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Effects of urea coated hydroxyapatite nanoparticles on biophysical parameters and oil profiling in two varieties of *Brassica juncea* L

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Abstract
Presence of nutrient in soil affect a crop in both qualitative as well as quantitative way. Use of slow releasing fertilizers is trending nowadays as these affect a crop positively. Urea coated hydroxyapatite nanoparticles are one of the slow releasing fertilizers. In the present investigation, urea coated hydroxyapatite nanoparticles were synthesized, and characterized. The soil in different pots were treated with these nanoparticles, and urea. Some pots (without) any treatment, were used as control. Effect of these treatments were observed on biophysical parameters and oil profiling in two varieties (GIRIRAJ, and NRCBH 101) of *Brassica juncea* L. Results revealed that in the presence of urea coated hydroxyapatite nanoparticles, plant height, number of branches per plant, number of seeds per plant and seed weight increased significantly. By oil profiling of seeds of these plants also showed positive effect of these nanoparticles. In both varieties, saturated fatty acids (non-essential) were decreased while MUFA, and PUFA (essential) fatty acids increased which is beneficiary to human's life.

Keywords: Urea coated hydroxyapatite nanoparticles, *Brassica juncea* L., biophysical parameters, oil profile etc

Introduction

Brassica juncea L. (also known as rapeseed mustard), is an oil seed crop. It belongs to family Cruciferae. It is one of the economically important crops which is grown in more than 50 countries in the world (Woods *et al.*, 1991) [14].
Oil contains different kinds of fatty acids. Saturated fatty acids are non-essential for body while monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are essential for body so these are considered as good fatty acids. Oils possessing more MUFA, more PUFA and low saturated fatty acids, are considered as good for health.
Quality and quantity of a crop depends upon availability of nutrients in soil. There are many essential micronutrients and macronutrients which are required for different metabolic pathways (Barita Y.*et al*., 2018) [15].
In soil, all essential nutrients are not present in optimum quantity in soil. So, there is need to add fertilizers in soil. Since long, various chemical fertilizers are being used to increase productivity of crops but these chemical fertilizers have harmful effects on environment. These may cause pollution, and affect lives of animals and humans after accumulating in their body by bio magnification process (Zebarth B.J.*et al*., 2009) [16].
Organic fertilizers are used to overcome hazards caused by chemical fertilizers. But may affect us negatively like higher concentration of nitrate may harm humans (Mensinga*et al*., 2003) [17]. Nowadays, use of slow releasing fertilizers are in trend. These slow- release fertilizers increase nutrient uptake proficiency of plants, lower continual application, decrease in money and labour, and upgrade storage and managing properties (Liu*et al*., 2011) [1].
Manufacturing of novel and innovative slow releasing fertilizers using nanotechnology is required to resolve the issues of nutrient losses because of their nano scale size and high surface to volume ratio (DeRosa*et al*., 2010; Kottegoda*et al.*, 2011; Gunaratne*et al*., 2016; Pulimi and Subramanian, 2016; Dimkpa and Bindraban, 2018; De Silva*et al*., 2020) [7, 3, 7, 6, 2, 5].
Urea coated hydroxyapatite nanoparticles are one of those slow releasing fertilizers. It has been studied that these nanoparticles release nitrogen slowly than conventional urea. These nanoparticles are widely renowned for their intrinsic biocompatibility and biodegradability, being the main component of human bones and teeth. So, use of these nanoparticles as fertilizers should not raise any concern on human and environmental health (Gomez-Morales, J.,*et al*., 2013; Tampieri A.,*et al*., 2016; Sprio S.,*et al*., 2017.) [8, 11, 9].
In the present investigation, effect of synthesized urea coated hydroxyapatite nanoparticles was observed on biophysical parameters and oil profiling in two varieties of *Brassica juncea* (GIRIRAJ and NRCBH 101).

Materials and Methods

1. **Synthesis and characterization of Urea coated hydroxyapatite nanoparticle**

Hydroxyapatite nanoparticle (HANPs) were synthesized using the method of Sandhofer*et al*., 2015. Urea coated Hydroxyapatite nanoparticles (UHANPs) were synthesized by using method of Kottegoda*et al*., 2013 [13]. For this, saturated urea solution, HANPs, and double distilled water were used. All the reagents were used of analytical grade. All solutions were prepared in double distilled water.
These synthesised nanoparticles were characterised by using X-Ray diffraction (XRD), Field Emission Scanning Electron Microscope (FESEM), and Fourier Transform Infra-Red (FTIR) techniques.

2. **Pot experiment**

a. **Site and climatic conditions of experimental area:** the present investigation was carried out at Jaipur

National university, Jaipur during November-Febuary, 2019-20. The research area is located at 26.835251 North latitude 75.824341 East longitude. The experimental site has semi-arid subtropical climate and characterized by very hot summer and mild winters.

b. **Soil collection and profiling:** the soil for experiment, was collected from Jaipur National University, Jagatpura, Jaipur. To know the exact nature and physico-chemical properties of the soil, a composite soil sample was taken before application of fertilizers and was subjected to mechanical and chemical analysis.

c. **Seed collection and processing:** Seeds of two varieties (Giriraj and NRC-BH 101) of *Brassica juncea L.* were obtained from Rajasthan Agricultural Research Institute (RARI), Durgapua, Jaipur, Rajasthan, India. Variety Giriraj was given name S1 while variety NRC-BH 101 was given name S2. Seeds of both the varieties were washed with running tap water. Those were surface sterilized with 5% NaOCl for 5 minutes and then washed repeatedly for two to three times with distilled water to prevent fungal/bacterial contamination. Treatment to soil and sowing of seeds Then seeds were surface sterilized in 0.01% mercuric chloride ($HgCl_2$) solution for 2 min followed by washing with autoclave water and dried on sterilized filter paper.

d. **Soil treatment and Sowing of seeds:** Seeds of both varieties of *Brassica juncea* L. were sown into plastic trays filled with peat (pH-5.5, EC-250 dS/m, N-300 mg/l, P-131 mg/l, K- 333.33 mg/l, organic matter- 2%). The seedlings were initially grown in a greenhouse and fertilized with 20N-20P-20K soluble fertilizers. About 1 month old seedlings were transplanted in pots. During the plant growing period, furrow irrigation was used. Insecticides were applied to avoid crop damage. Weeds were kept under control manualy. The experiments were conducted in earthen pots filled with the soil and pots were arranged in a completely randomized factorial design with three replicated each. Soil portions equivalent to 1 kg of dried soil were individually contaminated (by mechanical mixing in pots) with control (no addition of N fertilizer) and 2 treatments (500 ppm of each of urea, and Urea coated hydroxyapatite nanoparticles). Seeds were sown at a rate of 3 in each pot and thinned to one seedling in each pot 2 weeks to the sowing time. Deionised water was added on alternative days throughout the experiment to keep the water content near to the field capacity. Treatment was given by spraying using a hand-held sprayer four times during the vegetation at 10-day intervals.

e. **Harvesting of plant material:** Plants were harvested at developmental stage which marks 90 days to the time of sowing. From the harvested plants, seeds were collected, and processed for further evaluation.

3. **Biophysical parameters:** after harvesting, biophysical parameters of plants like plant height, number of primary branches per plant, branches per plants, number of secondary branches per plant, number of siliquas per plant, number of seeds per siliqua, and 1000 seed weight were recorded in all plants.

4. **Oil profiling:** fatty acids in the seeds were determined by gas-chromatography- Flame ionisation detector technique. Seeds were weighed in a suitable glass test tube (50-100 mg) and 200 mg of anhydrous sodium sulphate was added to remove the moisture. After 5 min, 2.0µl of sulfuric acid in methanol (2.5%) was added and incubated at 80°C on water-bath for 15 minutes. Above mixture was cooled down to room temperature and 200 mg of sodium chloride was added to it. After few minutes, 2.0µl hexane was added to above reaction mixture and allowed to vortex shaking for about 2 minutes. This mixture was transferred to centrifuge tube and centrifuged for 10 minutes at 3000 rpm. After the process of centrifugation, the upper layer (hexane fraction) was taken with the help of micropipette and transferred to glass vial for GC analysis. For analysis, analytical column of TG-WAXMS (30 m x 0.25 mm ID x 0.25) was used. 1µl sample was injected with 1.0 ml/min flow rate. After completion of chromatographic run the peaks observed in sample chromatogram were integrated. Results were generated as area percentage and on basis of area percentage of different components; saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids (in % or g/100 g) were calculated.

Results and Discussion

Characterisation of nanoparticles

XRD pattern of the synthesised nanoparticles indicated the presence of peaks due to UHANPs. The results of FESEM studies revealed that the average particle size of UHANPs ranges from 23-32 nm. It was alos observed that FESEM results are in good agreement with size distribution of UHANPs measured by XRD.by the FTIR studies, structure of UHANPs were confirmed. Results of characteristic analysis of these nanoparticles are shown in table 1 and Graph 1, and Graph 2.

Table 1: The main compound diffraction peaks corresponding with d spacing by XRD method.

Compound name	Obs. Max	d (Obs. max)	Net height	FWHM	Intensity
	2-Theta°	Angstrom	cts	2-Theta°	%
Urea	22.4899	3.95344	2224.09	0.1476	100.00
Calcium phosphate complex	24.8876	3.57773	263.77	0.1476	11.86
Calcium phosphate complex	26.1309	3.41026	116.77	0.2952	5.25
Urea	29.5101	3.02699	335.07	0.0886	15.07
Calcium phosphate	31.8967	2.80575	448.80	0.1181	20.18
Urea	35.7779	2.50978	176.94	0.1771	7.96

Soil profiling: the results showed that the percentage of fine sand in the soil was more in comparison to other components. Thus, on the basis of the above fractional analysis, the soil is categorized as a sandy loam having low aggregation. The results for the mechanical analysis of soil are mentioned in Table 2.

The results revealed that the soil selected for the experiment, is deficient in total Nitrogen, low in organic carbon, medium in phosphorous, rich in potash and the soil was slightly alkaline in nature. The results for chemical analysis of the soil are presented in Table 3.

Table 2: Mechanical Analysis of the Soil.

S.No.	Soil separates	Percentage
1.	Coarse sand	0.30
2.	Fine sand	59.74
3.	Silt	21.12
4.	Clay	18.40
5.	Soil Moisture at sowing	11.25
6.	Field capacity (Moisture)	16.40
5.	Texture class	Sandy loam

Table 3: Chemical Properties of the Soil.

S.no.	Components	Content
1.	Available N (Kg ha^{-1})	174.40
2.	Available P_2O_5 (PPM)	36
3.	Available Potash (PPM)	260
4.	pH (1: 2 Soil-Water Suspension)	7.86
5.	Electrical Conductivity (dsm^{-1} at 250° C)	0.11
6.	Organic Carbon (%)	0.68
7.	Zinc (PPM)	0.60
8.	Iron (PPM)	4.92
9.	Copper (PPM)	0.24
10.	Manganese (PPM)	1.24

Biophysical parameters: results for the effect of urea coated hydroxyapatite nanoparticles on biophysical attributes of GIRIRAJ and NRCBH 101 varieties of *Brassica juncea* are shown in Table 4 and Table 5 respectively.

Results revealed that UHANPs showed positive impact on all the parameters (plant height, number of primary branches per plant, number of branches per plant, nu. Of siliqua/plant, no. of seeds/plant, total no. of seeds/plant, and 1000 seed weight). For variety GIRIRAJ, plant height with treatment of UHANP increased 17.16% as compared to control, and 9.79% as compared to urea. No. of branches/plant increased by 1.26% and 5.75% as compared to c and urea respectively. Branches/plant increased by 27.52% and 21.02% as compared to C and urea respectively. Number of siliquas per plant and number of seeds per siliqua were increased at higher level so total number of seeds per plant were increased by 39.93% and 32.88%. 1000 seed weight was also increased in UHNAP treated plants by 19.94% and 17.57% respectively.

Similar trend was observed in NRCBH 101. Plant height was increased by 16.92% and 10.94%, treated plants produced more primary branches and more total branches on plants. Number of siliquas per plant, and number of seeds per siliqua were also higher is treated plants. So, total number of seeds were increased by 29.03% and 21.51%. 1000 seed weight was also higher in treated plants by 17.01% and 12.31%.

Table 4: Effect of Urea coated hydroxyapatite nanoparticles on biophysical parameters in *Brassica juncea* (variety GIRIRAJ).

Treatment	Plant height (cm)	Number of primary branches/plant	Branches/plant	No. of siliqua/ plant	No. of seeds/ siliqua	Total number of seeds/plant	1000 seed weight (g)
Control	134	3.99	4.2	130.06	8.2	1066	3.96
Urea	143	5.73	5.08	139.7	8.93	1241.27	4.04
UHANP	157	9.03	15.76	319.33	16.67	5323.23	4.75

Table 5: Effect of urea coated hydroxyapatite nanoparticles on biophysical parameters in *Brassica juncea* (variety NRCBH 101).

Treatment	Plant height (cm)	Number of primary branches/plant	Branches/plant	No. of siliqua/ plant	No. of seeds/ siliqua	Total number of seeds/plant	1000 seed weight (g)
Control	130	3.56	3.89	129.44	7.45	964.32	3.82
Urea	137	4.76	4.78	138.4	8.63	1194.39	3.98
UHANP	152	8.34	9.67	305.06	12.34	3764.44	4.47

Oil profiling: Presence of different fatty acids in all seed samples are shown in Table 6, and Figure 3.

Results revealed that in plants treated with UHANPs, fatty acids contents varied significantly than control and urea treated plants. Results of presence of various fatty acids are shown in Figure 4-9.

For GIRIRAJ variety, Total saturated fatty acids were decreased by 57.88 % and 2.91 % than Control and urea, MUFA and PUFA increased by 2.81 % and 0.13% than control and urea respectively.

For variety NRCBH 101, Total saturated fatty acids were decreased by 53.40% and 2.91% than Control and urea, MUFA and PUFA increased by 2.81% and 0.13% than control and urea respectively.

Total content (%) of saturated, MUFA, and PUFA are shown in Table 7.

Table 6: Effect of urea coated hydroxyapatite nanoparticles on oil composition (%) in two varieties of *Brassica juncea* L.

Name of fatty acid	Type of fatty acid	NRCBH101 control	NRCBH 101 urea	NRCBH 101 UHANP	Giriraj control	Giriraj Urea	Giriraj UHANP
Myristic acid (C14:0)	Saturated	.074	.06	.061	.069	.102	.074
Palmitic acid (C16:0)	Saturated	2.938	1.033	1.135	2.592	2.195	1.601
Stearic acid (C18:0)	Saturated	1.184	.899	.852	.968	.8	.853
Arachidic acid (C20:0)	Saturated	.764	.742	.71	.803	.887	.714
Behenic acid (C22:0)	Saturated	1.347	1.177	1.107	1.225	1.189	1.036
Lignoceric acid (C24:0)	Saturated	.834	.744	.658	.646	.641	.681
Palmitoleic acid (C16:1)	MUFA	.146	.161	.149	.249	.213	.179
Oleic acid (C18:1)	MUFA	8.505	9.228	9.528	7.816	8.532	9.392
Gandoleic acid (C20:1)	MUFA	6.08	4.495	4.253	5.348	4.293	4.15
Eruic acid (C22:1)	MUFA	51.119	51.497	50.209	49.396	48.409	48.47

Nervonic acid (C24:1)	MUFA	2.087	2.151	2.417	2.037	2.378	2.237
Linoleic acid (C18:2)	PUFA	13.906	14.925	15.261	14.566	15.088	15.25
Linolenic acid (C18:3)	PUFA	10.337	11.9	12.76	13.392	14.294	14.558
Eicosandienoic acid (C20:2)	PUFA	.679	.988	.9	.893	.979	.805

Table 7: Effect of urea coated hydroxyapatite nanoparticles on different types of fatty acids in oil from two varieties of *Brassica juncea* L.

Plants	Total saturated	Total MUFA	Total PUFA
Control GIRIRAJ	7.141	67.937	24.922
UREA Giriraj	4.655	67.532	27.813
UHANP Giriraj	4.523	66.556	28.921
Control NRCBH 101	7.141	67.937	24.922
Urea NRCBH 101	4.655	67.532	27.813
UHANP NRCBH 101	4.523	66.556	28.921

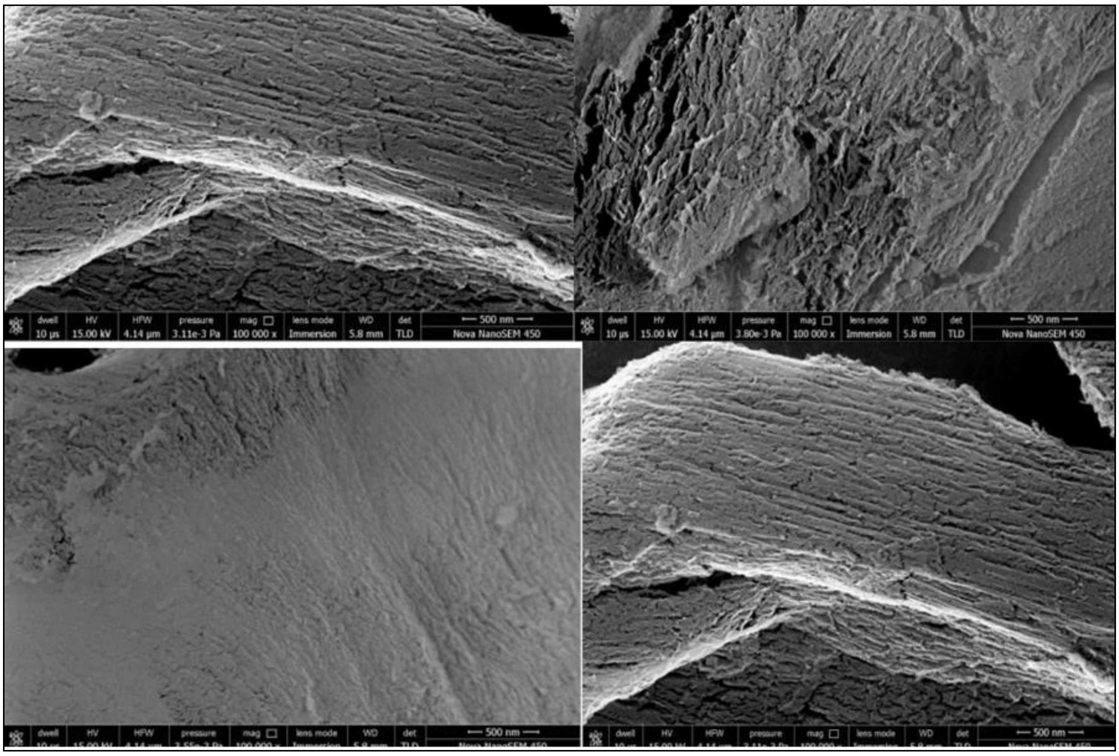

Fig 1: FESEM images of synthesized Urea coated hydroxyapatite nanoparticles.

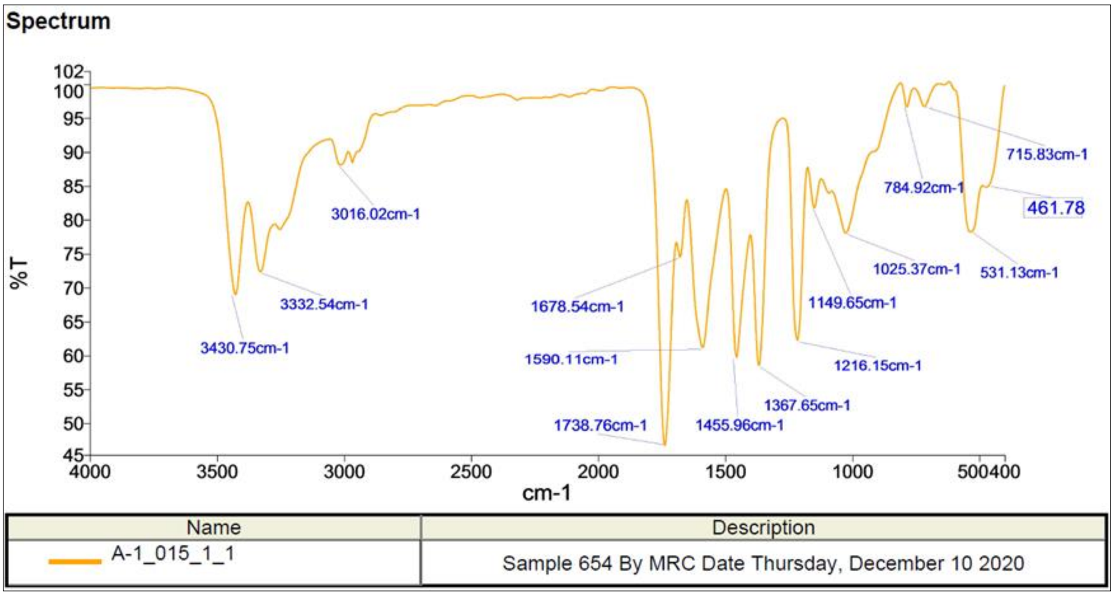

Fig 2: FTIR Spectrum of the synthesized urea coated hydroxyapatite nanoparticles.

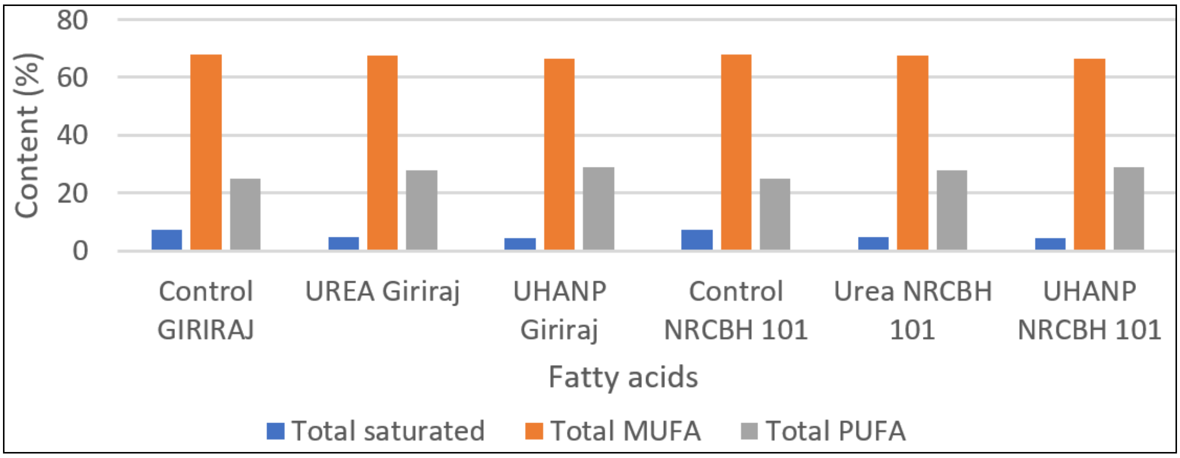

Fig 3: Effect of urea coated hydroxyapetite nanoparticles on fatty acid content in two varieties of *Brassica juncea* L.

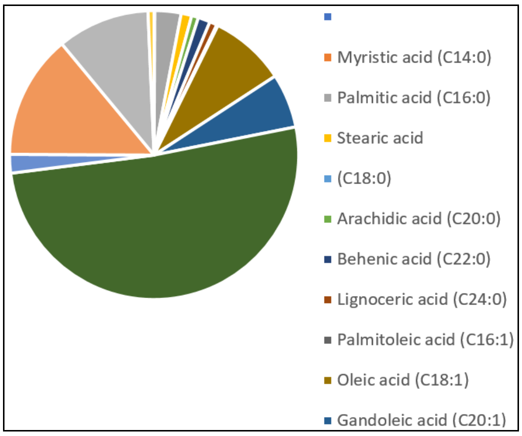

Fig 4: Fatty acid content (%) in control plants of *Brassaica juncea* (varietyNRCBH101)

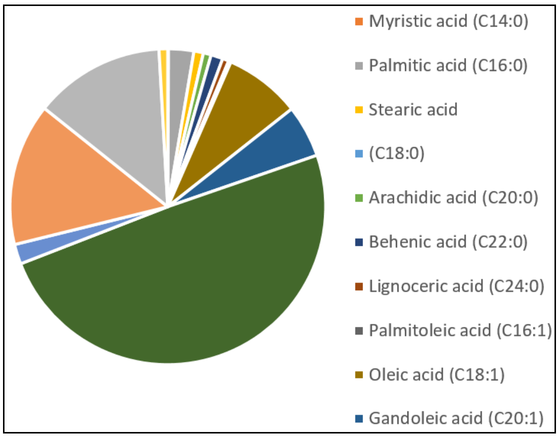

Fig 7: Fatty acid content (%) incontrol plants of *Brassaica juncea (*variety GIRIRAJ)

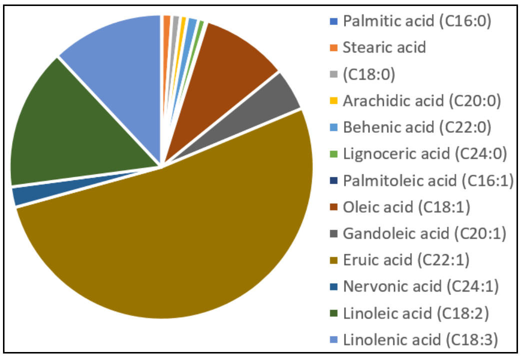

Fig 5: Fatty acid content (%) in urea treated plants of *Brassaica juncea (variety* NRCBH 101)

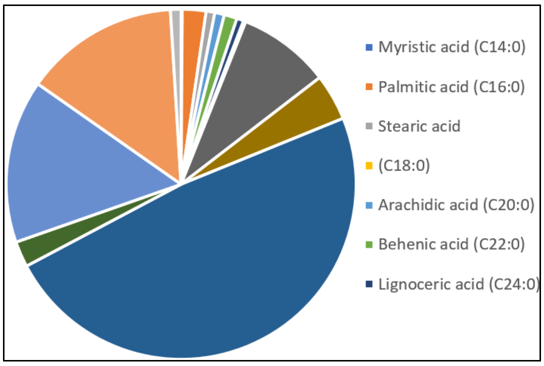

Fig 8: Fatty acid content (%) in urea treated plants of *Brassaica juncea* (variety GIRIRAJ)

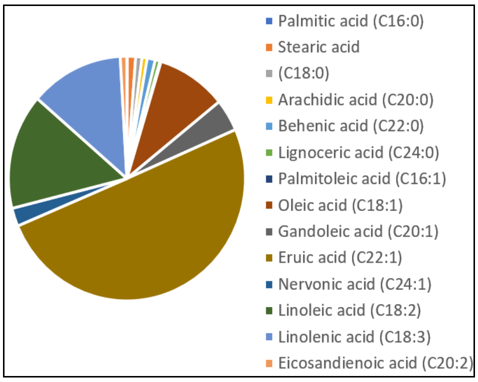

Fig 6: Fatty acid content (%) inUHANPs treated plants of *Brassaica juncea (*variety NRCBH 101)

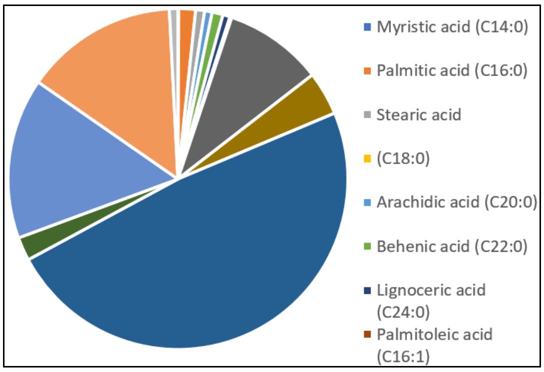

Fig 9: Fatty acid content (%) inUHANPs treated plants of Brassaica juncea (variety GIRIRAJ)

Discussion

Brassica juncea are mainly grown for its seeds. Seeds are used for edible oil. Saturated fatty acids in any oil are non-essential for body. Higher quantity of saturated fatty acids may cause life threatening diseases. MUFA and PUFA are essential fatty acids. These are good for body.

Results of the present studies revealed that plants treated with urea coated hydroxyapatite nanoparticles produced large number of seeds and seeds have more weight also. So, in these treated plants, economic yield may be higher. It was also observed in the present study, that in oil of seeds in saturated fatty acid content decreased while MUFA and PUFA increased which showed good quality of the oil may be obtained by this treatment.

Conclusion

From the present investigation, it can be concluded that treatment of urea coated hydroxyapatite nanoparticles can cause production of good crop of *Brassica juncea* in both qualitative and quantitative manner.

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References

1. Liu XW, Lu JW, Li XK. Dry matter accumulation and N, P, K absorbtion and utilization in direct seeding winter oilseed (*Brassica napus* L.) *China Agric Sci*,2011:44(23):4823-4832. http://dx.doi.org/10.3864/j.issn.0578-1752.2011.23.008)
2. Dimkpa CO, Bindraban PS. Nanofertilizers: New Products for the Industry? Journal of Agricultural and Food Chemistry,2018:66(26):6462-6473. https://pubs.acs.org/doi/10.1021/acs.jafc.7b02150
3. Kottegoda N, Munaweera I, Madusanka, Karunaratne VA. green slow-release fertilizer composition based on urea-modified hydroxyapatite nanoparticles encapsulated wood. *Current Science*,2011:101(1):6. https://www.jstor.org/stable/24077865
4. DeRosa MC, Monreal M. Schnitzer R, Walsh, Sultan Y. Nanotechnology in fertilizers. *Nature Nanotechnology*,2010:5(2):91. https://doi.org/10.1038/nnano.2010.2
5. De Silva M, Siriwardena DP, Sandaruwan C, Priyadarshana G, Karunaratne V, Kottegoda N. Ureasilica nanohybrids with potential applications for slow and precise release of nitrogen. *Materials Letters*,2020:272:127839. https://doi.org/10.1016/j.matlet.2020.127839
6. Pulimi M, Subramanian S. Nanomaterials for soil fertilisation and contaminant removal. In Nanoscience in food and agriculture 1, ed. S. Ranjan, N. Dasgupta and E. Lichtfouse, *Cham: Springer International Publishing*, 2016, 229-246. https://dx.doi.org/10.1007/978-3-319-39303-2_8
7. Gunaratne GP, Kottegoda N, Madusanka I, Munaweera C, Sandaruwan WMGI, Priyadarshana A*et al*. Two new plant nutrient nanocomposites based on urea coated hydroxyapatite: *Efficacy and plant uptake.* Indian Journal of Agricultural Sciences,2016:86(4):494–499. https://www.cabdirect.org/cabdirect/abstract/20163140090
8. Gómez-Morales J, Iafisco M, Delgado López JM, Stéphanie S, Drouet C. Progress on the preparation of nanocrystalline apatites and surface characterization: Overview of fundamental and applied aspects. *Prog. Cryst. Growth Charact. Mater*,2013:59:1-46. http://dx.doi.org/10.1016/j.pcrysgrow.2012.11.001
9. Sprio S, Sandri M, Ruffini A, Adamiano A, Iafisco M, Dapporto M*et al*. Tissue Engineering and Biomimetics with Bioceramics. *In Advances in Ceramic Biomaterials; Palmero, P., Cambier, F., De Barra, E., Eds.; Woodhead Publishing: Cambridge, UK*,2017, 407-432. https://www.istec.cnr.it/wp-content/uploads/2020/03/CV_Panseri_ENG.pdf
10. Tampieri A, Iafisco M, Sprio S, Ruffini A, Panseri S, Montesi M*et al*. Hydroxyapatite: From Nanocrystals to Hybrid Nanocomposites for Regenerative Medicine. *In Handbook of Bioceramics and Biocomposites; Antoniac, I., Ed.; Springer: Cham, Switzerland*,2016, 119-144. https://doi.org/10.1007/978-3-319-12460-5_6
11. Tampieri A, Iafisco M, Sprio S, Ruffini A, Panseri S, Montesi M*et al*. Hydroxyapatite: From Nanocrystals to Hybrid Nanocomposites for Regenerative Medicine. *In Handbook of Bioceramics and Biocomposites; Antoniac, I., Ed.; Springer: Cham, Switzerland*, 2016, 119-144. https://doi.org/10.1007/978-3-319-12460-5_6
12. Sandhöfer B, Meckel M, Delgado-López JM, Patrício T, Tampieri A, Rösch F*et al*. Synthesis and preliminary *in vivo* evaluation of well-dispersed biomimetic nanocrystalline apatites labeled with positron emission tomographic imaging agents. ACS Appl. Mater. Interfaces,2015:19:10623:10633. https://doi.org/10.1021/acsami.5b02624
13. Kottegoda N, Munaweera I, Samaranayake L, Gunasekara S, De Alwis A, Karunaratne V*et al*. Cellulose based sustained release macronutrient composition for fertilizer application (US20110296887A1), 2013. https://patentscope.wipo.int/search/en/detail.jsf?docId=EP77001057
14. Woods DL, Capcara RK. The potential of mustard (*Brassica juncea* (L.) Coss) as an edible oil crop on the Canadian Prairies. Can. J. Plant Sci,1991:71:195-198. https://doi.org/10.4141/cjps91-025
15. Barita Y, Prihastanti E, Haryanti S, Subagio A, Nagadiwiyana. The influence of granting NPK fertilizer and nanosilic fertilizers on the growth of Ganyong plant (Canna edulis Ker.). *IOP Conf. Series:* Journal of physics: Conf. series,2018:1025:012054. https://iopscience.iop.org/article/10.1088/1742-6596/1025/1/012054.
16. Zebarth BJ, Drury CF, Tremblay N, Cambouris AN. Opportunities for improved fertilizer nitrogen management in production of arable crops in eastern Canada: A review. *Can.* J. Soil Sci,2009:89:113-132. https://doi.org/10.4141/CJSS07102
17. Mensinga TT, Speijers GJ, Meulenbelt J. Health implications of exposure to environmental nitrogenous compounds. *Toxicol. Rev*,2003:22:41-51. https://doi.org/10.2165/00139709-200322010-00005