Chapter

Synthesis, Characterization of Dichlorofluorescein Silver Nanoparticles (DCF-SNPs) and Their Effect on Seed Germination of *Vigna radiata*

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Abstract

The main objective of this study was to investigate whether dichlorofluorescein (DCF) is adequate for the formulation of stable dichlorofluorescein-induced silver nanoparticles under the boiling method to analyze their effects on the seed germination of Mung seeds *(Vigna radiata)*. Preliminary dichlorofluoresceine nanoparticles (DCF-SNPs) synthesis evidence by noticing the solution color transformed from a light green color to a dark brown color. The 2.5 ml of dichlorofluoresceine (DCF) solution was found sufficient for the formulation of dichlorofluoresceine induced silver nanoparticles at boiling conditions. Purified dichlorofluoresceine nanoparticles (DCF-SNPs) measure an average diameter of 293 nm where the majority of nanoparticles were around 159 nm in size with the surface load of-9.35 mV zeta potential value. The impact of dichlorofluorescein silver nanoparticles (DCF-SNPs) on the germination percentage of *V. radiata* has shown that, the 25% concentration of DCF-SNPs is excellent for the growth of Mung seeds (*V. radiata*). Overall, the dichlorofluorescein silver nanoparticles may be constructive for improving the percentage of seed germination at 25% of its concentration and may also be useful for fluorescent measurement using the confocal microscopy technique. Hence, dichlorofluorescein silver nanoparticles (DCF-SNPs) are proposed as an efficient detection system for nanoparticles in agrochemicals for plants.

Keywords: dichlorofluoresceine (DCF), *Vigna radiata*, dichlorofluoresceine silver nanoparticles (DCF-SNP), Zeta potential, confocal microscopy studies

1. Introduction

Dyes are colored entities that are chemically attached to the matrix fiber and accumulate during the drying period, providing color through the systematic absorption of light and increasing the speed of the fiber dyeing process [1]. There are two types of dyes which are natural or organic dyes and chemically synthesized dyes. In this chapter we are going to deal with organic dyes.

1.1 Organic dye

Natural dyes are derived from natural resources and, are typically categorized as plant, animal, mineral and microbial dyes, though plants are the key sources of natural dyes [2]. Examples of natural and organic coloring dyes are blue dye which derived from plant leaves, while red dye, Madder and Morinda from roots. Brazil Wood an old-world dye comes from wood of plant. Safflower and saffron dye made from their flowers, however, rhizomes of turmeric use to make dye. On the other hand yellow dye derived from roots, leaves, stems and flowers [2]. The organic dyes are colored as they appear in the visible light spectrum (400–700 nm) comprising of one chromosphere, a conjugate framework with a dual bond alternating arrangement with a single bond and an electron resonance that stabilizes in organic compounds [3]. Based on the chemical structure and characteristics properties, dyes are classified as *Azo* dyes, *Anthraquinone* dyes, Nitro dyes, *Diphenlmethane* dyes, *Triphenylmethane* dyes, *Xanthene* dyes, *Phthaleins* dyes, *Indigoid* and *Thionidigoid* dyes [4]. These organic coloring powders are smaller, thicker, finely fragmented crystalline solids, which are water soluble, whereas they are insoluble in application media such as ink or paint for the use of optical media, image sensors [1], photosensitizer and coloration [5]. Improved color functionality has been studied in recent times by adding functional properties such as antimicrobial, UV protection, insect repellent, etc. [6]. In addition, organic coloring is a major supplier for floral, dried flowers, pesticides, ice-melt, deicing, reservoir, lake, water tracing, leak detection, greenhouse, livestock, seed treatment, crop protection, fertilizer staining, food, cosmetic and environmental issues [7]. The multitude application of organic dyes has enable to use them for coloring, detection, biosensor, tracking in the test samples. One of the best examples of tracking dye is dichlorofluorescein (DCF).

1.2 Dichlorofluoresceine (DCF)

Fluorescein based titrations shows effective results at 0.005 N concentrations of chloride and if the formulation has neutral alkaline conditions [8]. 2′,7’-Dichloro- and 2′,7′-difluorofluoresceins are superior alternatives to underivatized fluorescein. Quadrupole ion trap mass spectrometer for the analysis of the gas phase properties of three charge states of fluorescein, indicating that dianions and cations do not emit detectable fluorescence in the gas phase. Monoanions, on the other hand, do fluorescence and are useful for experiments [9]. Dichlorofluorescein (DFC) is a natural, crystalline organic, coloring agent that substitutes for chloride at 2 and 7 positions and originated in the fluorescein family. **Figure 1** illustrates the molecular

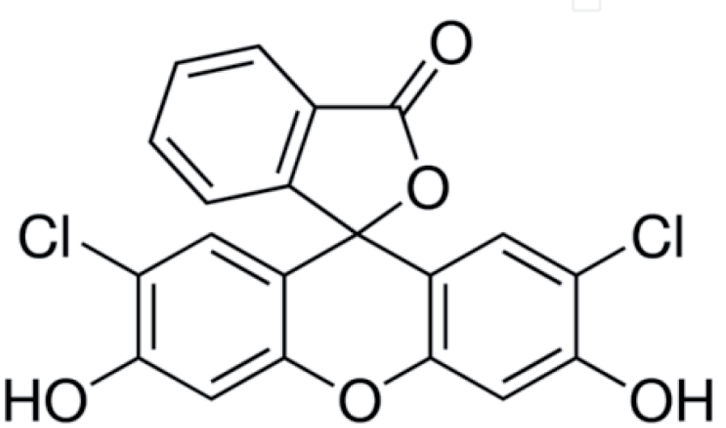

Figure 1.
Demonstrates the molecular structure of Dichlorofluorescein with empirical formula as $C_{20}H_{10}cl_2O_5$ [10].

structure of dichlorofluorescein. The molecular weight of dichlorofluorescein (PubChem SID: 24894041) is 401.20 g/mol, a melting point of 280 °C and it is used as an indicator which is not prone to soluble, absorbs, disintegrates or infuses silver or halide ion, but changes color at the end of precipitate due to absorption phenomena [8]. It was therefore used as a quantitative agentometry titrant indicator, consisting of a known concentration of silver nitrate, to estimate the molarity of chloride in the sample as specified in the Fajans method [11].

1.2.1 Dichlorofluorescein (DCF) applications

Organic Dichlorofluorescein (DCF) applications are even less documented, however it has demonstrated application as an indicator for halide titration argentometry, gaseous dianion studies, targeting it as a probe for imaging, heamotherapy and histological applications [8, 10, 12]. Generally, non fluorescent 2′ 7’ Dichlorodihydrofluorscein diacetate is used in human hepatocellular carcinoma cells to monitor the oxidation of 2′,7′-dichlorofluorescin-diacetate (DCF-DA) to an extremely fluorescent 2′,7′ dichlorofluorescein (DCF) compound due to the presence of reactive oxygen species using a fluorometric microplate assay [13]. And thereby, 2′,7′ dichlorofluorescein (DCF) may be very significant in the assessment of the human organ culture model. In the same way, organic dyes can be used to research the tracing of in the host body of insects, plants and animals [12]. DCF can be the reducing agent for metallic salt (iron, copper, zinc, silver, gold, etc.) for the formation of metallic nanoparticles for various agriculture applications.

1.3 Silver nitrate (AgNO3)

Silver nitrate (AgNO3) is an inorganic compound that appears to have a colorless, white crystalline composition with molecular weight of 169.873 g/mol. In its solid state, it has a density of 4.35 grams per cubic centimeter and its density in the liquid state at a temperature is 210 °C corresponds to 3.97 g/cm^3. The melting and boiling points of silver nitrate are 414 °F and 824 °F respectively. In 1800s, silver nitrate was used for the treatment of ulcerations and infected wounds and stomach ulcers [14–15]. Silver nitrate is a chemical with a wide range of applications including antiseptic, suturing, eye disease disinfectant, burned wounds, wart and granulation tissue reduction, ulceration, dental cavity retention, etc. [16]. Other noteworthy medical applications of silver include wire or coated suture topical therapy for osteocutaneous fistulae, and foil coverings for burn wounds [14]. Silver nitrate compound is a productive source of creation for many other silver compounds used for the medical, biotechnological, nanotechnological, pharmaceutical as well as several other industries. **Figure 2** shows the silver nitrate molecular structure. Silver nitrate is frequently used chemical in several areas in agriculture including control growth, flowering development, dormancy and by spraying it on the growth tip of plants [17].

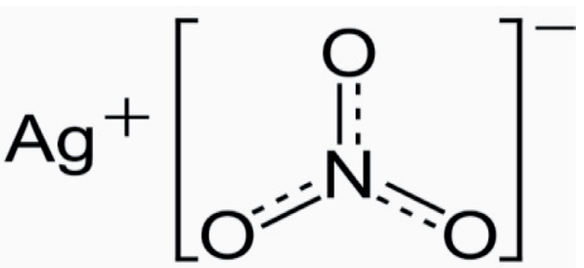

Figure 2.
Shows the molecular structure of silver nitrate [16].

Silver nitrate has a long use in nanotechnology for acting as a protective layer in stabilizing nanoparticles from further agglomeration. During the reduction of metal salt formation narrow size of nanoparticles are obtained. It absorbs on the surface of particle provide stabilization & diffusion barrier in the growth of particle [18]. Therefore, this silver salt are the best source for silver nanoparticles synthesis.

1.4 Silver nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) range from 1 to 100 nm in size, which are fundamentally synthesized by physical, chemical and biological approaches. Silver nanoparticles were being used as antimicrobial agents in a wide variety of applications, which include disinfecting medical instruments and home appliances to water treatment [19]. Even though the other biological properties of silver nanoparticles such as antimicrobial, antifungal, anti-inflammatory, anti-cancer and anti-angiogenesis [20] already have enabled them to be extensively used in the fields of medicine and dentistry, diagnostics, therapeutic, medical care, health and food applications [21]. The other medical applications, including wound repair, bone healing, dental applications, vaccine adjuvant, antidiabetic agent, and biosensing [22]. The techniques of synthesis of metal silver nanoparticles have certain benefits as well as drawbacks. Therefore, depending on the application, the selection of the procedure is presumed and therefore, depending upon the application the selection of the methodology is considered. A well recorded manuscript available in the literature on the physical, chemical and biological preparation of silver nanoparticles. Nanoparticles have proved to be efficient agrochemical agents in order to improve the crop productivity, reducing the pests, increasing the nutrient uptake, inhibiting the pathogens and act as 'magic bullets' serving as herbicides, pesticides and fertilizers etc. [23–24]. The biological activity of AgNPs depends on factors including surface chemistry, size, size distribution, shape, particle morphology, particle composition, coating/capping, agglomeration, and dissolution rate, particle reactivity in solution, efficiency of ion release, and cell type, and the type of reducing agents used for the synthesis of AgNPs are a crucial factor for the determination of cytotoxicity [20]. The physicochemical properties of nanoparticles enhance the bioavailability of therapeutic agents after both systemic and local administration and other hand it can affect cellular uptake, biological distribution, penetration into biological barriers, and resultant therapeutic effects [20]. There are several methods for creating nanoparticles, including co precipitation, hydrothermal synthesis, inert gas condensation, ion sputtering scattering, micro emulsion, microwave, pulse laser ablation, sol–gel, sono chemical, spark discharge, template synthesis, and biological synthesis. We shall now briefly look into the methods for the synthesis of nanoparticles [25].

1.5 Synthesis of silver nanoparticles

There are large number of mrthods for bthe synthesis of silver nanoparticles. While in the present study we will discuss using microorganisms, plant extract and we proposed to use DCF for silver nanoparticles synthesis.

1.5.1 Silver nanoparticles synthesis using microorganism

There are various microorganism that have been explored for the synthesis of silver nanoparticles due to their advantage of reliable and ecofriendly process. The microorganisms used for reducing and capping the silver salts produces the various size, shape and morphology of silver nanoparticles. Novel *Nocardiopsis species, Brevibac teriumfrigoritolerans* strain, *Klebsiella pneumoniae,*

Sr no	Plants	Size (nm)	Plant's part	Shape
1.	*Abutilon indicum*	7–17	Leaves	Spherical
2.	Acalyphaindica	0.5	Leaves	—
3.	Acalyphaindica	20–30	Leaves	Spherical
4.	*Acorus calamus*	31.83	Rhizome	Spherical
5.	*Allium sativum*	4–22	Leaves	Spherical
6.	*Aloe vera*	50–350	Leaves	Spherical,
7.	Alternanthera dentate	50–100	Leaves	Spherical
8.	*Argyreia nervosa*	20–50	Seeds	—
9.	Boerhaaviadiusa	25	Whole plant	Spherical
10.	*Brassica rapa*	16.4	Leaves	—
11.	*Calotropis procera*	19–45	Plant	Spherical
12.	*Carica papaya*	25–50	Leaves	circular,
13.	Centellaasiatica	30–50	Leaves	Spherical
14.	*Citrus sinensis*	10–35	Peel	Spherical
15.	Cocciniaindica	10–20	Leaves	—
16.	Cocous nucifera	22	Inflorescence	Spherical
17.	Cymbopogancitratus	32	Leaves	—
18.	*Datura metel*	16–40	Leaves	Quasilinear
19.	Eclipta prostrate	35–60	Leaves	pentagons,
20.	Eucalyptus hybrid	50–150	Peel	spherical
21.	Ficuscarica	13	Leaves	—
22.	*Garcinia mangostana*	35	Leaves	—
23.	Melia dubia	35	Leaves	Spherical
24.	Memecylonedule	20–50	Leaves	hexagonal
25.	*Moringa oleifera*	57	Leaves	—
26.	Musa paradisiacal	20	Peel	—
27.	*Nelumbo nucifera*	25–80	Leaves	triangular
28.	Nelumbo nucifera	25–80	Leaves	triangular
29.	*Passiflora foetida*	—	Leaves	Coral
30.	Pistaciaatlantica	10–50	Seeds	Spherical
31.	Pogostemonbenghalensis	>80	Leaves	—
32.	*Portulaca oleracea*	<60	Leaves	—
33.	Premnaherbacea	10–30	Leaves	Spherical
34.	Psoraleacorylifolia	100–110	Seeds	—
35.	Swieteniamahogani	50	Leaves	—
36.	Tea extract	20–90	Leaves	Spherical
37.	*Thevetia peruviana*	10–30	Latex	Spherical
38.	Trachyspermumammi	87, 99.8	Seeds	—

Sr no	Plants	Size (nm)	Plant's part	Shape
39.	*Tribulus terrestris*	16–28	Fruit	Spherical
40.	*Vitex negundo*	5 & 10–30	Leaves	Spherical
41.	*Vitis vinifera*	30–40	Fruit	circular,
42.	Ziziphoratenuior	8–40	Leaves	Spherical

Table 1.
Green synthesis of silver nanoparticles by different researchers using plant extracts [31].

Escherichia coli, and Pseudomonas jessinii are some of the examples of micro organisms used for silver nanoparticles synthesis [26]. Besides, the production of silver nanoparticles form *Bacillus clausii* cultured form *Enterogermina* is explored [27]. The aqueous extract of cynobacterial *Oscillatoria limnetica* fresh biomass was used for the green synthesis of AgNPs and it takes about 30–60 hours for the reduction and stabilizing the synthesize of metallic nanoparticles that ranges from 3.30–17.97 nm in size [28].

1.5.2 Silver nanoparticles synthesis using plants

The plants part being organic and eco friendly are extensively used for synthesis of silver nanoparticles (AgNPs). The plant are the hot spots for the phytochemicals and secondary metabolites that are used constantly for various medicinal purposes including antimicrobial, antifungal, anti inflammatory, wound healing, antidiabatic etc. The plant sources such as leaves, stem, roots, flowers possessing the medicinal properties are used for the formulation of silver nanoparticles which carries the specific medicinal compound to reduce and capped the silver salt and present at the outer layer of silver to make them stable [29]. Therefore, the plant based silver nanoparticles possess the duel properties from silver and one from capped compounds from plants. The different plant leaf extracts for examples pine, ginkgo, magnolia, mango, neem, *oscimum scantum*, are used for their extracellular synthesis of silver nanoparticles. The biological silver nanoparticles production has the faster synthesis rates than the chemical methods and potentially be used in various foods, agriculture, chemical and medical application. The aqueous peel extract of *Annona squamosa* has been used successfully for synthesis of silver nanoparticles of irregular spherical in shape with the average particle size of 35 nm, at room temperature [30]. Green synthesis of silver nanoparticles by different plant extracts are described in **Table 1**. There are some of the synthesis methods that are constantly used for nanoparticles synthesis such as high temperature, pressure, sunlight condition, in autoclave [29]. In contrast, there are other publication that performed the synthesis process at room temperature.

Diversity of compounds, polymers, exopolysaccharides, proteins, lipids and other compounds such as natural dyes could be a good source for reducing metal salts to form stable stained nanoparticles which can have various applications such as pesticides, nutrients, hormones delivery for sustainable agriculture. In the light of the above addressed interesting information on dichlorofluorescein, silver nitrate, silver nanoparticles, it is evident that silver nanoparticles have an enormous application in various fields. It was noticed that none of the papers reported the synthesis of silver nanoparticles using any dye. The direct use of dichlorofluorescein (DCF) for the reduction of silver salt can also produce silver nanoparticles and could be used for the study of absorption and biotransformation in seeds and plants. The aim of this research is therefore to conduct the synthesis and

characterization of dichlorofluorescein induced dichlorofluorescein silver nanoparticles (DCF-SNPs) under boiling method to evaluate their effects on the seed germination of *Vigna radiata* and to propose that they would be used for real-time transformation in the living plant using confocal microscopy.

2. Materials and methods

The materials and the methodology adopted for the synthesis of dichlorofluorescein silver nanoparticles (DCF-SNP), characterization and application are described below. Material required: Requirement specification for the formulation of silver nanoparticles utilize dichlorofluorescein, NaOH, distilled water, AgNO3 (1 mm), conical flask, beaker, aluminum foil, volumetric flask, etc. Silver nitrate (AgNO3): silver nitrate was used for the synthesis of dichlorofluorescein silver nanoparticles (DCF-SNPs) and was then used with the seed germination assay. Instruments for study: The following instruments such as Fourier transform infrared spectroscopy (FTIR), Zeta Potential (ZP) and Nanoparticles tracking analysis (NTA) were used for characterization of synthesized dichlorofluorescein silver nanoparticles (DCF-SNPs) [32].

2.1 Preparation of dichlorofluorescein (DCF) solution

Dichlorofluorescein (DCF) solution is prepared using 10 mg concentrate in 100 ml of distilled water. The solution is entirely blended until it becomes a greenish liquid. A 2.5 mL of the prepared dichlorofluorescein (DCF) solution was used for the preparation of 100 mL silver nanoparticles.

2.2 Synthesis of silver nitrate ($AgNO_3$) solution

The silver nitrate solution is prepared by using 1.698 g silver nitrate powder in 100 ml of distilled water to form 100 mM concentration. From his prepared 100 ml of silver nitrate solution; 1 ml is used for preparation of 1 mM silver nitrate solution for silver nanoparticles synthesis. The complete process is performed under absence of light.

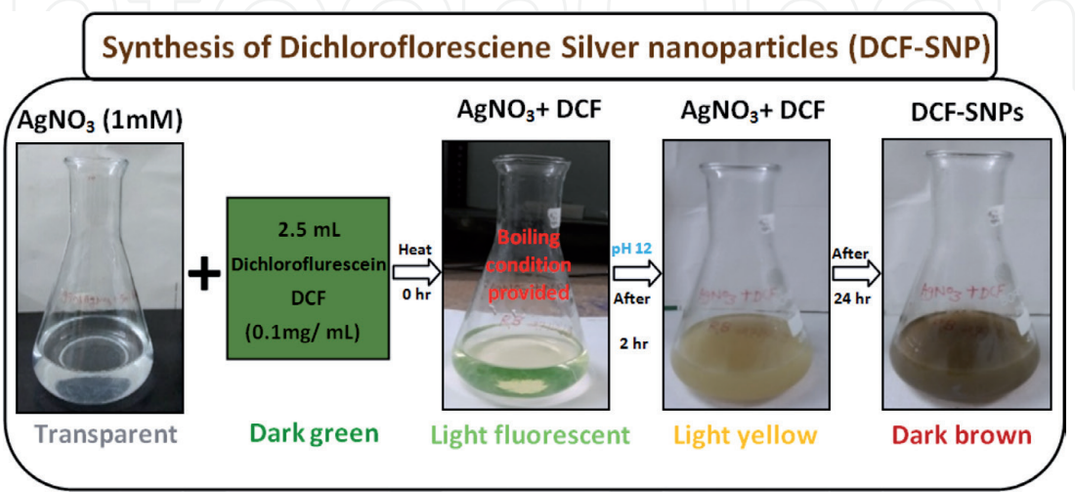

Figure 3.
Displays the synthesis of dichlorofluorescein silver nanoparticles (DCF-SNP) employing AgNO3 and dichlorofluorescein under heating conditions. The change in color of the reaction mixture was noticed from light green to dark brown, demonstrating the formation of silver nanoparticles.

2.3 Synthesis of dichlorofluorescein silver nanoparticles

The dichlorofluorescein silver nanoparticles (DCF-SNPs) is prepared by mixing 97.5 ml of AgNO3 (1 mm) and 2.5 ml of dichlorofluorescein in conical flask at constant stirring. The complete reaction process is carried out under boiling condition for 1–2 min, the pH of the mixture is adjusted to 12 and the reaction completes after 24 hours. The chemical constituents or the functional group that are presents in the Dichlorofluoresceine (DCF) structure reduced the silver salt to form the silver nanoparticles called as dichlorofluorescein silver nanoparticles (DCF-SNPs). The complete process of synthesis of dichlorofluorescein silver nanoparticles (DCF-SNPs) using $AgNO_3$ and dichlorofluorescein at 0 hr., 2 hr. and 24 hr. and observed changes in color from light green to dark brown, confirming the synthesis of silver nanoparticles synthesis is given in **Figure 3**.

3. Characterization of DCF-SNP

Various instruments are used to characterize the synthesized dichlorofluorescein silver nanoparticles. These are some of the techniques which we used in our investigations, such as FTIR analysis, that are used to classify a functional group that has a reducing and stabilizing properties. Finally, zeta potential and nanoparticles tracking analysis are used to track the surface charge and the size of the silver nanoparticles.

3.1 Fourier transform infra red (FTIR)

Dichlorofluorescein silver nanoparticles (DCF-SNPs) are used in the FTIR analysis to recognize the main functional groups involved in the reduction and capping of silver salt for the development of stable dichlorofluorescein silver nanoparticles (DCF-SNPs). In the FTIR instrument, the radiation falls on the sample and causes changes in the vibration and rotational motion of the molecules at a wavelength of 4000–440 cm − 1 consisting of near and far infrared frequencies and the FTIR spectrum of the formulation was recorded.

3.2 Nanoparticles tracking analysis (NTA)

The size of the synthesized dichlorofluorescein silver nanoparticles (DCF-SNPs) is calculated using Nanosight (LM-20, UK) at the Department of Biotechnology, Sant Gadge Baba Amravati University, India. Dichlorofluorescein silver nanoparticles (DCF-SNP) were diluted in 0.5 ml nuclease-free water and injected into the sample chamber. The instrument parameters are calibrated as specified and the device is analyzed nanoparticles samples to calculate the size of the nanoparticles. The data are summarized on the computer screen and are retrieved in PDF format for analysis.

3.3 Zeta-potential analysis (ZP)

The zeta potential characterization of dichlorofluorescein silver nanoparticles (DCF-SNPs) was tested by the Department of Biotechnology, Sant Gadge Baba Amravati University, Maharashtra, India. A zeta potential is used to assess the potential surface charge of Dichlorofluorescein Silver nanoparticles (DCF-SNP) using a zetasizer (nano ZS, malvern instrument Ltd., UK). In particular, the liquid samples of the dichlorofluorescein silver nanoparticles DCF-SNP (5 ml) were diluted with double distilled water (50 ml) using NaCl as an electrolyte suspension solution 2 M NaCl). In addition, the samples are injected into the sample slot of Zeta

potential instrument and the good and important results data are reported in PDF formats. In each case, an average of three different measurements made while the values of the zeta potential ranged from +30 mV to-30 mV.

3.4 Effect of dichlorofluorescein silver nanoparticles (DCF-SNPs) on seeds germination of *Vigna radiata*

Dichlorofluorescein silver nanoparticles (DCF-SNP) was used to investigate its effect on the germination of Mung Bean (*Vigna radiata*) seeds. Mung bean seeds were purchased from the market and stored in a dry place under room temperature in the dark. Here, four separate concentrations of 25 per cent, 50 per cent, 75 per cent and 100 per cent (v/v) of dichlorofluorescein silver nanoparticles (DCF-SNPs) dispersion were prepared in distilled water. The Whatman no.3 filter paper is layered on the sterile Petri dishes of 12 cm diameter and germination test analysis was carried out. In this test, the seeds were surface sterilized with 0.1% Hgcl2 solution and rinsed three times with distilled water In total, 10 seeds of Mung Bean (*V. radiata*) were placed in the respective Petri dishes. The solution of each concentration was transferred to each Petri dish and the treatment was administered daily at only enough doses to hydrate the seeds. The petri dish was then placed in a dark seed germinator and held at 25 °C. Seed with root tip 1 mm and above was considered germinated. Percent germination and root and shoot length (in mm) were recorded every 24 hours up to 72 hours. The root length was determined from the region under the hypocotylis to the end of the root cap. The length of the shoot was measured to the nearest millimeter of the root hypocotyl transission zone to the middle of the cotyledon. The length of the shoot and root was calculated with the aid of a string and a scale [33].

4. Results and discussions

Dichlorofluorescein silver nanoparticles (DCF-SNPs) are used by different analytical methods to characterize them at the nanoscale level. The technique used for characterization were fourier transform infrared spectroscopy (FTIR), zeta potential (ZP) analysis and nanoparticles tracking analysis (NTA) using NanoSight LM-20. In addition, the synthesized dichlorofluorescein silver nanoparticles (DCF-SNPs) was used to successfully test its effect on the germination of Mung Bean (*V. radiata*) seeds. Essentially, the characterization of the synthesized dichlorofluorescein silver nanoparticles (DCF-SNP) was primarily carried out by observing the change in color of the reaction mixture from light green to dark brown. In addition different nanoparticles characterization techniques are used to chemically characterize nanoparticles. In the UV–visible spectrum analysis a single, strong, and broad surface plasmon resonance (SPR) peak was observed at 419 nm that confirmed the synthesis of Dichlorofluoresceine Silver nanoparticles. In same way, [34] UV–vis results displayed that the SPRs becomes sharper and shifts towards lower wavelength (*Escherichia hermannii* = 438 nm, *Citrobacter sedlakii* = 441 nm), shows that the particle size of AgNPs decreased. The work of [35] suggested that the green synthesis of gallic acid-coated silver nanoparticles UV–vis absorption spectrum shows typical and narrow absorption peak at approximately 400 nm.

4.1 Visualization of (DCF-SNPs)

Dichlorofluorescein silver nanoparticles (DCF-SNP) were successfully fabricated using AgNO3 (1 mM) and DCF (2.5 per cent) by boiling the reactants at 12 pH. The reaction is observed for color shift and is found to be dark brown in color

after 24 hours. The synthesized dichlorofluorescein silver nanoparticles DCF-SNP before and after the reaction and color shift are shown in **Figure 4**.

The silver nanoparticles have been synthesized successfully using DCF has been synthesized by mixing 97.5 ml of $AgNO_3$ (1 mM) and 2.5 ml of dichlorofluoresceine in conical flask. It takes 75 sec to boil and the color changes from orangish to greenish color. However, [34] uses silver nitrate ($AgNO_3$) of 10–3 M concentration to the reaction vessels containing the bacterial isolate supernatants and reaction completes after 24 hours.

4.2 Fourier transforms infrared spectroscopy (FTIR) analysis

The FTIR measurement were carried out in order to identify the involvement of different functional groups present in dichlorofluorescein compound solution responsible for the bioreduction of Ag + and the capping of dichlorofluorescein silver nanoparticles (DCF-SNPs). The observed FTIR intense bands for Dichlorofluorescein compound solution were compared with the standard IR band ranges and this enables to identify the functional group at 1042.809 cm^{-1}, 1074.148 cm^{-1}, 1153.629 cm^{-1}, 1262.412 cm^{-1}, 1373.712 cm^{-1}, 1472.978 cm^{-1}, 1634.676 cm^{-1}, 1997.814 cm^{-1}, 2113.782 cm^{-1}, 2204.477 cm^{-1}, 2261.689 cm^{-1}, 2300.828 cm^{-1} and 3262.457 cm^{-1} for possibly capped the dichlorofluorescein silver nanoparticles. The FTIR spectrum of dichlorofluoresceine silver nanoparticles (DCF-SNPs) is shown in **Figure 5** and the details in terms of wave number, bond and intensity are given in (**Table 2**). **Table 3** illustrates a rather more specific FTIR spectrum of dichlorofluorescein silver nanoparticles (DCF-SNPs) including that of the area covered, peak height from left to right edge and centre.

The fourier transform infrared spectroscopy results confirms that absorption bands at 1042.809 cm^{-1}, 1074.148 cm^{-1}, 1153.629 cm^{-1}, 1262.412 cm^{-1}, 1373.712 cm^{-1}, 1472.978 cm^{-1}, 1634.676 cm^{-1}, 1997.814 cm^{-1}, 2113.782 cm^{-1}, 2204.477 cm^{-1}, 2261.689 cm^{-1}, 2300.828 cm^{-1} and 3262.457 cm^{-1} as the wave numbers for the functional groups amine, tertiary alcohol, aromatic ester, alkane, conjugated alkene, alkyne and isocynate that have taken part reducing the silver salt to form

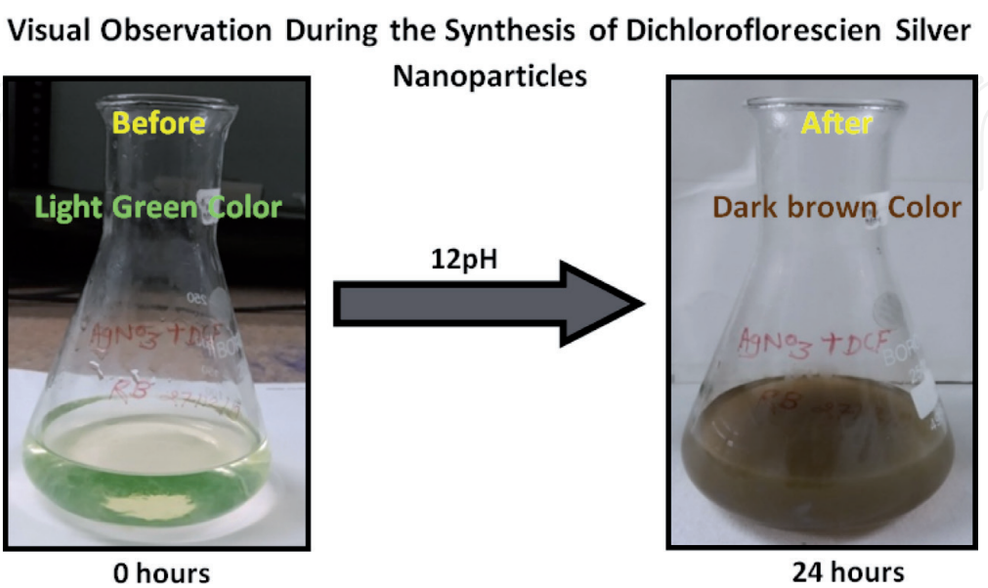

Figure 4.
Visual observation of color change during the dichlorofluorescein silver nanoparticles (DCF-SNPs) nucleation stage.

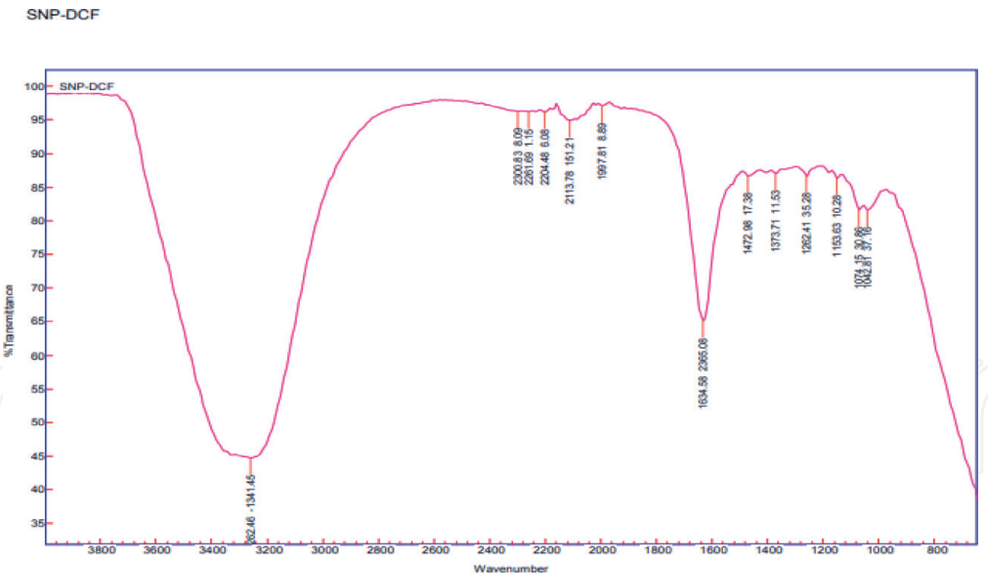

Figure 5.
Shows the FTIR spectrum of dichlorofluorescein silver nanoparticles (DCF-SNPs) which has been reduced via dichlorofluorescein (DCF) solution.

Sr. No.	Wavenumber (cm^{-1})	Bond	Functional group	Intensity
1.	1042.809	C-N stretching	Amine	Medium
2.	1074.148	C-N stretching	Amine	Strong
3.	1153.629	C-O stretching	Tertiary alcohol	Strong
4.	1262.412	C-O stretching	Aromatic easter	Strong
5.	1373.712	O-H bonding	Alcohol	Medium
6.	1472.978	C-H bonding	Alkane	Variable
7.	1634.676	C-C stretching	Conjugated alkene	Medium
8.	1997.814	C=C=C stretching	Allene	Medium
9.	2113.782	C C stretching	Alkyne	Weak
10.	2204.477	C C stretching	Alkyne	Weak
11.	22161.689	N=C=O stretching	Strong, broad	Isocynate
12.	2300.828	—	—	—
13.	3262.457	O-H stretching	Strong, broad	Alcohol

Table 2.
Shows the specifics of the FTIR spectrum of dichlorofluorescein silver nanoparticles (DCF-SNPs) in terms of wave number (cm^{-1}), bond, functional group and bonding strength.

dichlorofluorescein silver nanoparticles (DCF-SNP). Similarly, [36] showed single aldehyde, OH stretching and aldehyde, amide, carbonyl, ethylene, methoxy compounds present in Cannonball Leaves extract and involved in the reduction of silver salt for the creation of silver nanoparticles using the FTIR spectrum.

4.3 Nanoparticles tracking (NTA) analysis

Nanoparticles tracking and analysis (NTA) was carried out using NanoSight LM-20 to determine the dispersion characteristics, i. e. size and distribution of silver nanoparticles of dichlorofluoresceine (DCF-SNPs). The nanoparticles

Peak Name	Area	Height	Left Edge	Right Edge	Center
Peakr1	−37.159	1.234	1052.004	1010.969	1042.809
Peak2	−30.857	1.572	1104.232	1066.927	1074.148
Peak3	−10.283	0.895	1160.189	1141.537	1153.629
Peak4	−35.279	1.485	1279.566	1234.800	1262.412
Peak5	−11.528	0.547	1384.020	1335.523	1373.712
Peak6	−17.379	0.721	1481.013	1451.169	1472.978
Peak7	−2365.076	25.540	1764.532	1563.085	1634.576
Peak8	−8.886	0.502	2014.477	1984.633	1997.814
Peak9	−151.214	2.514	2137.584	2070.434	2113.782
Peak10	−6.076	0.452	2215.924	2197.272	2204.477
Peak11	−1.151	0.104	2268.151	2245.768	2261.689
Peak12	−8.086	0.027	2365.145	2297.996	2300.828
Peak13	1341.449	4.760	3290.312	2932.183	3262.457

Table 3.
Shows the more specific FTIR spectrum of dichlorofluorescein silver nanoparticles (DCF-SNPs) including the area covered, peak height from left to right edge and Centre.

tracking and analysis measure the size of individual nanoparticles in a suspension by their brownian motions from which the intensity of particle size distribution are obtained. The size of dichlorofluorescein silver nanoparticles (DCF-SNPs) from nanoparticles tracking and analysis was found to be less than 293 nm. The size distribution histogram of dichlorofluoresceine silver nanoparticles using nanoparticles tracking and analysis (NTA) is represented in **Figure 6** and the 3-D plot of dichlorofluoresceine silver nanoparticles size distribution intensity can be seen in **Figure 7**.

It could be seen extremely obviously from the histogram that the synthesized dichlorofluorescein silver nanoparticles (DCF-SNPs) ranged from 66 nm to 293 nm in size. The very few dichlorofluoresceine silver nanoparticles (DCF-SNPs) are

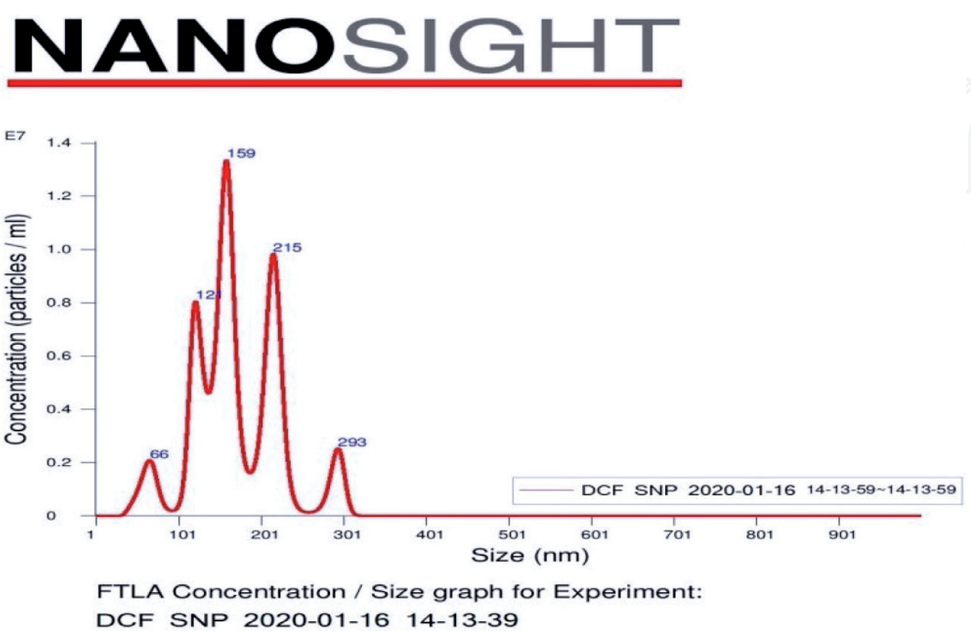

Figure 6.
Shows the size distribution histogram of dichlorofluoresceine silver nanoparticles using nanoparticles tracking and analysis (NTA).

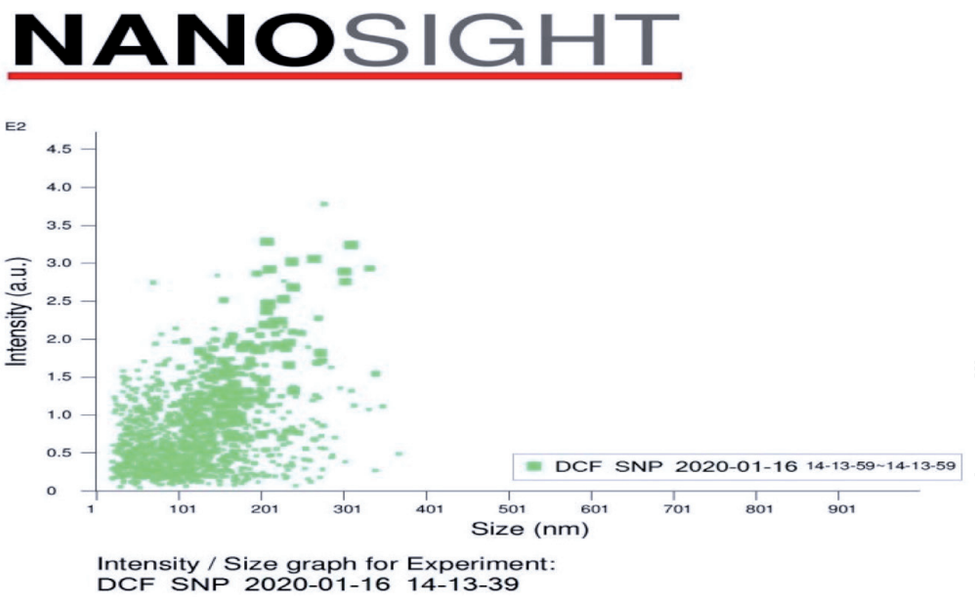

Figure 7.
Shows the 3-D plot for size distribution intensity of dichlorofluorescein silver nanoparticles (DCF-SNPs).

112 nm, 159 nm, 215 nm and 293 nm in size. However the substantial majority of dichlorofluorescein silver nanoparticles (DCF-SNP) are 159 nm wide.

The synthesized dichlorofluorescein silver nanoparticles (DCF-SNPs) intensity of their size distribution in nanoscale clearly demonstrates that most dichlorofluorescein silver nanoparticles (DCF-SNPs) are similar in diameter (159 nm) and few are clustered in scales above 300 nm. The synthesized dichlorofluorescein silver nanoparticles (DCF-SNPs) are therefore stabled formulated which could be used for chemical analysis in agriculture.

4.4 Zeta potential (ZP) analysis

The synthesized dichlorofluorescein silver nanoparticles (DCF-SNPs) has a zeta potential value of-9.35 mV implying that the dichlorofluorescein silver nanoparticles (DCF-SNP) have a high negative surface load. The zeta variance efficiency was found to be 6.10 and 0.0196, reflecting that the zeta potential synthesized dichlorofluorescein silver nanoparticles are significant and could be used to associate other materials or molecules to achieve secondary effects. **Figure 8** displays the zeta potential graph for dichlorofluorescein silver nanoparticles (DCF-SNP).

The dichlorofluoresceine silver nanoparticles (DCFs) are in ranged between 40–293 nm size in diameter confirmed by analyzing nanoparticles tracking and analysis (NTA). The size distribution and zeta potential of dichlorofluoresceine silver nanoparticles were determined by DLS and it is confirmed that the dichlorofluoresceine silver nanoparticles obtained are colloidal in nature, with average diameter approximately 159 nm and the corresponding average zeta potential for dichlorofluoresceine silver nanoparticles as −9.35 mV. In contrast, the work of Saeb et al. (2014) obtained silver nanoparticles using bacterial isolates gave the highest value of zeta potential of −30 mV, which indicates a good stability. However, unexpectedly, this zeta potential value was drastically decreased to −18.3 mV and − 9.5 mV after 30 and 90 days respectively.

4.5 Effect of DCF-SNPs on seeds germination of *Vigna radiata*

The effect of dichlorofluorescein silver nanoparticles (DCF-SNPs) on seed germination of Mung bean (*V. radiata*) was conducted and the root and shoot

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -9.35	Peak 1:	-9.35	100.0	6.10
Zeta Deviation (mV): 6.10	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0196	Peak 3:	0.00	0.0	0.00
Result quality Good				

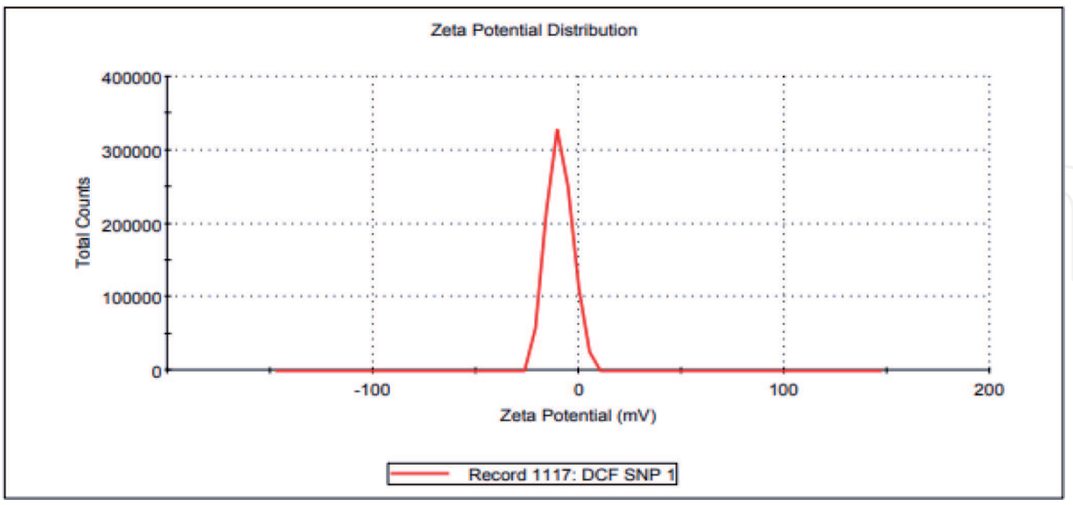

Figure 8.
Shows the zeta potential graph for surface charge on the dichlorofluoresceine silver nanoparticles (DCF-SNPs).

lengths were recorded every 24 hours. In the present experiment, the production of radicals exceeding 1 cm is interpreted to be positive growth otherwise indicated as negative. The effect of dichlorofluorescein silver nanoparticles (DCF-SNPs) on seed germination of Mung bean is shown in the **Figure 9**.

From the **Figure 9** it is noted that as the concentration of dichlorofluoresceine silver nanoparticles increases the root and shoot length decreases as compared to controls once. After 96 hrs, the Mung beans treated with 25% of dichlorofluoresceine silver nanoparticles (DCF-SNPs) shows growth in root and shoot length when compared with the positive control. However, the concentration of DCF-SNPs increases from 50% to 100%, there was inhibition of growth observed when compared to 25% of dichlorofluoresceine silver nanoparticles (**Figure 10**).

The effect of dichlorofluoresceine silver nanoparticles on seed germination of the Mung bean (*V. radiata*) was carried out and the size of root and shoot lengths were recorded after every 24 hour. Percentage of seed germination were substantially influenced by the addition of dichlorofluorescein silver nanoparticles (DCF-SNPs). The 25% concentration of DCF-SNPs treated Mung seeds indicates an excellent growth at 72 hours. In specific, the seed germination rate increases from 75% to 95%. While, as the concentration increases from 50% to 100%, the growth rate get varies or unpredicted. Our results are similar to the work of the [33] found that the as the concentration of ZnO NPs increased there was decrease in germination of seeds. Control showed statistically significant difference and could not improve shoot length. Besides, There was an increase in germination significantly with zinc oxide nano particles treated seeds at different concentrations viz., 20 mg shown 100%, 40 mg-95%, 60 mg-90%, 80 mg-90% and 100 mg of ZnO NPs shown 85% germination in Mung bean seeds [33].

The [37] study the effect of silver nanoparticles on the seed germination and plant growth and found that the highest germination rate for corn seeds, was 6.5 seeds/day, which was observed after exposure to 1.5 mg/ml of silver nanoparticles and the highest germination percentage (73.33%) and highest germination rate (1.59 seeds/day) for watermelon were recorded at 2 mg/ml silver nanoparticles.

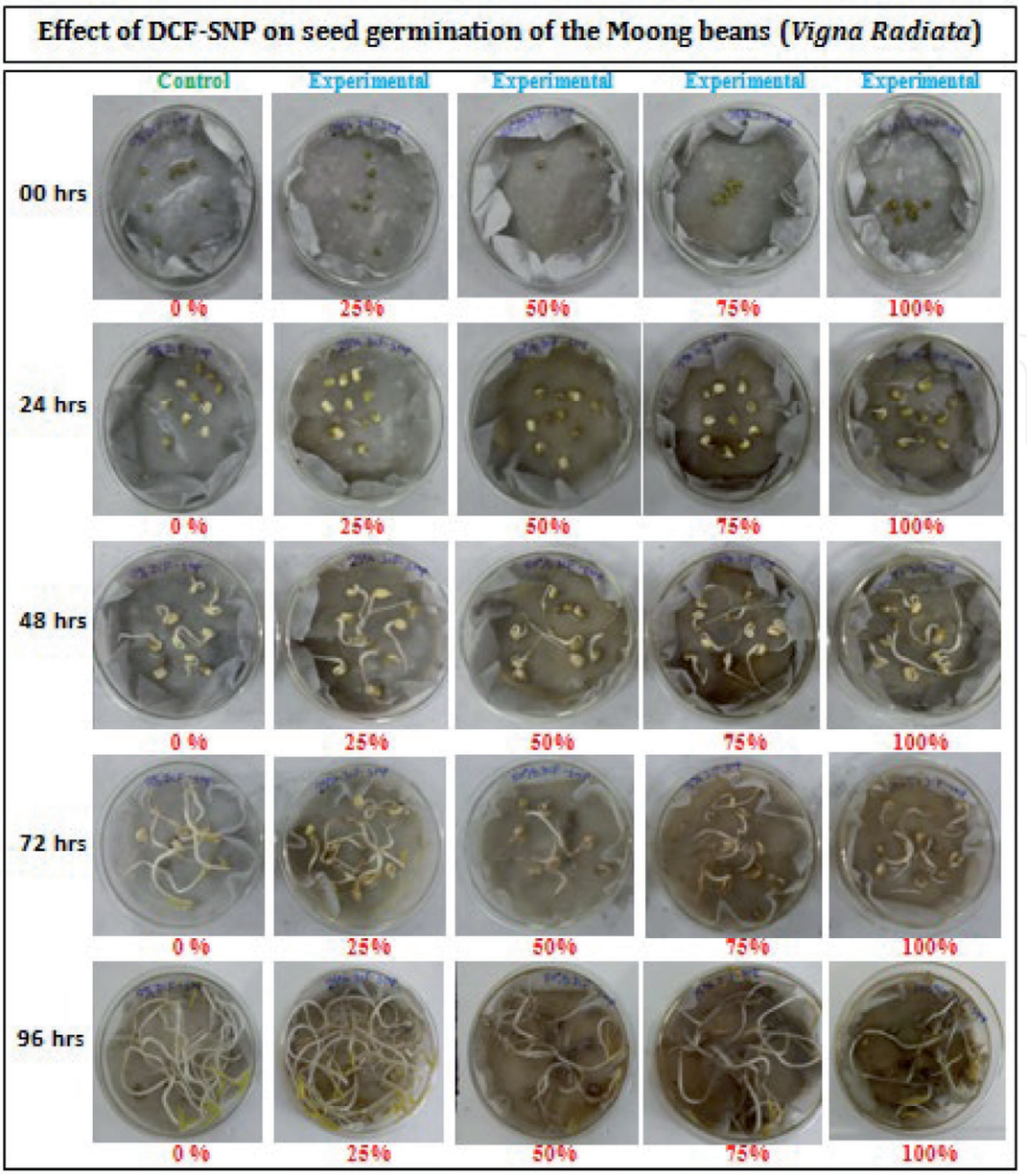

Figure 9.
Shows the time dependent toxicity effect of varying concentrations of dichlorofluoresceine silver nanoparticles (DCF-SNPs) on mung beans.

Due to interactions of dichlorofluorescein silver nanoparticles, the percentage of germination and length of root and shoot has indeed been affected. The average length of root are measure after 24 hrs, 48 hrs and 72 hrs and found to be 6.6 mm, 22.2 mm and 41.7 mm respectively. Similarly, the average length of shoot are measure after 48 hrs and 72 hrs and found to be 2.4 mm and 9 mm respectively. The experiment showed the average length of root and shoot at 72 hours was highest which is 41.7 mm and 9 mm respectively (**Table 4**).

In the present study, the dichlorofluoresceine silver nanoparticles showed unpredicted effects on root and shoot length when treated with the various concentrations of dichlorofluoresceine silver nanoparticles (DCF-SNPs). The higher concentration of nanoparticles may be attributed to toxic level of nanoparticles which has been seen in present experimentation that above certain level of concentration the seedlings respond in different way and causes subsequent declines in growth. The work of [33] evidence the same results their study stating that at low concentrations the ZnO nanoparticles shows good effect on root and shoot was more prominent.

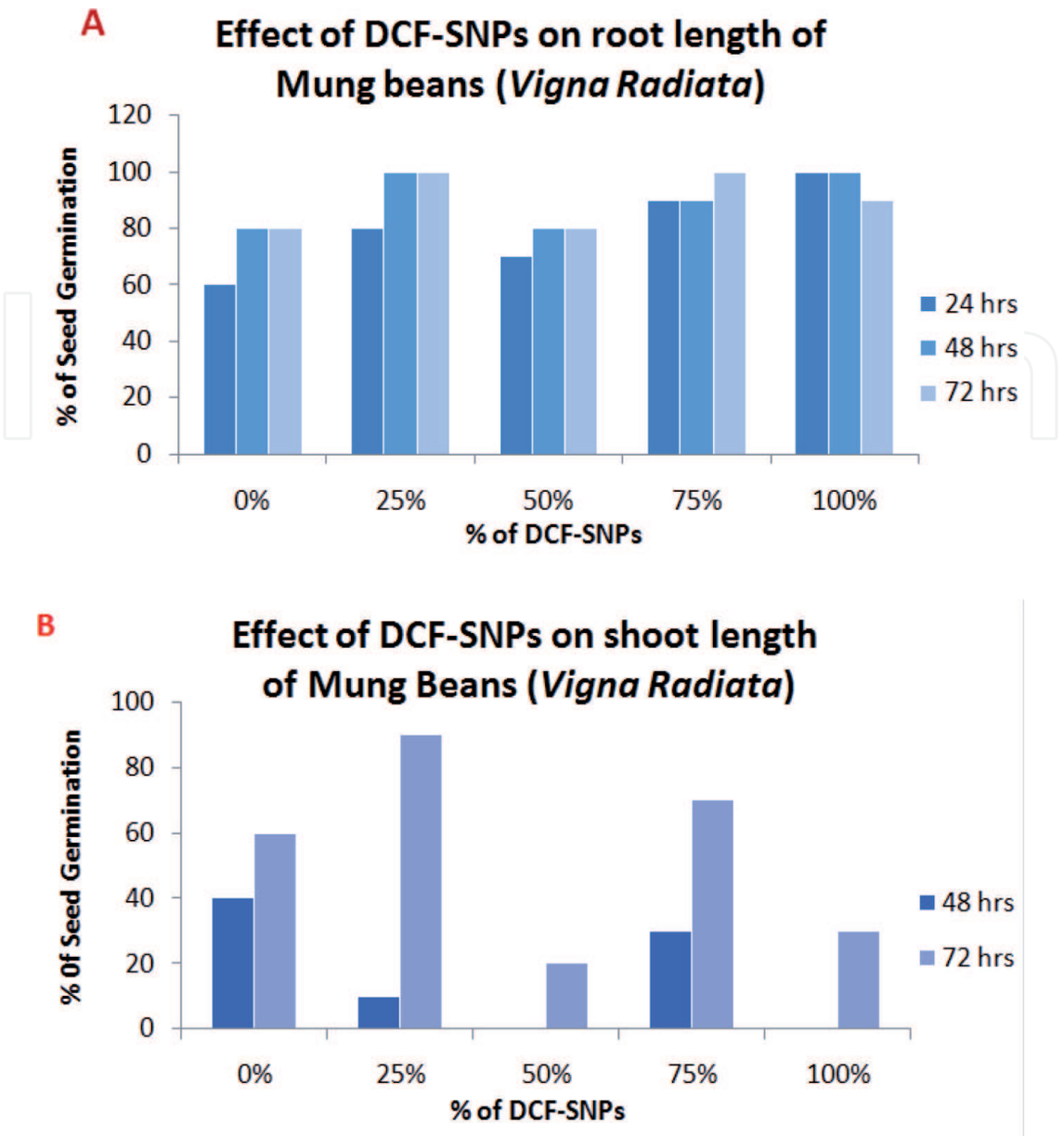

Figure 10.
Shows the dichlorofluorescein silver nanoparticles effect on a: Root length and B: Shoot length of mung beans (V. radiata) seed after 24 hr., 48 hr., 72 hr.

Sr. no	Conc. of DCF-SNPs	Average root length			Average shoot length		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
1.	0%	7 mm	16.1 mm	49.8 mm	—	4 mm	17.5 mm
2.	25%	7.1 mm	24.1 mm	50.2 mm	—	2 mm	16.3 mm
3.	50%	6 mm	22.3 mm	34.5 mm	—	—	2.5 mm
4.	75%	7.1 mm	27.4 mm	42.2 mm	—	1.3 mm	3.7 mm
5.	100%	6 mm	21.4 mm	32 mm	—	—	5.3 mm
6.	Total Avg.	6.6 mm	22.2 mm	41.7 mm		2.4 mm	9 mm

Table 4.
Indicates the average percentage of the observed root and shoot length of germinated mung bean after 24 hrs, 48 hrs, 72 hrs.

Therefore, the 25% concentration of dichlorofluoresceine silver nanoparticles (DCF-SNPs) though show positive effects on the seed germinations of mung beans it could have an advantage of using as the tracking the bio active compound,

fertilizers, pesticides, hormone, minerals transfer into the plant system. As the dichlorofluoresceine silver nanoparticles (DCF-SNPs) is a organic dye it could be coupled with the non toxic materials or polymer that could have avoid harmless to plants and at the same time assist to deliver bio active essential compound in plants. This bio uptake, biotransformation, and bioaccumulation of Fluorescent dichlorofluoresceine silver nanoparticles (DCF-SNP) could be studied using the Confocal Laser-Scanning Microscopy. A study done by [38] already used Confocal laser scanning microscopy (CLSM), Leica TCS SP2 microscope (Leica Inc., Buffalo Grove, IL) to visualize the fluorescent Zein nanoparticles translocation in sugar cane.

5. Conclusion

In the current study, the synthesized dichlorofluoresceine silver nanoparticles (DCF-SNPs) were synthesized successfully by boiling method. In addition, dichlorofluoresceine silver nanoparticles (DCF-SNPs) were characterized by different techniques for measuring the particles size, morphology, functional group and surface charge. Moreover, the different concentrations of dichlorofluoresceine silver nanoparticles (DCF-SNPs) effects on germination of mung beans (*V. radiata*) and the length of root and shoot were studied. The synthesized dichlorofluoresceine silver nanoparticles (DCF-SNPs) are less than 159 nm size that interact and activates growth related gene. Therefore, 25% concentration of dichlorofluoresceine silver nanoparticles (DCF-SNP) is tested positive for shoot and root growth as compare to control. The 25% concentration of dichlorofluoresceine silver nanoparticles (DCF-SNPs) is good dye for conjugation with other bioactive compounds and useful for tracking the bio active compounds, bio uptake, biotransformation and bioaccumulation into the plant system using confocal laser scanning microscopy.

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Conflict of interest

The authors declare no conflict of interest.

Footnotes

The FTIR analysis of dichlorofluorescein silver nanoparticles (DCF-SNPs) were done at the Narsamma Hirayya Arts Commerce & Science college, Amravati, Maharashtra, India.

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References

[1] Gürses A. Chapter 2 Dyes and Pigments: Their Structure and Properties. SpringerBriefs in Green Chemistry for Sustainability. 2016;13-29. DOI : 10.1007/978-3-319-33892-7_2.

[2] Saxena S and Raja ASM. Natural Dyes: Sources, Chemistry, Application and Sustainability Issues. Textile Science and Clothing Technology. 2014; 37-80. DOI: 10.1007/978-981-287-065-0_2.

[3] WHO (2010). IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Some Aromatic Amines, Organic Dyes, and Related Exposures. Lyon (FR): International Agency for Research on Cancer; 2010. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 99.) General Introduction To The Chemistry Of Dyes.

[4] Singh K, Kumar P and Srivastava R. An Overview of Textile Dyes and Their Removal Techniques: Indian Perspective. Poll Res. 2017;36(4): 790-797.

[5] Verma S and Gupta G. Natural dyes and its applications: A brief review. International Journal of Research and Analytical Reviews. 2017;4(4):57-60.

[6] Pargai D, Jahan S and Gahlot M. Functional Properties of Natural Dyed Textiles. Chemistry and Technology of Natural and Synthetic Dyes and Pigments. 2020;1-19. DOI: 10.5772/intechopen.88933.

[7] Organic dye and pigments. ORCO, Colorant solution for all industries [Internet]. 2020. Available from: https://www.organicdye.com/agriculture/.

[8] Kolthoff I M, Lauer W M and Sunde CJ. The use of Dichlorofluorescein as an Adsorption Indicator for the Argentometric Titration of Chlorides. Journal of American Chemical Society. 1929; 51, 11: 3273-3277. DOI:https://pubs.acs.org/doi/abs/10.1021/ja01386a014.

[9] Yao H and Jockusch RA. Fluorescence and electronic action spectroscopy of mass-selected gas-phase fluorescein, 2′,7′-dichlorofluorescein, and 2′,7′-difluorofluorescein ions. Journal of physical chemistry. 2013;117(6):1351-9. DOI: 10.1021/jp309767f. Epub 2013 Jan 23.

[10] Bambach K and Rider T H. Volumetric Determinations of Halides: Use of Dichlorofluorescein as an Adsorption Indicator. Industrial & Engineering Chemistry Analytical Edition. 1935; 7 (3): , 165-166. https://doi.org/10.1021/ac50095a012.

[11] Merck. Molecular structure of 2′,7′-dichlorofluorescein [Internet]. 2020. Available from: https://www.sigmaaldrich.com/catalog/search?term=111843-78-8&interface=CAS%20No.&N=0+&mode=partialmax&lang=en&region=IN&focus=product.

[12] Buccigrossi V, Laudiero G, Russo C, Miele E, Sofia M, Monini M, Maria F R, Guarino A. Chloride secretion induced by rotavirus is oxidative stress-dependent and inhibited by Saccharomyces boulardii in human enterocytes. PloS one. 2014;9(6): e99830. DOI: 10.1371/journal.pone.0099830. eCollection 2014.

[13] Sickinger A N. Cellular DCF-DA assay. Oxidant generating capacity as a metric to allow grouping of nanomaterials and prediction of human health effects. Standard operation procedure (SOP): 2015; Wp4 1. Vol. 1 p.p. 1-6. Availablefrom:https://www.nanopartikel.info/files/projekte/nanOxiMet/SOP/nanOxiMet_SOP_DCF-DA_V1.pdf.

[14] Politano AD, Campbell KT, Rosenberger LH, and Sawyer RG. Use of Silver in the Prevention and Treatment of Infections: Silver Review. Surgical

Infections. 2013;14(1):8-20. DOI: 10.1089/sur.2011.097.

[15] National Library of Medicine, National Centre For Biotechnology Information. Silver nitrate Pubchem: (2020). Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Silver-nitrate.

[16] Gao SS, Zhao IS, Duffin S, Duangthip D, Man Lo EC and Chu CH. Revitalising Silver Nitrate for Caries Management. International Journal of Environmental Research and Public Health. 2018; DOI:15,80; doi:10.3390/ijerph15010080.

[17] Karakaya D and Padem H. Effects of silver nitrate application on cucumbers (*Cucumis sativus* L.) morphology. African Journal of Biotechnology. 2012; 11(72):13664-13669. DOI: https://doi.org/10.5897/AJB11.3230.

[18] Muzamil M, Khalid N, Aziz MD and Abbas SA. Synthesis of silver nanoparticles by silver salt reduction and its characterization. Materials Science and Engineering. 2014; 60:1-9. DOI: 10.1088/1757-899X/60/1/012034.

[19] Kholoud MM, El-Nour A, EftaihaA, Al-WarthanA, Reda A.A. Ammar. Synthesis and applications of silver nanoparticles. Arabian Journal of Chemistry. 2010;3:135-140. DOI:10.1016/j.arabjc.2010.04.008.

[20] Zhang, Xi-Feng et al. "Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches." International journal of molecular sciences vol. 17,9 1534. 2016; doi:10.3390/ijms17091534.

[21] Burdușel, A. C., Gherasim, O., Grumezescu, A. M., Mogoantă, L., Ficai, A., & Andronescu, E. Biomedical Applications of Silver Nanoparticles: An Up-to-Date Overview. Nanomaterials (Basel, Switzerland). (2018): 8(9), 681. https://doi.org/10.3390/nano8090681.

[22] Xu L, Yi-Yi Wang, Huang J, Chun-Yuan Chen, Zhen-Xing Wang and Xie H. Silver nanoparticles: Synthesis, medical applications and biosafety. Theranostics. 2020;10(20):8996-9031. DOI:10.7150/thno.45413.

[23] Anand R and Bhagat *M. silver* nanoparticles (AgNPs): as nanopesticides and nanofertilizers. MOJ Biology and Medicine. 2019; 4(1): 19-20. DOI: 10.15406/mojbm.2019.04.00107.

[24] Lade BD and Gogle DP. Nanobiopesticide: synthesis and Applications in Plant Safety, Nano-biotechnology Applications in Plant Protection, Chapter 10, Volume 2 (Eds: Kamel Abd-Elsalam, and Ram Prasad, *Springer Nature, USA* under "*The Nanotechnology in the Life Sciences*" series; 2019. 169-189, volume 2, DOI: 10.1007/978-3-030-13296-5 9.

[25] Rane AV, Kanny K, Abitha VK and Thomas S. Methods for Synthesis of Nanoparticles and Fabrication of Nanocomposites. Synthesis of Inorganic Nanomaterials. 2018;121-139. DOI: https://doi.org/10.1016/b978-0-08-101975-7.00005-1.

[26] Baltazar-Encarnación E, Escárcega-González CE, Vasto-Anzaldo XG,Cantú-Cárdenas ME andMorones-Ramírez JR. Silver Nanoparticles Synthesized through Green Methods Using *Escherichia coli* Top 10 (Ec-Ts) Growth Culture Medium Exhibit Antimicrobial Properties against Nongrowing Bacterial Strains. Journal of Nanomaterials. 2019;1-9. DOI: https://doi.org/10.1155/2019/4637325.

[27] Mukherjee K, Gupta R, Humar G, Kumari S, Biswas S and Padmanabhan P. silver nanoparticle form *Bacillus clausii* and computational profiling of nitrate reductase enzyme involved in production. Journal of Genetic Engineering and Biotechnology. 2018. 16 (2); 527-536. https://www.sciencedirect.com/science/article/pii/S1687157X18300313.

[28] Hamouda, R.A., Hussein, M.H., Abo-elmagd, R.A. *et al.* Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium *Oscillatoria limnetica*. *Science Report.* 9, 13071 (2019). https://doi.org/10.1038/s41598-019-49444-y.

[29] Bipin D. Lade and Arti S. Shanware (January 21st 2020). Phytonanofabrication: Methodology and Factors Affecting Biosynthesis of Nanoparticles, Smart Nanosystems for Biomedicine, Optoelectronics and Catalysis, Tatyana Shabatina and Vladimir Bochenkov, IntechOpen, DOI: 10.5772/intechopen.90918.

[30] Kumar R, Roopan SM, Prabhakarn A, Khanna VG, Chakroborty S. Agricultural waste *Annona squamosa* peel extract: biosynthesis of silver nanoparticles. Spectrochim Acta A Mol Biomol Spectrosc. 2012 May;90:173-6. doi: 10.1016/j.saa.2012.01.029. Epub 2012 Jan 28. PMID: 22336049.

[31] Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. Journal of Advanced Research. 2016;7(1):17-28. DOI: https://doi.org/10.1016/j.jare.2015.02.007.

[32] Nano Composix. Zeta Potential Analysis Of Nanoparticles, version 1.1. Nanocomposix, San Diego, 2012; CA, pp 1-6.

[33] Jayarambabu N, Kumari B S, Rao K V and Prabhu YT. Germination and Growth Characteristics of Mungbean Seeds (*Vigna radiata* L.) affected by Synthesized Zinc Oxide Nanoparticles. International Journal of Current Engineering and Technology. 2014; 4 (5); http://inpressco.com/wp-content/uploads/2014/09/Paper593411-3416.pdf.

[34] Saeb AT, Alshammari AS, Al-BrahimH, Al-RubeaanKA. Production of silver nanoparticles with strong and stable antimicrobial activity against highly pathogenic and multidrug resistant bacteria. The scientific world journal. 2014 ;2014:704708. DOI: 10.1155/2014/704708.

[35] Li D, Liu Z, Yaun Y and Niu F. Green synthesis of gallic acid-coated silver nanoparticles with high antimicrobial activity and low cytotoxicity to normal cells. Process Biochemistry. 2015; 50(3): DOI: 10.1016/j.procbio.2015.01.002.

[36] Devraj P, Kumari P, Chirom A and Renganathan A. Synthesis and Characterization of Silver Nanoparticles Using Cannonball Leaves and Their Cytotoxic Activity against MCF-7 Cell Line. Journal of Nanocomposites. 2013;1-5. DOI: https://doi.org/10.1155/2013/598328.

[37] Almutairi Z and Alharbi A. Effect of Silver Nanoparticles on Seed Germination of Crop Plants. International Journal of Nuclear and Quantum Engineering. 2016;9:594-598.

[38] Alisha Prasad, Carlos E. Astete, Andreea E. Bodoki, McKenzie Windham, Ede Bodoki, and Cristina M. Sabliov. Journal of Agricultural and Food Chemistry. 2018 66 (26), 6544-6551.DOI: 10.1021/acs.jafc.7b02487.