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# Efficacy of IAA, $GA_3$ and Riboflavin for Morpho-biochemical and Yield Attributes of Fenugreek (*Trigonella foenum-graecum* L.) in Pakistan

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## ABSTRACT

**Background:** Fenugreek is an important winter leafy vegetable in Pakistan with high nutritional values. Its seeds are used as spices and whole plant is used in many home remedies and medicine throughout the world. Its foliage and seed productivity is much low in the region. Plant growth regulators (PGRs) and vitamins (riboflavin) can be useful for the enhancement of plant productivity.

**Methods:** Treatments of indole acetic acid (IAA), gibberellic acid ($GA_3$) and riboflavin (50 mmol $L^{-1}$ each) were applied as foliar spray after 14 days of sowing on fenugreek variety Kasuri Methi. Experiment was arranged in a completely randomized design (CRD) with six replicates.

**Result:** Result showed that $GA_3$ and IAA has significantly enhanced the root and shoot development, foliage growth and ions concentrations, peroxidase (POD) and catalases (CAT) activities. PGRs also increased the pod and seed yield of fenugreek. Riboflavin did not show any significant effect on fenugreek except increased the antioxidant activities at seedling stage. $GA_3$ was more effective as compared to IAA for the enhancement biomass production, yield and biochemical attributes of fenugreek. It was determined that IAA and $GA_3$ can be used to enhance the foliage biomass production and yield but $GA_3$ is superior over IAA in fenugreek. These outcomes can be useful for economic benefits with high production of this leafy vegetable.


**Key words:** Fenugreek, Growth, PGRs, Vitamin, Yield.

## INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an annual leafy vegetable and oldest known cultivated medicinal plant that belongs to family Fabaceae (Khorshidian *et al*., 2016). Due to high concentration of niacin, ascorbic acid, protein and potassium present in fenugreek, it is considered as high nutritious vegetable (Mullaicharam *et al*., 2013). Fenugreek seeds and leaves are widely consumed in subcontinent of Indo-Pak as well as in many countries as a food and spices (Bukhari *et al*., 2008).

Plant growth regulators (PGRs) are natural substances produced in the plants as well as produced synthetically for exogenous application (Javid *et al*., 2011). PGRs control the cell differentiation and division, senescence of plants, root growth and shoot growth (Al-Whaibi *et al*., 2012). Among PGRs, IAA plays a vital role in the growth and development of plant. IAA is called key auxin which helps in control different physiological processes of plants for example cell division, cell enlargement, differentiation of tissue that show various responses to light and gravity (Spaepen *et al*., 2007). Since the time of discovery of gibberellic acid is consider as important substance to regulate or control the height of the plant and it stimulates the elongation and growth of the shoot. $GA_3$ promoted the growth of plant such as no. of leaves, leaf area and production of seed in cowpea (Emongor and Ndambole, 2011).

Vitamins are called basic growth regulators, because they are use in cellular metabolism of the plants. In numerous enzymatic reactions they play part as cofactor (Miret and Munne-Bosch, 2014) Vitamins have numerous biochemical and metabolic functions. Riboflavin indicates significant effect on physiological attributes, free amino acid, carbohydrates and protein contents (Azooz *et al*., 2013). Riboflavin (vitamin B2) is essential for the metabolism of nutrient and protection of antioxidant (Ashoori and Saedisomeolia, 2014).

In Pakistan, PGRs are not used in vegetables for crop improvements that's why less information is available to enhance the growth, yield and quality fenugreek by IAA and $GA_3$. For this purpose, this study was aimed to find out the efficacy of IAA, $GA_3$ and riboflavin in fenugreek for better productivity of this vegetable.

## MATERIALS AND METHODS

Pot experiments were performed at University of Gujrat,

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Pakistan during 2018-2019. Fenugreek seeds variety Kasuri Methi were obtained from Pakistan Agriculture Research council (PARC) Islamabad, Pakistan. Seeds were sown in pots containing 10kg sandy clayey soil. The soil was mixed with organic manure with 1:1 ratio; no synthetic fertilizer was used and no disease was observed during the experiment. Following treatments of IAA, $GA_3$ and riboflavin were applied as foliar spray in one dose after 14 days of sowing:

T0=control (water)
T1= IAA (50 mmol $L^{-1}$)
T2= $GA_3$ (50 mmol $L^{-1}$)
T3= Riboflavin (50 mmol $L^{-1}$)

The proposed concentrations were economically viable for fenugreek. Experiment was arranged in a completely randomized design (CRD) with six replicates. Four plants were taken for data collection at seedling stage (60 days after sowing) and vegetative (120 days after sowing) for biomass production (root length and shoot length, root dry biomass and shoot dry weights, numbers of branches and leaves, leaf area), photosynthetic pigments (chlorophyll "a" and "b" and carotenoids contents), antioxidants pursuits i.e. peroxidase and catalase. The number of pods and seed weight were determined at maturity.

Chlorophyll a, b and total chlorophyll and carotenoids were estimated by Arnon (1949) method. Total carbohydrates were estimated with the Anthrone method. Soluble protein was estimated following Bradford (1976). Estimation of POD and CAT activities was determined using Chance and Maehly (1955) procedure. Root and shoot dry samples about 1 g was digested with sulphuric acid and $H_2O_2$ by using the method Wolf (1982) for determination of ions of potassium ($K^+$), sodium ($Na^+$) and calcium ($Ca^{2+}$). Concentrations of $K^+$, $Na^+$ and $Ca^{2+}$ were measured by using flame photometer (model PFP7, Jenway Staffordshire, UK).

Minitab was used for analysis of data (Version: 19.2.0, Coventry, UK) and means values were compared using Tukey's Test.

## RESULTS AND DISCUSSION

### Root and shoot development

Results indicated that IAA, $GA_3$ and riboflavin had highly notable effect on root length and root fresh weight but it was non-significant for root dry weight in fenugreek (Table 1). Higher root length was observed by the treatment of 50 mmol $L^{-1}$ $GA_3$ at both growth stages. Higher root fresh biomass was noted by application of 50 mmol $L^{-1}$ riboflavin at seedling stage and higher root fresh biomass was present with 50 mmol $L^{-1}$ $GA_3$ at vegetative stage (Fig 1A-C). IAA and $GA_3$ effects were highly significant on shoot length and shoot fresh and dry weight in fenugreek but riboflavin had highly significant results for shoot length at seedling stage and shoot fresh weight at vegetative stage (Table 1). Higher shoot length and shoot fresh weight was observed with the treatment of 50 mmol $L^{-1}$ $GA_3$ at both growth stages (Fig 1A-C).

**Table 1:** Means squares (MS) from the Analysis of Variance (ANOVA) for root and shoot development of Fenugreek due to f IAA, $GA_3$ and Riboflavin.

| Source of Variance | df | Root length | | Root fresh weight | | Root dry weight | | Shoot length | | Shoot dry weight | | Shoot fresh weight | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Seedling stage | Vegetative stage | Seedling stage | Vegetative stage | Seedling stage | Vegetative stage | Seedling stage | Vegetative stage | Seedling stage | Vegetative stage | Seedling stage | Vegetative stage |
| IAA | 1 | 7.801*** | 98.350*** | 0.077** | 0.051*** | 1.125ns | 0.001ns | 14.178*** | 283.815** | 0.005** | 1.711** | 0.195** | 13.521*** |
| $GA_3$ | 1 | 7.22*** | 4.727** | 0.021** | 0.001** | 1.125ns | 5.281ns | 22.278*** | 263.925** | 0.001** | 2.101*** | 0.061 *** | 17.111*** |
| Riboflavin | 1 | 4.651** | 8.302 ns | 2.812** | 0.016* | 25.004ns | 2.812ns | 6.038** | 13.132 ns | 2E-04ns | 0.101ns | 0.007ns | 5.121*** |
| InteractionIAA× $GA_3$ | 1 | 0.101ns | 33.415** | 0.018ns | 0.002ns | 1.253ns | 3.781ns | 0.428 ns | 104.762 ns | 85.584ns | 0.245ns | 0.031ns | 0.501ns |
| IAA× Riboflavin | 1 | 0.72ns | 42.090** | 0.077** | 0.001ns | 25.004ns | 0.002* | 0.508*** | 906.315*** | 0.002ns | 2.645*** | 0.112ns | 6.661*** |
| $GA_3$×Riboflavin | 1 | 0.101ns | 49.252*** | 0.018ns | 0.002ns | 53.005ns | 3.781ns | 0.428ns | 104.762ns | 85.584ns | 0.245ns | 0.031ns | 0.501ns |
| IAA× $GA_3$×Riboflavin | 1 | 7.22*** | 11.640ns | 0.021ns | 0.001ns | 1.125ns | 5.281ns | 22.278*** | 263.925* | 0.001ns | 2.101*** | 0.061ns | 17.111*** |
| Error | 16 | 0.438 | 3.030 | 0.008 | 0.002 | 1.687 | 4.489 | 0.535 | 39.734 | 9.501 | 0.132 | 0.036 | 0.136 |
| Total | 23 | | | | | | | | | | | | |

ns= non-significant; *, **, *** = significant at P≤ 0.05, 0.01, 0.001.

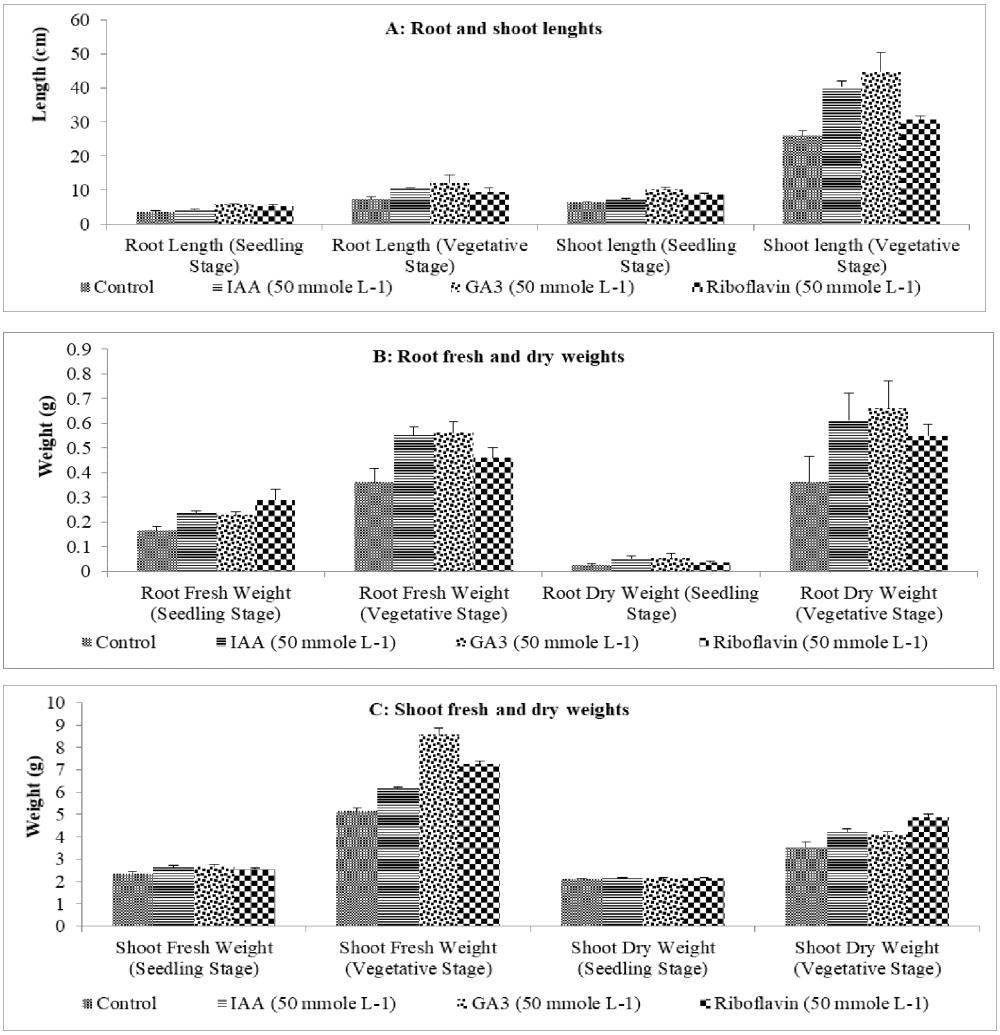


**Fig 1:** Effect of Indole acetic acid, Gibberellic acid and Riboflavin on morphological attributes of fenugreek at seedling and vegetative stages.

### Foliage growth

Foliage growth of fenugreek *i.e*. leaf area, no. of branches and leaves were significantly improved with the treatment of $GA_3$ and IAA and riboflavin (Table 2). It was noted that higher leaf area/plant was observed at growth (seedling stage) by 50 mmol $L^{-1}$ $GA_3$ and at vegetative stage was obtained by the treatment of 50 mmol $L^{-1}$ IAA (Fig 2A). Maximum number of leaves and branches was noted by 50 mmol $L^{-1}$ IAA at seedling stage. At vegetative stage, 50 mmol $L^{-1}$ riboflavin increased the number of leaves and 50 mmol $L^{-1}$ $GA_3$ enhanced the growth of branches (Fig 2B).

### Photosynthetic pigments

It was noted IAA and $GA_3$ improved contents of photosynthetic pigments in fenugreek *i.e*. chl. a, b and carotenoids while the effects of riboflavin was non-significant on photosynthetic pigments (Table 2). At seedling stage, higher chl-a was present at 50 mmol $L^{-1}$ of $GA_3$. However at vegetative stage, higher contents of chl-a and carotenoid was calculated with 50 mmol $L^{-1}$ $GA_3$ (Fig 2C). Higher carotenoid and chl-b contents were noted with the applications of 50 mmol $L^{-1}$ riboflavin (Fig 2C).

### Ion contents

It was observed that IAA, $GA_3$ and riboflavin had highly significant increasing effects on ion accumulations of potassium ($K^+$) and calcium ($Ca^{2+}$) in root and shoot of fenugreek except for sodium ($Na^+$) contents (Table 3). IAA applied in 50 mmol $L^{-1}$ showed the highest accumulation of potassium ($K^+$) and calcium ($Ca^{2+}$) ions in root and shoot of fenugreek at both growth stages except for $Na^+$ (Fig 3). Accretion of $Ca^{2+}$, $Na^+$ and $K^+$ was higher in shoot as compared to roots in fenugreek (Fig 3).

### Antioxidant activities

Results for antioxidant activities showed that peroxidase (POD) and catalases (CAT) were significant increased with the treatment level of gibberellic acid and indole acetic acid. Riboflavin had significantly increasing results for superoxide dismutase (SOD), POD and CAT at vegetative

**Table 2:** Means squares (MS) from the Analysis of Variance (ANOVA) for foliage growth and photosynthetic pigments of Fenugreek due to f IAA, $GA_3$ and Riboflavin.

| Source of Variance | df | Leaf area/plant | | Number of leaves/plant | | Number of branches/plant | | Chlorophyll-a | | Chlorophyll-b | | Carotenoid contents | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Seedling stage | Vegetative stage | Seedling stage | Vegetative stage | Seedling stage | Vegetative stage | 2.101** | 0.023* | 6.480** | 1.757** | 8.006* | 3.003* |
| IAA | 1 | 0.071** | 2.486** | 10.125** | 750.781*** | 9.031* | 12.501* | 3.781*** | 0.014** | 1.512** | 5.281* | 3.125* | 3.781* |
| $GA_3$ | 1 | 0.264** | 2.726*** | 32.001** | 87.781*** | 1.531*** | 24.512** | 1.125ns | 0.001ns | 1.280** | 2.257*** | 1.253ns | 1.378ns |
| Riboflavin | 1 | 0.062** | 0.064** | 28.125* | 148.781ns | 0.031ns | 2.531* | 6.050** | 0.003ns | 5.005ns | 7.812ns | 1.250ns | 7.812ns |
| Interaction IAA×$GA_3$ | 1 | 7.812* | 0.324ns | 18.101ns | 57.781ns | 2.531ns | 3.125ns | 8.041*** | 0.019ns | 1.250ns | 3.315*** | 3.231* | 2.278ns |
| IAA×Riboflavin | 1 | 0.075ns | 6.125*** | 15.125ns | 140.281ns | 9.031* | 45.120*** | 6.055** | 0.003ns | 5.005ns | 7.812ns | 1.250ns | 7.812ns |
| $GA_3$×Riboflavin | 1 | 7.812ns | 0.324ns | 18.101ns | 57.781ns | 2.531ns | 3.125ns | 3.781*** | 0.014ns | 1.512ns | 5.281ns | 3.125ns | 3.781ns |
| IAA×$GA_3$×Riboflavin | 1 | 0.264ns | 2.726ns | 32.001ns | 87.781ns | 1.531ns | 24.512** | 6.791 | 0.047 | 1.402 | 1.207 | 6.770 | 6.760 |
| Error | 16 | 0.081 | 0.379 | 15.145 | 43.968 | 1.156 | 2.583 | 2.101** | 0.023* | 6.480** | 1.757** | 8.006* | 3.003* |
| Total | 23 | | | | | | | | | | | | |

ns= non-significant; *,**, *** = significant at P≤ 0.05, 0.01, 0.001.

**Table 3:** Means squares (MS) from the Analysis of Variance (ANOVA) for ionic contents of Fenugreek due to f IAA, $GA_3$ and Riboflavin.

| Source of Variance | df | Sodium ($Na^+$) ions in root | | Sodium ($Na^+$) ions in shoot | | Potassium ($K^+$) ions in root | | Potassium ($K^+$) ions in shoot | | Calcium ($Ca^{2+}$) ions in root | | Calcium ($Ca^{2+}$) ions in shoot | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Seedling stage | Vegetative stage | Seedling stage | Vegetative stage | Seedling stage | Vegetative stage | Seedling stage | Vegetative stage | Seedling stage | Vegetative stage | Seedling stage | Vegetative stage |
| IAA | 1 | 1.579ns | 3.465ns | 9.735ns | 0.017ns | 6.133*** | 24.745*** | 0.023ns | 13.158** | 11.257*** | 15.521*** | 0.005*** | 0.087*** |
| $GA_3$ | 1 | 0.367ns | 0.106ns | 0.243ns | 0.425ns | 0.711*** | 4.651*** | 0.014** | 1.711* | 1.960*** | 2.856*** | 0.001** | 7.031* |
| Riboflavin | 1 | 0.596* | 0.487ns | 1.106* | 2.559* | 0.717*** | 12.325*** | 0.001 ns | 1.281ns | 3.962*** | 5.746*** | 0.002* | 0.039** |
| Interaction IAA×$GA_3$ | 1 | 0.058* | 0.338ns | 1.069*** | 0.538** | 0.664*** | 0.536*** | 0.003* | 1.557** | 0.465*** | 0.594** | 0.003** | 0.002* |
| IAA×Riboflavin | 1 | 0.005ns | 0.044ns | 0.226ns | 0.826* | 0.658*** | 0.387*** | 0.019* | 1.030ns | 0.008*** | 0.004ns | 0.005*** | 0.031*** |
| $GA_3$×Riboflavin | 1 | 0.058* | 0.338ns | 1.069*** | 0.538** | 0.664*** | 0.536*** | 0.003 ns | 1.557ns | 0.465*** | 0.594** | 0.003** | 0.002* |
| IAA×$GA_3$×Riboflavin | 1 | 0.367*** | 0.106* | 0.243ns | 0.425* | 0.711*** | 4.651*** | 0.014 ns | 1.711ns | 1.960*** | 2.856*** | 0.001ns | 7.031ns |
| Error | 16 | 0.008 | 0.021 | 0.064 | 0.040 | 0.024 | 3.187 | 0.047 | 1.520 | 4.020 | 0.051 | 3.625 | 4.718 |
| Total | 23 | | | | | | | | | | | | |

ns= non-significant; *,**, *** = significant at P≤ 0.05, 0.01, 0.001.

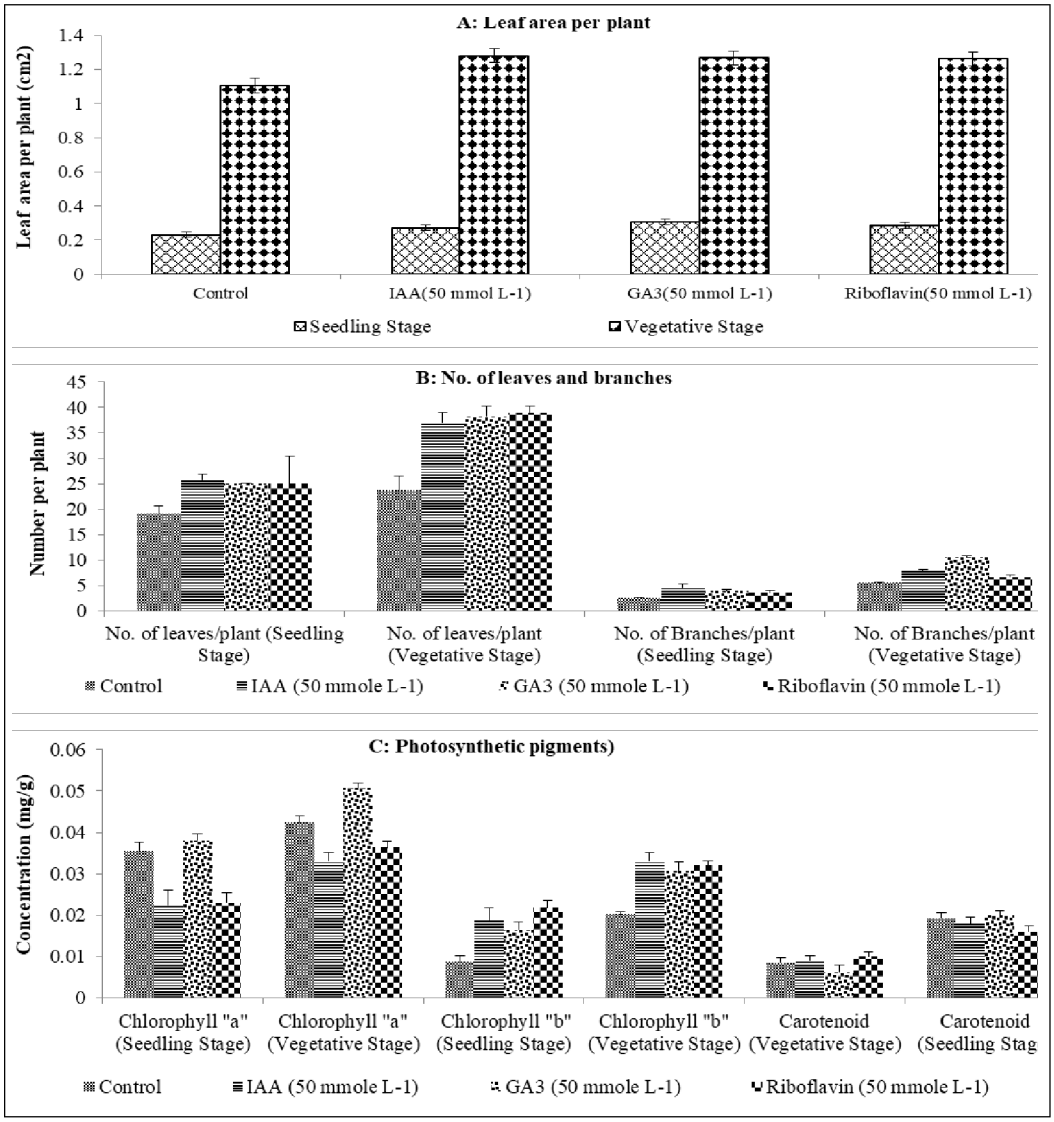


**Fig 2:** Effect of Indole acetic acid, Gibberellic acid and Riboflavin on various morphological and photosynthetic pigments of fenugreek at seedling and vegetative stages.

stage (Table 4). POD and catalases activities were increased by the treatment of 50 mmol $L^{-1}$ of $GA_3$ as compared to other treatment at seedling stage. SOD activity was increased at vegetative stage by 50 mmol $L^{-1}$ of $GA_3$ while POD and CAT activities were improved by 50 mmol $L^{-1}$ of IAA at vegetative stage (Fig 4A).

### Yield attributes

ANOVA revealed that the application of $GA_3$ and IAA had significant results for number of pod and seed yield per plant of fenugreek while, there was non-significant effect by the application of riboflavin (Table 4). In most of cases, interactions were also non-significant except IAA× $GA_3$. Number of pods per plant were improved by the application of 50 mmol $L^{-1}$ IAA while, higher seed yield was obtained by the application of 50 mmol $L^{-1}$ $GA_3$ (Fig 4B-C).

As results indicated that IAA and $GA_3$ enhanced the biomass production including root and shoot development and foliage growth. It was due to the auxins that regulated numerous physiological processes *e.g*. tissue differentiation, cell division and cell enlargement (Spaepen *et al*., 2007). Correspondingly, Sevik and Guney (2013) described that $GA_3$ and IAA applications have improved the length of root and shoot length in *Melissa officinalis*. In many studies, it was noted that IAA and $GA_3$ are effective to increase the plant productivity (root and shoot development) in Arabidopsis (Zúñiga *et al*., 2013). In present study riboflavin also enhanced the root and shoot growth only for few cases. El-lethy *et al*. (2011) stated that riboflavin enhanced that root and shoot fresh biomass by foliar application in *Pelargonium graveolens* and *Hibiscus sabdariffa.* It was also noted by noted Sharma *et al*. (2017) that 1.86 mg/L NAA was useful for shoot development in pea. Chl. content was enhanced by IAA treatment in barley (Eleiwa *et al*., 2013). Similarly,

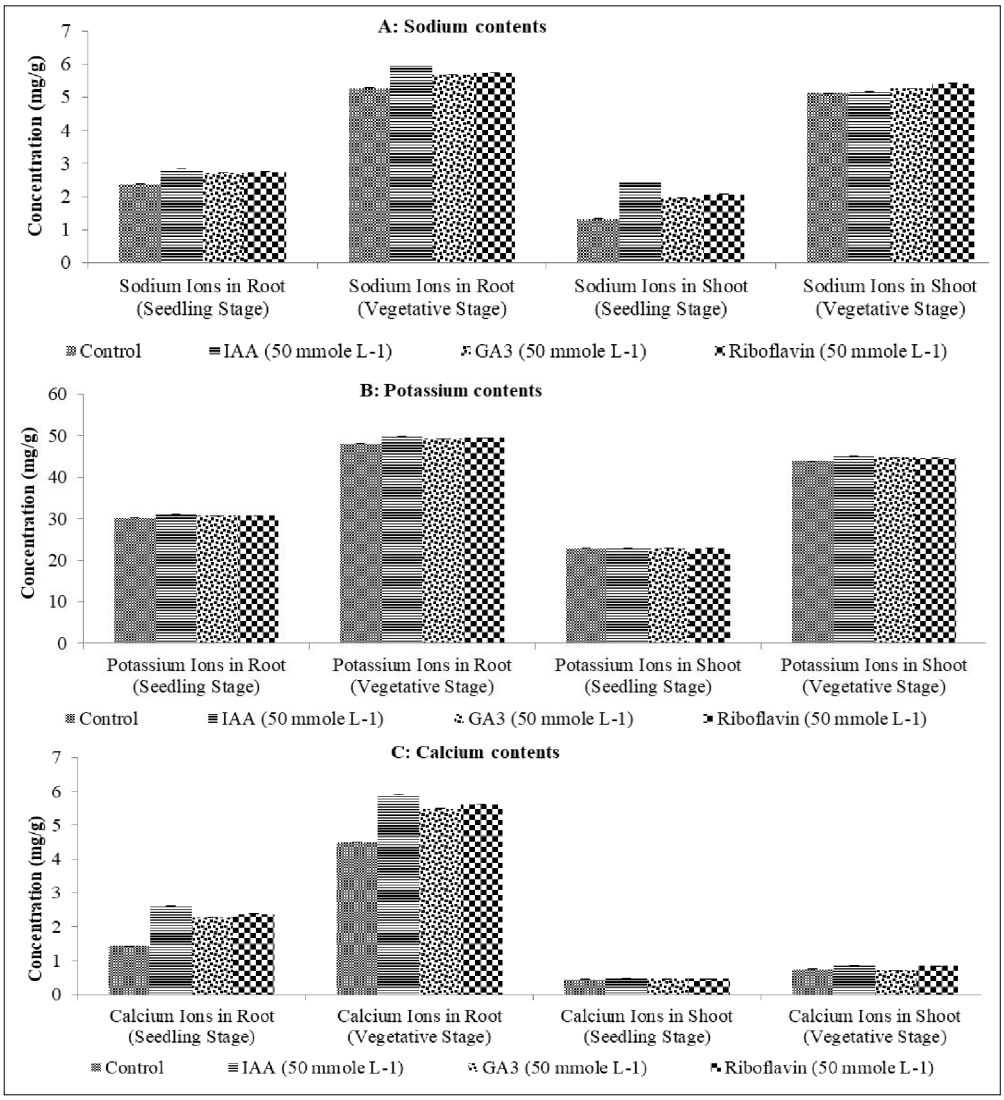


**Fig 3:** Effect of Indole acetic acid, Gibberellic acid and Riboflavin on ionic contents in fenugreek at seedling and vegetative stages.

**Table 4:** Means squares (MS) from the Analysis of Variance (ANOVA) for antioxidant activities and yield of fenugreek due to f IAA, $GA_3$ and Riboflavin.

| Source of Variance | df | SOD at seedling stage | SOD at vegetative stage | POD at seedling stage | POD at vegetative stage | CAT at seedling stage | CAT at vegetative stage | Number of Pods/ plant | Seed yield/ plant |
|---|---|---|---|---|---|---|---|---|---|
| IAA | 1 | 0.005ns | 1.402ns | 0.016 * | 0.475* | 0.017* | 0.088* | 0.0281* | 0.781* |
| $GA_3$ | 1 | 5.821ns | 0.036ns | 9.112 ** | 0.060* | 0.005* | 0.022* | 9.031* | 3.781** |
| Riboflavin | 1 | 0.002ns | 0.537* | 5.951ns | 0.045* | 0.002ns | 0.019* | 11.281ns | 0.031ns |
| Interaction IAA×$GA_3$ | 1 | 9.802ns | 0.050ns | 0.008ns | 0.056* | 7.801ns | 0.006* | 3.781* | 0.281* |
| IAA×Riboflavin | 1 | 9.921ns | 0.100ns | 0.011ns | 0.073* | 0.003ns | 0.007* | 69.031ns | 7.031ns |
| $GA_3$×Riboflavin | 1 | 9.802ns | 0.050ns | 0.001ns | 0.056* | 7.801ns | 0.006** | 3.781ns | 0.281ns |
| IAA×$GA_3$×Riboflavin | 1 | 5.281ns | 0.036ns | 0.012ns | 0.060* | 0.005ns | 0.002** | 9.031ns | 3.781ns |
| Error | 16 | 0.003 | 0.627 | 0.013 | 2.533 | 0.002 | 3.756 | 4.177 | 1.781ns |
| Total | 23 | | | | | | | | |

ns= non-significant; *,**, *** = significant at P≤ 0.05, 0.01, 0.001.

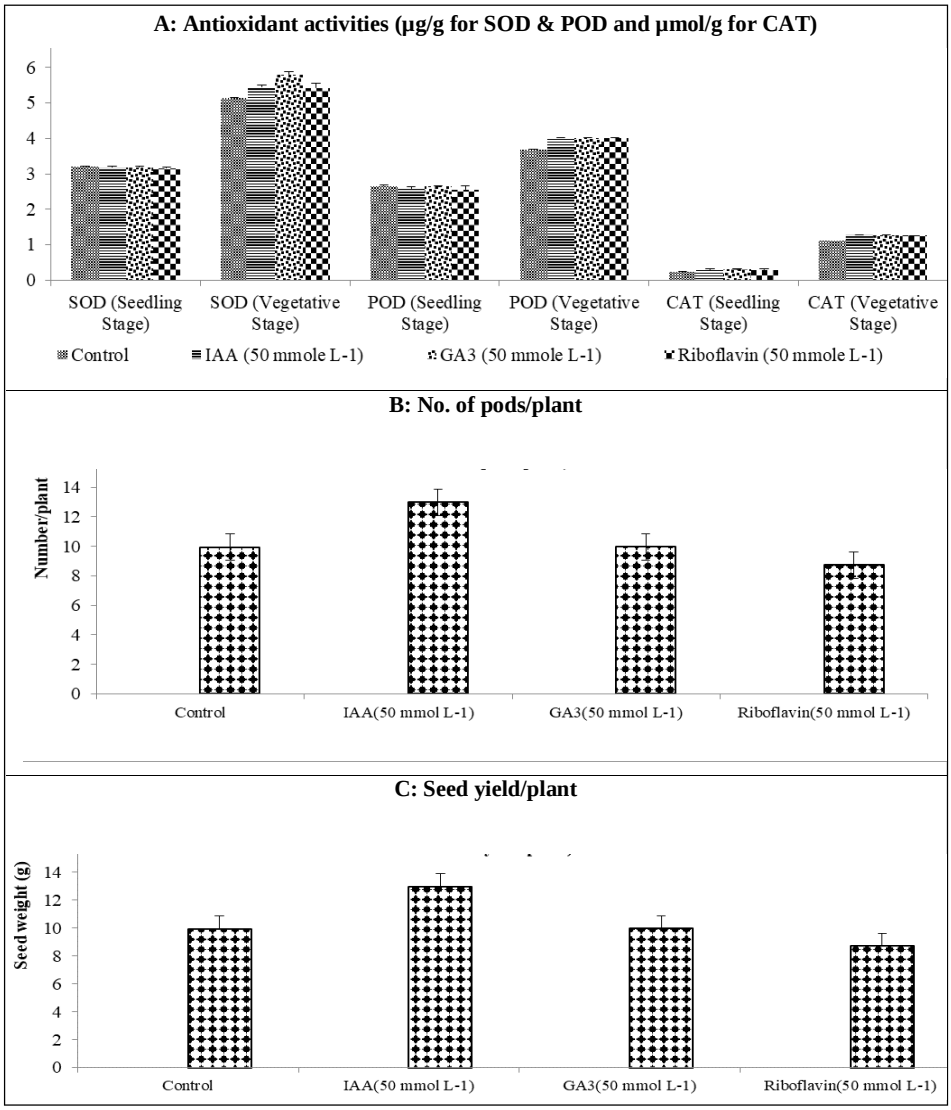


**Fig 4:** Effect of Indole acetic acid, Gibberellic acid and Riboflavin on antioxidant activities and yield of fenugreek.

Afroz *et al*. (2005) noted that $GA_3$ increased chlorophyll carotenoid contents in mustard. Riboflavin enhanced the contents of chlorophyll with foliar spray in *Pelargonium graveolens* (El-lethy *et al*., 2011).

Results showed that IAA and $GA_3$ exerted positive effect in increasing the conc. of ion contents in root and shoot of fenugreek. Ions concentration *i.e*. Ca, K, Cl and Na was enhanced by the application of indole acetic acid in maize and date palm (Kaya *et al*., 2010). Change in antioxidant activities resulted strong defense mechanism and to cope under any stress conditions. Ali *et al*. (2014) reported that enzyme activities such as SOD, POD and CAT was improved by IAA in rose. Similarly, Mohammadi *et al.* (2014) noted that IAA also enhanced that antioxidant enzyme activities such as catalase and peroxidase in okra. Similarly, riboflavin exerts regulatory effect on activities of antioxidant in *Pelargonium graveolens* (El-lethy *et al*., 2011). Similarly, Pal (2019) showed faster plant growth, plant yield and quality for garden peas when treated with PGR, particularly IAA and NAA. Increase in yield and seed quality were noted by the applications of IAA and $GA_3$ (Khandaker *et al.,* 2018).

## CONCLUSION

It was concluded that gibberellic acid ($GA_3$) and indole-3-acetic acid (IAA) can be used to enhance the foliage biomass production and yield but $GA_3$ is superior over IAA in fenugreek. These outcomes can be useful for economic benefits with high production of this leafy vegetable.

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