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Research Article

Carbon Nanotubes Treated Grains

Qualitative Assessment of MWCNT- Treated Grains of Some Food Cereals

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Abstract: The advancement in nanotechnology and its utilisation in agriculture increases agricultural output. To protect crops from numerous plant diseases and pests, excessive chemical, pesticide, and fungicide use can be replaced with nanoparticles. The first step of plant growth, seed germination, is thought to be the most delicate time in a plant's life cycle. The effects of carbon nanotubes on seed quality and seed content both before and after treatment are discussed in this study. Rice, wheat, and oat grains were compared with the seeds harvested from MWCNT (multiwalled carbon nanotubes) treated plants at three different concentrations (70, 80, and 90µg/ml) and indicated no significant changes in various components such as carbohydrates, protein, crude fat, crude fibre, moisture and ash contents when compared to untreated grains. Grain primed with MWCNT before sowing resulted in no change in the above components, but an increase in mineral accumulation was found. Here, we noticed a significant increase in grain yield. The treatment of MWCNT enhanced the growth of grains count by two folds compared to the control plant. The wheat grain had a moisture content: 9.3-9.38%, ash content: 1.31-1.35%, crude fat: 0.9-0.93%, crude fibre: 1.1-1.13%, protein content: 9.2-9.38%, carbohydrate content: 77.96-78.04%. The rice grain had a moisture content: 9.98-10.25%, ash content: 0.42-0.45%, crude fat: 1.00-1.02%, crude fibre: 0.9-0.93%, protein content: 4.42-4.49%, carbohydrate content: 82.92-83.03%. The oat grain had a moisture content: 8.95-9.3%, ash content: 1.74-1.8%, crude fat: 2.1-2.3%, crude fibre: 5.1-5.27%, protein content: 9.5-9.59%, carbohydrate content: 71.78-72.23%. The study revealed that the examined MWCNT concentrations might efficiently enhance the grain yield without altering the grain quality.

Keywords: Wheat, Rice, Oats, MWCNT, Protein Content, Grain Yield

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1. INTRODUCTION

In an economy, agriculture is a valuable sector that fulfils the basic needs of humans and feeds the world. Cereals are a staple food that provides energy, protein, B vitamins and minerals to the world population. Wheat and rice are the most important cereal grain and staple food in many countries (Soto-Gómez et al. 2022)[1]. With the increase in the population, the demand for food is increasing at a fast rate. Due to climate change, low nutrient use efficiency, diminished soil organic matter, stagnation of produce (crop yield), and decreased water availability and land, the agriculture field is going through many problems (FAO 2017)[2]. It seemed to be challenging to feed the growing population by producing on a shrinking landscape, with minimum input costs and environmental hazards (Anonymous, 2009)[3]. To provide food to such a big population, there must be a technology to provide more yield in less time. To conquer the challenges like sustainability and food security, it is essential to use modern technology such as nanotechnology. These days, nanotechnology helps improve agriculture productivity without affecting the environment by using various shapes and sizes of nanoparticles[4,5]. Nanotechnology in food and agriculture systems covers many forms in packaging material [6], food safety [7], delivery systems [8], treatment of diseases, sensors for the detection of pathogens [9], increasing the productivity in a shorter time [10]. Nanotechnology has the potential to change the agriculture sector with the use of advanced tools to increase the absorption of,[11,12] precision farming methods,[13-15] and detection of diseases, [16, 17] control of [18], and pest control[19]. Nanotechnology plays an important role in every stage of agricultural production, processing, storage and transport. Nanotechnology has the potential to diminish pollution by the use of sorbents, filters or catalysts to make agriculture more environmentally friendly (Joseph and Morrison, 2006;[20- 22]. Using nanodevices and nanoparticles has made it possible to change the scenario of the agriculture sector[23]. Previously, amongst all nanoparticles, carbon nanotubes (CNTs) were widely used by a group of researchers to enhance the yield and nutrient content of various food products[24-26]. For example, [27] reported that tomato seeds treated with carbon nanotubes improved the germination rate and biomass due to the penetration of carbon nanotubes inside the seeds and hence increased the water uptake as compared to untreated seeds. Wang and his co-workers in 201228 demonstrated that of-MWCNTs considerably enhance the cell elongation in the root and augment the activity of dehydrogenase enzyme, which leads to rapid root growth and improved biomass.[29] described the effect of MWCNTs and C60 fullerenes on tomatoes and corn. MWCNTs showed an increase in the total biomass of tomatoes and corn, whereas C60 fullerenes negatively affected the biomass production of tomatoes and corn. Rao and Srivastava (2014)[30] investigated approximately the promising outcomes of functionalized multiwalled carbon nanotubes on peanuts, garlic and wheat. Low-dose MWCNTs seemed to be beneficial, enhancing water absorption, determined to boost the germination system via reducing germination time and better biomass production. Zhai et al. (2015)[31] revealed that Maize (*Zea mays*) exposed to MWCNTs at a concentration ranging from 10-50 mg/L for 18 days resulted in accelerated growth, dry biomass and water transpiration rate as compared to maize control. Thus, Carbon nanoparticles can improve the germination rate and yield of products used in a rainfed agricultural system. The effect of foliar application of MWCNTs was recently tested on the *Salvia verticillata* plant. The results revealed the MWCNTs at low dosages improved the production of the pharmaceutically important secondary metabolites by altering the ROS generation (Rahmani et al. 2020)[32]. Recently, Cai et al. 2022[33] reported improved soluble sugar and protein content, plant height, fresh weight, dry weight, leaf width and leaf area by enhancing the activities of soil enzymes in MWCNT's treated *Brassica campestris* plants. In *Zea mays,* MWCNTs at 100 mg/L concentration enhanced the fresh and dry weight of the shoot and root, chlorophyll content, and transpiration rate. Moreover, activities of carbon and nitrogen metabolic enzymes such as sucrose phosphate synthase (SPS), sucrose synthase (SS), phosphoenolpyruvate carboxylase (PEPC), glutamate synthetase (GOGAT), nitrate reductase (NR) and glutamine synthase enzymes leading to the improved content of carbohydrates, soluble proteins and nitrogen in *Zea mays* (Hu et al. 2021)[34]. So far, many researchers have found incredible applications of nanoparticles in the agriculture sector like an increase in the productivity of agricultural produce in a shorter period with lesser hazards to the environment (Joshi et al., 2018a; Joshi et al., 2018b; Joshi et al. 2020)[35, 37]. Hence, it was important to examine the nutritional value of nano-agro grains to determine whether the use of nanoparticles is affecting the nutritional value.

2. MATERIALS AND METHODS

2.1 Procurement of raw material (MWCNT and Grains)

MWCNTs were purchased from Sigma Aldrich (USA), with a length of 3-6μm, an outer diameter of 10±1 nm, an inner diameter of 4.5±0.5 nm and ≥98% carbon basis. MWCNTs are naturally hydrophobic and insoluble in water, so the functionalization of MWCNTs with nitric acid (HNO_3) was compulsory to understand their behaviour and activities in cereal crops Fig 1. Grains of wheat (HD 2956), rice (Pusa basmati 1121) and oats (Kent) were procured from the Grain Market Sector 26 Chandigarh.

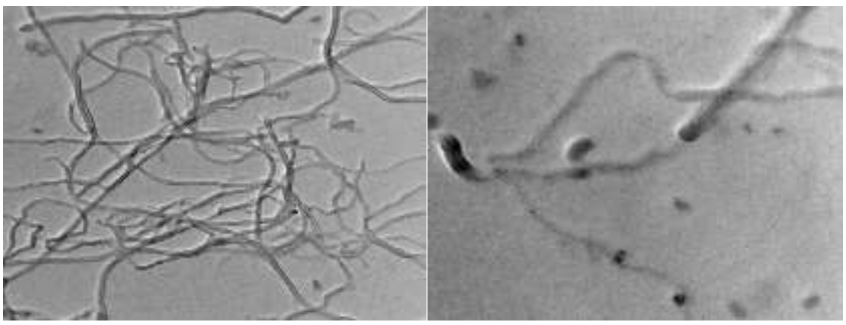

Fig 1. (A) TEM images of MWCNT and (B) Functionalized MWCNT

2.2 Priming of grains

Grains were soaked in MWCNTs (functionalized) solution for 6 hours of different concentrations of (0, 70, 80and 90 µg/ml of distilled water). The grain samples primed with MWCNTs were denoted as C0 (control), MW70, MW80 and MW90. Grain priming is a presoaking treatment to decrease the germination time and increase the germination rate. It is also important to allow the penetration of CNTs inside the grains.

2.3 Proximate analysis of grains

All three types of grains were harvested from the MWCNTs treated (70, 80 and 90µg/ml) and untreated (control) plants and thus designated as harvested grains. In addition, the direct control (untreated) and MWCNTs treated (70, 80 and 90µg/ml) grains were primed and hence termed as primed grains and were denoted as C0, MW70, MW80 and MW90.

2.4 Determination of moisture content

In processing and testing foods, the moisture content is one of the most important parameters to determine the quality. Moisture content is directly related to the shelf life of the food product. For example, the moisture content of flour generally ranges from 11-14% (Syeda et al., 2012) [38]. If moisture content goes beyond this range, fungi and mould will grow due to their increased enzymatic activity. Higher moisture content results in loss of nutrients, especially protein and fat (Honey, 1994)[39], so moisture content must be known in determining the nutritional value of a food. The moisture level was calculated according to the method used by AACC, 2000[40]. Two grams of sample were weighed in an empty dish and placed in an oven at 135 ˚C for two hours. After that sample was placed for 30 minutes in a desiccator and again weighed till a constant weight was obtained.

$$\boldsymbol{Moisture}(\%) = \frac{\boldsymbol{Original\ sample\ weight - dried\ sample\ weight}}{\boldsymbol{Original\ sample\ weight}} \times \mathbf{100} \ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

2.5 Determination of ash content

Ash content is an indicator of non-organic or mineral content in food stuffs. The Ash content of the flour has a significant effect on flour colour. Ash content also indicates the performance of milling. Ash content in the samples was estimated through standard analysis methods (AACC, 2000)[40]. Two grams of dried sample were placed in a weighted crucible set at 600˚C for 2 hours until white ash was obtained. Then, the crucibles were removed and cooled in desiccators for 30 minutes and reweighted.

$$\boldsymbol{Ash}(\%) = \frac{\boldsymbol{Ashweight}}{\boldsymbol{sampleweight}} \times \mathbf{100} \ldots\ldots\ldots\ldots\ldots\ldots \text{(2)}$$

2.6 Determination of crude fat

A standard method by AOAC, 2000[41] was used to calculate the Crude fat by means of solvent extraction. Five grams of samples were weighed and transferred to an extraction thimble covered with cotton plugs to avoid the loss of the sample. Next, thimbles were placed in a soxhlet extractor connected with a condenser and flask filled with solvent petroleum ether (boiling point 60-80˚C). Afterwards, samples were heated for 14 hours, and thimbles were removed from the apparatus, dried in a hot air oven, and then cooled and weighed.

$$\boldsymbol{CrudeFat}(\%) = \frac{\boldsymbol{Weightoffat}(g)}{\boldsymbol{Weightofsample}(g)} \times \mathbf{100} \ldots\ldots\ldots\ldots\ldots\ldots \text{(3)}$$

2.7 Determination of crude fibre

Fibre is a carbohydrate, so it plays a crucial role in keeping the digestive system healthy. The crude fibre content was also measured by the suggested standard method (AOAC, 2000) [41]. One gram of the defatted sample was weighed and shifted to a beaker; 200 ml of 1.25% sulphuric acid (H_2SO_4) was added. Samples were boiled on low flame for exactly 30 minutes. Contents were filtered through a muslin cloth placed over a glass funnel. The sample on the cloth was washed several times with boiling water until it became acid-free. The sample was transferred to the beaker containing 200 ml of 1.25% sodium hydroxide (NaOH) and boiled for 30 minutes. The sample was washed back with boiling water until it became alkali free. All insoluble matter was transferred to a crucible and dried at 100˚C in a hot air oven, cooled and weighed. After that crucible was placed in a muffle furnace set at 550˚C for one hour. The crucible was cooled in desiccators and reweighed.

$$\boldsymbol{Crude\ fiber}(\%) = \frac{\boldsymbol{Weightofcruciblewithcontentsafterignition - weightofcrucible}}{\boldsymbol{Weightofsample}\ (g)} \times \mathbf{100} \ldots\ldots\ldots \text{(4)}$$

2.8 Determination of protein

Total protein content was determined using the Lowry method with slight modifications (Lowry et al., 1951)[42].5 ml of phosphate buffer was added to 5 gm of powdered sample and centrifuged at 2000 rpm for 5 minutes. After centrifugation, the supernatant was used for the estimation. Next, 50 mg of BSA (Bovine Serum Albumin) was dissolved in distilled water and made the final volume to 50 ml. 10 ml of BSA stock solution was diluted to 20 ml of distilled water and used as a working solution. Next, 2ml, 4ml, 6ml, and 8ml of the working solution were pipette out in test tubes. Finally, 1 ml of the sample was pipette out into another test tube to make the final volume of 10 ml using distilled water. A blank was prepared by adding 10 ml of distilled water to a test tube. Afterwards, 5 ml of reagent C was added to all the test tubes and allowed to stand for 10 minutes. Then, all the test tubes were added to reagent D of 0.5 ml and mixed well. The test tubes were incubated for 30 minutes, and the absorbance and OD value was observed for each test tube at 680 nm. Bovine serum albumin was used as a standard protein to prepare the standard curve.

2.9 Determination of carbohydrates

The values of crude fat, ash, moisture, protein and crude fibre were added and deducted from 100. This will evaluate the content of available carbohydrates.

Carbohydrate = 100- Moisture content + crude fiber + crude fat + Ash content + protein

3. RESULTS AND DISCUSSION

The proximate composition of harvested and primed grains treated with different concentrations of MWCNTs are shown in Table 1 and Table 2, and their graphical trend is in fig 2-19. The moisture content of the harvested rice grains was compared with MWCNTs-treated wheat and oat grains. Previously Joshi et al. 2018 a, Joshi et al. 2018b; Joshi et al. 2020)[35-37] reported the moisture content of harvested rice grains C0, MW70, MW80 and MW90 was 10.24%, 10.1%, 10.23% and 10.2% respectively. In contrast, the moisture content of primed rice grains at the same treatments was found to be 10.25%, 9.98%, 10.2% and 10%, respectively, indicating the effectiveness of MWCNT. Whereas the moisture content of harvested wheat grains collected from plants treated with C0, MW70, MW80 and MW90 was 9.38%, 9.3%, 9.3% and 9.35%, respectively, whereas that of primed wheat grains was 9.35%, 9.25%, 9.32% and 9.3%, respectively. There was no change from controls in MWCNT-treated plants. The values were supported by the work of Saxena et al. (1995) [43] and Gandhi et al. (2001)[44,] who reported the moisture content of wheat flour from 6-9.85%. In oat, the moisture content for harvested grain, taken from the plants treated with C0, MW70, MW80 and MW90 µg/ml of MWCNTs was found to be 9.28, 9.26%, 9.2% and 9.28% respectively, whereas, in primed rice grains, it was 9.3%, 8.95%, 9.3% and 9.28%, respectively, indicating no effect of MWCNTs. It was documented that ash content for harvested rice grains from C0, MW70, MW80 and MW90 µg/ml of treated plants was 0.42%, 0.44%, 0.43% and 0.42%, whereas in primed rice grains for the same treatments was 0.42%, 0.43%, 0.45% and 0.42%, respectively (Joshi et al. 2020,) [35- 37]. On the other hand, the ash content for harvested wheat grains C0 MW70, MW80 and MW90 µg/ml of MWCNTs was found to be 1.32%, 1.34, 1.37% and 1.34%, whereas the ash content of primed wheat grains was 1.33%, 1.33%, 1.35% and 1.31%, respectively, indicating no effect of MWCNT. Oko et al. (2011)[45] observed the ash content in the 0.50 to 2.00% range. Ash content of harvested oat grains C0, MW70, MW80 and MW90 was found to be 1.77%, 1.8%, 1.79% and 1.74%, while for primed oat grains, the values were 1.76%, 1.78%, 1.77% and 1.74%, respectively, suggesting no effect of MWCNT. The crude fat content of harvested wheat grains, taken from C0, MW70, MW80 and MW90 treated plants were 0.92%, 0.93%, 0.92& and 0.92%, whereas those of primed wheat grains, given the same treatments, were 0.93%, 0.91%, 0.9% and 0.93%, respectively, indicating no effect of MWCNT. Results were found to be similar as reported by Saeid et al., (2015) [46]in the range of 0.893-1.387. The crude fat content of harvested rice grains from C0, MW70, MW80 and MW90 treated plants were 1.02%, 1.02%, 1.00% and 1.01%, whereas it was 1.02%, 1.01%, 1.02% and 1.01%, respectively for the primed wheat grains under the same treatments. The crude fat content of harvested oat grains collected from C0, MW70, MW80 and MW90 treated plants was 2.3%, 2.1%, 2.1% and 2.3%, whereas, in the case of primed oat grains, it was 2.3%, 2.2%, 2.2% and 2.2%, respectively. The crude fibre content of harvested wheat grain from the plants treated with C0, MW70, MW80 and MW90 was 1.12%, 1.12%, 1.1% and 1.11%, respectively, whereas, in the case of primed wheat grains, these values were 1.12% 1.11%,1.13% and 1.12%, respectively. The crude fibre content of harvested rice grain collected from plants, which have been treated with C0, MW70, MW80 and MW90, was 0.93%, 0.91%, 0.92% and 0.92%, whereas crude fibre of primed rice grains at similar concentrations were 0.92% 0.9%, 0.93% and 0.91%, respectively. The crude fibre content in the case of oat grains harvested from the C0, MW70, MW80, and MW90 treated plants was 5.3%, 5.2%, 5.2% and 5.3%, respectively, whereas, in the case of primed oat grains, it was 5.2%, 5.27%, 5.1% and 5.25%, respectively. The protein content in rice grains was 4.47%, 4.47%, 4.49% and 4.42 %, with C0, MW70, MW80 and MW90 treatments, whereas in the case of primed rice grains, it was 4.48%, 4.46%, 4.45% and 4.47%, indicating no effect of MWCNT(Joshi et al. 2020)[37]. The protein content of harvested wheat grains from the treated plants with C0, MW70, MW80 and MW90 was found to be 9.3%, 9.24%, 9.3%, and 9.2 %, whereas the protein content of primed wheat grains treated with the same concentrations was 9.38%, 9.29%, 9.2% and 9.3%, respectively. The protein content in wheat flour is similar to the result obtained by Sidhu *et al.* (1990); Singh *et al.* (2000)[47, 48], which is in the range of 8-10%. The protein content of harvested oat grains collected from C0, MW70, MW80, and MW90 treated plants was recorded to be 9.57%, 9.5%, 9.58% and 9.53%, whereas, in primed grains, these values were 9.58%, 9.57%, 9.59% and 9.56%, respectively. The values were similar to the work of Sterna *et al.* (2016), [49]who reported the protein content in the 9.70-17.30% range. Carbohydrate content in wheat grains harvested from the plants treated with C0, MW70, MW80 and MW90 was 77.96%, 78.07, 78.01% and 78.08%, whereas in the case of primed wheat grains, at the same concentrations, it was 77.89%, 78.01%, 78.01% and 78.04%, respectively. In rice grains harvested from C0, MW70, MW80, and MW90 treated plants. Carbohydrate content was 82.92%, 83.06, 82.93% and 83.03%, whereas, in primed rice grains, it was 82.91%, 83.22%, 82.95% and 83.19%, respectively. The Carbohydrate content of harvested oat grains from the plants treated with C0, MW70, MW80 and MW90 was 71.78%, 72.14, 72.13% and 71.84%, whereas, in primed oat grains, it was 71.86%, 72.23%, 72.04% and 71.97%, respectively.

Table 1. Proximate nutritional value of harvested grains of wheat, rice and oat plants treated with MWCNT at different concentrations of 0, 70, 80 and 90µg/ml.

Parameters	Wheat 0	Wheat 70	Wheat 80	Wheat 90	Rice 0	Rice 70	Rice 80	Rice 90	Oat 0	Oat 70	Oat 80	Oat 90
Moisture (%)	9.38	9.3	9.3	9.35	10.24	10.1	10.23	10.2	9.28	9.26	9.2	9.28
Ash (%)	1.32	1.34	1.37	1.34	0.42	0.44	0.43	0.42	1.77	1.8	1.79	1.74
Crude fat (%)	0.92	0.93	0.92	0.92	1.02	1.02	1.00	1.01	2.3	2.1	2.1	2.3
Crude fibre (%)	1.12	1.12	1.1	1.11	0.93	0.91	0.92	0.92	5.3	5.2	5.2	5.3
Protein (%)	9.3	9.24	9.3	9.2	4.47	4.47	4.49	4.42	9.57	9.5	9.58	9.53
Carbohydrate (%)	77.96	78.07	78.01	78.08	82.92	83.06	82.93	83.03	71.78	72.14	72.13	71.84

Table 2. Proximate nutritional value of primed grains of wheat, rice and oat plants treated with MWCNT at different concentrations of 0, 70, 80 and 90µg/ml.

Parameters	Wheat 0	Wheat 70	Wheat 80	Wheat 90	Rice 0	Rice 70	Rice 80	Rice 90	Oat 0	Oat 70	Oat 80	Oat 90
Moisture (%)	9.35	9.25	9.32	9.3	10.25	9.98	10.2	10	9.3	8.95	9.3	9.28
Ash (%)	1.33	1.33	1.35	1.31	0.42	0.43	0.45	0.42	1.76	1.78	1.77	1.74
Crude fat (%)	0.93	0.91	0.9	0.93	1.02	1.01	1.02	1.01	2.3	2.2	2.2	2.2
Crude fibre (%)	1.12	1.11	1.13	1.12	0.92	0.9	0.93	0.91	5.2	5.27	5.1	5.25
Protein (%)	9.38	9.29	9.2	9.3	4.48	4.46	4.45	4.47	9.58	9.57	9.59	9.56
Carbohydrates (%)	77.89	78.01	78.01	78.04	82.91	83.22	82.95	83.19	71.86	72.23	72.04	71.97

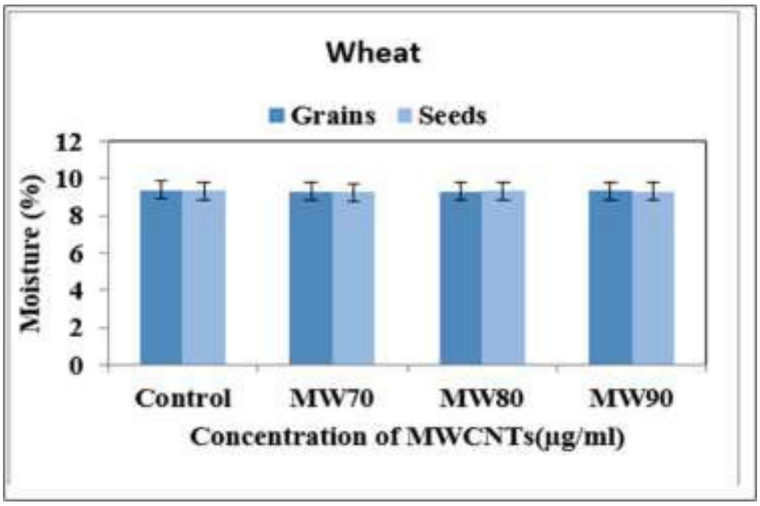

Fig 2. Proximate moisture content of wheat flour at different concentrations of MWCNT.

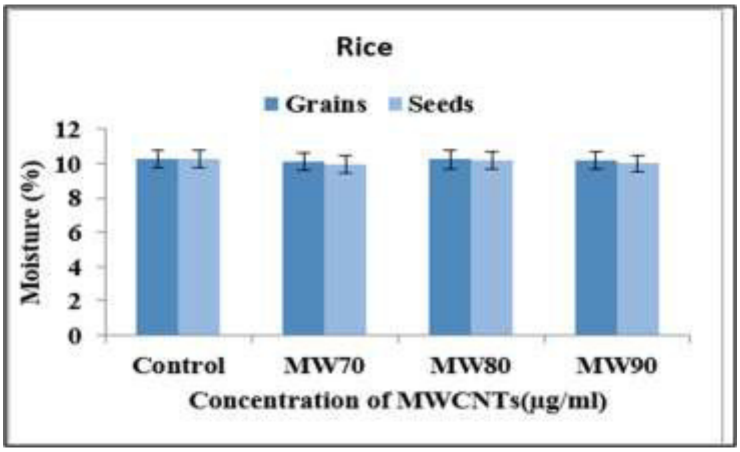

Fig 3. Proximate moisture content of rice flour at different concentrations of MWCNT.

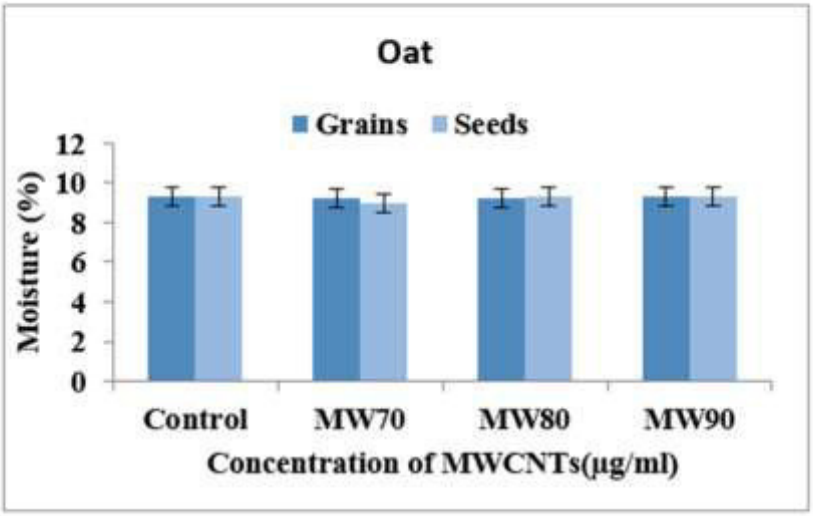

Fig 4. Proximate moisture content of oat flour at different concentrations of MWCNT.

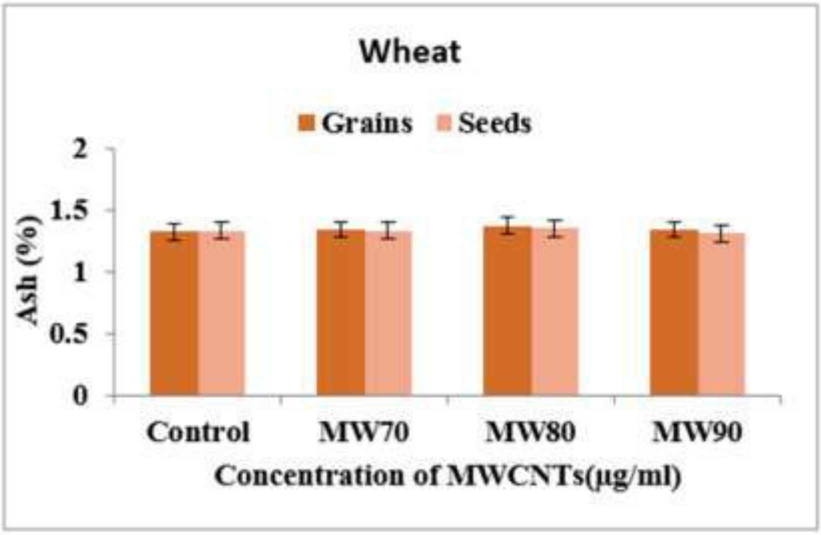

Fig 5. Proximate ash content of wheat flour at different concentrations of MWCNT.

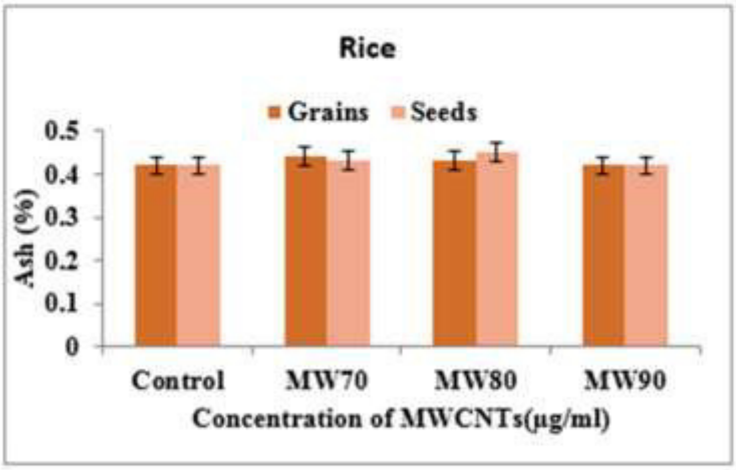

Fig 6. Proximate ash content of rice flour at different concentrations of MWCNT.

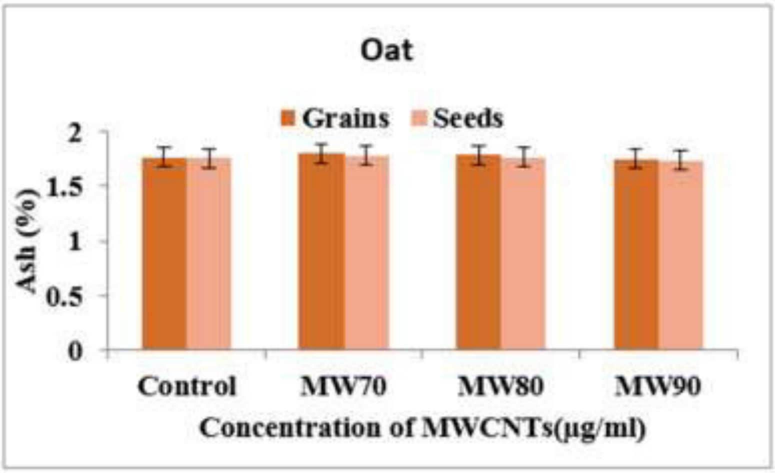

Fig 7. Proximate moisture content of oat flour at different concentrations of MWCNT.

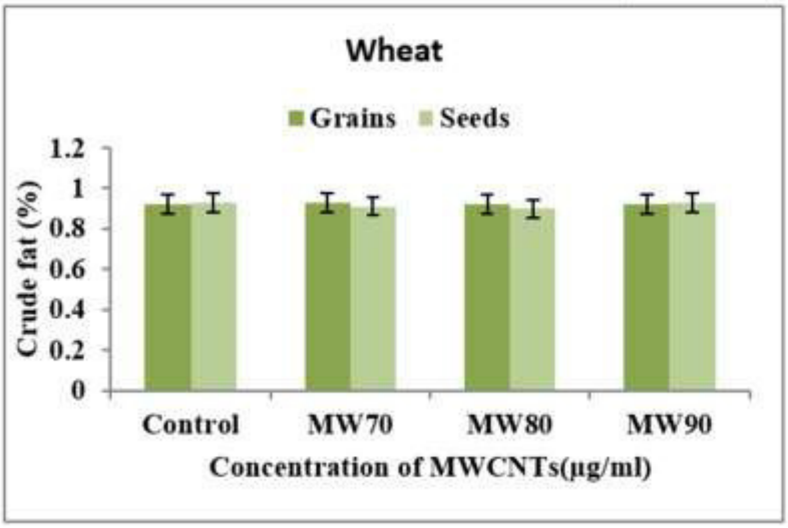

Fig 8. Proximate crude fat content of wheat flour at different concentrations of MWCNT.

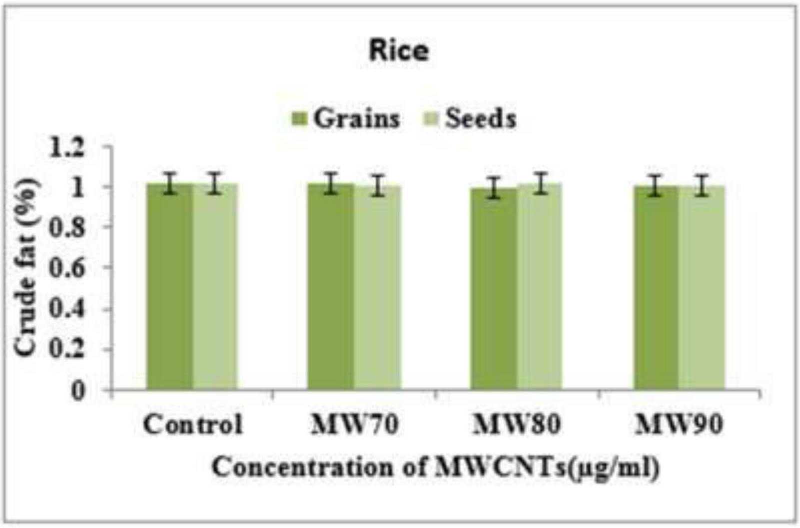

Fig 9. Proximate crude fat content of rice flour at different concentrations of MWCNT.

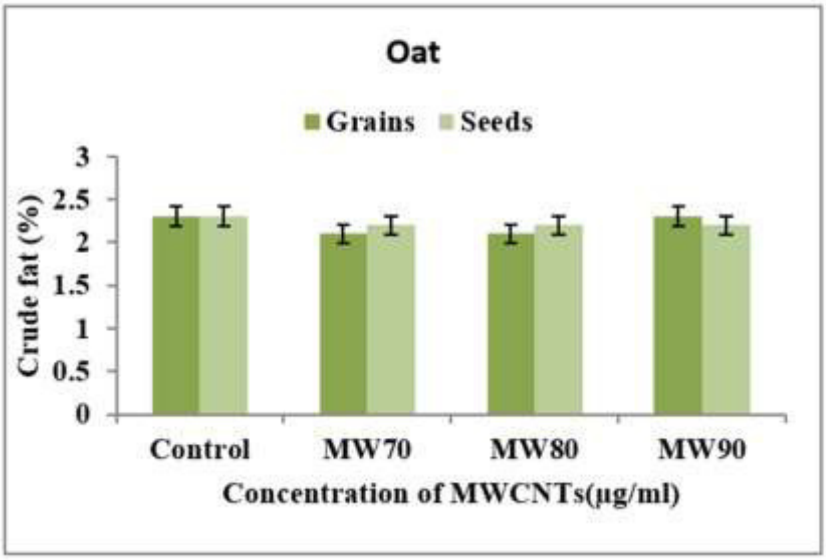

Fig 10. Proximate crude fat content of oat flour at different concentrations of MWCNT.

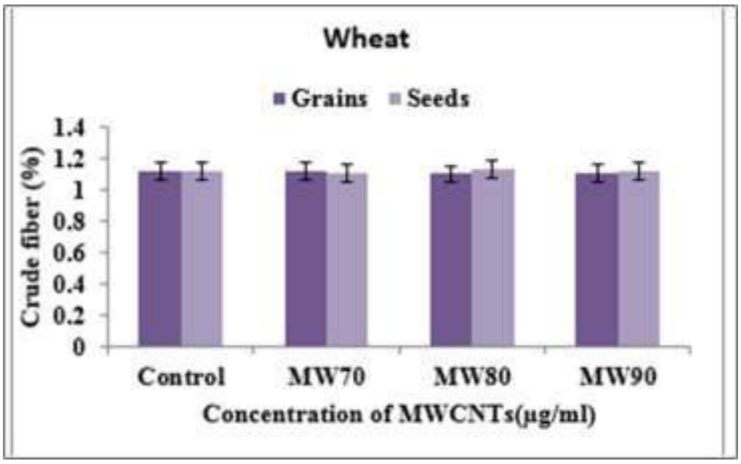

Fig 11. Proximate crude fiber content of wheat flour at different concentrations of MWCNT.

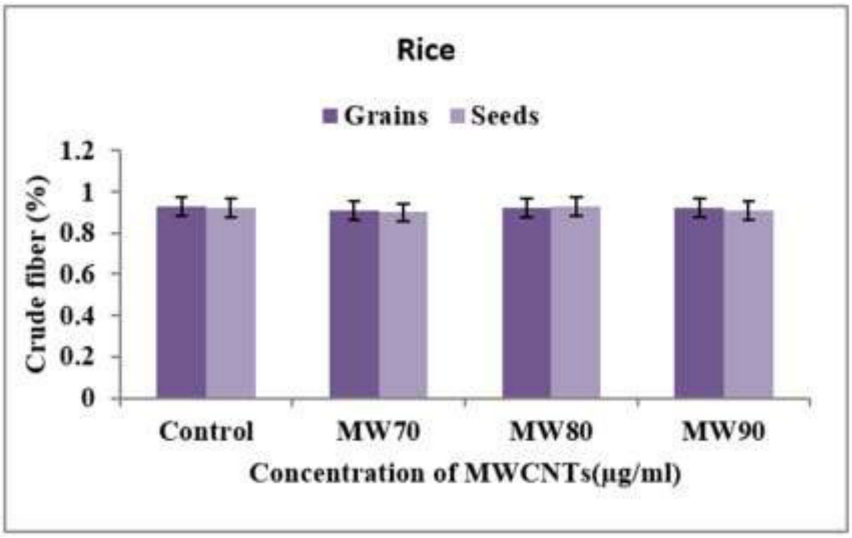

Fig 12. Proximate crude fiber content of rice flour at different concentrations of MWCNT.

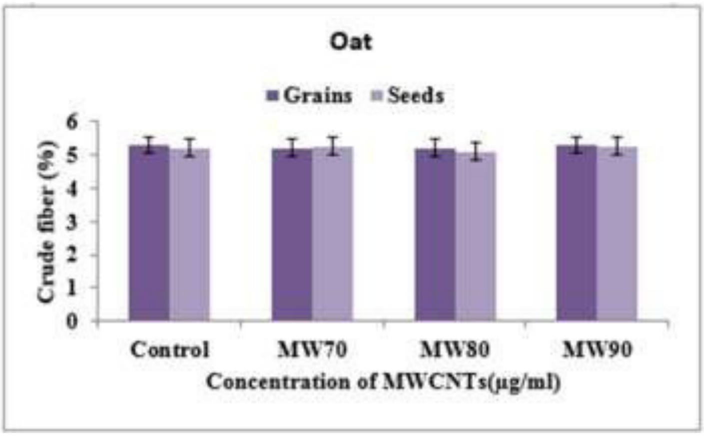

Fig 13. Proximate crude fiber content of oat flour at different concentrations of MWCNT.

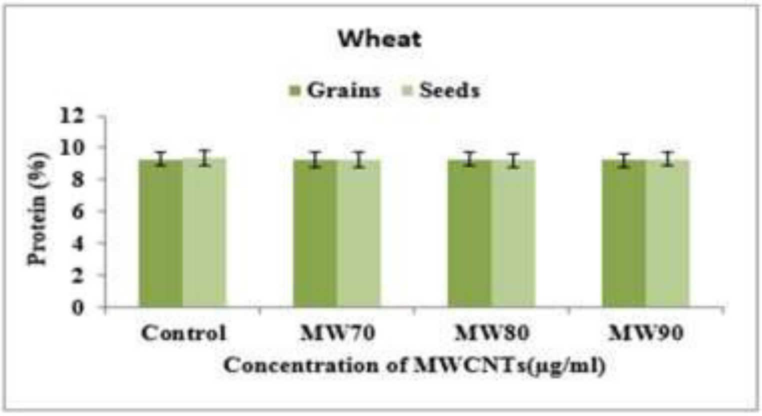

Fig 14. Proximate protein content of wheat flour at different concentrations of MWCNT.

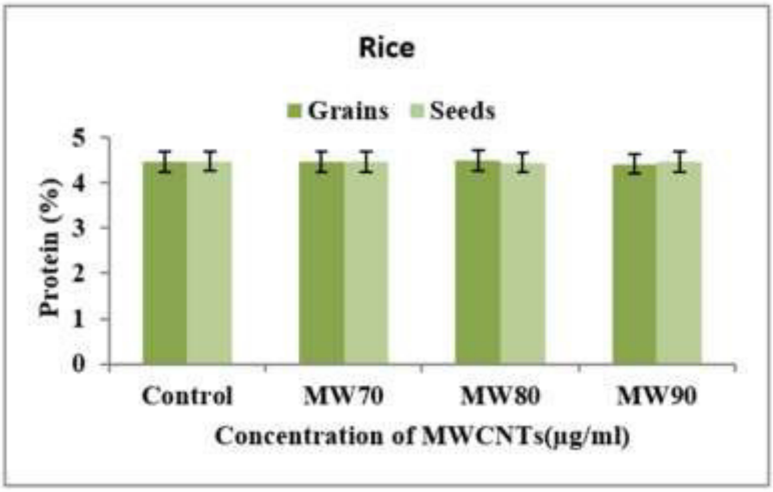

Fig 15. Proximate protein content of rice flour at different concentrations of MWCNT.

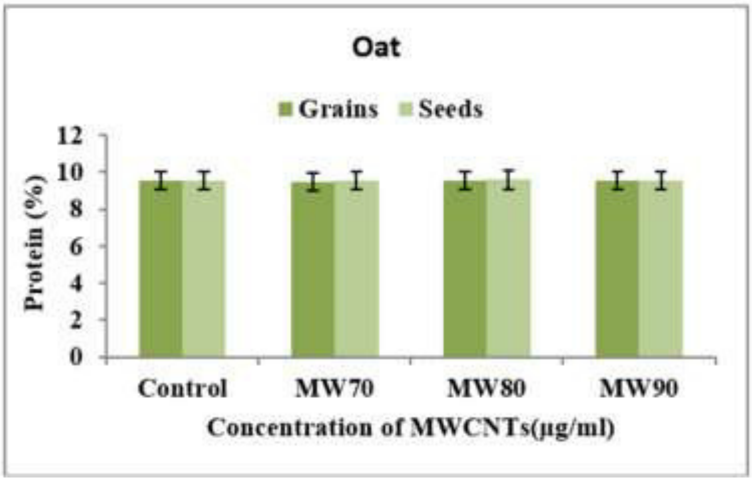

Fig 16. Proximate protein content of oat flour at different concentrations of MWCNT.

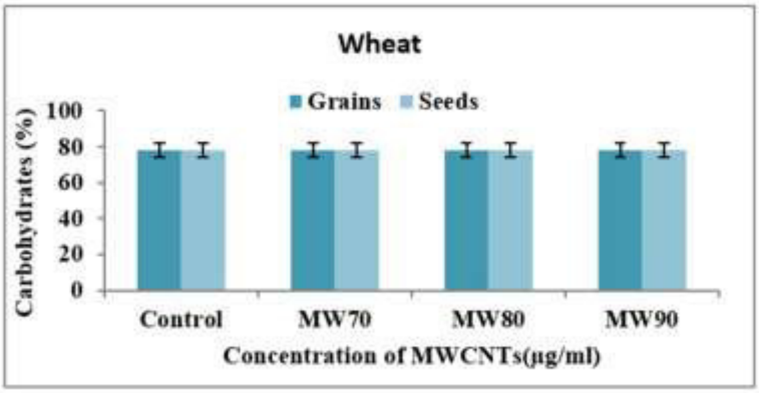

Fig 17. Proximate Carbohydrate composition of wheat flour at different concentrations of MWCNT.

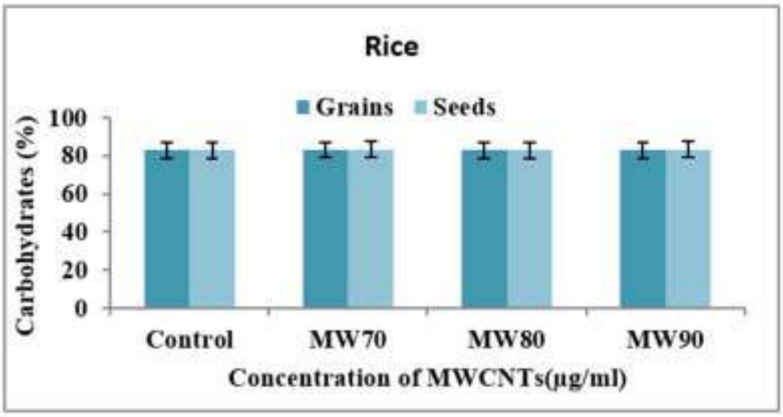

Fig 18. Proximate Carbohydrate composition of rice flour at different concentrations of MWCNT.

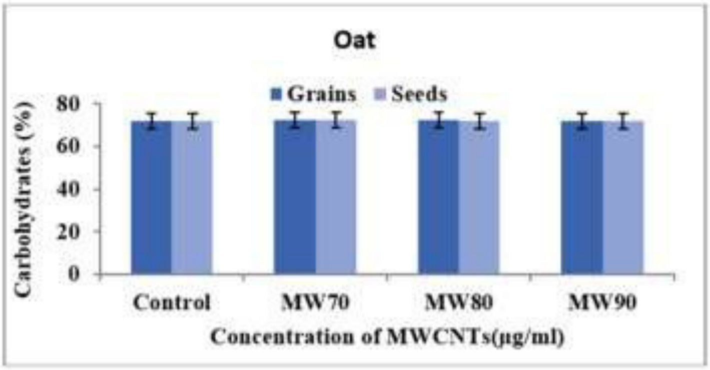

Fig 19. Proximate carbohydrate composition of oat flour at different concentrations of MWCNT.

4. CONCLUSION

Wheat, Rice, and Oats are the most important cereal grains in the world. Wheat and rice are considered a staple food, whereas oat contains a higher amount of β-glucan, which has several health benefits like reducing cancer, hypertension, and cholesterol. Demand for food is increasing with an increase in population, but due to limited resources, production is decreased. Many researchers found that incorporating nanomaterials in cereal grains has resulted in maximum output with minimum input. The study revealed that seed priming with different concentrations of MWCNT does not considerably affect the varied components such as carbohydrates, proteins, fat, and fibre. Still, there was an increase in mineral accumulation. In the case of grains collected from MWCNT-treated plants of all the cereals, no detrimental effect was noticed on the grain composition, signifying that the nutritional status of grains was unaltered with nanotubes treatments, as a result, was secure to use for these cereals. The study has shown that the MWCNT (at tested concentrations) could efficiently enhance the yield while preserving the grain's quality.

5. AUTHOR CONTRIBUTION STATEMENT

Anjali Joshi, Neha Verma and Gaurav Verma performed experimentation; Simranjeet Kaur, Avneesh Kumar, and Vajinder Kumar conceived the idea; Anjali Joshi, Simranjeet Kaur, Avneesh Kumar, and Narender Yadav helped in writing the manuscript thoroughly.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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