Effect of Gold and Silver nanoparticles, BVG nano and Chelated micronutrients on growth and biochemical parameters on *Trigonella foenum graecum* L.

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Abstract

Fenugreek (Trigonella foenum-graecum L.), is annual herbaceous legume crop belonging to family Fabacecae known as Fenugreek and is considered as one of the oldest multipurpose medicinal herbs. It is a native of South eastern Europe and Western Asia and widely cultivated in India which harbours great diversity of fenugreek. It is commonly found growing in the Mediterranean region of the world. Due to its use in daily vegetable need; an attempt was made to study the effect of some eco-friendly and highly cost-effective fertilizers on growth and biochemical parameters. The plastic pots (20 X 20 X 30 cm) of around 2 kg soil capacity were used for growing the Fenugreek seeds. An experiment was carried in three replicate manner with control. Four different sets namely Gold nano particles, Silver nano particles, BVG nano and Chelate were used for experimentation. Leaflet extract of a fern Nephrolepis exaltata (L.) was used to obtain nano particles and these nano particles were sprayed on leaves for further investigation.

Apart from these synthesized nano particles, a readymade product of nanoparticles with brand name BVG nano and chelated micronutrients were used for comparative studies with respect to growth and biochemical parameters in fenugreek plants. Morphological parameters like root length, shoot length, number, surface area, fresh and dry weight of leaves were studied. Biochemical parameters included qualitative and quantitative tests. Qualitative tests were performed to check presence or absence of alkaloids, flavonoids, phenolics, saponins, steroids, tannins and glycosides etc. Quantification of chlorophyll a, b and total chlorophylls, protein, total free amino acids, carbohydrates, reducing sugars, vitamin C and phenol was done using suitable methods.

Keywords: Nano particles, Gold, Silver, BVG, Chelate, Nephrolepis.

Introduction

Fenugreek is commonly grown herbaceous legume crop for its importance and utilization in diet as green leafy vegetable. It belongs to family Fabacecae and commonly known as Fenugreek or Methi in Marathi. It is commonly grown in the Mediterranean region of the world. In India, it is an important versatile Rabbi season crop mainly grown for seed spice in Maharashtra, Rajasthan, Gujarat, Madhya Pradesh and Haryana etc. Although it is a rabbi crop it, is grown during all seasons throughout India. It was named *Trigonella* from Latin language that means ‘‘little triangle” due to its yellowish-white triangular flowers.[2]

Fenugreek like other legumes is a worthy source of dietary protein for feeding by man and animals. From earliest times, Greeks and the Romans used it as medicine, spice and cattle fodder and so it was and still known as Greek hay.

It is also used to treat a variety of health problems in Egypt, Greece, Italy, and South Asia[1]. Seeds of fenugreek are used as a yellow dye, in cosmetics and for medicinal commitments. Fenugreek improves soil fertility as a nitrogen fixer or green manure[17]. Seeds are used as a condiment for flavouring of foods and leaves as vegetable. It has also got medicinal importance, therefore used for cure of flatulence, dysentery, diarrhoea, enlargement of liver and spleen, rickets, diabetes, and many others[9]. It is assumed that the class of flavonoids which fenugreek contains, may play a substantial role in the prevention of cancer[29].

Fenugreek seed contains 20% protein, 50% carbohydrate, 5% fat and 25% dietary fibers lipids, cellulose starch, ash, calcium, iron and β-carotene. It also has been found to contain vitamin C, niacin, potassium, and diosgenin (a compound that has properties similar to estrogen). Other active constituents in fenugreek are alkaloids, lysine and L-tryptophan as well as steroidal saponins, therefore it is used to in artificial flavouring and in the production of hormones[1].

Green fenugreek is a good source of iron (Fe) as well as other minerals for human beings[8]. *Trigonella foenum-graceum L*. is a medicinally important plant possessing anti-diabetic, anti-cancerous, anti-microbial properties)[20]. Crop production can be improved by improving the metabolic activity and nutritional status of crop plants.

‘Nano’ in its literal term means small. In nanotechnology, a sub classification of ultrafine particle is with length in two or three dimensions greater than 1nm and smaller than 100 nm and which may or may not exhibit a size-related intensive property. Nanoparticle can be synthesized broadly by three methods namely physical, chemical, biological and one technique called hybrid technique available to

synthesize nanoparticles. The technique to be used depends upon the material of interest, type of nanostructure viz. zero dimensional, one dimensional or two-dimensional material, size, quantity etc.

Nowadays nano particles are being used in agriculture as source of fertilizers. It is well-established fact that nano particles have tremendous applications with respect to all agricultural purposes. In food processing, nanoparticles help in improve consistency, food texture and nutritional value of food. It also protects aroma, flavour and other ingredients in food.

BVG Agrow Magic Nano, developed by BVG Life Sciences Ltd, a BVG Group Company, is an all-in-one growth booster for crops that helps increase the yield of all agricultural crops by speeding up photosynthesis and increasing nutrient absorption. When applied to roots, it breaks down nutrients into nano particles which increase their absorption by the roots. When applied on leaves, it opens up their pores and increase photosynthesis. Simply put, it increases the hunger of the crops and support it by increasing nutrient absorption. It visibly enhances growth, hardness and yield. Increase the BRIX (Sugar content) of produce. This action leads to better quality and growth in yield of the crops. It is 100% green, herbal, eco-friendly, 100 % biodegradable and a 100 non-carcinogenic yet very effective and efficacious product.

Chelate is a chemical compound of a metal ion and a chelating agent. A chelating agent is a substance whose molecule can form several bonds to a single metal ion. Chelating agents are organic molecules that can trap or encapsulate certain metal ions like Ca, Mg, Fe, Co, Cu, Zn, and Mn. These organic molecules can form various bonds with a single metal ion. Natural chelating agents are organic substances, either applied or produced by plants or microorganisms. The most important substances having this nature are hydroxamate siderophores, organic acids, and amino acids.

Amino acids increase health, lead to vigorous growth, and produce greater yields. Chelated fertilizer can be useful to solve specific problems. It might help growing acid- loving plants in alkaline soil. It could solve a nutrient deficiency problem. Chelated micronutrients make ions soluble, prevent precipitation, reduce toxicity of metal ions and suppress plant pathogen. Considering importance of fenugreek in diet and as an important agricultural crop, this plant is selected for investigation with respect to some commonly used fertilizers and few recently demanding fertilizers. In present investigation an emphasis has been given to study growth, biochemical parameters and comparative studies with respect to Fenugreek plant.

Material and Methods

Gold and silver nanoparticles were synthesized from the leaves of *Nephrolepis exaltata*. For synthesizing gold nanoparticles, 1 g matured leaves of *Nephrolepis exaltata* were crushed in 10 ml distilled water. The extract was later filtered through muslin cloth. 1ml of this extract were further added in 100 ml distilled water. To this solution, 100 mg of chloro auric acid ($HAuCl_4$) were added to it. This solution was incubated at room temperature for 24 hrs. The gold nanoparticles were settled at the bottom of the flask. These gold nanoparticles were analyzed using SEM, Nano Sight and Zeta Potential Report.

Similarly, silver nano particles were synthesized by crushing 1g matured leaves in 10 ml distilled water. The extract was later filtered using muslin cloth. 1ml of this extract was further added in 100 ml distilled water. To this solution 100 mg of silver nitrate were added. This solution was incubated at room temperature for 24 hrs. The silver nanoparticles were settled at the bottom of the flask. These silver nanoparticles were analyzed using SEM, Nano Sight and Zeta Potential Report. 0.5 ml of all four nanoparticles were added in 499.5 ml distilled water. These four solutions were further used for further experimentation.

BVG nano and chelated micro fertilizers were bought from the market with brand name BVG agrow magic and Yara respectively.

Seed germination was studied using five Petri plates with wet blotting paper to grow the seeds with the respective solutions of nanoparticles.10 seeds of fenugreek were placed in each Petri plate. First Petri plate was considered as control seeds while second, third, fourth and fifth plates were of silver nano particles, gold nano particles, BVG nano and chelate treated seeds respectively. In all Petri plates, 2 ml solution of all respective nano particles and fertilizers was added to study germination percentage. After 2 days the germinating seeds were observed. This process was repeated for 3 times and after interval of 2 days, root length and percent seed germination were recorded.

For studying morphological and biochemical parameters, the plants were grown in Botanical Garden of Nowrosjee Wadia College, Pune. The plants used for this experiment were *Trigonella foenum-graecum* L. *Pusa* variety. *Trigonella* seeds were bought from market. The plastic pots (20 X 20 X 30 cm) of around 2 kg soil capacity were used for growing the *Trigonella* seeds. At bottom of the pots, a small hole was made to remove excess of water and the pots were filled completely with soil. Approximately 1 liter of tap water was added to each pot. Soil was mixed properly to prevent lumps. A hole of approximate diameter 1 cm was made in soil and seed was placed in it. The seed were then covered with soil.

Four sets of nanoparticles along with its three replicates and a controlled one were made for further experimentation. All the pots were filled with garden soil. In each pot, around 10-12 seeds were grown. The pots were watered alternate days. The seedling emerged after 2-3 days. After 10 days of germination, nanoparticles were sprayed only to the foliar parts of the plant in respective pots except in controlled pots.

The pots were placed in shade net for further observation with respect to morphological parameters, later each set was used for further growth parameters like root length, shoot length, number of leaves, surface area of leaves, fresh weight and dry weight etc. The growth parameters were measured by using measuring tape, surface area measured by using graph paper, fresh weight directly measured using weight balance and dry weight measured after drying leaves in oven after drying for four days at 50^0C.

Qualitative testes for presence or absence of Alkaloids, Flavonoids, Phenolics, Saponins, Steroids, Tannins and Glycosides were carried out by using suitable tests. Biochemical analysis for chlorophyll, vitamin C and reducing sugars, phenolic, free amino acid, water soluble proteins etc.

Estimation of Chlorophyll: Estimation of chlorophylls was carried out by Arnon[3]. Chlorophyll extract was prepared from fresh leaves of *Trigonella* one g by grinding in a mortar and pestle together with 10 ml of ice cold 80% acetone. The homogenate was centrifuged at 3000 rpm for 2 minutes. The absorbance of the supernatant was recorded at 663 nm, 645 nm and the concentration of chlorophyll a, chlorophyll b and total chlorophyll was calculated using following equations:

Chlorophyll−a=(12.7×A663−2.69A645) ×v1000×w
Chlorophyll−b=(22.9×A645−4.68×A663) ×v1000×w
Total chlorophyll=20.2×A645+8.02×A663v1000×w

Estimation of Proteins: Estimation and quantification of proteins were done by Lowry et al[12]. The fresh leaves of *Trigonella* from control and experimental plants were cut into small pieces separately and one g plant material was extracted with 5 ml of water. The extract was centrifuged at 10,000 rpm for 15 min. The supernatant was discarded and the pellet was dissolved in 2 ml of 1.0 N NaOH solution. This was used as a sample and 0.2 ml was taken for the estimation of proteins. The working standard of BSA and plant extract was taken in a series of test tubes and final volume was adjusted to 1 mL in each tube. Then 5 ml of reagent C was added in all the tubes and the mixture incubated for 10 min. This was followed by addition of 0.5 ml of Folin- Ciocalteau and incubated at dark for 30 min.

The blue colour developed in the reaction mixture was read at 660 nm on UV-visible spectrophotometer. Bovine serum albumin fraction V (BSA) was used at the concentration of 50 mg and dissolved in distilled water and used as a standard protein to prepare the standard graph. The amount of protein was calculated with the help of standard graph.

Estimation of Carbohydrate: One g plant material was used to grind in 80% ethanol with the help of mortar and pestle. The working standard of sucrose and plant extract was taken in a series of test tubes and final volume was adjusted to 1 ml in each tube. Then 4 ml anthrone was added in all the tubes and the mixture incubated for 8 min. Cool the mixture rapidly and take OD at 630 nm.

Estimation of reducing sugars: The reducing sugars were estimated by using dinitro salicylic acid (DNSA) reagent. Weigh one g leaves of *Trigonella* and extract the sugars with hot 80% ethanol twice (5 ml each time). Pipette out 0.5 ml of the extract in test tubes and adjust the final volume up to 3 ml with distilled water in all the tubes. Then add 3 ml of DNSA reagent and the contents were heated in a boiling water bath for 5 minutes.

One ml of 40% Rochelle salt solution was added in the warm contents. After cooling the contents, the intensity of dark red colour was recorded at 510 nm on UV-visible spectrophotometer. D-glucose at the concentration of 100 µg per ml was used to prepare the standard graph. The amounts of reducing sugars were calculated with the help of standard graph.

Vitamin C: Vitamin C content from the leaves of *Trigonella* was estimated following the method of Birch et al.[5] Initially working standard and stock standard solutions were prepared. 5ml of working standard was taken into a conical flask; in this 10 ml, 4% oxalic acid was added. This solution was titrated against dye (42 g Na_2CO_3 dissolved in 52 mg of 2-6-dichloro phenol indophenols and final volume raised up to 200 mL with distilled water). The amount of dye consumed was equivalent to amount of ascorbic acid. The amount of vitamin C was calculated.

Estimation of phenol: Estimation of phenol was done by using method of Malik and Singh[13]. For estimation, weigh exactly one g of the plant sample and grind it with a mortar and pestle in 80% ethanol. The working standard of catechol and plant extract was taken in a series of test tubes and final volume was adjusted to 3 ml with the help of distilled water. Then add 0.5 ml of Folin-ciocalteau reagent. Incubate the mixture for 3 min. Then add 2 ml of 20% Na_2CO_3 in each tube. Then boil for 1min.Then take absorbance at 650 nm.

Results and Discussion

For studying growth parameters, five Petri plate were used with wet blotting paper to grow the seeds with the respective solution of nanoparticles. Seed germination rate was recorded after 6 days. The seed germination rate in controlled seeds was between 4.21 and 4.42 cm. Highest germination was obtained in seed treated with gold nanoparticle (5.36 cm) while lowest germination rate was found in seeds treated with chelate (3.61 cm).

Root length in controlled plants was in between 3.27 and 3.30 cm. It was more in experimental plants as compared with controlled plants. It was highest 3.84 cm in plants treated with gold nano particles. Shoot length also showed similar results like root length and it was recorded more in all experimental plants than controlled plants. Shoot length was highest 34.2 cm in plants treated with gold nano

particles. Root length in all experimental plants was more than control plants.

Highest root length among all four experimental plants, plants treated with gold nanoparticle showed highest result (3.42 cm). Gold nanoparticle increases absorption area of the roots so that more nutrients are absorbed by the plant roots and hence resulted well as compared to control plants. Similar results were obtained by Mehmet et al[16] and Seyed et al[25] (Fig. 1). Shoot length in all experimental plants was more than control plants. Highest shoot length among all four experimental plants was in plants treated with gold nanoparticle. Gold nanoparticle increases absorption area of the roots so that more nutrients are absorbed by the plant roots and hence resulted well as compared to control plants. This was probably due to increase in water use by plant and evaporative was lost. Our results agree with the results of Bonvissuto et al[6] and Miedema et al[18] (Fig. 2).

The number of leaves and surface area of experimental plants were more as compared to controlled plants. Average number of leaves in controlled plants was in between 6.75 and 6.86. The results of number of leaves were highest 9.00 in experimental plants treated with silver nanoparticles and lowest 8.10 in plants treated with chelate. Similar trend was observed with respect to the surface area of leaves. It was recorded more in all experimental plants than controlled plants. It was maximum 2.70 cm^2 in plants treated with gold nano particles. Number of leaves and leaf surface area were more in experimental plants than in controlled plants. number of leaves were more in plants treated with silver nanoparticle.[9]

Surface area of leaf of plants treated with gold nanoparticle was 3.70 $cm.^2$ When these plants absorb the nano particles, it leads to the increase in root and shoot length number of leaves followed by leaf surface area. Due to availability of nutrients to the plant roots and fast mobility of nano particles in plants, there is positive impact over growth and that has resulted in increased number and surface area of leaves.

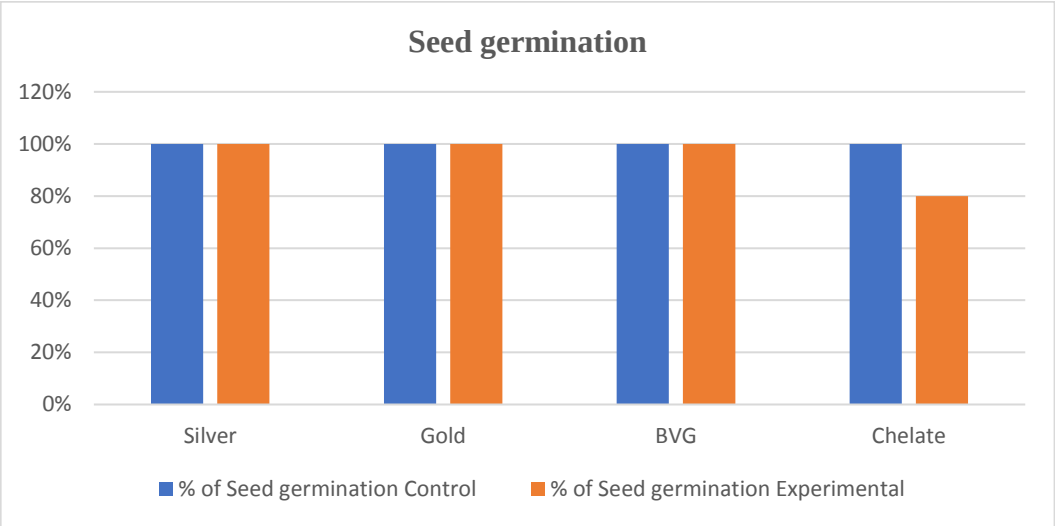

Fig. 1: Effect of Silver nanoparticles, Gold nanoparticles, BVG nano and Chelate on Seed germination of *Trigonella foenum graecum* L.

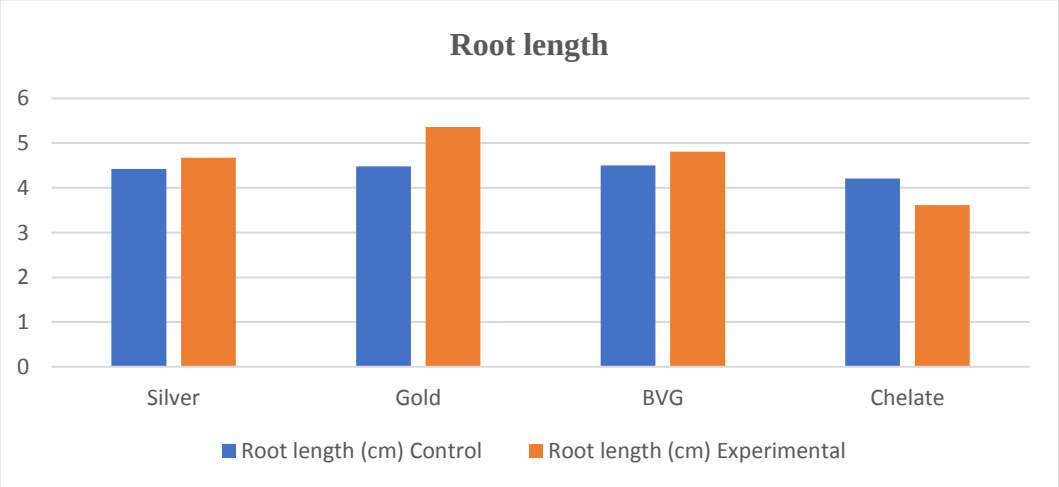

Fig. 2: Effect of Silver nanoparticles, Gold nanoparticles, BVG nano and Chelate on Root length of *Trigonella foenum graecum* L.

The silver nanoparticles improved growth in plant leaf. At high concentration, it become toxic, causing symptoms such as chlorosis and necrosis, stunting, leaf discoloration and inhibition of root growth[14,23]. Similar results were obtained by Saeideh et al[22] in *Vigna radiata* as in fig. 3.

The fresh weight increased in all experimental plants as compared with controlled plants. Fresh weight of controlled plants was in between 0.22 and 0.26 g whereas it was highest 0.55 g in plants treated with silver nanoparticles. Similar trend was observed with respect to dry weight showing highest 0.11 g dry weight in plants treated with silver nano particles. The fresh weight in all controlled plants was comparatively less than in experimental plants.

Because the photosynthetic efficiency resulted in formation of sugars in large amount, this resulted in increase in storage content which leads to increase in fresh weight of all experimental plants. The rate of metabolism increases when there is availability of nutrients or any fertilizer to the plants.

Nano particles have highest mobility in plants due to its size and that is the reason of vigorous growth. The growth vigour resulted in more fresh weight of all experimental plants than controlled plants. More fresh weight will show comparatively and proportionately higher weights of the plants. Our results corroborate with Saeideh et al[22].

Saponins were present in controlled as well as in experimental plants in all sets of silver nanoparticle, gold nanoparticle, BVG nano and chelate. Similar result was recorded for glycosides as well as steroids showing appearance of green colour. Phenols, alkaloids, flavonoid and tannins were absent in all controlled and experimental pots. Saponins were present in controlled as well as experimental plants in all sets of silver nano particles, gold nano particles, BVG nano and chelate. Parallel results were recorded for glycosides as well as steroids as in table 1. Our results match with the findings of Basu et al[4], Kumar et al[11], Suleyman et al[27] and Thinagaran et al[28].

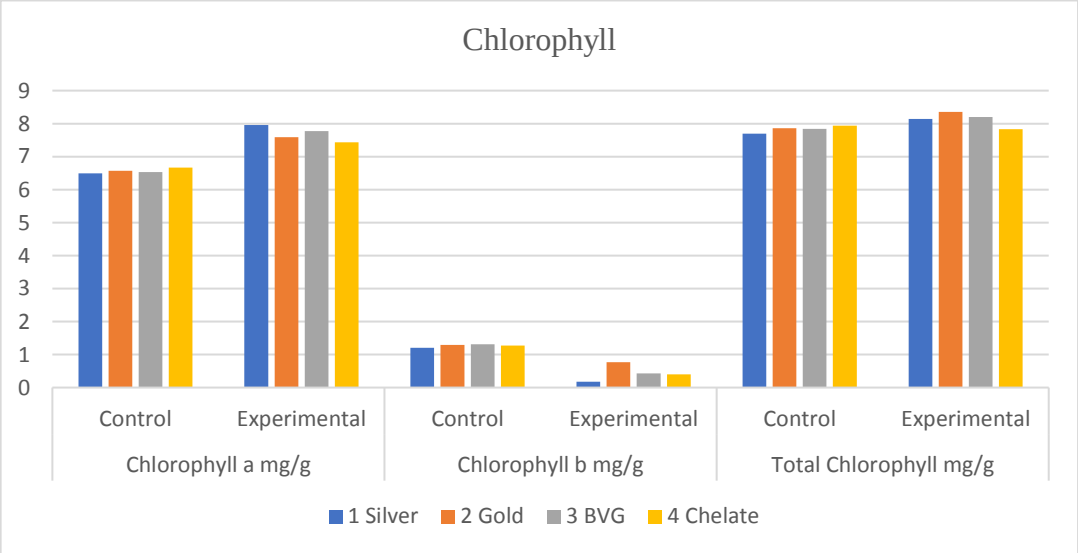

Fig. 3: Effect of Silver nanoparticles, Gold nanoparticles, BVG nano and Chelate on Chlorophyll of *Trigonella foenum graecum* L.

Table 1
Effect of Silver nanoparticles, Gold nanoparticles, BVG nano and Chelate on Phytochemical content of *Trigonella foenum graecum* L.

Test	Control		Silver nanoparticle		Gold nanoparticle		BVG Nano		Chelate		Observation
	D.W	Ethanol	D.W	Ethanol	D.W	Ethanol	D.W	Ethanol	D.W	Ethanol	
Saponin	+	+	+	+	+	+	+	+	+	+	Appearance of foam
Glycoside	+	+	+	+	+	+	+	+	+	+	Appearance of Bluish green colour
Steroid	+	+	+	+	+	+	+	+	+	+	Appearance of Green colour.
Phenol	-	-	-	-	-	-	-	-	-	-	-
Alkaloid	-	-	-	-	-	-	-	-	-	-	-
Flavonoid	-	-	-	-	-	-	-	-	-	-	-
Tannin	-	-	-	-	-	-	-	-	-	-	-

Chlorophyll a content in controlled plants was ranging between 6.49 mg/g and 6.67 mg/g. The amount of chlorophyll a was recorded lowest in experimental plants of chelate and highest was in plants treated with silver nanoparticles. Chlorophyll b content was ranging between 1.21 to 1.31 mg/g in controlled plants whereas it was recorded highest in plants treated with gold nano particles and lowest in plants treated with silver nanoparticles.

In experimental plants, chlorophyll b was less as compared with controlled plants. Total chlorophyll contents were in between 7.70 to 7.95 mg/g in controlled plants. On the other hand it was recorded maximum 8.36 in plants treated with gold nanoparticles. It was minimum 7.70 in plants treated with silver nano particles. Total chlorophyll content was high in experimental plants than in controlled plants. Among all experimental plants, maximum chlorophyll content was observed in plants treated with increase in chlorophyll content might be due to the beneficial effect of nano particles due to its contribution in supplying additional plant nutrients and increasing availability of native soil nutrients. Increased availability of nutrients to the plant roots results in high photosynthetic efficiency and hence there was increased chlorophyll content in experimental plants. Similar findings were recorded by Saeideh and Rashid[22] as in fig 5.

The protein content in all experimental plants was considerably more than controlled plants It was lowest in control plants (3.5mg/g) and highest in experimental plants (9.6 mg/g). The results of free amino acid content were superior 12.1 µg/g in plants treated with silver nanoparticles and it was inferior in 4.4 µg/g in plants treated with chelate. The protein content of the fenugreek leaves was significantly more in experimental plants than controlled plants due to nano particles. More growth due to more nutrients results in higher rate of metabolism.

This ultimately increase in photosynthetic efficiency has ultimate effect over protein content in Fenugreek plants. The values of protein obtained from the present study were analogous to those reported by Mathur and Choudhry[15] and Naidu et al[21]. Protein is an essential component of diet needed for survival of animals and human beings; their basic function is to supply the adequate amount of required amino acids as in fig. 6.

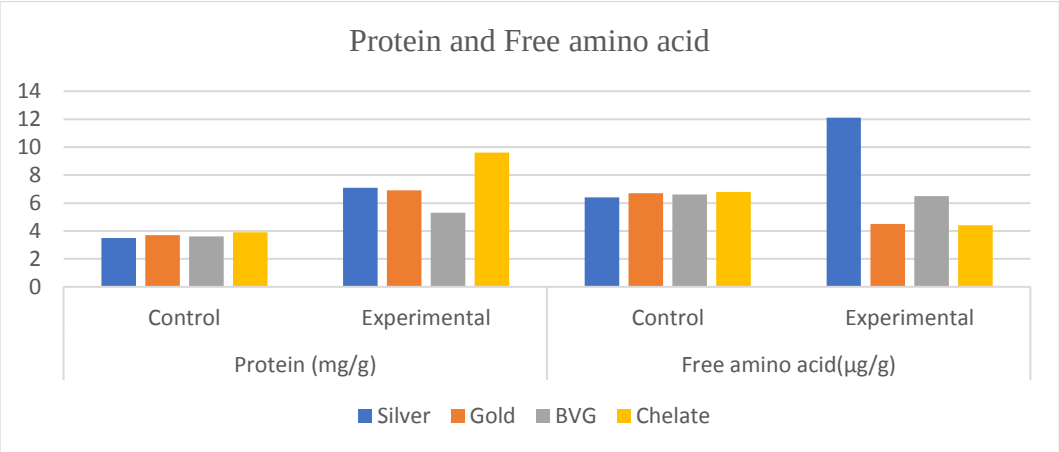

Fig. 4: Effect of Silver nanoparticles, Gold nanoparticles, BVG nano and Chelate on Protein and Free amino acid of *Trigonella foenum graecum* L.

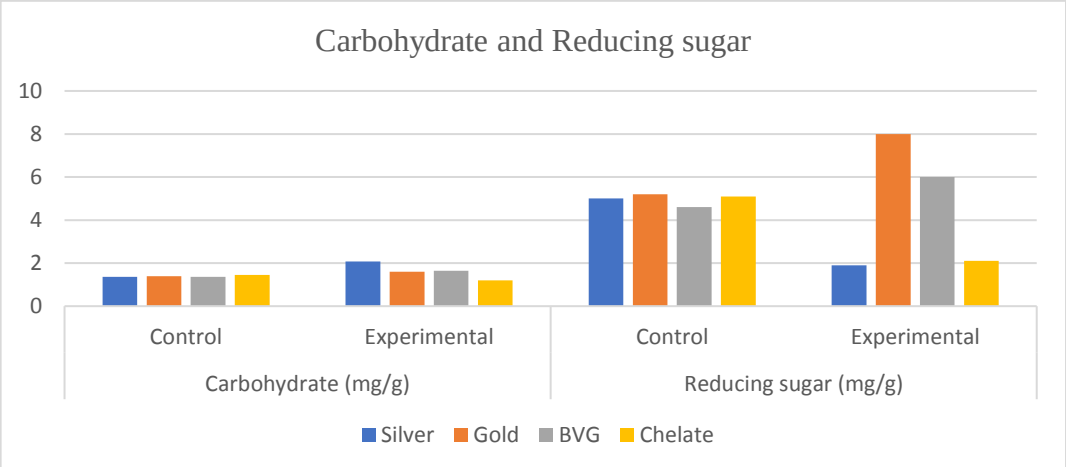

Fig. 5: Effect of Silver nanoparticles, Gold nanoparticles, BVG nano and Chelate on Carbohydrate and Reducing sugar of *Trigonella foenum graecum* L.

The carbohydrate content in all experimental plants was considerably elevated than control plants except in plants treated with chelate. The carbohydrate content was lowest in all control plants ranging from 1.36 to 1.45 mg/g and highest in plants treated with silver nanoparticle (2.08 mg/g). Reducing sugars were recorded more on all experimental plants as compared with controlled plants. Maximum reducing sugar content was found in highest plants treated with gold nanoparticle (8 mg/g) and lowest in control plants (2.10 mg/g) in plants treated with chelate fertilizers, increase in chlorophyll content resulted in increase in the rate of photosynthesis.

Therefore, rate of photosynthesis is directly proportional to the carbohydrate content. Exposure of gold nanoparticles to the plants caused a decrease in overall growth of *Trigonella* attributed to increase in stress. Our findings are matching with the findings of Mehmet et al[16] as in fig. 7.

An amount of vitamin C considerably increased in all experimental plants than control plants. It was 12.9 mg/g in plants treated with gold nano particles and chelate and lowest 12.7 mg/g in BVG nano. Phenols were ranging between 5.3 and 5.5 mg/g in controlled plants and on the other hand in experimental plants, it was ranging between 6.0 to 8.0 mg/g. It was highest 8.0 in plants treated with chelate fertilizer and lowest in gold nano particles. Naturally plants can produce vitamin C for its healthy growth. All the experimental plants contained high amount of ascorbic acid which leads to healthier growth as compared to controlled plants. This was due to increase in biochemical compounds and morphological parameters in all experimental plants than in controlled plants. Similar results were recorded by Buba et al[7] in *Trigonella foenum* graecum L. as in fig. 8.

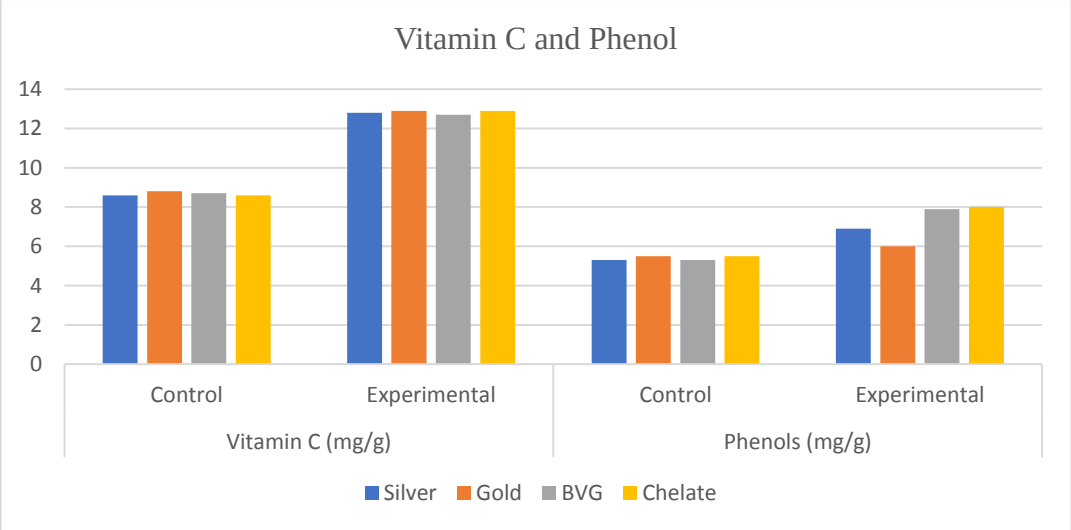

Fig. 6: Effect of Silver nanoparticles, Gold nanoparticles, BVG nano and Chelate on Vitamin C and Phenol of *Trigonella foenum graecum* L.

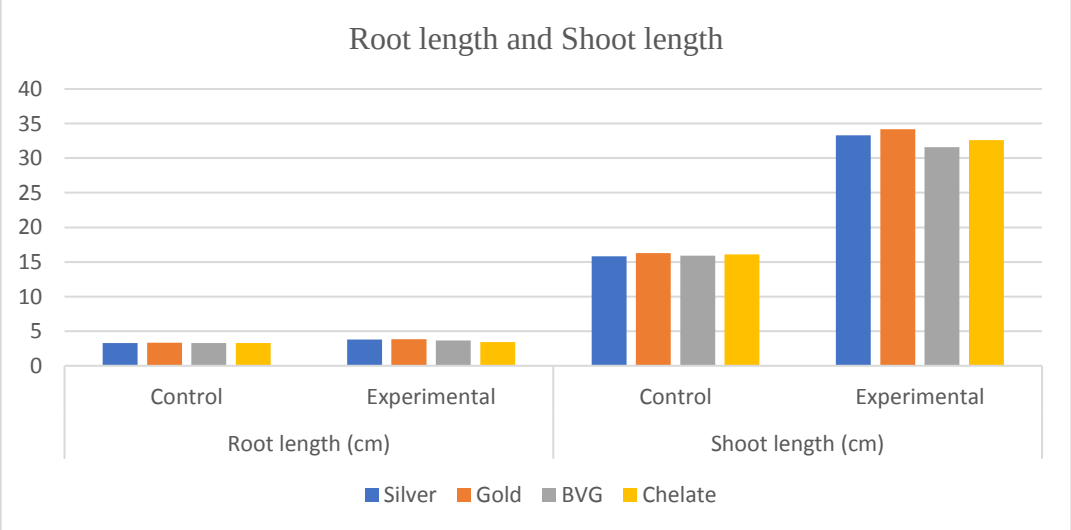

Fig. 7: Effect of Silver nanoparticles, Gold nanoparticles, BVG nano and Chelate on Root length and Shoot length of *Trigonella foenum graecum* L.

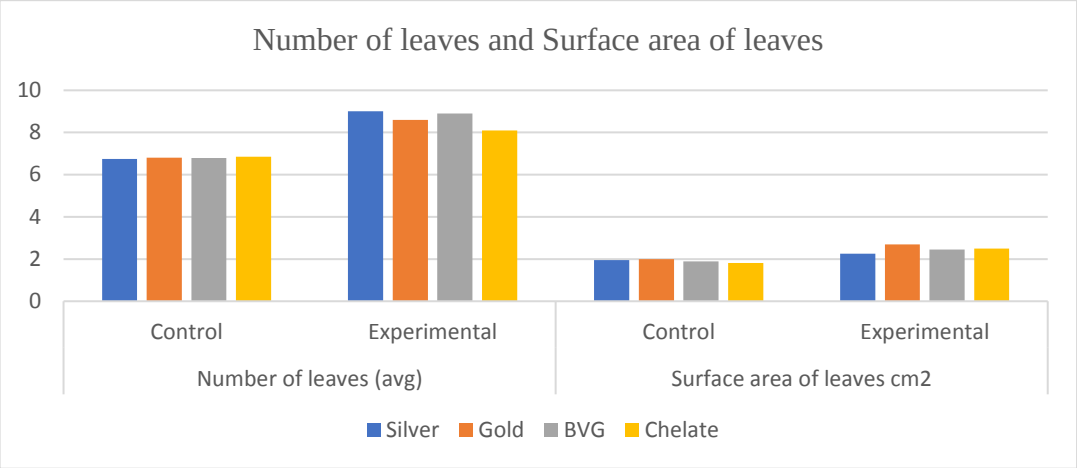

Fig. 8: Effect of Silver nanoparticles, Gold nanoparticles, BVG nano and Chelate on number of leaves and Surface area of leaves of *Trigonella foenum graecum* L.

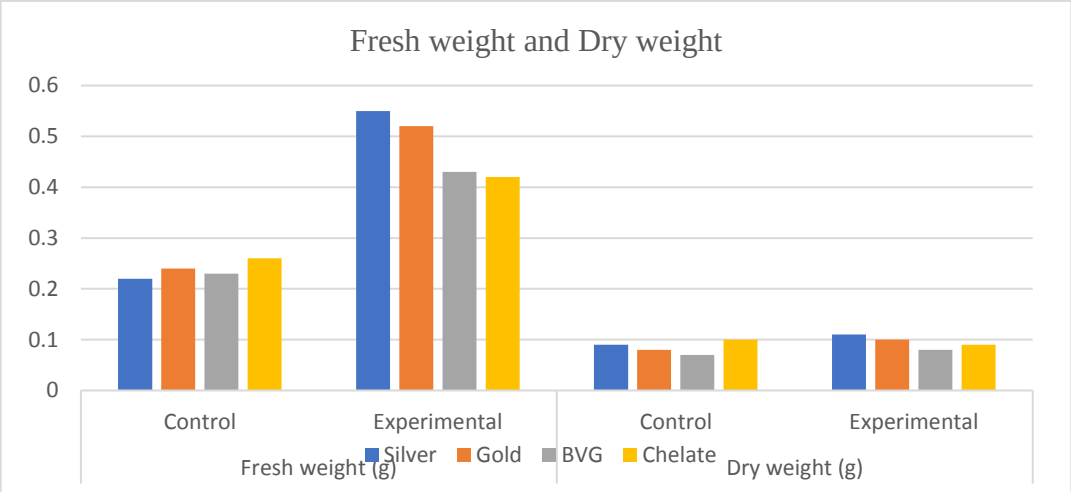

Fig. 9: Effect of Silver nanoparticles, Gold nanoparticles, BVG nano and Chelate on Fresh weight and Dry weight of *Trigonella foenum graecum* L.

Conclusion

From present investigation it was evident that gold and silver nano particles have positive effect on growth parameters of Fenugreek plants. Germination percentage, root length, reducing sugars were comparatively more in plants treated with gold nanoparticles. Amount of proteins was maximum in plants treated with chelated micronutrients whereas free amino acids and carbohydrates were more in plants treated with silver nano particles.

Amendment of Gold, Silver nanoparticles, BVG nano Chelated micronutrients did not show substantial effect on vitamin C and phenolic contents. Fresh weight and dry weight of the plants treated with silver nano particles had highest effect. It can be concluded that nano particles have substantial effect with respect to growth parameters in Fenugreek plants.

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