. *International Journal of Agriculture and Biology* 7(2): 210-213.
- Wien, H.C. and Y. Zhang. 1991. Prevention of flower abscission in bell pepper. *Journal of American Society for Horticulture Science* 116(3): 516-519.
- Zahir, Z.A., M. Arshad, M. Azam and A. Hussain. 1997. Effect of an auxin precursor tryptophan and Azotobacter inoculation on yield and chemical





- composition of potato under fertilized conditions. *Journal of Plant Nutrition* 20(6): 745-752.
- Zahir, Z.A., M.A.U.R. Malik and M. Arshad. 1999. Effect of auxins on the growth and yield of rice. *Pakistan Journal of Agricultural Science*. 36: 3-4.
- Zahir, Z.A., M.A.U.R. Malik and M. Arshad. 2000a. Improving crop yields by the application of an auxin precursor L-tryptophan. *Pakistan Journal of Biological Sciences* 3(1): 133-135.
- Zahir, Z.A., S.A. Abbas, M. Khalid and M. Arshad. 2000b. Substrate dependent microbially derived plant hormones for improving growth of maize seedlings. *Pakistan Journal of Biological Sciences* 3(2): 289-291.

