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Synthesis of Graphene oxide composites for Growth Enhancement of Pearl millets Seed Germination with statistical analysis

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Abstract
In this work, we have examined the impact of graphene oxide composites on pearl millet seed germination. We have used the ultrasonication approach to synthesize graphene oxide composites with copper oxide and silver. FTIR (fourier transform infrared spectroscopy), FESEM (field emission scanning electron microscopy), PXRD (powder x-ray diffraction spectroscopy), and EDX/EDS (energy dispersive x-ray analysis) are used to assess the morphology of both composites. The impact of time and concentration on the rate of Pearl Millets seed germination is tested using the synthesised composites. After the seeds were immersed in the graphene oxide composite solution for 24, 48, 72, and 96 hours, respectively, observations were made. The analysis of data is done using SPSS version-26 and we have conducted full factorial analysis to know the best set of time, type of catalyst and number of samples which gave better results in the growth of pearl millets.
Keywords- *Graphene oxide, pearl millets, seed germination*

INTRODUCTION
In addition to providing food for the world's rapidly expanding population of about 7.5 billion people, the agricultural sector is important to developing economies [1]. Since 90% of food crops are developed from seed, the seed is a critical input for sustained agricultural productivity and production. A healthy agro seed contributes to efficient farming methods by producing seedlings that are stronger, healthier, and more viable. A number of issues confronting agriculture today include shifting environmental conditions such as drought, salinity, soil heavy metal buildup, and climate shifts, among others, which can have a negative impact on seed germination, seedling growth, and eventually crop yield [2]. In order to solve these relevant challenges with agro seeds, new methods and approaches are continuously being developed. New products based on nanomaterials are being launched to transform contemporary agricultural methods. These materials' unique physicochemical characteristics and high surface area-to-volume ratio allow them to satisfy growing demand because of their high reactivity [3]. Materials with sizes between 1 and 100 nm are the focus of nanotechnology [4-8]. Furthermore, a material's reactivity might be affected by a greater amount of the nanomaterial contacting surrounding components when its surface area per mass is increased [9]. Compared to equivalent amounts of materials at a greater scale, nanoparticles have a substantially bigger surface area [10]. Research on the use of nanotechnology for seed treatment is still in its infancy. Because they work better than traditional agrochemicals and are therefore both ecologically friendly and economically feasible, nanoagrochemicals for seed treatment are becoming more and more popular.
Nanotechnology can improve the effectiveness of agricultural inputs and make a substantial contribution to the sustainable development of nanoscale agrochemicals for seed treatment. There is evidence that nanoparticles improved seed biomass production and germination. Nanomaterials including silver, gold, copper, palladium, selenium, zinc oxide, magnesium oxide, titanium dioxide, and iron oxide have been

demonstrated in the literature to enhance agricultural yields and encourage seed germination[11-12]. Porous, biogenic, metallic, metal oxide, and polymeric nanoparticles will propel the next-generation seed agrochemical revolution [13]. Nanomaterials can act as seed guardians in addition to aiding in seed germination. They can shield seeds from pests, fungus, and bacteria. Nanomaterials have occasionally been shown to exhibit toxicity that varies with size and concentration, such as lowering germination rates and producing phytotoxicity in seedlings [14]. Controlling the physicochemical characteristics of nanoparticles, which dictate their biological response, including size, shape, surface charges, composition, and concentration, might lessen their toxicity. When nanoagrochemicals are used to treat seeds, there is a greater chance that they may leak into the soil and environment. Concerns regarding these nanoagrochemicals' safety, exposure levels, and toxicological effects on the environment and human health are raised when they are used in real-world field settings [15]. Once in the environment, nanoagrochemicals can change physically, chemically, and biologically depending on their makeup and the presence of organic and inorganic components. The stability, reactivity, toxicity, and selectivity of the nanoagrochemicals may be impacted, and their target may change, when they are converted or aggregated [16].

2-Experimental Section: The chemicals used are obtained from Sigma Aldrich- 4-nitrophenol, Graphite flakes, Potassium Permanganate($KMnO_4$), Hydrogen Peroxide(H_2O_2), Sulphuric acid(H_2SO_4), Silver nitrate($AgNO_3$)and Sodium Borohydride($NaBH_4$).

2.1-Characterization: The powder properties of the prepared material were determined using PXRD, which is detected using Cu K radiation with a wavelength of 0.15418 nm. Additionally, produced materials underwent FT-IR analysis to identify and track the peaks of various functional groups. The Thermo-Gravimetric measurements were made using the STA 6000 from Perkin Elmer, and they were taken in a nitrogen atmosphere at a 10°C heating rate. The Tecnai G2 20 S-TWIN [FEI] is a 200 Kv TEM that offers high resolution with a point resolution of 0.24. The sample's elemental composition is provided by EDS/EDX. The morphology of the synthesized material was examined using Field Emission Scanning Electron Microscopy. **Preparation of Metal Oxide decorated reduced Graphene Oxide Nanoparticles:** Graphene oxide is prepared as reported in earlier. 1g of Graphene Oxide is added to 200ml of distilled water in a beaker. Then, it is sonicated for 10 min. After that, CuO(400 mg) and distilled water (100 ml) are added to another beaker and sonicated for 10 minutes. After sonication, both solutions are mixed with the addition of sodium borohydride(20mg). The reaction mixture is left to stir for 4 hours at room temperature. After that, it is filtered, washed multiple times with ethanol, and dried in an oven for 8 to 10 hours at 80°C to produce Copper oxide-decorated reduced Graphene Oxide (rGO-CuO). For the preparation of reduced Graphene oxide and decorated silver composite, we have prepared 1 g graphene oxide is added in 50 ml distilled water solution. The resulting solution is sonicated for 20 minutes to prepare homogenous reaction mixture. Then silver nitrate is added in the reaction mixture and reaction mixture is stirred for 10 minutes. After that sodium borohydride is added dropwise in the reaction mixture. At last the reaction mixture is washed, filtered and dried in an oven at 80 degree Celsius[17] **Seed Germination experiment:** All the material were laminar air flowed for 15 minutes. The seeds were washed using mercuric chloride solution for 2 minutes. Then again seeds were washed using distilled water for 3 to 5 times. Batch samples of reduced graphene oxide and metal oxide composites were prepared and seeds were soaked in 10 ml of solution for 24 hours. Then the growth root and shoot was observed after 24 hours, 48 hours and 72 hours respectively. Petri plates were taken, one petri plate have 5 seeds in it.

RESULTS AND DISCUSSION

3.1-Powder X-ray diffraction patterns

In the figure 6 the peaks at 2θ = 28.72°(002), 69.82°(113) and 72.90°(311) which are almost similar to the standard XRD data. The peak at 28.72 clearly shows the reduction of GO for the incorporation of CuO.

The peaks at 38.99°, 50.53° and 69.82° arises due to CuO particles. The crystalline size, porosity and percent cryatallinity is already calculated in our previously published literature with same catalyst. Figure 5 shows the PXRD pattern of the synthesised catalyst is shown below. The plane (200) is represented by a strong 2θ = 43.21° value in the PXRD pattern of GO. Furthermore, a little peak reflecting the (002) plane was seen at 2θ = 24.93°. As graphite oxidised, oxygen-containing functional groups were added, increasing the interlayer gap in graphene oxide (GO) relative to graphite. AgNPs showed a significant diffraction peak that was in good accord with previously published research, with a 2θ value of 39.11° corresponding to the (111) plane[17-18]

3.2-FESEM The fesem figures 1 and 2 below make it very evident that copper oxide particles are dispersed throughout the graphene oxide surface like paper. CuO has a diameter ranging from 6 to 10 nm. On GO sheets, CuO particles typically have a thickness of around 20 nm. Furthermore, figures displays fesem of the GO-Ag nanocomposite. It shows the deposition of AgNPs on the GO surface, Figurs 3 and 4 shows several GO layers. They also display SEM micrographs of the Ag/GO nanocomposite. AgNPs stick to the GO plates and have very round forms and in some places they are aggregated. AgNPs, however, have a tendency to be loosely grouped together.

ImageJ software was used to measure the diameter of AgNPs on the GO surface. According to the Ag/GO nanocomposite's SEM micrographs, the AgNPs' distribution diameters varied from 28 to 90 nm, with an average diameter of around 55 nm[17-19]

3.3- Energy Dispersive Spectroscopy An enticing confirmation of the proper placement of CuO on rGO sheets is provided by the mapping (table 1). The nanocomposite's element composition is as follows: Oxygen 6.22 weight percent, Copper 15.70 weight percent, and Carbon 78.08 weight percent. An enticing confirmation of the proper placement of Ag on rGO sheets is provided by the mapping (table 2). The nanocomposite's element composition is as follows: Oxygen 9.53 weight percent, Ag 5.57 weight percent, and Carbon 84.9 weight percent respectively[17-19]

Table-1

element	wt%	at%
C	78.08	91.09
Cu	15.70	3.46
O	6.22	5.45

Table-2

element	wt%	at%
C	84.9	57.52
Ag	5.57	33.88
O	9.53	8.6

3.4-FTIR In the figure 7 the peaks present in Graphene oxide are 1264 cm^{-1}(C-O epoxy), 1067cm^{-1}(C-O alkoxy) and 1721cm^{-1}(C=O carbonyl). In the nanocomposite catalyst, the peaks of the OH functional group and the C=C double bond vanished, and the intensity of the C=O, C-OH, and C=O peaks decreased, confirming the reduction of graphene oxide during preparation. This suggests that graphene oxide will be reduced in addition to the nanocomposite's synthesis. Figure 8 displays the FTIR spectra of the synthesised GO-Ag nanocomposite, which range from 4000 to 400 cm-1. Intermolecular H-bonding was represented by an adsorption band that emerged in GO at 3675 cm^{-1} and vanished in the GO-Ag nanocomposite. Other bands also showed up at 2979, 1392, and 1046 cm^{-1}, which stand for C-H, C-O, and C-O, respectively. The intercalation of AgNPs on the GO surface was indicated by a reduction in the intensity of the C-O and -OH bands following doping. Following doping, these peaks showed a blue shift[17-19].

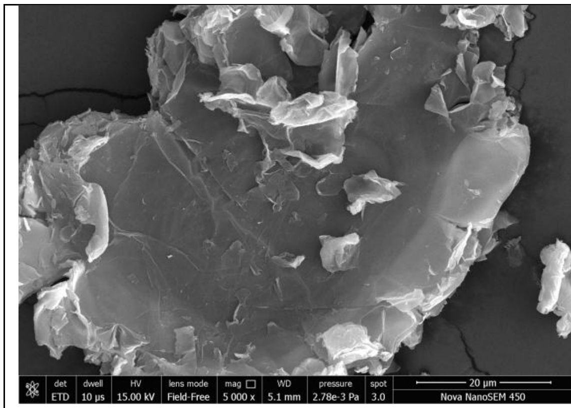

Figure 1- rGO-CuO

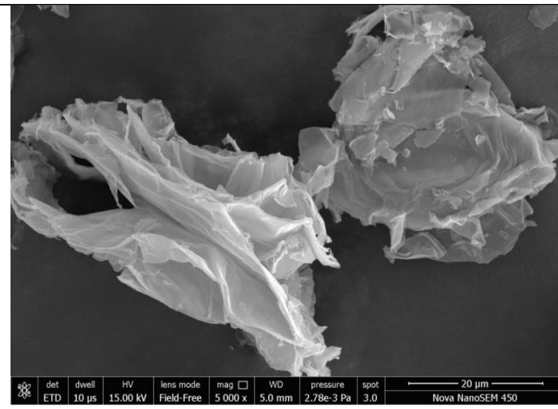

Figure 2- rGO-CuO

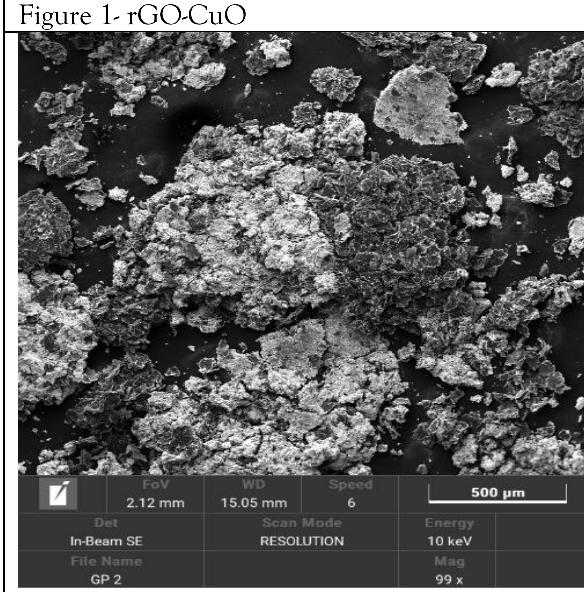

Figure 3- rGO-Ag

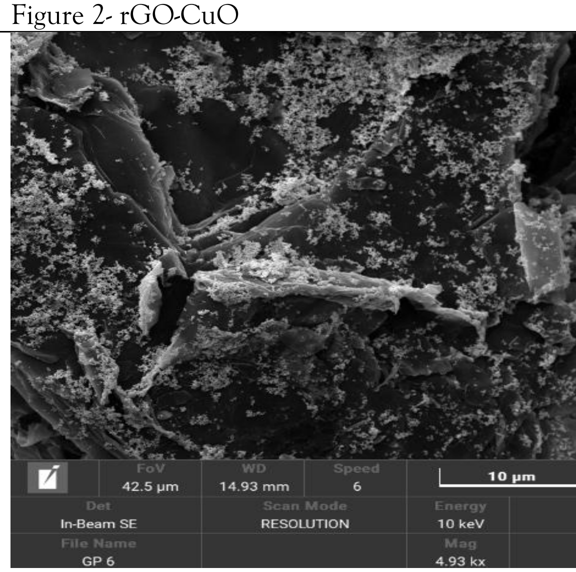

Figure 4- rGO-Ag

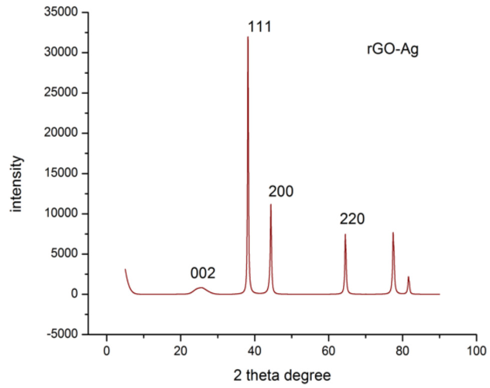

Figure 5- PXRD of rGO-Ag

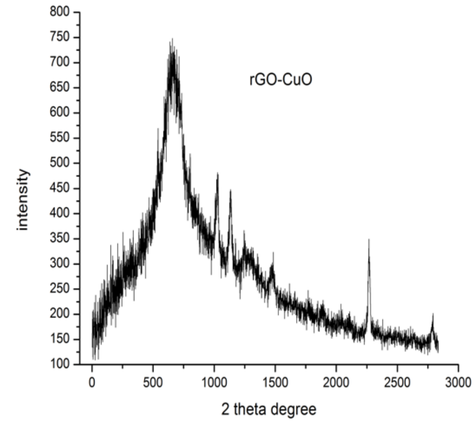

Figure 6- PXRD of rGO-CuO

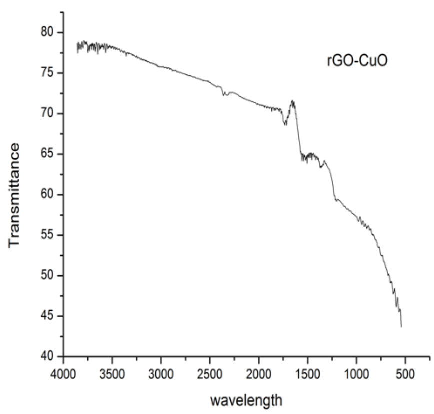

Figure 7- FTIR of rGO-CuO

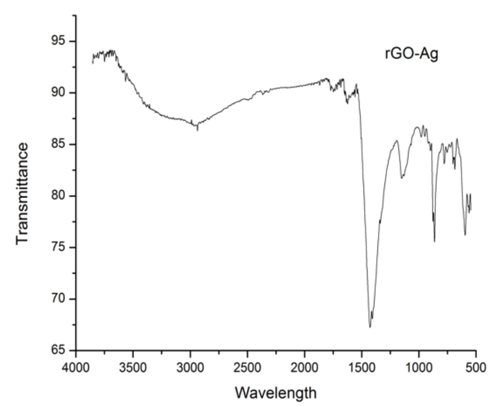

Figure 8- FTIR of rGO-Ag

3.5-Seed germination experiment

Table 3- Seed germination of Pennisetum glaucum after 24, 48, 72 and 96 hours.

After 24 hours			
sample	rGO-CuO	rGO-Ag	Controlled
1	0 root and shoot	1 root and shoot	3 root and shoot
2	0 root and shoot	0 root and shoot	1 root and shoot
3	0 root and shoot	0 root and shoot	3 root and shoot
4	0 root and shoot	0 root and shoot	1 root and shoot
After 48 hours			
sample	rGO-CuO	rGO-Ag	Controlled
1	1 root and shoot	1 root and shoot	1 root and shoot
2	1 root and shoot	0 root and shoot	1 root and shoot

3	0 root and shoot	0 root and shoot	2 root and shoot
4	0 root and shoot	0 root and shoot	2 root and shoot
After 72 hours			
sample	rGO-CuO	rGO-Ag	Controlled
1	1 root and shoot	1 root and shoot	1 root and shoot
2	1 root and shoot	1 root and shoot	1 root and shoot
3	1 root and shoot	1 root and shoot	2 root and shoot
4	0 root and shoot	0 root and shoot	2 root and shoot
After 96 hours			
sample	rGO-CuO	rGO-Ag	Controlled
1	1 root and shoot	1 root and shoot	3 root and shoot
2	2 root and shoot	1 root and shoot	4 root and shoot
3	1 root and shoot	1 root and shoot	2 root and shoot
4	1 root and shoot	1 root and shoot	2 root and shoot

The composites of Graphene oxides can impact the seed germination of Pennisetum glaucum (pearl millet) in a way that depends on the concentration and exposure time. Studies shows that at low concentrations GO composites can have positive impact on seed germination of pearl millet. Similarly we have also used 10 ml of GO composites solution to soak the seeds and our exposure is also limited. We have taken the readings up to 96 hours and observed that after 24 hours(figure-9) the penetration of nanoparticle has already began and we can see 1 root and shoot(table-3) in the first petri-plate whereas in the controlled system seed germination started after 24 hours respectively. Furthermore after second day onwards we can see seed germination in the petri-plates of both the composites. GO composites can improve germination under stress conditions like salinity, others indicate that high concentrations can be toxic. GO composites can promote germination, but this effect might be dose-dependent and potentially time-dependent, with longer exposure times leading to decreased germination in some cases.

Figure 9- Seed germination of Pearl millets

3.7- Statistical Analysis

Using SPSS version-26, a statistical study has been conducted using the analysis of variance technique. In the statistical analysis, we have seen the effect of catalysts on the seed germination of bajra seeds. Four levels of samples are taken, three levels of root and shoot are taken and two levels of catalysts are taken respectively. There were 32 observations and they were repeated three times. Therefore total observations are 96 for the analysis.

Following hypotheses will be tested by using 3^3 full factorial experiments:

The following statistical hypotheses are developed in order to accomplish the aforementioned goals-
To test the individual effects of the parameters quantity of sample, catalyst, time, root and shoot.
Ho_1: Various samples have no significant difference among them.
$H1_1$: Various samples compounds have significant difference among them.
To test the pairwise effects of the parameters quantity of samples, catalyst, time, root and shoot.
Ho_2: Various samples, catalyst,time(T) have no pairwise significant difference among them.
$H1_2$: Various samples, catalyst, time(T) have pairwise significant difference among them.
To test the three factor interaction of the parameters quantity of samples, catalyst, time, root and shoot.
Ho_3: Various samples, catalyst, time have no three factor interaction among them.
$H1_3$: Various samples, catalyst, time have three factor interaction among them.

Table 4- statistical analysis

Tests of Between-Subjects Effects

Dependent Variable: time

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	49.061[a]	18	2.726	2.958	.001	.409
Intercept	117.035	1	117.035	127.034	.000	.623
sample	.431	3	.144	.156	.926	.006
cat	.026	1	.026	.028	.867	.000
root	46.525	2	23.262	25.250	.000	.396
sample * cat	.192	3	.064	.070	.976	.003
sample * root	.235	5	.047	.051	.998	.003
cat * root	1.294	1	1.294	1.405	.240	.018
sample * cat * root	.522	3	.174	.189	.904	.007
Error	70.939	77	.921			
Total	336.000	96				
Corrected Total	120.000	95				

a. R Squared = .409 (Adjusted R Squared = .271)

Table 5- Multiple comparison

Multiple Comparisons

Dependent Variable: time

	(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Tukey HSD	1.00	2.00	.0000	.27708	1.000	-.7276	.7276
		3.00	.0000	.27708	1.000	-.7276	.7276
		4.00	.0000	.27708	1.000	-.7276	.7276
	2.00	1.00	.0000	.27708	1.000	-.7276	.7276
		3.00	.0000	.27708	1.000	-.7276	.7276
		4.00	.0000	.27708	1.000	-.7276	.7276
	3.00	1.00	.0000	.27708	1.000	-.7276	.7276
		2.00	.0000	.27708	1.000	-.7276	.7276
		4.00	.0000	.27708	1.000	-.7276	.7276
	4.00	1.00	.0000	.27708	1.000	-.7276	.7276
		2.00	.0000	.27708	1.000	-.7276	.7276
		3.00	.0000	.27708	1.000	-.7276	.7276
LSD	1.00	2.00	.0000	.27708	1.000	-.5517	.5517
		3.00	.0000	.27708	1.000	-.5517	.5517
		4.00	.0000	.27708	1.000	-.5517	.5517
	2.00	1.00	.0000	.27708	1.000	-.5517	.5517
		3.00	.0000	.27708	1.000	-.5517	.5517
		4.00	.0000	.27708	1.000	-.5517	.5517
	3.00	1.00	.0000	.27708	1.000	-.5517	.5517

		2.00	.0000	.27708	1.000	-.5517	.5517
		4.00	.0000	.27708	1.000	-.5517	.5517
	4.00	1.00	.0000	.27708	1.000	-.5517	.5517
		2.00	.0000	.27708	1.000	-.5517	.5517
		3.00	.0000	.27708	1.000	-.5517	.5517

Based on observed means.

Using SPSS version-26, a statistical study has been conducted using the analysis of variance technique. In the statistical analysis, we have seen the effect of catalysts on the seed germination of bajra seeds. Four levels of samples are taken, three levels of root and shoot are taken and two levels of catalysts are taken respectively. The tables 4 and 5 clearly shows that samples(petri plates) one, two and four with catalyst reduced graphene oxide copper oxide shows more number of root and shoot than shown in the petri plates containing the catalyst reduced graphene oxide and silver. Pairwise comparison of sample and catalyst shows that rGO-CuO catalyst is significant with samples one, two and four. Pairwise comparison of sample and root shows that rGO-CuO is significant with time that is the growth is shown after 24 hours with the same catalyst. Pairwise comparison of catalyst and root shows that with the increase in amount of catalyst and time the growth increases. Therefore it is concluded from the analysis(three level interaction) that type of catalyst –rGO-CuO, sample one, two and four and time 24 hours gave the best results for the growth of bajra seeds in comparison to other catalysts.

CONCLUSION

This work has demonstrated that the process of synthesis of GO composites with copper oxide and silver. The synthesized composites are used to study the rates of seed germination in pearl millets. It was found that after 48 hours considerable amount of root and shoot are observed in the petri plates of both composites of graphene oxides respectively.

REFERENCES

1) Berners-Lee, M., Kennelly, C., Watson, R., & Hewitt, C. N. (2018). Current global food production is sufficient to meet human nutritional needs in 2050 provided there is radical societal adaptation. Elem Sci Anth, 6, 52.

2) Imran, Q. M., Falak, N., Hussain, A., Mun, B. G., & Yun, B. W. (2021). Abiotic stress in plants; stress perception to molecular response and role of biotechnological tools in stress resistance. *Agronomy*, *11*(8), 1579.

3) Chan, K. K., Yap, S. H. K., & Yong, K. T. (2018). Biogreen synthesis of carbon dots for biotechnology and nanomedicine applications. *Nano-micro letters*, *10*, 1-46.

4) Neme, K., Nafady, A., Uddin, S., & Tola, Y. B. (2021). Application of nanotechnology in agriculture, postharvest loss reduction and food processing: food security implication and challenges. *Heliyon*, *7*(12).

5) Mittal, D., Kaur, G., Singh, P., Yadav, K., & Ali, S. A. (2020). Nanoparticle-based sustainable agriculture and food science: Recent advances and future outlook. *Frontiers in Nanotechnology*, *2*, 579954.

6) Shang, Y., Hasan, M. K., Ahammed, G. J., Li, M., Yin, H., & Zhou, J. (2019). Applications of nanotechnology in plant growth and crop protection: a review. *Molecules*, *24*(14), 2558.

7) Prasad, R., Bhattacharyya, A., & Nguyen, Q. D. (2017). Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. *Frontiers in microbiology*, *8*, 1014.

8) Usman, M., Farooq, M., Wakeel, A., Nawaz, A., Cheema, S. A., ur Rehman, H., ... & Sanaullah, M. (2020). Nanotechnology in agriculture: Current status, challenges and future opportunities. *Science of the total environment*, *721*, 137778.
9) Guha, T., Gopal, G., Kundu, R., & Mukherjee, A. (2020). Nanocomposites for delivering agrochemicals: A comprehensive review. *Journal of Agricultural and Food Chemistry*, *68*(12), 3691-3702.
10) Nile, S. H., Baskar, V., Selvaraj, D., Nile, A., Xiao, J., & Kai, G. (2020). Nanotechnologies in food science: applications, recent trends, and future perspectives. *Nano-micro letters*, *12*, 1-34.
11) Jiang, M., Song, Y., Kanwar, M. K., Ahammed, G. J., Shao, S., & Zhou, J. (2021). Phytonanotechnology applications in modern agriculture. *Journal of Nanobiotechnology*, *19*, 1-20.
12) Manzoor, N., Ali, L., Ahmed, T., Noman, M., Adrees, M., Shahid, M. S., ... & Zaki, H. E. (2022). Recent advancements and development in nano-enabled agriculture for improving abiotic stress tolerance in plants. *Frontiers in plant science*, *13*, 951752.
13) Sarkar, M. R., Rashid, M. H. O., Rahman, A., Kafi, M. A., Hosen, M. I., Rahman, M. S., & Khan, M. N. (2022). Recent advances in nanomaterials based sustainable agriculture: An overview. *Environmental Nanotechnology, Monitoring & Management*, *18*, 100687.
14) Murali, M., Gowtham, H. G., Singh, S. B., Shilpa, N., Aiyaz, M., Alomary, M. N., ... & Amruthesh, K. N. (2022). Fate, bioaccumulation and toxicity of engineered nanomaterials in plants: current challenges and future prospects. *Science of the Total Environment*, *811*, 152249.
15) Rajput, V., Minkina, T., Mazarji, M., Shende, S., Sushkova, S., Mandzhieva, S., ... & Jatav, H. (2020). Accumulation of nanoparticles in the soil-plant systems and their effects on human health. *Annals of Agricultural Sciences*, *65*(2), 137-143.
16) Meena, R. S., Kumar, S., Datta, R., Lal, R., Vijayakumar, V., Brtnicky, M., ... & Marfo, T. D. (2020). Impact of agrochemicals on soil microbiota and management: A review. *Land*, *9*(2), 34.
17) Pandey, G., Singh, N., Rajput, N., Saini, M. K., Kothari, S. L., Prasad, J., ... & Chauhan, M. S. (2024). Comparative study of NiO/CuO/Ag doped graphene based materials for reduction of nitroaromatic compounds and degradation of dye with statistical study. *Scientific Reports*, *14*(1), 2077.
18) Thi, P. T., Van Khai, T., Diem, T. X., Nghia, C. N. T., Ngan, T. T. T., Tri, L. M., ... & Hien, N. M. (2021). Synthesis of Ag/GO nanocomposite with promising photocatalytic ability for reduction reaction of p-nitrophenol. *Materials Research Express*, *8*(10), 105009.
19) Kumari, S., Sharma, P., Yadav, S., Kumar, J., Vij, A., Rawat, P., ... & Majumder, S. (2020). *A novel synthesis of the graphene oxide-silver (GO-Ag) nanocomposite for unique physiochemical applications. ACS Omega 5: 5041–5047.*
20) Sudarvizhi, A., Siddiqha, Z. A., & Pandian, K. (2014). Single step synthesis of graphene oxide protected silver nanoparticles using aniline as reducing agent and study its application on electrocatalytic detection of nitrite in food samples. *J Chem Applied Biochem*, *1*, 101.