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IMPACT OF BIOGENIC CARBON NANOFIBER ON SEED GERMINATION AND SEEDLING GROWTH OF JOWAR (SORGHUM BICOLOR) AND CHICKPEA (CICER ARIETENUM)

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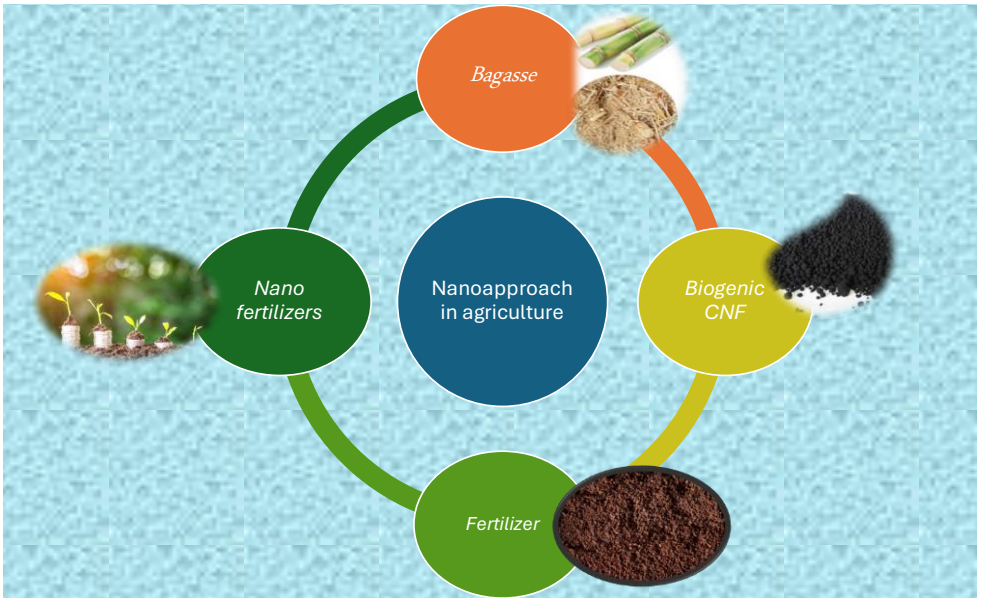
Abstract
The present work was started with an intention to use biogenic Carbon Nanofiber (CNF) for slow release of fertilizer NPK. Prior to using CNF loaded with fertilizer, it was imperative to know the desired level of CNF to be used that is suitable for plant growth. Hence, the aim of current research is to investigate the impact of CNF on seed germination and seedling growth using Jowar (Sorghum bicolor) a monocot and Chick pea (Cicer arietenum) a dicot crop. The parameters, to observe the seedling growth in presence of different concentrations of CNF (0.5 to 15 ppm CNF in Soilrites); included percentage germination and seedling vigor, seedling growth (shoot and root length as well as number of leaves and lateral root), mitotic index, auxin level, and primary metabolites of the seedlings. Presence of CNF in soil was inhibitory to Jowar seeds , where as it promoted germination percentage of Chickpea. Once the seed germinated seedlings of both Jowar and Chickpea showed positive response in morphogenic growth, in Mitotic Index, Chlorophyll content as well as primary metabolites. Chickpea was found to be more synergistic to CNF than Jowar.

Keywords Carbon nanofibers, Cicer arietenum, CNF, Nanotechnology, Seed-germination, Seedling-growth. Soil-toxicity. Sorghum bicolor.



Graphical abstract representing Nanotechnology in agriculture

INTRODUCTION

Nanotechnology has spread its tentacles for application in many fields of the science. Agriculture is being one of them, especially due to insurmountable need for food. Use of fertilizer has given rise to the successful Green Revolution in India and many countries. However, excessive use of fertilizer has resulted into many adverse effects on soil. Hence the emphasis is now not being limited to "Ability to invent" demands focus on "Sustainability".

Use of nano-fertilizer is one such step, so has to have controlled release of fertilizer or nutrients. The advent of newer and sustainable technologies is the pressing priority. Nanotechnology is one such alternative (Sharon et al 2010) [1].

In the present work our emphasis has been to use Carbon nanofiber (CNF) to deliver fertilizer/nutrient to the agricultural crops. CNF is a chemically inert material, having high surface area and many dangling bonds, which offers enhanced adsorption capacity of chemicals and water. Hence, it has been envisaged as a material for slow release of nutrients and fertilizers (Zhang et al 2014; Sharon and Sharan 2021)[2,3].

Present work started with an intention to use CNF synthesized from an agro-waste bagasse for controlled delivery of NPK to agricultural crops. However, prior to adding CNF as a slow-release carrier of fertilizers in soil, it was thought desirable to study the impact of CNF on the soil as well as on the plant growth.

The aim of present work was to study the impact of CNF on the early stages of growth of a plant viz. seed germination to seedling growth on Jowar (Sorghum bicolor) a monocotyledon millet and Chickpea (Cicer arietenum) a dicotyledon pulse. A biogenic CNF synthesized from Bagasse as precursor was used for this purpose.

MATERIALS AND METHODS

Carbon nanofiber (CNF) – synthesized by standard CVD method under pyrolytic condition using bagasse as precursor (Sharon et al 2007) [4] was sourced from wcRnb Solapur.

Characterization of CNF was done by:

SEM - The morphological observations of procured Carbon Nano Material synthesized from Bagasse was carried out by using a Hitachi (S-4700) SEM by placing the carbon samples on conductive carbon tape.

X-Ray Diffraction (XRD) Analysis of carbon nanomaterials was performed with a powder X-ray

PHILIPS PW 1710 diffractometer with Cu Kα source; to calculate the crystallographic parameters. The scanning angle (2θ) was usually set between 50 to 890. The step size was 0.01700 and continuous scan mode was used. The tube voltage was set at 40 kV giving 30mA generator current.

Raman Spectroscopy - Raman spectra were measured in a backscattering geometry at room temperature using Ar ion laser (488nm), with JASCO, NRS-2100.

Surface Area of CNF was analyzed using standard method of adsorption of Methylene blue (Kahr and Madsen 1995) [5].

Soilrite soil – was obtained from an ISO certified company for studies. Soilrite is a mixture of horticulture grade expanded perlite, Irish Peat moss and exfoliated vermiculite in equal ratio of 1/3:1/3:1/3. Prior to use Soilrite was dried over-night at 450C, to remove moisture.

Plant Material – Seeds of organically grown Jowar (Sorghum bicolor) and Chickpeas (Cicer arietenum) were obtained from local shop, selling agro-related material. They were potted separately in 300 gm Soilrite, to which six different concentrations i.e. 0 ppm (Control), 0.5 ppm, 1.0 ppm, 5ppm,10 ppm, and 15 ppm of CNF was added.

Prior to sowing the seeds, they were soaked in distilled water for 4 hours. Seeds were sown at a depth of 2cm in soil. An adequate supply of light and known amount of water was maintained all through the experiments.

Growth Record – included

i. Seed Germination from the soil containing different amount of CNF was recorded regularly by visual observation.

ii. Seedling Growth - by 9th day all the seeds had germinated. Therefore, on 11th day of germination length of shoot and root, number of leaves, fresh and dry weight of seedling was recorded.

iii. Mitotic index was recorded from the root meristem.

iv. AUXIN - Indole Acetic Acid (IAA) Estimation. IAA from the seedlings was extracted with peroxidase free diethyl ether at 00C temperature. Nonacidic and acidic fraction~ were separated. IAA was estimated from acidic fraction. For IAA analysis Gordon and Webber's (1951) [6] reagent was used (Salkowski reaction) and readings were taken in Spectrophotometer at 530 nm.

v. Chlorophyll Estimation - Chlorophyll content was analyzed by Arnon's method. 0.5 g finely cut fresh leaves were ground, to which 10ml of 80% acetone was added and then centrifuged at 10000 rpm for 5mins. The supernatant was transferred, and the centrifugation procedure was repeated till the residue becomes colourless. Then all the extracts were pooled together and condensed. The absorbance of the solution was read at 645nm and 663nm against the solvent (acetone) blank. The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation:

Chlorophyll a: 12.7(A663) – 2.69(A645)

Chlorophyll b: 22.9(A645) – 4.68(A663)

Total Chlorophyll: chl a + chl b was noted.

vi. Primary Metabolites assessed in the seedlings were (a)Total Carbohydrates using phenol sulphuric acid method, (b) Reducing sugar was assessed using FSSAI lab method using Fehling's regent (c) Total Protein analysis was done by Kjeldahl method, and (d) Free Amino acids were recorded using Paper chromatography.

vii. Total Carbohydrates estimation was done in root, stem, leaf, and seeds of Chickpea and Jowar seedlings; using phenol sulphuric acid method (Tamboli et al., 2020) [7]. To 100mg of glucose, 5ml 2.5N HCl was added and boiled on a water bath for 3hrs to hydrolyse. Cooled to room temperature and a sufficient quantity of solid sodium carbonate (Na2CO3) was added till effervescence ceased, indicating complete neutralization. It was filtered and the volume was made up to 100ml and 0.2, 0.4, 0.6, 0.8, 1ml of working standards was pipetted out in the series of test tube. Then 0.2 ml of the sample solution was taken and made up to 1ml with water. A blank was set with all reagents without sample.1ml phenol was added to each tube. To this 5ml 96% H2SO4 was added and shaken well. After

10min these tubes were placed in a water bath at 25-30°C for 20 min. In hot acidic medium glucose gets dehydrated to hydroxymethyl furfural. This forms a green-colored product with phenol. The colour intensity was measured at 490 nm. The percentage of total carbohydrate content present in the sample was determined by the formula mentioned below:-

Absorbance corresponds to 0.1 ml of test sample = x mg of glucose

100 ml of sample solution contain = x/0.1 *100 mg glucose = % of total Carbohydrate present.

viii. Estimation of Reducing Sugar

Reducing sugar was assessed using FSSAI lab method using Fehling's reagent and the results obtained were below detection limit (BDL).

ix, Estimation of Total Proteins

Total Protein analysis was done by Kjeldahl method using Automated Biokjgel (Protein estimation Machine) (IS: 7219:1973 RA 2005). The process involved four steps:

1. Digestion of dried powder of seedlings was done by adding 30 gm potassium sulphate, 0.5 gm anhydrous cupric sulphate, 10 ml concentrated H2SO4 to 1 gm powder. It was placed in digestion unit and locked with a bio-scrub fume neutralizer, and digested at 250 °C for 10 min, 300 °C for 10 min, and 350 °C for 10 min, 420 °C for 75 min.

2. Distillation was carried out automatically after the sample cooled down to room temperature. Prior to distillation 40% NaOH 40 ml in the tube and 4% Boric acid 25 ml in the receiver was added by machine. It took 9min. to distil the sample.

3. Titration was done manually by removing the receiver flask to which 2-3 drop of

Methyl red Indicator was added and titrated with 0.1 N HCl /0.1 N H2SO4; the endpoint showed pink color, the estimation of proteins was completed using Kjeldahl method.

x. Estimation of free Amino acids

Free Amino acids were recorded using paper chromatography method for the presence of 18 amino acids – lysine, valine, tyrosine, aspartic acid, leucine, arginine, methionine, alanine, asparagine, ornithine, threonine, proline, isoleucine, histidine, serine, 4-aminobutyric and phenylalanine. The samples were dissolved in alcohol and the solvent used in mobile phase was Butanol: Acetic acid and Water in the ratio 4:1:5 respectively. Based on Rf values results are presented in table 3A and 3B.

RESULTS AND DISCUSSIONS

SEM images showed peculiar tubular fibrous structure (Figure 1). Advantages of using plant derived precursors is that during pyrolytic carbonization their basic anatomical structures are not destroyed; resulting into some interesting channel types as well as porous structures. These structures are otherwise difficult to synthesize. These carbon materials possess suitable adsorption properties for both liquid and gases which we wished to explore so that it can be used as nutrient of fertilizer carrier.

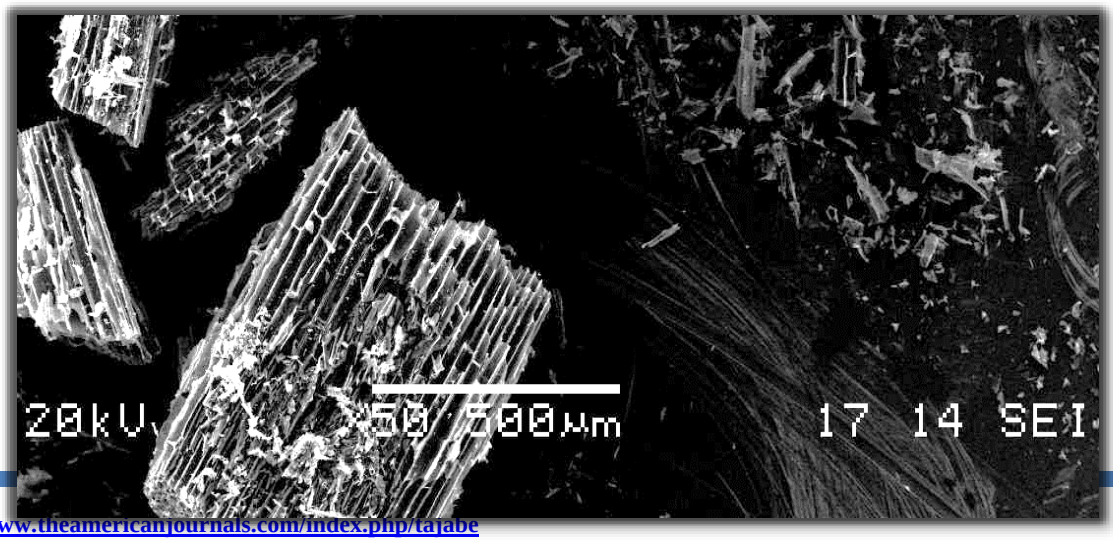


Figure 1: SEM image of CNF synthesized from Bagasse.

XRD spectrum- (Figure 2A) of CNF obtained from Bagasse, shows a broad diffraction peak at 24.10 is that of (002), and 45 º of (100) plane of graphitic carbon respectively. The graphitic peaks are weak suggesting more amount of amorphous carbon i.e. lacking a defined ordered structure, they are chemically inert so not a good conductor of electricity, but it is suitable for present work where conductivity or hardness is not a concern. Amorphous carbon lacks a well-defined crystal structure. It is made up of disordered, randomly arranged carbon atoms.

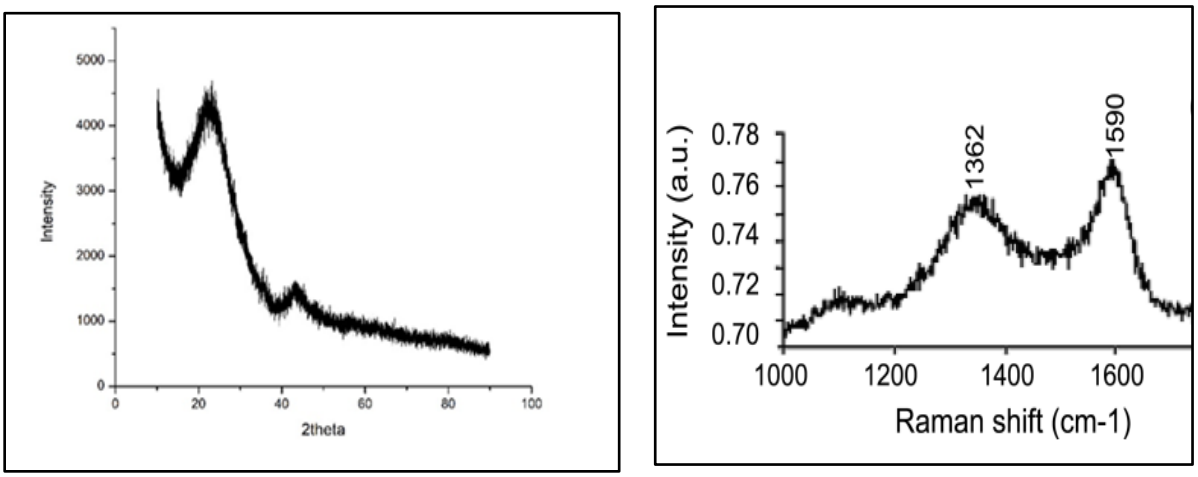


Figure-2: (A) XRD pattern of CNF and (B) RAMAN spectrum of CNF

Synthesized from Bagasse

Raman Spectra (Figure 2B) shows D band as well as G band peaks at 1362 cm−1 and 1590 cm−1 respectively and thus confirming the graphitic nature (Figure 2). D band shows defect in graphitic carbon, whereas G band indicates typical Raman peaks of graphitic carbon. The peak at 1362 cm−1 is associated with vibrations of carbon atoms with dangling bonds in plane terminations of disordered graphite. The peak at 1590 cm−1 is attributed to the Raman active E2g in plane vibrational mode and is related to the vibration of sp2 bonded carbon atoms in a two-dimensional hexagonal lattice.

Methylene Blue analysis showed that sample has a surface area of 94.61 m2/g.

Seed Germination percentage as recorded on 9th day showed that the response to CNF varied for two tried varieties. Jowar seeds showed (Tabe 1A) inhibition in germination in all tried concentrations of CNF whereas chickpea exhibited enhanced seed germination at all tried concentration except highest concentration 15 ppm of CNF, (Tabe 1B) which was similar to the control. Seed germination's primary requirement is water absorption. Though process of water absorption by germinating monocot and dicot seeds is similar in many ways, but there are some differences based on the structure and anatomy of these types of seeds. Water is absorbed primarily through the micropyle, a small pore in the seed coat, by both monocot and dicots. However, dicot seeds can also

absorb water through small openings in the seed coat called lenticels, especially in leguminous seeds like chickpea, thus availing more water to the seed, which might have been the reason for better seed germination rate of chickpea.

Seedling growth

Length of Seedlings- So far as the primary growth of seedlings i.e. the length is concerned, for Jowar seedling there was an enhanced growth in the length of Jowar seedling at all the tried concentrations of CNF, maximum being at 0.5 ppm. The length of Chickpea seedling was enhanced at 0.5 ppm CNF only.

TABLE 1A – Growth of 11days old Jowar Seedling, Grown on CNF Containing Soil

CNF (ppm) IN SOIL	% SEED GERMINATED	SEEDLING GROWTH				LEAF NUMBER PER PLANT
		Mean length (cm)	Mean Fr. Wt. (mg)	Mean Dry Wt. (mg)	% Dry Wt.	
0.0	83.3	12.75	206	30	14.56	3.0
0.5	60.6	14.8	272	45	16.54	4.0
1.0	63.3	14.0	300	60	20.00	4.0
5.0	63.3	14.2	310	120	38.70	4.0
10	63.3	14.0	332	100	30.12	4.0
15	73.3	13.9	386	130	33.67	4.0

TABLE 1B – Growth of 11days old Chickpea Seedling, Grown on CNF Containing Soil

CNF (ppm) IN SOIL	% SEED GERMINATED	SEEDLING GROWTH				LEAFLET NUMBER PER PLANT
		Mean length (cm)	Mean Fr.Wt. (mg)	Mean Dry Wt. (mg)	% Dry Wt.	
0.0	70.0%	33.0	1203	183	15.2	81
0.5	90.0%	34.4	1393	207	14.86	83
1.0	83.3%	32.2	1438	182	12.65	81
5.0	86.6%	30.3	1565	186	11.88	81
10	86.6%	29.8	1560	186	11.92	78.
15	70.0%	29.0	1320	159	12.04	75.

Weight of Seedlings -As compared to control both fresh and dry weight of Jowar seedlings, showed increase in all tested concentration in Jowar. Percentage of dry weight was also above control was also enhanced in all tested concentrations, maximum being at 5.0 ppm CNF,

In Chickpea, though fresh weight was enhanced in all tested concentrations, maximum being at 0.5 ppm; but the ratio of fresh to dry weight percentage was reduced.

Leaf number in the plants were slightly enhanced in Jowar at all the tried concentrations, whereas in Chickpea the leaflet number showed an increase only at 0.5 ppm CNF as compared to the control.

TABLE 2A - Impact of CNF on Mitotic Index, and Chlorophyll Content of Jowar Seedlings

Conc. of CNF (ppm)	Mitotic Index	Chlorophyll Content (chl a+chl b) mg/g
0.0	3.54	6.448
0.5	3.04	5.043
1.0	4.42	4.039
5.0	3.00	4.039
10	2.80	1.473
15	2.95	3.937

TABLE 2B - Impact of CNF on Mitotic Index, and Chlorophyll content of Chickpea Seedlings

Conc. of CNF (ppm)	Mitotic Index	Chlorophyll Content (chl a + chl b) mg/g
0.0	O.45	4.439
0.5	0.62	4.848
1.0	0.67	4.841
5.0	0.78	5.765
10	1.68	5.765
15	1.42	3.937

Chlorophyll is the main primary metabolite synthesizer (Ying et al 2018) [10] and important source of energy (Baker 2008) [11], for the growth of plants. Hence, Chlorophyll a and b were measured (Table 3). Jowar plants grown in soil containing CNF. There was a positive response in chlorophyll content in Chickpea but not in Jowar.

Mitotic Index is an indication of the rate of cell division in a tissue. In Jowar highest mitotic index was observed at 1.0 ppm CNF, whereas it was highest at 1.0 ppm. In the present work it is very difficult to correlate MI with growth. There would be many parameters involved in it. All it can be said that dicot Chickpea had higher MI at all tested concentrations of CNF than control in Jowar.

Total Protein content has shown the best response

to all tested concentrations of CNF by both Jowar and Chickpea (Table 2A & B). Protein content in growing seedlings was monitored as it provides insights into their overall health, growth status, and ability to cope with environmental challenges. Moreover, optimal protein levels ensure for robust seedling development. Proteins are not only the building blocks of cells, tissues, and organs, they play a fundamental role in essential processes like cell division, elongation, and differentiation; through enzyme system and as carriers or channels for the transport of nutrients and other molecules within the plant.

Free Amino Acids very low level of free amino acids in early stage of seedlings are often noted because they rely more on stored nutrients from the seed until they establish robust root systems and initiate active photosynthesis. Moreover, the level of free amino acids in seedlings can vary depending on their developmental stage. In Jowar Tyrosine and Leucine was noted whereas Chickpea seedings had only Leucine. Both Leucine and Tyrosine, are essential amino acid, plays several important roles in the growth of seedlings and protein synthesis. Leucine is also known for its role in gene expression regulation, energy metabolism, stress response, and secondary metabolite production. Its multifaceted roles make it indispensable for the growth, development, and adaptation of seedlings to their environment. Some of the specific roles of Tyrosine in growing seedlings are; specialized metabolite biosynthesis, hormone synthesis, redox regulation, and UV protection. It contributes to stress tolerance, and adaptation to environmental conditions. An increased level of IAA in Jowar may be attributed to the presence of Tyrosine in Jowar (Table 3A).

Carbohydrate content in growing seedlings provides insights into their metabolic status, energy reserves, and ability to sustain growth under varying environmental conditions. It helps assess the balance between photosynthetic carbon assimilation and carbohydrate utilization, guiding cultivation practices to optimize seedling health and productivity. Impact of CNF was clearly stimulatory in both Jowar and Chickpea seedling; but in Chickpea it was slightly inhibitory at 15 ppm CNF.

Reducing Sugar almost negligible levels of reducing sugars may be indicative of impaired nutrient uptake by the seedlings. Factors such as soil pH imbalance, nutrient imbalances or deficiencies, or root damage could affect the plant's ability to absorb essential nutrients from the soil, leading to reduced sugar production.

TABLE 3 A - Impact of CNF on Primary Metabolites, and Auxin content of Jowar seedlings

Conc. of CNF (ppm)	Total Protein g/100gm	Free Amino Acids D - in cm; Rf- in cm	Total Carbohydrate g/100 gm	IAA µg/lit
0.0	11.3	**ND**	43.59	12.096
0.5	11.8	*Tyrosine* D=3.7 Rf=0.60	44.21	19.907
1.0	12.5	*Tyrosine* D=3.7 Rf=0.60	45.95	16.386
5.0	14.53	*Tyrosine* D=3.6 Rf =0.58	51.32	16.855
10	16.8	*Leucine* D=5.0 Rf= 0.81	46.66	16.895
15	17.9	*Tyrosine* D= 3.5 Rf= 0.56	47.42	16.825

TABLE 3B - Impact of CNF on Primary Metabolites, and Auxin of Chickpea

Conc. of CNF (ppm)	Total Protein g/100 gm	Free Amino Acids D= cm Rf= cm	Total Carbohydrate g/100 gm	IAA content µg/lit
0.0	14.73	**ND**	42.40	30.813
0.5	15.4	**ND**	41.81	29.726
1.0	16.71	*Leucine* D=3.4 Rf= 0.79	62.71	29.645
5.0	17.78	*Leucine* D=3.4 Rf= 0.79	44.14	29.244
10	18.12	*Leucine* D=3.5 Rf=0.81	47.50	20.943
15	19.19	*Leucine* D=3.4 Rf=0.79	40.90	17.603

ND = Not Detected

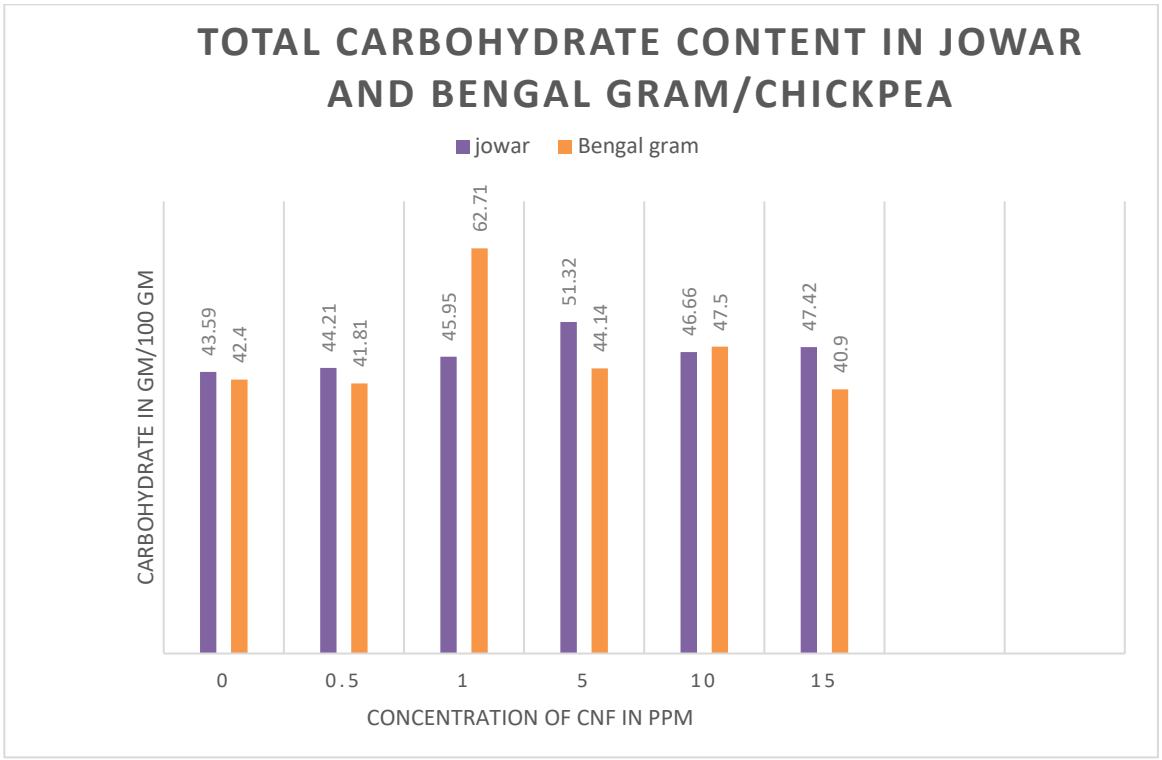


Figure 3: Histogram representing total carbohydrate content of jowar and Bengal gram in g/100gm of sample.

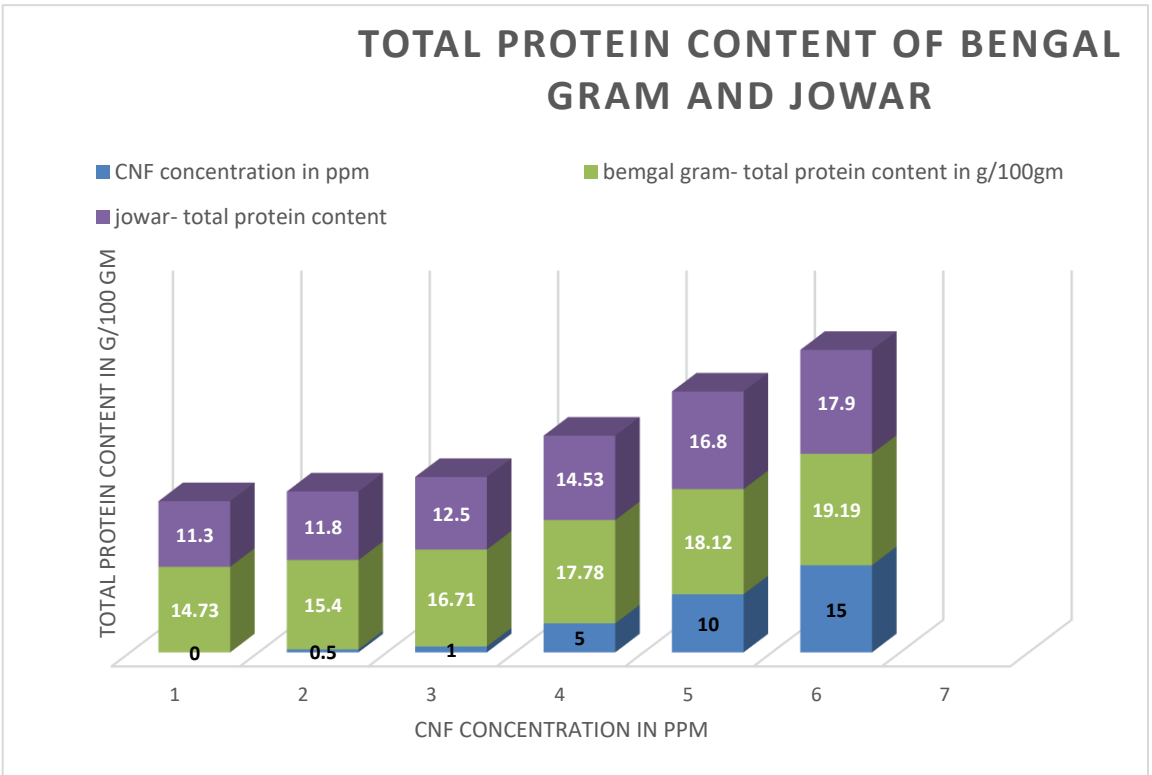


Figure 4: Histogram representing the total protein content of Bengal gram and jowar in g/100 gm

of sample

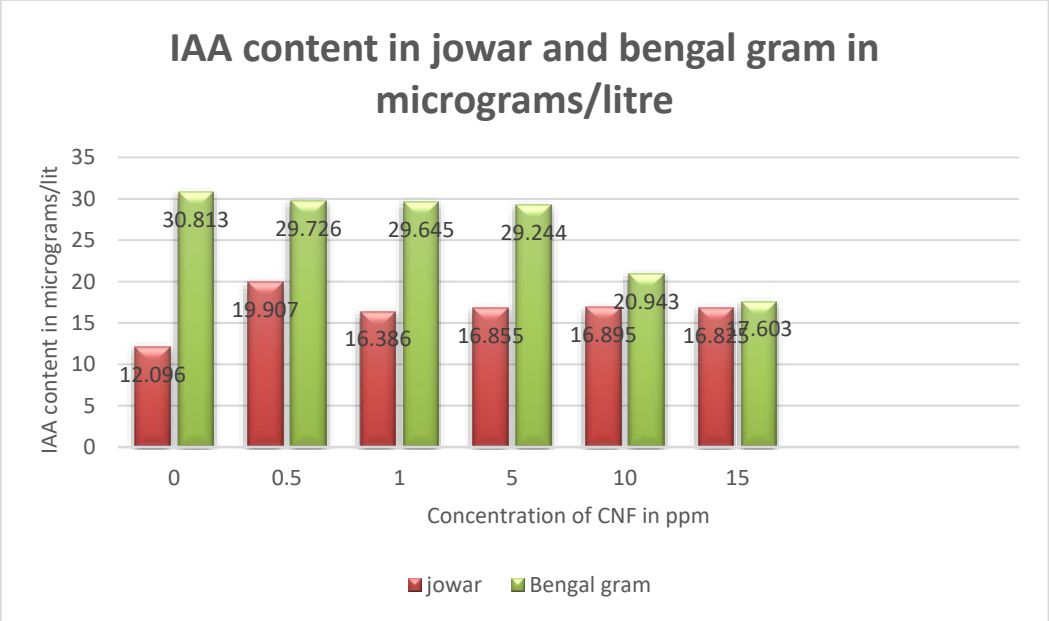


Figure 5: Histogram representing Indole acetic acid (IAA) content in Bengal gram and jowar in µg/lit

CONCLUSION

In our quest to find out whether CNF is suitable and safe material to be used as for controlled release of NPK, we studied its impact on the seed germination and early stages of growth of a monocot (Jowar) and a dicot (Chickpea) seedling in soil containing CNF. Except for seed germination percentage, which was inhibited in Jowar; both the plants used in the present study has shown that seedling growth, it's primary metabolites and auxin content as well as mitotic index was promoted in presence of CNF. However, lower concentrations were more suitable. Results showed that Chickpea was more synergistic than Jowar.

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