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Impact of plant growth regulators and organic fertilizer on growth and biochemical composition of *Trigonella foenum-graceum* L.

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Abstract
The present experiment was carried out to assess the effect of organic manure and Plant growth regulators on morphological changes and biochemical composition of fenugreek. The experiment comprising of Control, Organic manure and three different Growth regulators such as IAA (Indole Acetic Acid), IBA (Indole Butyric Acid) and NAA (Naphthalene Acetic Acid). These three growth regulators are taken in four different combinations such as NAA+IAA, IAA+IBA, IBA+NAA and NAA+IBA+IAA. The hormones were prepared with distilled water and treated to the plants. The plants were irrigated with tap water regularly for 30 days. After 30 days the fresh plants were taken for the further analysis like Morphological studies and Biochemical analysis. The results reveals that the morphological parameters such as root length, shoot length, leaf area, fresh weight and dry weight are increased in the treatment of NAA+IBA, and in biochemical analysis chlorophyll, carotenoids, total sugar, starch, protein and amino acid contents were increased in plants treated with organic manure, when compared to all others treatments.

Keywords: plant growth regulators, fenugreek, bio fertilizers, chlorophyll, aminoacid

1. Introduction
Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop, self-pollinating and dicotyledonous plant belonging to the Leguminacae (Fabaceae) family. This crop is native to an area extending from Iran to northern India and widely cultivated in China, India, Egypt, Ethiopia, Morocco, Ukraine, Greece, Turkey, etc. Fenugreek leaves and seeds are consumed in different countries around the world for different purposes such as medicinal uses, making food, roasted grain as coffee-substitute, controlling insects in grain storages, and perfume industries. Several intrinsic and extrinsic factors affect on growth, development and secondary metabolites biosynthesis and accumulation of medicinal and aromatic plants. Fenugreek possesses pharmacological properties such as antimicrobial, carminative, emollient, febrifuge, laxative, restorative, uterine tonic, expectoral, galactogogue, anti-carcinogenic, anti-inflammatory, antiviral, antioxidant, demulcent and hypotensive (Moradi kor and Moradi, 2013) [21]. Organic manures are carbon-based compounds that increase the productivity and improve quality of plants (Leu, 2007) [16]. They have various benefits over chemical fertilizers. Organic fertilizers are low priced and eco-friendly inputs that have tremendous prospect of supplying nutrients which can reduce the over dependence on chemical fertilizer (Bajeli *et al*., 2016) [4]. The use of PGRs in the field of agriculture has become commercialized in some advanced countries like Europe, USA and Japan. PGRs can be divided into five classes as auxin, gibberellins, cytokinins, abcisic acid, and ethylene. Naphthalene acetic acid (NAA) belongs to synthetic forms of auxins. Auxins play key role in cell elongation, cell division, vascular tissue, differentiation, root initiation, apical dominance, leaf senescence, leaf and fruit abscission, fruit setting and flowering. Growth regulators are organic substances besides nutrients, synthesized in plants, causing alteration in their cellular metabolism. Synthesis of some plant hormones is adversely affected by environmental factors, which causes restriction on physiological processes of the plant and ultimately, limits their growth potential (Copur *et al.*, 2010) [9]. The application of these hormones in low concentration regulates growth, differentiation and development, either by promotion or inhibition (Naeem *et al.*, 2004) [24], and allows physiological processes to occur at their normal rate (Gulluoglu, 2004) [13]. Major plant growth regulators (PGRs) significantly enhanced fiber yield in cotton (Copur *et al.*, 2010) [9], protein content in pea (Bora and Sarma, 2006) [5] chemical constituents in *Croton* (Soad *et al.*, 2010) [31], fruit size in *Molina* (Vwioko and Longe 2009) [33], seed germination rate in black gram and horse gram (Chauhan *et al.*, 2009) [6], floral buds in *Jojoba* (Prat *et al.*, 2008) [26] and other growth parameters in different plants. Thus, to overcome the production constraints, chemical manipulation could be done to improve yield and growth parameters. Among PGRs, auxin and play vital role in regulating developmental processes within plant bodies (Gou *et al.*, 2010) [11]. Auxin promotes cell elongation, especially of shoots, and induces apical dominance and rooting, cell growth of stem, leaves and other aerial parts by causing cell elongation, and increase in inter nodal length. The present study is aimed to analyze the growth and biochemical composition of PGRs and organic fertilizer treated fenugreek.

2. Materials and Methods
The fenugreek seeds are sowed in each pot. The continuous irrigation were giving daily using tap water. The temperature was maintained upto 27˚ C under green shade net. Weeds were controlled by hand-hoeing when necessary. The plants

were treated with plant growth regulators and organic fertilizers by soil drenching method. The Electrical conductivity (EC) level of soil was maintained constant throughout the experiments. The experiments were carried out in Fenugreek at different concentrations of plant growth regulators and organic fertilizers i.,e treatment 1 - control, treatment 2 - NAA + IBA, treatment 3 - NAA + IAA, treatment 4 - IBA + IAA, treatment 5 - NAA+ IBA+ IAA, treatment 6 - Organic fertilizer (Panchagavya) in order to determine the viable range of treatments. The control plants were treated with tap water. The experimental yard was roofed with transparent polythene sheet at a height of 3m from the ground in order to protect the plants from rain. The Plant growth hormones (Himedia, Mumbai, India) were prepared with distilled water. Plants were harvested after 30 days and separated into leaf, stem and root was used for determining growth and biochemical contents. The standard methods were followed for all the parameters.

2.1 Morphological Parameters

Shoot length was recorded by measuring the height of the plant from the surface of the soil to the tip of the topmost leaf. The root length was measured from the point of first cotyledonary node to the tip of longest root. The total leaf area was calculated by measuring the length and width and number of leaves and multiplied by a correlation factor (0.66) derived from the method of Yoshida *et al.,* (1972) [34]. For the estimation of fresh weight, the plant samples are collected and weighed and noted. They were dried in a hot air oven at 80ºC for 24 hours. Then, the dry weight was taken by using an electronic balance.

2.2 Pigments

The chlorophyll content of young and mature leaves was determined following the method by Arnon (1949) [2]. The carotenoid content of young and mature leaves was determined following the method by Davis, (1965) [10].

2.3 Biochemical Parameters

The total sugar content was estimated by Nelson (1944) [25]. The estimation of starch content was determined by Clegg (1956).Free amino acid contents of different parts of the plant were determined following the method by Moore and Stein (1948) [22]. Protein content of different parts of the plant were determined by following the method of Lowry *et.,al* (1951) [18].

3. Results and Discussion

In *Trigonella foenum-graceum*, the shoot length was observed to be higher in treatment 6 (organic fertilizer). This results resembles the results of F.B.Mukthar, (2008) [23] whose experiment was on phosphorous content of *Abelmoschus esculentus*. The plants treated with organic fertilizer results in possession of high phosphorous content. The root length is found to be increased in the plants in treatment 6 (organic fertilizers). Likewise the results of Sangeetha v *et al.,* (2010) [27] proved that organic fertilizers improves the leaf growth and development in *Arachis hypogeal* (Table No. 1). The number of leaves is found to be increased and in control and the plants treated with organic fertilizers. This results resembles the results of F.B.Mukthar (2008) [23] whose experiment was on number of leaves of *Hibiscus sabdariffa.* The plants treated with organic fertilizer results in higher leaf count (Table No.2). The prominent increase in the number of roots were showed in treatment 2(NAA + IBA). The results are closely related to the results of D.B. Kachave *et al.,* (2003) [14] whose experiment was on the total soluble solid of juice on the kagzi lime. Their results states that the plant growth hormone NAA results in increasing of total soluble solid of juice when compared to others. Refer table 2. On 30th DAP the leaf area is found to be increased in the treatment 4 (IBA + IAA) when compared to all other treatments. Similarly the research work of Jutta Ludwig Muller (2000) [17] on *Zea mays* increases induction of adventitious roots by using IBA (Table No.3). A prominent gain of fresh weight observed in the treatment 6 (organic fertilizer) as reported in Jen Hshuan chen k *et al.,* (2004) [7] experiment on cabbage using organic fertilizer gives higher total growth. The significant increase of dry weight with lager extent was found in the treatment 6 (organic fertilizer).

S. Thirusenduraselvi e*t al*.,(2007) [32] reported the same where the rate of germination of the bitter gourd increases by the foliar application of the organic fertilizer (Table No. 4).The increased chlorophyll content was recorded maximum in the leaves of treatment 6 (organic fertilizer). These results are found to be similar with the results of M.N. Ali *et al.,* (2011) [1] whose experimental results shows highest carbon content in green gram. The increased chlorophyll content were found in stems of treatment 6 (organic fertilizer). According to P.Ganesh *et al.,* (2011) [15] chlorophyll content increases in *Vigna mungo* by the foliar application of organic fertilizer (Table No. 5). The carotenoid content of *Trigonella foenum-graceum* was significantly increased in the leaves of treatment 6 (organic fertilizer) M.N. Sreenivasa *et al.,* (2008) [32] reported that the nutrient content of *Lycopersicon esculentum* increases on treating it swith the organic fertilizers. The carotenoid content of *Trigonella foenum-graceum* was significantly increased in stems of treatment 6 (organic fertilizer) Results of Bindumathi mohan *et al.,* (2008) [20] stated that the increase in yield of *Lycopersicon esculentum* is due to the action of organic fertilizer (Table No. 6).

The total sugar content is increased in leaves of treatment 6 (organic fertilizer). Results of Saritha.M *et al.,* (2013) [29] states that the germination percentage of *Cyamopsis tetragonoloba* increased in the plants treated with organic fertilizer. The total sugar content is increased in stems of treatment 6 (organic fertilizer). The experimental results of S.Sarkar *et al.,* (2008) [30] states that the photosynthetic pigments of vegetable crops by the foliar application of the organic fertilizers (Table No. 7). Starch content was increased in leaves of treatment 4(IBA + IAA). *Arabidopsis thaliana* treated with IAA increased number of root segments which was proven by the research of Jutta Ludwig muller (2000) [17]. There was a prominent change observed in starch content of *Trigonella foenum-graceum* leaf and stem. Starch content was increased in stems of treatment 5 (IBA + IAA + NAA). Similarly the work by S S Meena *et al.,* (2006) [19] on vegetative growth of *Coriandrum sativum* shows that the growth is higher in seeds treated with NAA (Table No. 8).

Amino acid content content were increased in leaves of

treatment 1 (control). Amino acid content was increased in stems of treatment 2 (IBA + NAA). Likewise the experimental study done by Ruchita Gour *et al.,* (2009) [12] using NAA in *Trigonella foenum-graceum* resulted in higher foliage growth (Table No. 9). Total protein content increased in *Trigonella foenum-graceum* in treatment 6 (organic fertilizer). Likewise S. Sanjutha (2008) [28] concluded that organic fertilizer increases the number of leaves in *Andrographis paniculata*. Total protein content increased in stems of *Trigonella foenum-graceum* in treatment 5 (IAA + IBA + NAA). The experimental results of Bairava *et al.,* (2012) [3] proves that the NAA increases the plant height in *Trigonella foenum-graceum* (Table No. 10).

Table 1: Effect of different treatments of plant growth regulators and organic fertilizer on shoot and root length of *Trigonella foenum-graceum*

Treatment	Shoot Length (cm)	Root Length (cm)
Control	9.5 ± 0.1	2.3 ± 0.15
NAA + IBA	9.4 ± 0.1	3.9 ± 0.1
NAA + IAA	9.1 ± 0.15	3.4 ± 0.1
IBA + IAA	9.0 ± 0.15	3.8 ± 0.1
NAA + IAA + IBA	8.7 ± 0.1	3.7 ± 0.1
Organic Fertilizer	10.1 ± 0.2	4.0 ± 0.2

Values are expressed as means ± S.D (n=3).

Table 2: Effect of different treatments of plant growth regulators and organic fertilizer on number of leaves and roots of *Trigonella foenum-graceum*

Treatment	Number of leaves	Number of roots
Control	8 ± 1.0	5.6 ± 1.5
NAA + IBA	7.6 ± 1.5	5.6 ± 1.5
NAA + IAA	7.0 ± 1.0	4.0 ± 1.0
IBA + IAA	6.6 ± 1.5	4.3 ± 0.5
NAA + IAA + IBA	6.3 ± 1.5	4.3 ± 1.5
Organic Fertilizer	8 ± 1.0	5.3 ± 1.5

Values are expressed as means ± S.D (n=3).

Table 3: Effect of different treatments of Plant growth regulators and Organic fertilizer leaf area of *Trigonella foenum-graceum*

Treatment	Leaf Area (cm^2)
Control	1.12 ± 0.04
NAA + IBA	0.96 ± 0.18
NAA + IAA	1.16 ± 0.07
IBA + IAA	1.44 ± 0.34
NAA + IAA + IBA	1.16 ± 0.2
Organic Fertilizer	0.99 ± 0.39

Values are expressed as means ± S.D (n=3).

Table 4: Effect of different treatments of Plant growth regulators and Organic fertilizer on fresh and dry weight of *Trigonella foenum-graceum*

Treatment	Fresh Weight (g)	Dry Weight (g)
CONTROL	2.40 ± 0.015	0.19 ± 0.015
NAA + IBA	2.11 ± 0.015	0.14 ± 0.03
NAA + IAA	2.18 ± 0.015	0.17 ± 0.015
IBA + IAA	2.34 ± 0.03	0.18 ± 0.017
NAA + IAA + IBA	2.04 ± 0.02	0.12 ± 0.02
Organic Fertilizer	2.7 ± 0.2	0.21 ± 0.015

Values are expressed as means ± S.D (n=3).

Table 5: Effect of different treatments of Plant growth regulators and Organic fertilizer on total chlorophyll of *Trigonella foenum-graceum*

Treatment	Leaf (mg/g fresh weight)	Stem (mg/g fresh weight)
Control	0.78 ± 0.0015	0.703 ± 0.0032
NAA + IBA	0.792 ± 0.001	0.742 ± 0.001
NAA + IAA	0.841 ± 0.0011	0.799 ± 0.0015
IBA + IAA	0.863 ± 0.002	0.805 ± 0.0015
NAA + IAA + IBA	0.878 ± 0.001	0.803 ± 0.0015
Organic Fertilizer	0.924 ± 0.001	0.896 ± 0.0015

Values are expressed as means ± S.D (n=3).

Table 6: Effect of different treatments of Plant growth regulators and Organic fertilizer on carotenoid content of *Trigonella foenum-graceum*

Treatment	Leaf (mg/g fresh weight)	Stem (mg/g fresh weight)
Control	0.713 ± 0.0028	0.628 ± 0.001
NAA + IBA	0.825 ± 0.0057	0.793 ± 0.001
NAA + IAA	0.881 ± 0.0015	0.803 ± 0.0015
IBA + IAA	0.841 ± 0.0011	0.798 ± 0.0015
NAA + IAA + IBA	0.921 ± 0.001	0.886 ± 0.001
Organic Fertilizer	0.947 ± 0.002	0.913 ± 0.001

Values are expressed as means ± S.D (n=3).

Table 7: Effect of different treatments of Plant growth regulators and Organic fertilizer on total sugar content of *Trigonella foenum-graceum*

Treatment	Leaf (mg/g fresh weight)	Stem (mg/g fresh weight)
Control	2.83 ± 0.02	2.1 ± 0.01
NAA + IBA	3.53 ± 0.01	3.11 ± 0.01
NAA + IAA	3.85 ± 0.01	3.20 ± 0.0057
IBA + IAA	3.96 ± 0.01	3.32 ± 0.005
NAA + IAA + IBA	4.71 ± 0.015	4.12 ± 0.015
Organic Fertilizer	5.21 ± 0.015	4.81 ± 0.015

Values are expressed as means ± S.D (n=3).

Table 8: Effect of different treatments of Plant growth regulators and Organic fertilizer on starch content of *Trigonella foenum-graceum*

Treatment	Leaves (mg/g fresh weight)	Stem (mg/g fresh weight)
Control	0.23 ± 0.015	0.194 ± 0.01
NAA + IBA	0.21 ± 0.020	0.183 ± 0.011
NAA + IAA	0.20 ± 0.02	0.203 ± 0.005
IBA + IAA	0.24 ± 0.01	0.192 ± 0.01
NAA + IAA + IBA	0.15 ± 0.015	0.244 ± 0.15
Organic Fertilizer	0.16 ± 0.005	0.225 ± 0.01

Values are expressed as means ± S.D (n=3).

Table 9: Effect of different treatments of Plant growth regulators and Organic fertilizer on amino acid content of *Trigonella foenum-graceum*

Treatment	Leaves (mg/g fresh weight)	Stem (mg/g fresh weight)
Control	0.23 ± 0.015	0.194 ± 0.01
NAA + IBA	0.21 ± 0.020	0.183 ± 0.011
NAA + IAA	0.20 ± 0.02	0.203 ± 0.005
IBA + IAA	0.24 ± 0.01	0.192 ± 0.01
NAA + IAA + IBA	0.15 ± 0.015	0.244 ± 0.15
Organic Fertilizer	0.16 ± 0.005	0.225 ± 0.01

Values are expressed as means ± S.D (n=3).

Table 10: Effect of different treatments of Plant growth regulators and Organic fertilizer protein content of *Trigonella foenum-graceum*

Treatment	Leaf (mg/g fresh weight)	Stem (mg/g fresh weight)
Control	0.323 ± 0.005	0.212 ± 0.01
NAA + IBA	0.411 ± 0.01	0.162 ± 0.015
NAA + IAA	0.390 ± 0.015	0.210 ± 0.01
IBA + IAA	0.221 ± 0.01	0.184 ± 0.01
NAA + IAA + IBA	0.320 ± 0.015	0.382 ± 0.04
Organic Fertilizer	0.527 ± 0.015	0.294 ± 0.01

Values are expressed as means ± S.D (n=3).

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