Changes in Growth and Trigonelline/Mucilage Production of Fenugreek (*Trigonella foenum- graecum* L.) under Plant Growth Regulators Application

Danesh Talab S (M.Sc.)[1], Mehrafarin A (Ph.D.)[2], Naghdi Badi H (Ph.D.)[2*], Khalighi-Sigaroodi F (Ph.D.)[3]

1- Department of Horticulture, Karaj branch, Islamic Azad University, Karaj, Iran
2- Cultivation & Development Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran
3- Pharmacognosy & Pharmaceutics Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran
* Corresponding author: Cultivation & Development Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, P.O.Box (Mehr Villa): 31375-1369, Karaj, Iran
Tel: +98-26-34764010-9, Fax: +98-26-34764021
Email: Naghdibadi@yahoo.com

Received: 12 Sep. 2013 **Accepted:** 7 Oct. 2013

Abstract

Background: Fenugreek (*Trigonella foenum - graecum* L.) is an annual medicinal plant belongs to the legume family and has anti-diabetic, anti-fertility, anticancer, anti-microbial, anti-parasitic and hypocholesterolaemic effects.

Objective: Evaluation of growth and trigonelline /mucilage content in fenugreek under application of plant growth regulators (PGR).

Methods: The experiment was conducted on randomized complete blocks design (RCBD) with 13 treatments and 3 replications. The treatments were consist of control (distilled water application), Giberrellic Acid (GA_3) 25 and 50 ppm and Naphthalene Acetic Acid (NAA) 25 and 50 ppm (for each concentration by soaking the seeds before planting, spray after planting, and soaking seeds before planting with spray after planting).

Results: Application of plant growth regulators significantly increased trigonelline and mucilage content of seed, shoots and pods dry weight per plant and 1000-seed weight. Application of plant growth regulators had no significant effect on the SPAD value. Mean comparisons showed that the highest shoot dry weight was related to NAA, GA_3 50 ppm (soaking seeds before planting with spray after planting), and also the highest pods dry weight, 1000-seed weight, mucilage and trigonelline content of seeds were obtained from GA_3 50 ppm (soaking the seeds before planting with spray after planting).

Conclusion: Application of GA_3 50 ppm as the soaking seeds before planting with spray after planting are effective to obtain maximum trigonelline and mucilage content in fenugreek.

Keywords: ***Trigonella foenum-graecum*** **L., Gibberellic acid, Naphthalene acetic acid, Trigonelline and mucilage content**

Introduction

Medicinal plants are rich source of secondary metabolites such as trigonelline alkaloid and mucilage which are potential source of drugs. Fenugreek is an annual crop belongs to the legume family. This crop is native to an area extending from Iran to northern India, but is now widely cultivated in China, north and east Africa, Ukraine and Greece [1, 2]. Due to its strong flavor and aroma, fenugreek in one of such plants whose leaves and seeds are widely consumed in Indo-Pak subcontinent as well as in other oriental countries as a spice in food preparations, and as an ingredient in traditional medicine [3]. Mucilage and trigonelline are considered as part of secondary metabolites fenugreek which are used in the treatment many diseases. Fenugreek is reported to have anti-diabetic [4, 5, 6, 7, 8, 9], anti-fertility, anticancer, anti-microbial, anti-parasitic and hypocholesterolaemic effects [10].

PGR are substances when used in small amount, modify substantially the growth of plant, both stimulatory and inhibitory. Plant growth regulators are small organic compounds that influence physiological responses of plant to environmental stimulants at very low concentration (generally less than 10^{-7}M). They are used extensively in agriculture, and biotechnology to modify plant growth and development. Auxins were the first plant hormones discovered. Naphthalene acetic acid is one of the synthetic auxins [11]. An increase in number of pods and grains pod^{-1} in gram was observed with 25 ppm NAA [12], seed and pod weight was increased with foliar application of 25 - 50 ppm NAA to chickpea thrice at 5-days interval, beginning at flowering stage [13]. Planofix (NAA) increased number of pods plant^{-1}, dry pod yield and 100 seed weight in groundnut (40 and 50 days after sowing) [14]. Giberrellic acid is one of the most important growth stimulating substances used in agriculture. Giberrellic acid is a chemical substance that occurs naturally in many plants. It regulates various important functions such as elongation of stems, creation of proteins and germination of seed plants [15]. Earlier studies have reported that GA application (at 50, 100, and 500 g m^{-3}) as foliar spray on transplanted cuttings increased plant height [16]. There are numerous physiological effects of auxin in plants that include: cell elongation, phototropism, geotropism, apical dominance, roots begin, ethylene production and root developmental as well as gibberellin stimulated cell division and cell elongation [17]. In some recent research, PGRs have been shown to improve herb yield in fenugreek [18], basil [19] and coriander [20, 21].

The objective of this research was to elucidate further the effect of PGRs and methods of their application on dry matter production and mucilage and trigonelline content of fenugreek seeds.

Materials and Methods

This experiment was carried out in 2011-12 at Institute of Medicinal Plants (ACECR) (56° 35′ N and 50° 58′ E; 1500 m elevation). The soil was loam-silt with N (0.071%), Phosphorous (48.9 mg.kg^{-1}), Potassium (33.6 mg.kg^{-1}), EC (2.71 ds.m^{-1}), and pH (8.3).

This study was done on the base of Randomized Complete Block Design (RCBD) with 13 treatment and 3 replications. The details of the treatments are mentioned in table 1.

Table 1- Treatments used in the experiment

Code	Treatments
C	Control :soaking seeds before planting and spray after planting with distilled water.
G_2D	GA_3 25 ppm: soaking* the seeds before planting.
G_2DS	GA_3 25 ppm: soaking seeds before planting with spray after planting.
G_2S	GA_3 25 ppm: spray** after planting.
G_5D	GA_3 50 ppm: soaking the seeds before planting.
G_5DS	GA_3 50 ppm: soaking seeds before planting with spray after planting.
G_5S	GA_3 50 ppm: spray after planting.
N_2D	NAA 25 ppm: soaking the seeds before planting.
N_2DS	NAA 25 ppm: soaking seeds before planting with spray after planting.
N_2S	NAA 25 ppm: spray after planting.
N_5D	NAA 50 ppm: soaking the seeds before planting.
N_5DS	NAA 50 ppm: soaking seeds before planting with spray after planting.
N_5S	NAA 50 ppm: spray after planting.

* Pre-plant soaking the seeds was for 8 hours in a solution with the desired concentration of growth regulators [21].
** Spraying the growth regulators on plants after planting (Spraying 20 days after sowing): the spraying was done until the drops start to fall [21]

Twenty seeds were sown at each pot. PGRs were sprayed in three stages including: 20, 32 and 44 days after planting. All operations were done regularly during the growing season and until before flowering. Plants were harvested at full maturity pods.

In order to measure total dry matter, some plants were selected randomly from each pot and then were placed in the electric oven of 75°C until the constant weight was gained. Morphological characteristics including shoot dry weight per plant (g), pod dry weight per plant (g) and 1000 seed weight (g) and phytochemical characteristics including content of mucilage (%) and trigonelline (mg g^{-1} DW) in seeds and SPAD value (SPAD) were determined.

Isolation and extraction of mucilage

Fenugreek seeds (200 g) were soaked in distilled water (1.5 L) at room temperature for 1h and then boiled under stirring condition in a water bath until the slurry was prepared. The solution was cooled and kept in a refrigerator overnight to settle out undissolved materials. The upper clear solution was decanted and centrifuged at 500 rpm for 20 minutes. The supernatant was separated and was concentrated at 60°C on a water bath to one third of its original volume. The solution was cooled to the room temperature and was poured into thrice the volume of acetone with continuous stirring. The precipitate was washed repeatedly with acetone and dried at 50 - 60°C under vacuum. The dried material was powderd and kept in a desiccator [22].

Quantitative analysis of trigonelline

For measurement of trigonelline in the seed samples, the Zheng and Ashihara method (2004) [23] was modified. The samples were ground with 80% methanol and magnesium oxide (MgO) in a mortar and pestle. After incubation at 60°C for 30 min, the homogenates were centrifuged and the supernatant was collected. After complete evaporation of methanol, the methanol-soluble extracts were dissolved in distilled water. The samples were filtered using a disposable syringe filter unit and the aliquots were used for determination of trigonelline (TG) by HPLC. The analyses of the samples were

carried out using a Knauer K2600A liquid chromatography (Germany), equipped with a Nucleosil C_{18} (150 mm × 4.6 mm I.D, 5 µm) column. A mixture of methanol: water (50:50 v/v) served as the mobile phase and pH of solution adjusted to 5.0 with 50 mM sodium acetate. The elution has been made in an isocratic mode at a flow rate of 1 mL. min^{-1} and the detection made at 268 nm by UV detector from the above mentioned company [24; 25]. One analysis requires 20 min. The retention time of this alkaloid was 4.4 min. Before carrying out HPLC analysis, we made calibration curve by using different concentration (0.1, 0.2, 0.5, 0.7 and 1.0 $mg.mL^{-1}$) of trigonelline in phase media. Then calibration curve made with trigonelline and the correlations were excellent for trigonelline (Figure 1). This process was performed according to United States Pharmacopoeia by cold extraction method as directed for alcohol soluble material, except to use water in place of alcoho [26].

Data analysis using SPSS software and the least significant difference (LSD) mean comparison test was performed.

Results

Plant growth and phytochemical characteristics were significantly influenced by PGRs and their method of application. Analysis of variance indicated that application of PGRs had significantly effects ($p \leq 0.01$) on shoot dry weight (Table 2) and highest shoot dry weight was observed in GA_3 50 ppm (soaking seeds before planting with spray after planting) and the lowest was obtained in control (Figure 2). The PGRs have significantly affected ($p \leq 0.01$) on 1000-seed weight and trigonelline content of seed (Table 2). Also, the pods dry weight and mucilage content were significantly different ($p \leq 0.05$) (Table 2). The highest pods dry weight, 1000-seed weight and trigonelline content were observed in GA_3 (soaking seeds before planting with spray after planting) and the lowest was obtained in control (Figure 3, 4, 5 and 6, respectively). Application of plant growth regulators had no significant effect on the SPAD value (Table 2).

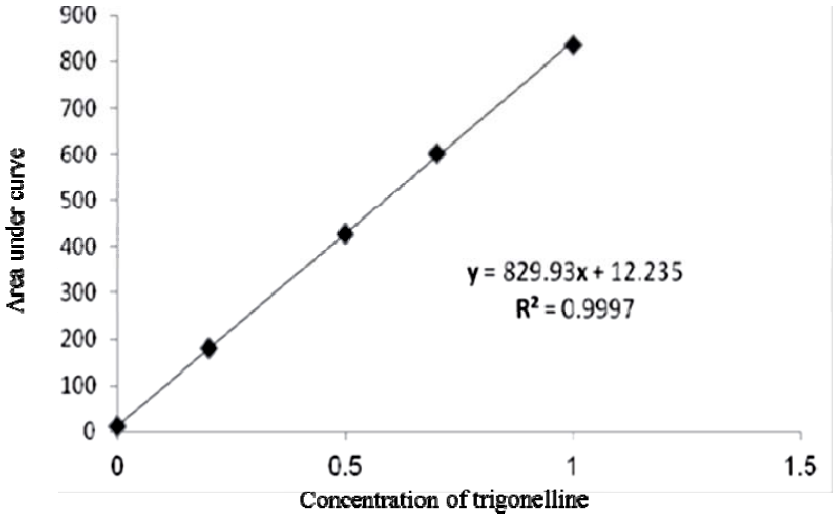

Figure 1- Calibration curve using trigonelline as standard

Table 2 - Analysis of variance for dry matter and trigonelline and mucilage content in fenugreek

SOV	df	Shoot dry weight per plant	Pods dry weight per plant	1000-seed weight	Mucilage content	Trigonelline content of seeds	SPAD Value
Block	2	0.00012	0.000031	0.049	79.241	1.943	8.110
Treatment	12	0.00077**	0.00010*	1.873**	21.250*	19.471**	81.664^{ns}
Error	24	0.00013	0.000038	0.481	9.133	5.521	59.051
CV (%)	-	8.65	12.85	10.13	13.92	12.42	17.98

*, **, ns shows significant at 5%, 1% and no significant, respectively

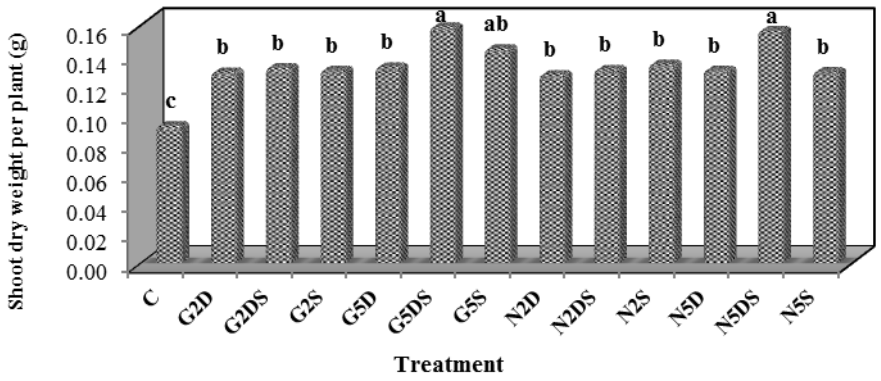

Figure 2- Effect of PGRs on shoot dry weight per plant of fenugreek by LSD test at 5% level of significance

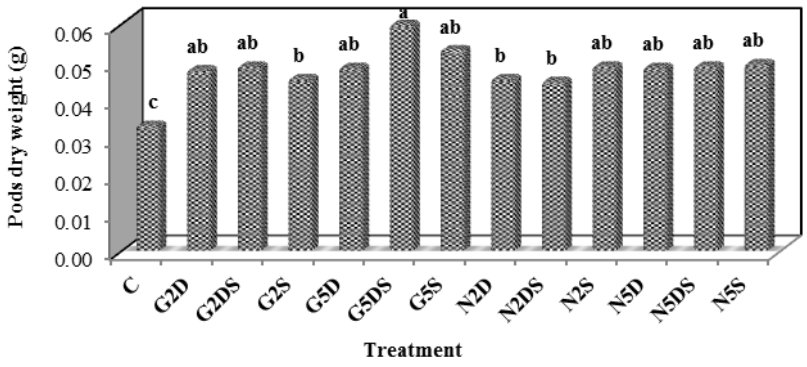

Figure 3- Effect of PGRs on pods dry weight per plant of fenugreek by LSD test at 5% level of significance

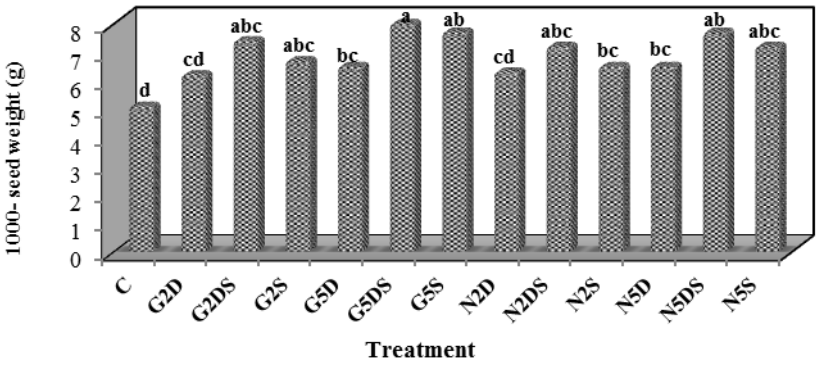

Figure 4 - Effect of PGRs on 1000-seed weight of fenugreek by LSD test at 5% level of significance

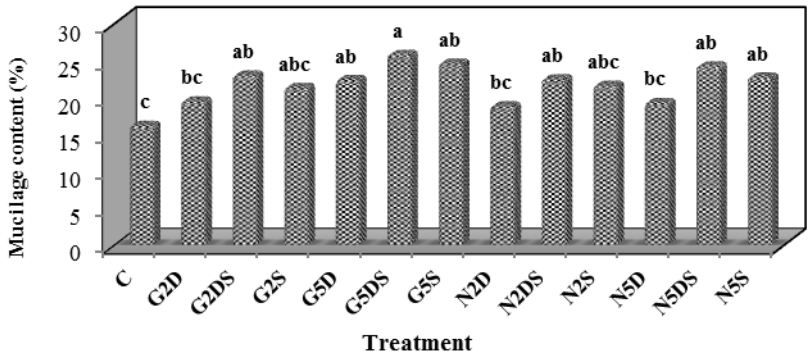

Figure 5 - Effect of PGRs on mucilage content of seed of fenugreek by LSD test at 5% level of significance

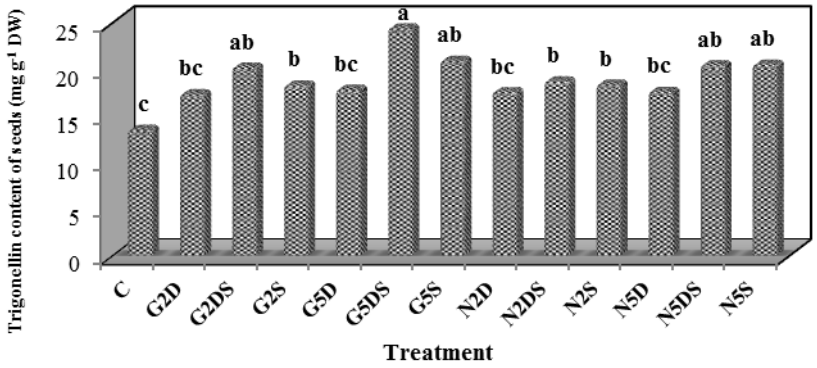

Figure 6 - Effect of PGRs on trigonelline content of seed of fenugreek by LSD test at 5% level of significance

Discussion

Plant growth regulators (PGRs) have crucial impact on primary and secondary metabolism of plants [27]. Among PGR_S there is strong evidence that GA_3 had constant effects on plants growth and development, and consequently their active principles content and yield. Application of 100 mg L^{-1} GA resulted in higher essential oil content of *Salvia officinalis* L. compared to control [28]. It is possible that GA_3 had the potential to accelerate the nutrients partitioning towards cells and active growth sites and consequently increases those nutrients absorption via increased root potential [27]. The phytochemical quantitative estimation of the fenugreek showed that the seeds were rich in trigonelline alkaloid and mucilage. GA enhances metabolic activity within pathways leading to accumulation of secondary metabolites, *e.g*. steroids [29], anthocyanin [30], and essential oil (terpenoid) production [31]. Srivastava and Srivastava (2007) reported that total alkaloid contents in leaves, stems, and roots of GA-treated plants (*Catharanthus roseus*) were significantly higher than in untreated plant parts [32]. Subroto and Doran (1994) reported that GA_3 positive effect in improvement the accumulation of steroidal alkaloids in the *Solanum aviculare* [33].

GA_3 is known to induce an influx of Ca^{2+} into the endoplasmic reticulum of guard cells, thereby initiating a process that leads to increase in stomatal activity [34]. A less stomatal resistance enables an easier exchange of gases [35]. With increasing CO_2, photosynthesis was increased and subsequently was improved performance such as seeds. In general, the GA_3 treatment may have also strengthened the sink potential of the developing pods and through enhancement of the duration rate of assimilate translocation to these reproductive structures caused the observed increase in pod dry weight and 1000-seed. It is during this critical growth phase that the basic infrastructure of the plant functioning is laid down, the effective dividends of which are reaped when the plant reaches harvest [36, 37]. Higa (1972) reported that application of auxin and gibberellin improves the evolutionary process of flowers and increased fruit set in saffron [38]. Vijayaraghavan (1999) showed that the grain yield of pearl millet (*Pennisetum glaucum* L.) was increased by the soaking seeds in GA_3 before planting [39]. The soaking seeds in GA_3 before planting of *Cucurbita pepo* L. and herb spray with NAA subsequently resulted maximum number of seeds per fruit [40].

Naphthalene acetic acid (NAA), a synthetic growth regulator is known to affect the growth, development and other physiological and biochemical processes of plants [41, 42, and 43]. Investigations in other countries have shown that foliar application of NAA improved yield, nutrient content and uptake in cotton [44], tomato [45], greengram [46] and fenugreek [47, 48]. The increased herb growth and yield resulting from PGR application could be due to the stimulation of cell division and elongation while increasing plasticity of cell wall [49, 50]. Similar results on herb growth and yield of celery (*Apium graveolens*) were noted by Mishriky [21, 51]. Plant growth and seed yield increased in fenugreek when phosphorus was applied 60 $kg.ha^{-1}$ and sprayed with naphthalene acetic acid (NAA) 20 ppm [52]. Varna (1990) showed that foliar application of plant growth regulators (NAA and CEPA) on cucumber seedlings increased the performance [53].

In general, maximum plant performance (pods dry weight, shoot dry weight and 1000-seed weight) and plant metabolites (mucilage and trigonelline content of seed per plant)

were obtained with soaking seeds before planting and spray after planting. The overall result is in conformity to the findings that plant growth regulators at different concentrations can have quite different effects in different plants and sometimes the same plant growth regulator at identical concentrations can have different effects on plant.

Conclusion

In this study, plant growth regulators (GA_3 50 ppm and NAA 50 ppm) had significantly positive effect on dry matter and mucilage and trigonelline content of fenugreek seeds. The results showed that the treatments of GA_3 50 ppm and NAA 50 ppm as soaking of seed before planting and plant spraying 20 days after planting, improved vegetative growth and mucilage and trigonelline content of fenugreek seeds. Ultimately, it can be concluded that seed priming with plant growth regulators had the positive effect on germination and plant establishment. Also, foliar application of plant growth regulators has a positive effect on synthesis of secondary metabolites.

References

1. Petropoulos GA. Fenugreek - The genus *Trigonella*, Taylor and Francis, London and New York. 2002.

2. Acharya S, Srichamroen A, Basu S, Ooraikul B and Basu T. Improvement in the nutraceutical properties of fenugreek (*Trigonella foenum - graecum* L.) Songklanakarin. *J. Sci. Technol*. 2006. 28 (1): 1 – 9.

3. Bukhari B, Bhanger M and Memon Sh. Antioxidative activity of extracts from fenugreek seeds (*Trigonella foenum-graecum*). *Pak. J. Anal. Environ. Chem.* 2008; 9 (2): 78 - 83.

4. Broca C, Breil V, Cruciani-Guglielmacci C, Manteghetti M, Rouault C, Derouet M, Rizkalla S, Pau B, Petit P, Ribes G, Ktorza A, Gross R, Reach G and Taouis M. The insulinotrophic agent 1D1101 (4-hydroxyisoleucine) activates insulin signaling in rat. *Am. J. Physiol. Endocrinol. Metab*. 2004; 287 (3): 463 - 71.

5. Devi BA, Kamalakkannan N and Prince PS. Supplementation of fenugreek leaves to diabetic rats-effect on carbohydrate metabolic enzymes in diabetic liver and kidney. *Phytother. Res*. 2003; 17 (10): 1231 - 3.

6. Hannan JM, Rokeya B, Faruque O, Nahar N, Mosihuzzaman M, Azad Khan AK and Ali L. Effect of soluble dietary fibre fraction of *Trigonella foenum-graecum* on glycemic, insulenimic, lipidemic and platelet aggregation status of Type 2 diabetic model rats. *J. Ethnopharmacol.* 2003; 88: 73 - 7.

7. Tahiliani P and Kar A. The combined effects of *Trigonella* and *Allium* extracts in the regulation of hyperthyroidism in rats. *Phytomed*. 2003; 10 (8): 665 - 8.

8. Thakaran S, Salimuddin and Baquer NZ. Oral administration of orthovandate and *Trigonella foenum-graecum* seed powder restore the activities of mitochondrial enzymes in tissues of alloxan-induced diabetic rats. *Mol. Cell Biochem*. 2003; 247 (1 - 2): 45 - 53.

9. Vats V, Yadav SP and Grover JK. Effect of *Trigonella foenum-graecum* on glycogen content of tissues and the key enzymes of carbohydrate metabolism. *J. Ethnopharmacol.* 2003; 85 (23): 237 - 42.

10. Al-Habori M and Raman A. Pharmacological Properties in Fenugreek-The genus *Trigonella* (1st edition) by G.A. Petropoulos (ed.), Taylor and Francis, London and New York. 2002; 10: 163 - 82.

11. Theint K and Htwe T. Synthesis of NAA (2- (1-Naphthyl) ethanoic Acid) from coal tar and application of plant hormone with soy bean and cow pea in aqueous medium. *International Conference on Chemical Processes and Environmental Issues (ICCEEI'2012).* 2012, pp: 15 - 6.

12. Bangal DB, Deshmukh SN and Patil VA. Note on the effect of growth regulators and urea on yield and yield attributes of gram (*Cicer arietinum* L.) Legume Res. 1982; 5 (1): 54 - 6.

13. Bangal DB, Deshmukh SN and Patil VA. Contribution of pod-wall in grain development of chickpea (*Cicer arietinum* L.) as influenced by foliar application growth regulators and urea. *Indian J. Pant Physiol*. 1983; 26 (3): 292 - 5.

14. Singh GS and Sharma B. Effect of plant growth regulators on groundnut productivity. *India J. of Ecol*. 1982; 9 (2): 281 – 5.

15. Yahaya SU and Gaya UH. Response of tomato (*Lycoperiscon Lycopersicum* (L) Karst) to various rates of giberrellic acid. *Bayero J. Pure and Applied Sci*. 2012; 5 (1): 33 – 7.

16. Sadowska A, Racka M, Staszkiewicz J and Sadowska M. Effect of some growth substances upon rooting of cuttings, yield and alkaloid content of *Catharanthus roseus. Acta Hort. (ISHS)*. 1984; 144: 99 - 102.

17. Arteca RN. Plant growth substances: principles and applications. Fathi G, Ismail pour B. *Jihad, Mashhad University Press*. 1950.

18. Deore BP and Bharud RW. Growth, yield and storability of fenugreek as influenced by foliar spray of growth substances. *J. Maharashtra Agricult. Univ.* 1990. 15 (2): 208 – 10.

19. Arularasu P and Sambandamurthi S. Effect of gibberellic acid, nitrogen and spacing on herbage yield and oil yield in *tulsi* (*Ocimum sanctum* L.). *South Indian Horticult.* 1999; 47: 370 – 2.

20. Badgujar CD and Warhal KN. Effect of seed soaking and wrapping on growth and yield of vegetable coriander. *J. Maharashtra Agricultural Univ.* 1988; 13 (3): 344 – 65.

21. Verma P and Sen NL. The impact of plant growth regulators on growth and biochemical constituents of coriander (*Coriandrum sativum* L.). *Journal of Herbs, Spices and Medicinal Plants* 2008; 14 (3 - 4): 144 - 53.

22. Sabale V, Patel V, Paranjape A and Sabale P. Isolation of fenugreek seed mucilage and its comparative evaluation as a binding agent with standard binder. *International Journal of Pharmaceutical Research* 2009; 1 (4): 56 - 62.

23. Zheng XQ and Ashihara H. Distribution, biosynthesis and function of purine and pyridine alkaloids in coffea arabica seedlings. *Plant Sci*. 2004; 166: 807 - 13.

24. Koshiro Y, Zheng XQ, Wang M, Nagai C and Ashihara H. Changes in content and biosynthetic activity of caffeine andtrigonelline during growth and ripening of coffea arabica and coffea canephora fruits. *Plant Sci*. 2006; 171: 242 - 50.

25. Mehrafarin A, Qavami N, Naghdi Badi H

and Qaderi A. Trigonelline alkaloid, avaluable medicinal metabolite plant. *J. Medicinal Plants* 2012; 8 (11): 12 - 29.

26. Mehrafarin A. Effect of methanol and nano-iron chelate fertilizer application on agronomical and medicinal yield of fenugreek (*Trigonella foenum-graecum* L.). Ph.D. tessise. 2013.

27. Hassanpouraghdam MB, Hajisamadi ASL B and Khalighi A. Gibberellic acid foliar application influences growth, volatile oil and some physiological characteristics of lavender (*Lavandula officinalis* Chaix.). *Romanian Biotechnological Letters* 2011; 16 (4): 6322 - 7.

28. Povh JA and Ono EO. Rendimento do oleo essencial de *Salvia officinalis* L. sob acao de reguladores vegetais. *Acta Sci. Biol. Sci*. 2006; 28: 189 - 93.

29. Ohlsson AB and Bjork L. Effects of gibberellic acid on cardenolide accumulation by *Digitalis lanata* tissue cultures grown in light and darkness. *J. Plant Physiol*. 1988; 133: 535 - 8.

30. Weiss D, Van Blokland R, Kooter JM, Mol JNM and Van Tunen AJ. Gibberellic acid regulates chalcone synthase gene transcription in the corolla of *Petunia hybrida*. *Plant Physiol*. 1992; 98: 191 - 7.

31. Singh P, Srivastava NK, Misra A and Sharma S. Influence of etherel and gibberellic acid on carbon metabolism, growth and essential oil accumulation in spearmint (*Mentha spicata*). *Photosynthetica* 1999; 36: 509 - 17.

32. Srivastava NK and Srivastava AK. Influence of gibberellic acid on $^{14}CO2$ metabolism, growth, and production of alkaloids in *Catharanthus roseus*. *Photosynthetica* 2007; 45 (1): 156 - 60.

33. Subroto MA and Doran PM. Production of steroidal alkaloids by hairy roots of solanum aviculare and the effect of gibberellic acid. *Plant Cell Tissue Organ Cult*. 1994; 38: 93 - 102.

34. Assmann SM and Armstrong F. Hormonal regulation of ion transporters: the guard cell system. In: Hooykaas P.J.J. Hall M.A. Libbenga K.R. (eds.), Biochemistry and molecular biology of plant hormones. Elsevier, Amsterdam. 1999, 337 – 61.

35. Arteca RN and Dong CN. Stimulation of photosynthesis by application of phytohormones to root systems of tomato plants. *Photosynth*. 1981; Res. 2: 243 – 9.

36. Shah SH, Ahmad I and Samiullah. Effect of gibberellic acid spray on growth, nutrient uptake and yield attributes during various growth stages of black cumin (*Nigella sativa*). *Asian J. Plant Sci*. 2006; 5: 881 – 4.

37. Shah SH. Physiological effects of pre-sowing seed treatment with gibberellic acid on *Nigella sativa* L. *Acta Bot. Croat*. 2007; 66 (1): 67 – 73.

38. Higa T. Studies on flower bud differentiation and develoment and artificial treatments on flowering and fruit set in Satsuma. Science Bulletin of the College of Agriculture University of Ryukyus. 1972, 19: 1 - 5.

39. Vijayaraghavan H. Effect of seed treatment with plant growth regulators on bhendi (*Abelmoschus esculentus* L.) grown under sodic soil conditions. *Madras Agricultural J*. 1999; 86: 247 - 9.

40. Sedghi M, Nemati A, Khandan Bejandy T and Namvar A. Effect of growth regulators on Seed characteristics and Performance of *Cucurbita pepo* L. *Medicinal Plants J*. 2010; 26 (1): 40 - 8.

41. Jahan N and Fattah QA. Effect of foliar treatments of NAA and IBA on reproductive and yield parameters of bitter gourd (*Momordica charantia* L.). *Dhaka University Journal of Biological Sciences* 1991; 6 (1): 69 - 71.

42. Chaudhuri D, Basuchaudhuri P and Gupta DKD. Effect of growth substances on growth and yield of rice. *Indian Agriculturist J*. 1980; 24: 169 - 75.

43. Iqbal M, Khan MQ, Uddin J, Rehman K and Munir M. Effect of foliar application of NAA on fruit drop, yield and physicochemical characteristics of guava (*Psidium guajava* L.) cv. Red Flesh. *J. Aric. Res*. 2009; 47 (3): 259 - 69.

44. Patel JK. Effect of tricontanol and naphthalene acetic acid (NAA) on lint yield, fibre quality and nitrogen, phosphorus and potash uptake in cotton (*Gossypium* sp). *Indian J. Agron*. 1992; 37: 332 - 7.

45. Alam SM and Khan MA. Fruit yield of tomato as affected by NAA spray. *Asian J. Plant Sci*. 2002; 1 (1): 24.

46. Raman R and Venkatrama K. Effect of foliar nutrition on NPK uptake, yield attributes and yields of greengram (*Vigna radiate* L.). *Crop Res*. 2006; 32 (1): 21 - 3.

47. Purbey SK and Sen NL. Effect of bioinoculates and bioregulators on yield and nutrient uptake by fenugreek (*Trigonella foenum-graecum* L.). *Indian J. Agric. Res*. 2007; 41 (2): 154 - 6.

48. Adam AMMG, Jahan N and Hoque S. Effects of naphthalene acetic acid on nutrient uptake by two varieties of rice (O*ryza sativa* L.). Dhaka Univ. *J. Biol. Sci*. 2012; 21 (1): 9 - 15.

49. Mohammed HM. Effect of GA_3, calcium, kinetin and ethylene on growth and cell wall composition of pea epicotyls. *Plant Physiol.* 1975; 56 (5): 622 – 5.

50. Paleg LG. Physiological effect of gibberellins. *Ann. Rev. Plant Physiol.* 1965; 16: 291 – 322.

51. Mishriky JE. Response of celery (*Apium graveolens* var. Dulce) to foliar application of gibberelic acid (GA_3). *Bull. Faculty Agricult. Cairo Univ., Egypt.* 1990; 41 (3): 777 – 84.

52. Purbey SK and Sen NL. Effect of bioinoculates and bioregulators on yield and nutrient *uptake* by fenugreek (*Trigonella foenum-graecum* L.). *Indian J. Agric. Res*. 2007; 41 (2): 154 - 6.

53. Varna RY. Effect of C.E.P.A and GA_3 on cucumber yield. *Horticultural Abstract*. 1990; 50: 11 - 2.