

## Effect of foliar application of chitosan on growth and yield in okra

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### Abstract

Pot and field experiments were conducted in two consecutive years at the pot-yard and experimental field of Bangladesh Institute of Nuclear Agriculture, Mymensingh, during March to June 2010 and 2011, to investigate the effect of foliar application of chitosan, a growth promoter, on morphological, growth, biochemical, yield attributes and fruit yield of okra cv. BARIdherosh-1. The experiment comprised of five levels of chitosan concentrations viz., 0 (control), 50, 75, 100 and 125 ppm. The chitosan was sprayed three times at 25, 40 and 55 days after sowing. The pot experiment was laid out in a completely randomized design and the field experiment in a randomized complete block design, both with four replicates. Results revealed that most of the morphological (plant height, leaf number plant<sup>-1</sup>), growth (total dry mass plant<sup>-1</sup>, absolute growth rate, relative growth rate), biochemical parameters (nitrate reductase and photosynthesis) and yield attributes (number of fruits plant<sup>-1</sup> and fruit size) were increased with increasing concentration of chitosan until 25 ppm, resulted the highest fruit yield in okra (27.9% yield increased over the control). However, the increment of plant parameters as well as fruit yield was not significant from 100 ppm of chitosan. Therefore, foliar application of chitosan at 100 or 125 ppm may be used at early growth stage to achieve a maximum fruit yield in okra.

**Keywords:** chitosan, foliar spray, fruit yield, growth okra.

**Abbreviations:** PGR: plant growth regulator; DAS: days after sowing; TDM: total dry mass; AGR: absolute growth rate; RGR: relative growth rate; Pn: photosynthesis; NR: nitrate reductase

### Introduction

Okra (*Hibiscus esculentus* L.) known as lady's finger is a heat loving plant of Malvaceae family and is one of the most important summer vegetable in Bangladesh. It is also popular home garden vegetable and a good source to fulfill the energy requirements of the body. It is also provides vitamin A, B, C, protein, amino acids, minerals and iodine (Hossain et al., 2006). This vegetable is quite palatable and linked equally by poor and rich. Total area under cultivation in Bangladesh is 12900 hectares and total production is about 40000 tons of green fruits (BBS, 2009). In spite of all our efforts to increase okra yield in the country, its yield is much lower (3.1 t ha<sup>-1</sup>) than that of other agriculturally developed countries (FAO, 2007). Despite significant annual increase in fertilizer use, its yield has stagnated and even declined in some cases. So far, different conventional approaches have been used to improve the yield of okra. These approaches are not much effective in many cases in improving the yield or narrowing down the gap between potential and farmer's obtained yields. This situation forces us to use non-conventional approaches such as biotechnology, genetic engineering and use of plant hormones. Application of plant growth regulator (PGR) seems to be one of the important practices in view of convenience, cost and labour efficiency. Recently, there has been global realization of the important role of PGRs in

agriculture for better growth and yield of crops. Developed countries like Japan, China, Poland, South Korea etc. have long been using PGRs to increase crop yield. Like other crop plants, the physiological mechanisms of okra growth are hormonally mediated. Additional supply of plant growth regulators control growth and yield in plants. Chitosan is derived from chitin, a polysaccharide found in exoskeleton of shellfish such as shrimp, lobster or crabs and cell wall of fungi (Wojdyla, 2001). Very few efforts were done to study the effect of chitosan on plant growth, development and productivity, which is mainly attributed to stimulation of plants immunity against microorganisms (bacteria and fungi) (ChunYan et al., 2003; Patkowska et al., 2006; Sereih et al., 2007; No et al., 2007; Gornik et al., 2008). Recently, some researchers reported that chitosan enhanced plant growth and development (Khan et al., 2002; Chibu et al., 2003; Gornik et al., 2008). They reported that application of chitosan increased key enzymes activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) and improved the transportation of nitrogen (N) in the functional leaves which enhanced plant growth and development. Research works of chitosan on growth, yield attributes and fruit yield of okra is almost rare. Considering the above facts, the present research work was undertaken to study the effect of chitosan on growth, morphological features, yield

attributes and yield in okra under sub-tropical (24°75' N and 90°50' E) conditions.

## Results and Discussion

### *Effect of chitosan on morpho-physiological parameters*

The effects of different concentrations of chitosan on morphological characters such as plant height and leaf number plant<sup>-1</sup>, growth parameters such as TDM production plant<sup>-1</sup>, AGR and RGR were significant (Table 1). Results showed that all morphological, yield attributes and fruit yield was greater in the field condition than pot culture condition (Tables 1 & 3). Results revealed that plant height and leaf number plant<sup>-1</sup> both at pot and field conditions and TDM. Moreover, TDM, AGR and RGR at field condition were greater in chitosan applied plants than control plants. All morpho-physiological parameters such as plant height, leaf number, TDM both at 70 and 85 DAS and AGR at 70-85 DAS increased with increasing concentration of chitosan until 125 ppm. RGR followed no regular pattern. However, plant height and leaf number both at pot and field conditions increased significantly till 100 ppm and thereafter increased but not significantly. The lowest value of the above parameters was recorded in control plants. TDM was greater in high concentration of chitosan (100 and 125 ppm) than low concentrated ones (50 and 75 ppm), which might be due to of AGR increase (Table 1). These results indicate that application of chitosan at early growth stages had tremendous effect on growth and development in okra. These results are consistent with El-Tantawy (2009), who reported that plant growth and development enhanced by the application of chitosan in tomato. Furthermore, Ke (2001) reported that application of carboxymethyl chitosan increased key enzymes activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease), which enhanced plant growth and development in rice. Similar phenomenon may be happened in the present experiment, resulting to increased TDM in chitosan applied plants than control.

### *Effect of chitosan on biochemical parameters*

The effects of different concentrations of chitosan application on NR activity in leaves and photosynthesis (Pn) were significant but no significant influence on Chlorophyll content of leaves was observed (Table 2). Apparently, chlorophyll content was increased in leaves of chitosan applied plants than control. Results revealed that NR and Pn increased with increasing concentration of chitosan up to 125 ppm. Furthermore, within the chitosan applied plants, there were no significant difference in NR and Pn. In contrast, the lowest chlorophyll, NR and Pn was recorded in control plants. These results indicate that foliar application of chitosan enhanced the biochemical activities. These results are consistent with Khan et al. (2002) who reported that application of chitosan increased Pn in leaves of maize and soybean. Again, El-Tantawy (2009) reported that application of chitosan increased photosynthetic pigment thereby the Pn increased.

### *Effect of chitosan on yield attributes and fruit yield*

There was significant variation in yield attributes and fruit yield both in pot and field conditions due to different levels of foliar application of chitosan on okra plant (Table 3). Results revealed that number of fruits plant<sup>-1</sup>, fruit length and diameter and single fruit weight were higher in chitosan

applied plants than control. Results showed that yield attributes and fruit yield increased significantly with increasing concentration of chitosan up to 100 ppm. The higher fruit yields both per plant and per hectare were recorded in 100 and 125 ppm chitosan treatments, in which the highest values recorded in 125 ppm (15.31 t ha<sup>-1</sup>). The fruit yield was higher in 100 and 125 ppm chitosan treatments due to production of higher number of fruits plant<sup>-1</sup> and increased fruit size. Again, the number of fruits plant<sup>-1</sup> increased in chitosan applied plants than control due to increase the plant height, resulting from increase in the fruit bearing nodes in okra. In contrast, the lowest fruit yield was recorded in control plants of both pot and field conditions due to production of fewer fruits and smaller fruit size (Table 3). However, the effect of foliar application of chitosan on okra plant was greater in pot culture (48.7% yield increased over control) than field condition (27.9% yield increased over control). Chibu et al. (2002) reported that application of chitosan at early growth stages increased plant growth and development, thereby increased seed yield in rice and soybean. Similar results were also observed by Boonlertnirum et al. (2005) in rice and Rehim et al. (2009) in maize and bean.

## Materials and methods

### *Site description*

The first experiment was carried out at the pot-yard of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh (24°75' N and 90°50' E), Bangladesh, during March to June 2010. The soil used for pot culture was collected from BINA experimental farm. The first experiment was repeated in the following year (2011) and the experiment was set up on March, 2011 at the experimental field of BINA, Mymensingh, Bangladesh. The experimental field was medium high belonging to the Sonatola Soil Series of Grey Floodplain soil (sandy loam soil) under the agro-ecological zone of Old Bahmaputra Floodplain (AEZ-9). The soil of the experiment was sandy loam having a total nitrogen of 0.06%, organic matter 1.15%, available phosphorus 18.5 ppm, exchangeable potassium 0.28 meq%, sulphur 18 ppm and pH 6.8.

### *Plant materials and experimental design*

The widely cultivated cultivar, BARI dherosh-1 was used as test crop. The pot culture experiment was laid out in a completely randomized design with four replications. Four seeds were sown in each pot and after 20 DAS, they were thinned to two seedlings which denoted as a replication. The field experiment was laid out in a randomized complete block design with four replications. The seeds were sown on 10 and 12 March for pot and field experiment, respectively.

### *Land preparation, fertilizer application and intercultural operation*

For pot experiment, the soil was thoroughly mixed with the given amounts of urea, triple superphosphate, muriate of potash, gypsum and cowdung at the rate of 10.0, 5.15, 5.00, 2.00 and 250 g pot<sup>-1</sup> corresponding to 200, 110, 100, 40 and 5000 kg ha<sup>-1</sup>, respectively. Total amount of TSP, MP, gypsum and cowdung were applied as basal dose during soil preparation. One third of urea was applied as top dress at 25 DAS and the remaining two third was applied in two equal splits at 45 DAS and 65 DAS. The pots were filled with 10

**Table 1.** Effect of foliar application of chitosan on morpho-physiological characters in okra *cv.* BARI dherosh-1.

Chitosan concentration (ppm)	Plant height (cm) at 110 DAS		Number of leaves plant <sup>-1</sup> at 110 DAS		Growth parameters at field condition			
	Pot	Field	Pot	Field	TDM plant <sup>-1</sup> (g) at		AGR (mg plant <sup>-1</sup> day <sup>-1</sup> )	RGR (mg g <sup>-1</sup> day <sup>-1</sup> )
					70 DAS	85 DAS		
0	97.7c	126.0c	22.9d	25.2d	25.8c	35.9e	673e	22.0c
50	104.2bc	135.6b	24.5cd	28.6c	27.5c	40.1d	840d	25.1ab
75	110.3b	141.2ab	26.3bc	32.4b	30.7b	43.8c	923c	23.4bc
100	120.3a	145.6a	28.5ab	34.0ab	37.0a	52.0b	1000b	22.7c
125	126.1a	148.1a	29.4a	36.0a	39.7a	58.4a	1247a	25.7a
F-test	**	**	**	**	**	**	**	**
CV (%)	5.12	3.53	5.67	6.18	5.57	3.72	5.22	5.75

In a column, figures having the same letter(s) do not differ significantly at  $p \leq 0.05$  by DMRT; \*\* = Significant at 1% level of probability.

**Table 2.** Influence of chitosan application on biochemical attributes in okra *cv.* BARI dherosh-1.

Chitosan concentration (ppm)	Chlorophyll (mg g <sup>-1</sup> fw)	NR ( $\mu\text{mol NO}_2^- \text{g}^{-1} \text{fw}$ )	Pn ( $\mu\text{mol CO}_2 \text{dm}^{-2} \text{s}^{-1}$ )
0	2.41	7.44c	20.16b
50	2.48	8.11bc	22.10a
75	2.57	8.61ab	21.92a
100	2.51	9.20a	22.54a
125	2.53	9.30a	22.63a
F-test	NS	**	*
CV (%)	5.22	5.55	4.51

In a column, figures having the same letter(s) do not differ significantly at  $p \leq 0.05$  by DMRT; NS = Not significant; \*, \*\* = Significant at 5% and 1% level of probability, respectively.

**Table 3.** Effect of chitosan application on yield attributes and fruit yield in okra *cv.* BARI dherosh-1

Chitosan concentration (ppm)	Number of fruits plant <sup>-1</sup>		Fruit length (cm)		Fruit diameter (cm)		Single fruit weight (g)		Fruit weight plant <sup>-1</sup> (g)		Fruit yield (t ha <sup>-1</sup> )
	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Field
0	14.9c	17.9d	12.8b	14.3b	1.14c	1.31	15.2b	17.0b	193d	243c	11.97c
50	16.1bc	19.4cd	13.2b	15.1ab	1.16bc	1.30	15.8ab	18.4ab	216c	286b	13.03c
75	17.0b	21.6bc	14.1a	15.8a	1.17abc	1.33	16.0a	18.1ab	240b	313b	14.85b
100	19.7a	22.7ab	14.5a	15.7a	1.20ab	1.36	16.4a	18.6a	280a	338a	15.21a
125	20.3a	23.1a	14.4a	15.8a	1.22a	1.35	16.3a	18.5a	287a	342a	15.31a
F-test	**	**	**	*	*	NS	*	**	**	**	**
CV (%)	5.53	7.04	2.74	4.08	2.88	2.89	2.83	2.62	4.81	5.73	5.60

In a column, figures having the same letter(s) do not differ significantly at  $p \leq 0.05$  by DMRT; NS = Not significant; \*, \*\* = Significant at 5% and 1% level of probability, respectively.

kg of prepared soils. For field experiment, the land was prepared properly with ploughing and laddering. The unit plot size was 3 m × 3 m. The row to row and plant to plant distances were 50 cm and 40 cm, respectively. Fertilizer doses and application methods were same to first experiment (pot experiment). Intercultural operations like irrigation, weeding, mulching and pest control of pot and field experiments carried out when necessary for normal plant growth and development.

#### Treatments

Five concentrations of chitosan *viz.*, 0, 50, 75, 100 and 125 ppm were applied three times with 15 days interval, started from 25 DAS (before flowering start phase). The growth regulator was sprayed by a hand sprayer at afternoon.

#### Parameters measured

Growth parameters such as TDM plant<sup>-1</sup>, AGR and RGR were recorded at 70 and 85 DAS. Plant samples (five plants) were oven dried at 80 °C ± 2 for 48 hours. The total dry matter plant<sup>-1</sup> was estimated by summing dry matter of

leaves, stem, roots and fruits dry weight per plant. AGR and RGR were determined following the methods of Hunt (1978). Chlorophyll content in leaves, NR activity in leaves and photosynthesis in leaf were determined at 65-70 DAS of upper 3-4 nodes leaves in the canopy. Chlorophyll was determined following the method of Yoshida et al. (1976). NR was estimated following the procedure of Stewart and Orebanjo (1979). Pn was measured by automatic photosynthesis meter (LICOR 6400, USA). Harvesting was done at different dates depending on fruit maturity (4-5 days old fruits).

#### Statistical analysis

All data were analyzed statistically as per the used design following the analysis of variance (ANOVA) and the mean differences were adjusted with Duncan's Multiple Range Test (DMRT) using the statistical computer package program, MSTAT-C (Russell, 1986).

#### Conclusion

It is concluded that foliar application of chitosan at vegetative stage enhanced the plant growth and development, which

resulted from increased fruit yield in okra. Among the concentrations, 100 and 125 ppm had superiority for plant growth, yield components and fruit yield over 50 and 75 ppm. Therefore, application of chitosan at the rate of 100 or 125 ppm may be recommended for okra cultivation. However, more experiments should be conducted in different locations and seasons to draw a valid conclusion regarding the chitosan foliar application for fruit yield improvement of okra.

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#### References

- BBS (2009) Hand book of Agricultural Statistics, December, 2009. Bangladesh Bureau of Statistics (BBS). Ministry of Planning, Govt. People's Repub. Bangladesh. p. 14
- Boonlertnirun S, Boonlertnirun K, Sooksathan I (2005) Proceedings of 43<sup>rd</sup> Kasetsart University Annual Conference, Thailand, 1-4 February. pp. 37-43
- Chibu H, Shibayama H (2003) Effects of chitosan application on the growth of several crops. In: Chitin and chitosan in life science. T. Uragami, K Kurita, and T. Fukamizo (eds.). Yamaguchi, Japan. pp. 235-239. ISBN 4-906464-43-0
- Chibu H, Shibayama H, Arima S (2002) Effects of chitosan application on the shoot growth of rice and soybean. Jap J Crop Sci 71: 206-211
- ChunYan L, GuoRui M, WenYing H (2003) Induction effect of chitosan on suppression of tomato early blight and its physiological mechanism. J Zhejiang Univ Agric Life Sci 29: 280-286
- El-Tantawy EM (2009) Behaviour of tomato plants as affected by spraying with chitosan and aminofort as natural stimulator substances under application of soil organic amendments. Pakistan J Biol Sci 12: 1164-1173
- FAO (2007) Production Year Book of 2006. No. 67. Food and Agriculture Organization (FAO), Rome, Italy p. 54
- Gornik K, Grzesik M, Duda BR (2008) The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and temperature stress. J Fruit Ornamental Plant Res 16: 333-343
- Hossain MS, Rahman MM, Rashid MR, Farid ATM, Kaium MA, Ahmed M, Alam MS, Uddin KMS (2006) Handbook on Agro-technology, 4<sup>th</sup> Edition, Bangladesh Agricultural Res. Inst., Gazipur-1701, Bangladesh. pp. 356-358
- Hunt R (1978) Plant growth analysis studies in biology. Edward Arnold Ltd. London. pp. 65-67
- Ke L, XiangYang L, LiSha P (2001) Effects of carboxymethyl chitosan on key enzymes activities of nitrogen metabolism and grain protein contents in rice. J Hunan Agric Univ 27: 421-424
- Khan WM, Prithiviraj B and Smiyh DL (2002) Effect of foliar application of chitin oligosaccharides on photosynthesis of maize and soybean. Photosynthetica 40: 621-624
- No HK, Meyers SP, Prinyawiwatkul W, Xu Z (2007) Applications of chitosan for improvement of quality and shelf life of foods: a review. J Food Sci 72: 87-100
- Patkowska E, Pieta D, Pastucha H (2006) The effect of biochikol 020 pc on microorganisms communities in the rhizosphere of Faba bean plants. Polish Chitin Sco Monograph 11: 171-178
- Rehim HAA, Hegazy EA, El-Barbary AM (2009) Radiation modification of natural polysaccharides for application in agriculture. Polymer 50: 1952-1957
- Russell DF (1986) MSTAT-C Package Programme. Crop and Soil Science Department, Michigan Univ, USA
- Serei A, Neven A, Abd-El-Aal S, Sahab AF (2007) The mutagenic activity and its effect on the growth of *Trichoderma harzianum* and *Fuzarium oxysporum*. J Appl Sci Res 3: 350-455
- Stewart GR, Orebamjo TO (1979) Some unusual characteristics of nitrate reduction in *Erythrina senegalensis*. New Phytol 83: 311-319
- Wojdyla AT (2001) chitosan in the control of rose disease: six years trials. Bull. Polish Acad Sci Biol Sci 49: 233-252
- Yoshida S, Forno DA, Cock JA, Gomes KA (1976) Laboratory manual for physiological studies of rice. 3<sup>rd</sup> ed., IRRI, Los Banos, Philippines