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Green Synthesis of Silver Nanoparticles from *Silybum Marianum* Leaf Extract and Their Impact on Legume Seed Germination

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ABSTRACT

This current study concerened with impact of AgNPs on seed germination of five legume species: mung bean, fenugreek, pea, bean and chickpea by soaking seed in different concentrations of biosynthesized AgNPs from *Silybum marianum* L. aqueous extract of vegetative parts. Experimentswere conducted in laboratories of AL Nahrain university. Characterization of NPs by (UV-Vis, FTIR, AFM, and SEM) was carried out at Scientific research commission laboratories. An absorption peak at 420nm, spherical to semi spherical shape, 49.8nm average diameter , FTIR of extract showed O-H hydroxyl and C=O carbonyl groups indicate to alcohols, phenolic compounds and flavonoids with N-H amines in amino acids and proteins, C-H_2 and C-H_3 groups heighlighting the prescence of terpenoids, lipids and aliphatic. After AgNPs synthesis, C-N of aromatic amines and O-H of phenolic compounds, these functional groups correspond to amino acids proteins and polyphenolic metabolities. SEM demonstrated homogenous distribution of AgNPs, diameters 46, 49, 64 and 79nm with average size approximately 59.5nm. Response of seed germination to AgNPs illustrated differences among tested species. Mung, bean, fenugreek and pea displayed high germination at concentrations (0.0, 0.1, 0.5 and 1.0 mg/ml), with range values 93.84 to 100%, especially mung bean and fenugreek reached 100% at 0.1and 0.5mg/ml, although pea reached 100% at 0.0, 0.5 and 0.1mg/ml. In comparision, bean recorded significantly lower between 68.75% at 0.0 and 0.1mg/ml to 81.25% at 0.5mg/ml, whereas chickpea lowest value, especially at 0.5mg/ml, then followed by 62.50% at 0.1 and 1.0 mg/ml

Keywords: Silver nanoparticles; *Silybum marianum*; *Trigonella foenum-graecum*; *C. arietinum, P. vulgaris, V. radiata, T. foenum-graecum, P. sativum;* seed germination.

1. Introduction

The application of nanotechnology in agriculture lead to open new frontiers for sustainable in crop production, especially through the advancement using of eco-friendly nanomaterials (Rahman *et al*., 2023; Shah, *et al*., 2020). Various approaches have used in metallic NPs synthesis, Green route considered as ecofriendly method (Singhal, *et al*., 2011). The green synthesis of NPs has received more attentions compaired to other techniques chemical and physical due to important attributes such as environmental-friendly, safety and using nontoxic. Green NPs synthesized are characterized with safer and more diversity in their size and shape, stable. Using plant extracts for formulation of ecofriendly NPs crust out the use of hazard chemicals with toxic effect. Also, to synthesize NPs with definite shape and size. Researchers employment aqueous media containing hydrolytic reagents for reduction, capping and stabilization of nanomaterials (Shah *et al*., 2020).

Milk thistle (*Silybum marianum* L.) one of well known medicinal plant. It has been the subject of numerous studies and research due to its pharmacological and phytochemical properties. It belongs to Asteraceae (Compositae), which consider as one of the largest families of flowering plants, only few species fall under *Silybum* genus. *S. marianum* is the most medicinally and economically significant (Chavan and Badhe, 2023: Valkova *et al*., 2020). It classified as annual or biennial herb and distinguished with its spiny leaves with prominent white veins and its strong upright stem (Valkova *et al*., 2020). An increasing in attention directed toward the vegetative parts (stems and leaves), which contain numerous bioactive compounds. Phytochemical investigations showed that leaves particulary rich in flavonoids and phenolic compounds, they significantly contribute in antioxidant activity (Iraqi *et al*., 2025). Flavonoids such as quarcetin, kaemferol and taxifolin with other phenolic compounds identified in the vegetative tissues (Chavan and Badhe, 2023). Leaves exibt stronger antioxidant potential in comparision to stems due to higher flavonoid continent. Morever, the vegetative parts contain secondary metabolites include phenolic acids, terpenoids and minor amounts of sulfur and nitrogen-containing compounds, the play an important role in biological and defense activity.

AgNPs gathered an important attention due to their unique physicochemical properities, include high surface area to volume ratio, modulation capacity plant physiological processes (Rahman *et al*., 2023; Shah, *et al*., 2020). The conventional physical and chemical synthesis methods for AgNPs by using ethylene glycol, sodium borohydride or hdrazine hydrate pose health concerns and health substantial. They used toxic reducing and capping agents generate hazardous by products and limiting biomedical applicability (Shah, *et al*., 2020; Salama *et al*., 2012).

Between different plants *S. marianum* one of plant species used for AgNPs synthesis, it known as medicinal herb Because it richment with bioactive polyphenolic compounds named silymarin. This mixture contain flavonoid taxifolin and lavonolignans such as silybin A, silybin B, isosilychristin, isosilybin A, isosilybin B, silychristin, silydianin(Sivalingam *et al*., 2023; Shah *et al*., 2020).

Shah *et al*., 2020 showed that *S. marianum* wild plant extracts mediated in AgNPs fabrication, characterized in highly crystalline, stable NPs with size 13.20-18.12nm. High Performance Liquid Chromatography (HPLC) analysis explained that silydianin act as primary capping agents, while silybin B was detected at concentrations til 2289mg/g DW. confirming silyarin components are directly contribute in the reduction and stabilization processes (Shah, *et al*., 2020; Salama *et al*., 2012). Several distinct advantages of biogenic AgNPs: non-toxic, cost effective, ecofriendly environment and biocompatibility compaired to chemically counterparts. Morever, natural plant extract capping agents enhancement the stability and dispersibility of NPs, prevent their aggregation without using synthetic stabilizers (Mohammadinjad *et al*., 2013).

The legumes botanically to the Fabaceae family, consider as the third largest family of angiosperms, from significant crops for human nutrition and play an important role in agricultural sustainability worldwide (Gepts *et al*., 2005). This family includes different species such as beans, chickpeas, peas, mung beans, fenugreek, soybeans, lentils and cowpeas, Which consumed as food in the world (Messina, 1999). Legumes are nutritionally and economically significant worldwide. They serve as important source of protein and soil fertility through biological nitrogen fixation (Rahman *et al*., 2023). Legumes are uniquely positioned among plant-based foods due to their special macronutrient composition, richment with protein and dietary fibers (Didinger and Thompson, 2021). Morever, legumes contain bioactive phytochemicals such as phenolic acids, phytosterols, saponins, flavonoids and bioactive peptides such as lunasin and Bowman-Birk protease inhibitors, which act as reducer risks of cardiovascular disease, certain cancers and type 2 diabetes (Arnoldi *et al*., 2015; Rochfort and Panozzo, 2007).

The application of biosynthesized AgNPs led to seed germination enhancement of various plant species. The effects dependent on concentration and species specific. Generally lower concentrations of AgNPs promote seed germination, seedling vigor and plant growth by the increasing water uptake through nanopore formation on seed coats, enhancing α-amylase activity for starch mobilization and activating antioxidant defense enzymes superoxide dismutase, catalase and ascorbate peroxidase (Husen, 2025; Rahman *et al*., 2023). Anoticeable particularly noteworthy response of leguminous crops such as *P. sativum*, *V. radiata*), *P. vulgaris* and *C. arietinum* showed, when they treated with AgNPs. Treatment of *P. sativum* for 3 hours with AgNPs, results demonstrated significantly enhancement of seed germination percentage (reaching 100% compared to 85% in controls). However, higher concentrations decreasing germination rates to 70% (Rahman *et al*., 2023). Similary effects of AgNPs are significant on common bean (*Phaseolus vulgaris*) and corn (*Zea mays*) (Salama *et al*., 2012).

According to the increasing world population and growing impact of climate change on agriculture, enhancing seed germination of crops become an important in modern agriculture. Nanobiotechnology, particularly using of biosynthesized AgNPs offers promising approach, applications it in agriculture consider as sustainable technique. Legumes regard nutritional and economic importance worldwide as important protein source and soil fertility factor through biological nitrogen fixation. This study aims to investigate the effect of biosynthesized AgNPs fabricated by using the aqueous extract of the vegetative parts of the plant Milk thistle (*Silybum marianum* L.) without using any toxic reducing agent, on the seed germination of five legume species: Chickpea (*Cicer arietinum*), Bean (*Phaseolus vulgaris*), Mung Bean (*Vigna radiata*), Fenugreek (*Trigonella foenum-graecum*) and Pea (*Pisum sativum*). This approach represents the application of nanotechnology in sustainable agriculture, which has become an urgent necessity in today's world.

2. Materials and methods

2.1 Biosynthesis of AgNPs

This research was carried out in the plant tissue culture Lab/ Department of Applied Biology Science/ College of Biotechnology/ AL- Nahrain University, Baghdad, Iraq.

The aqueous extract of *S. marianum* of vegetative parts (leaves and stems) was prepared and biosynthesis of AgNPs was completed according to the Halwani (2017) method with some minor modifications.

2.2 Preparation of Silybum marianum 25% Aqueous Extract

Fresh vegetative parts (stems and leaves) of *S. marianum* were collected from AL Nahrain University gardens. Leaves were thoroughly washed under tap water to remove the pollutants and contaminated then rinsed with deionized distilled water (D.D.W.), 25gm of fresh leaves were mixed with 100ml of D.D.W with heating at 55-60 C◦ for 45min, the components were cooled to room temperature and left for overnight, then filtered through Whatman No. 1 filter paper. Thus, the pure aqueous extract obtained, which stored at 4◦C until use.

2.3 Synthesis of AgNPs

10ml of 25% *S. marianum* aqueous extract was gradually added to 100 ml of 2mM silver nitrate (AgNO3), heated on magnetic stirrer hot plate at 45-60 C◦ for 30-45 min. An observation color change of the mixture from pale yellow to brown, thus preliminary evidence of the formation of AgNPs. The mixture was kept at dark room temperature with shaking for 24 hours AgNPs solution centrifiguated three times to purified AgNPs at 15,000rpm

for 15 min, the supernatant was discarded, precipitate washed with D.D.W and centrifugated. Finally the precipitate collected and dried to obtain the crystal phase of AgNPs.

2.4 Characterization of AgNPs

Using Ultraviolet-visible spectroscopy (UV-Vis), Atomic Force Microscopy (AFM), Fourier-Transform Infrared Spectroscopy (FTIR) and Scanning Eectron Microscope (SEM).

Preparation of AgNPs Concentrations

Firstly: the stock concentrated was prepared, then preparation of other concentrations (0.1, 0.5 and 1.0mg/ml), in addition using D.D.W. as control treatment (0.0mg/ml) for comparasion.

2.5 Soaking the seeds in different concentrations of AgNPs

Seeds were prepared from approved sales centers to ensure that they are free and pure of pathogens and pollutants, after seed sterilization, the seeds were rinsed in D.D.W. three times and dry by air then soaked in different concentrations of AgNPs(0.0 , 0.1, 0.5 and 1.0 mg/ml) for 24 hours, then transfered to sterile petri dishes. Where one layer of wet filter paper with D.D.W. was placed with 10 seeds of Mung Bean (*Vigna radiata*),Pea(*Pisum sativum*), Fenugreek(*Trigonella foenum-graecum*), Chickpea(*Cicer arietinum*) and Bean (*Phaseolus vulgaris)* respectively per dish at equal distances between each seed with two replicate for every concentration, then they were placed in the growth chamber under sterile conditions where the conditions were 23 ± 1 C ° and light for 16 hours/day, the seeds were monitored for 7 days, taking into account that the filter paper did not dry out, and the percentage of germinated seeds was calculated according to the percentage germination equation.

Germination percentage (GP%) = GN× 100 / SN.

GN: total number of germinated seeds.

SN: total number of tested seeds.

2.6 The Experimental Design

Fourty experimental units were used since the design followed the Randomized Blocks Completely Design (RBCD) in two factors, Firstly, the plant species factor, which has five levels representing legume plants (*V. radiata* mung, *P. sativum, T. foenum-graecum, C. arietinum* and *Phaseolus vulgaris*), while the second factor, which represents the concentrations of AgNPs, has four levels 0.0, 0.1, 0.5, 1.0 mg/ml, with two replicate for every concentration.

2.7 Statistical Analysis

The results were statistically analyzed using a completely randomized block design (RBCD) to study the impact of different factors on the seed germination percentage. Significant differences were compared using the Least Significant Difference (LSD) test to compare the arithmetic means of the coefficients at a probability level of 0.05 (SAS, 2012).

3. Results and Discussion

3.1. Characterization

3.1.1. Visual observation:

The rapid color change from pale yellow to brown was the first indicator of the successful AgNPs bio-manufacture from an aqueous extract of *Silybum marianum* fresh vegetative parts (leaves and stems). As noticed in (Fig.1A-C), Initially the 2Mm $AgNO_3$ solution colorless, while the color of *S. marianum* aqueous extract was light yellow due to the presence of naturally phytochemicals. After mixing $AgNO_3$ solution with plant extract, the reaction mixture gradually changed from light yellow to brown indicating the reduction of Ag^+ ions to Ag^0 as shown in fig.1. The bioactive compounds in the aqueous extract of *S. marianu* act as reducing, capping and stabilizing agents. Extract contains phenolic acids, flavonoids, proteins, tannins and antioxidants molecules donate electrons to reduce Ag^+ ions to $Ag^0$and preventing aggregation of NPs through capping mechanisms. The current observation is agreement with different studies about AgNPs biosynthesis combined with visible color change from pale yellow to brown (Nasirvand *et al*., 2023). Also, Alatar *et al*. (2024) and Shareef *et al*. (2024) showed that in green synthesis of AgNPs performed by AgNO3 reaction transformation the color from light yellow to reddish brown.

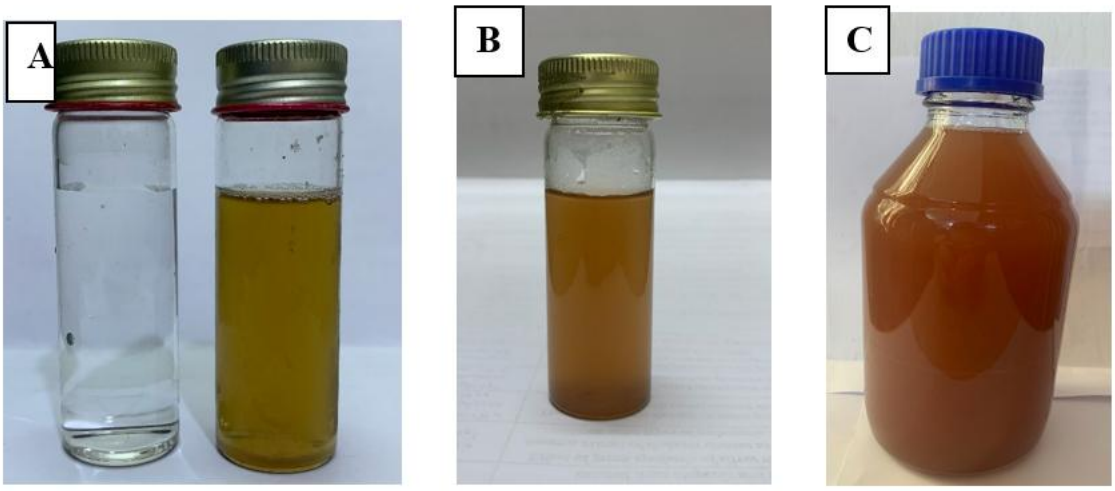


Fig. 1: Color change of aqueous extract of *S. marianum* vegetative parts within synthesis of AgNPs

3.1.2. UV-Vis Spectrscopy:

Fig. 2 illustrated the Uv-Vis spectrum, broad absorbtion band with maximum absorbance at 420 nm, which is characteristic of LSPR(Localized Surface Plasmon Resonance) of AgNPs. This indicate the reducation of Ag^{+} ions to element Ag^{0} and AgNPs formation (Shah e*t al*., 2020; Vanlalveni *et al*., 2021). SPR band of spherical AgNPs, which synthesized from plants falls within 400-450nm. A λmax near 420nm indicates the formation of good colloidal stability, small and well-dispersed spherical NPs (Ijaz *et al*., 2021; Vanlalveni *et al*., 2021). Multiple phytochemicals participate in nucleation, growth and stailization processes during green synthesis resulting NPs with variations in diameter (Vanlalveni *et al*., 2021; Akintelu *et al*., 2020). The single broad peak at 420nm suggests that the phytochemicals in *S. marianum* extract efficiently stabilized AgNPs surface (Yousaf *et al*., 2021). Fresh stems and leaves of *S. marianum* rich with phytochemicals, such as tanins, phenolic compounds, amino acids, proteins, reducing sugars and terpenoids, which act dual reducing and stabilizing function enhances NPs stability and prevents agglomeration (Shah e*t al*., 2020; Vanlalveni *et al*., 2021). Vegetative tissues of *S. marianum* contain high concentration of phenolic acids such as chlorogenic, ferulic and caffeic acids, together with flavonoids including kaempferol and quercetin derivatives, they possess strong reducing power for NPs fabrication and antioxidant activity. Also, their hydroxyl functional groups bind to AgNPs surface lead to increase stability and protect NPs from oxidation and aggregation (Shah e*t al*., 2020).

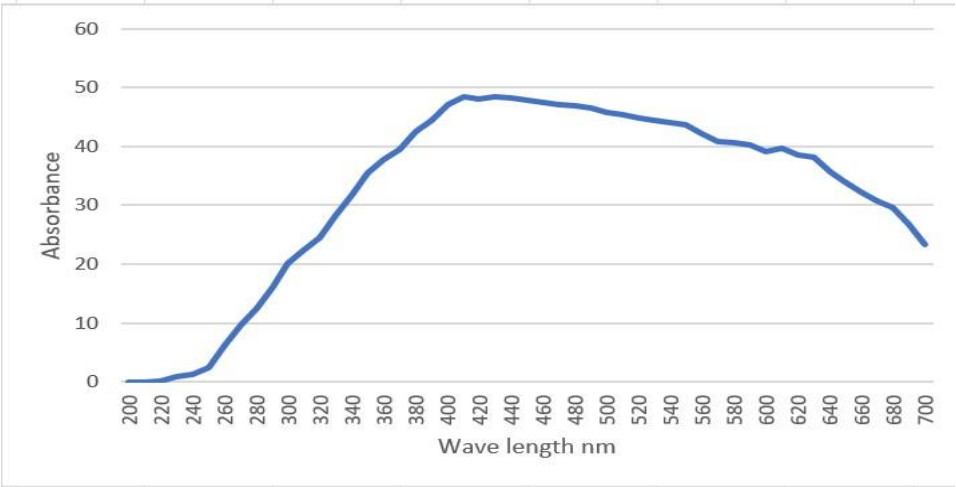


Fig. 2: UV visible spectrum of AgNPs synthesized using *S. marianum* aqueous extract

3.1.3. Atomic Force Microscope:

AFM is powerful technique of nanoscale characterization, which employed to characterize surface morphology, topography, particle size, surface distribution of NPs and high resolution information about NPs morphology, surface roughness and height profile. Fig.3A-C showed an average data of AgNPs size is 49.8nm. The 3D image showed that AgNPs were uniformly distributed, the scanned surface appeared as discrete nanosized elevations with homogenous height distribution. An absence of large agglomerations emphases the stability of NPs during the process of biosynthesis due to the phytochemicals adsorbed on NPs surface (Vanlalveni *et al*., 2021). 2D image demonstrated that AgNPs were spherical to semi spherical with slight

particle dimensions variations. The granularity analysis showed that an average diameter of AgNPs was 49.8nm, which falls within an optimum nanoscale range commonly noticed with plant mediated AgNPs and agrees with value of AgNPs from medicinal plants (Vanlalveni *et al*., 2021). The narrow particle size distribution appeared in the granularity histogram confirms nucleation followed by controlled NPs growth during Ag^{+2} ions reduction. This occurs when using plant extracts rich in natural reducing agents. Fresh stems and leaves of *S. marianum* rich with flavonoids (kaempferol and quercetin derivatives), phenolic acids (caffeic, chlorogenic and ferulic acids), proteins, amino acids, tannins and reducing sugars, which reduce Ag^{+2} to AgNPs and act as natural capping agents and prevent agglomeration (Shah *et al.,* 2020).

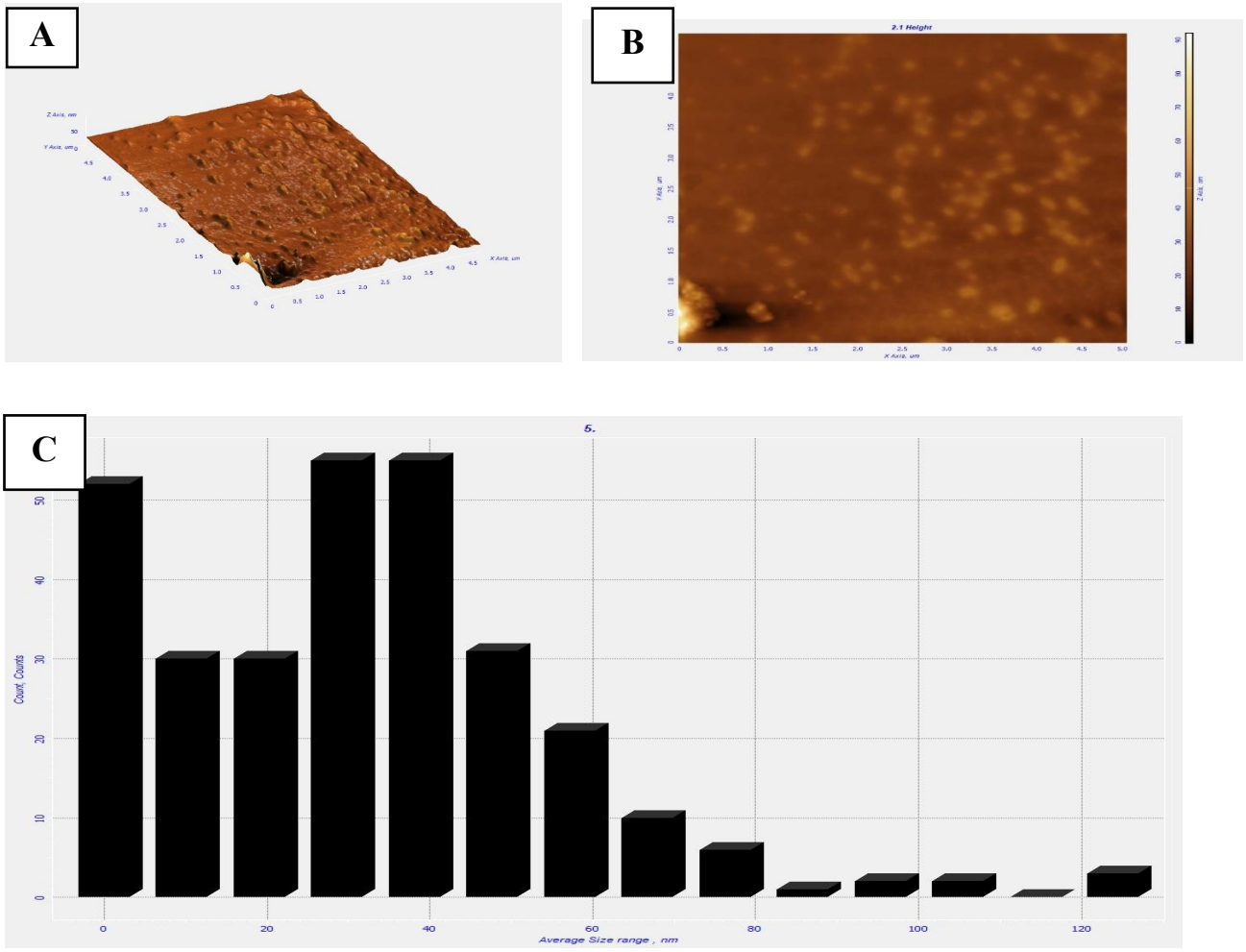


Fig. 3: AFM images (A) 3D , (B) 2D and (C) Granularity accumulation of AgNPs

3.1.4. FTIR Spectroscopy:

FTIR was conducted to discover the functional groups in the extract and identify their roles in synthesis and stabilization of AgNPs. FTIR shown in Fig.4 A-B. FTIR of *S. marianum* extract display several characteristic absorption bands aligned to different phytochemical. The band of broad absorption at nearly 3300-3400cm^{-1} was appointed to O-H vibrations of hydroxyl groups present in alcohols, phenolic compounds and flavonoids, together with N-H extending of amino acids and proteins. These compounds, which contain hydroxyl groups are well known with antioxidant properities and play necessary role in reducing Ag^{+2}ions to Ag^{0}(Shah *et al*., 2020; Vanlalveni *et al*., 2021). An absorption band noticed near 2920-2935cm^{-1}associated to asymmetric C-H_2 vibrations of aliphatic and C-H_3 groups, highlighting the prescence of terpenoids, lipids and aliphatic phytochemicals, which act in NPs stabilization by forming protective organic layer on surface of NPs An absorption band noticed near 2920-2935cm^{-1} similar to asymmetric C-H_2 vibrations of aliphatic and C-H_3 groups, highlighting the prescence of terpenoids, lipids and aliphatic phytochemicals, which act in NPs stabilization by forming protective organic layer on surface of NPs. Obvious absorption peak around 1630-1650cm^{-1} was Associated to C=O stretching vibration of amide I groups from enzymes and proteins or C=C stretching of aromatic rings Available in phenolic acids and flavonoids. After synthesis of NPs, this band showed aslight shift and reduction in intensity, proposing that the carbonyl in the biomolecules Took part in the reduction of of Ag ions and act as capping agents. The region within 1380-1450cm^{-1} of absorption bands were assigned to C-N Stretching modes of aromatic amines and O-H bending of phenolic compounds. These functional groups correspond to amino acids, proteins and polyphenolic metabolities assist with NPs stabilization via cooperation with metallic surface (Khan *et al*., 2017). Intermediate bands between 1000 and 1100 cm^{-1} reflect to C-O and C-O-C stretching vibrations of ethers, alcohols, polysaccharides and glycosidic compounds. The detected shifts in these peaks after

the formation of AgNPs demonstrate that functional groups, which containing oxygen participated in binding and stabilizing the NPs. These interactions restrict crystal growth and enhance the colloidal stability of NPs by reducing aggregation (Vanlalveni *et al*., 2021).

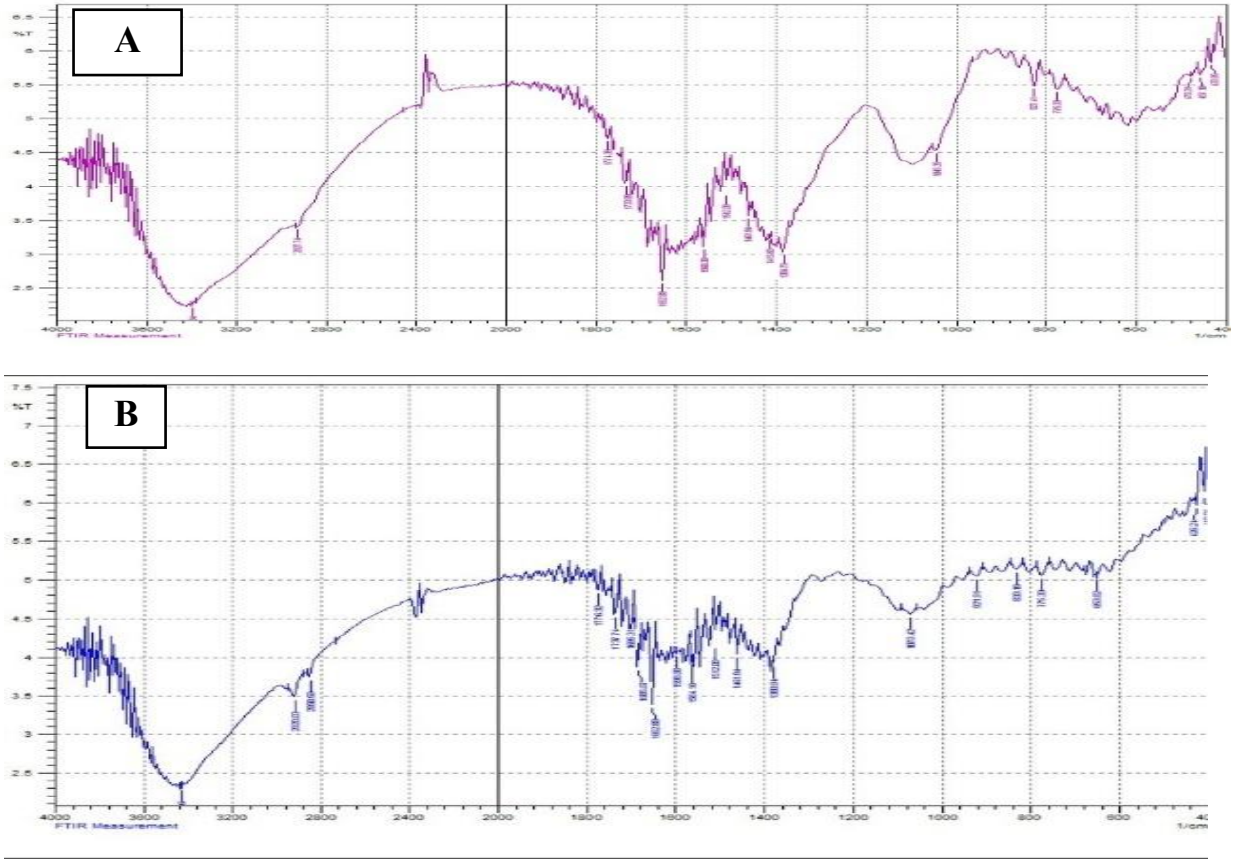


Fig. 4: FTIR of (A) *S. marianum* extract (B) AgNPs

3.1.5. Scanning Electron Micrroscopy:

SEM technique used to investigate the morphology, particle size and surface characteristics of AgNPs. The Fig. 5 demonstrated spherical to semi spherical morphology of NPs and relative homogenous distribution, they appeared well dispersed throughout the scanned area with slight agglomeration in some regions. Same observations were observed by (Dhir *et al*., 2024; Abada *et al*., 2024).

SEM image showed diameters were 46, 49, 64 and 79nm with average particle size approximately 59.5. This size range agrees with Dhir *et al*. (2024) and Zuhrotun *et al*. (2023). NPs exhibited relatively smooth external surfaces with no evidence of irregular bulk structure or large crystalline fragments, which indicate controlled nucleation and crystal growth during biosynthesis of AgNPs. Moderation variation of particle diameter suggests slightly polydisperse population, because different phytochemicals together participate in reduction and stabilization (Abada *et al*., 2024).

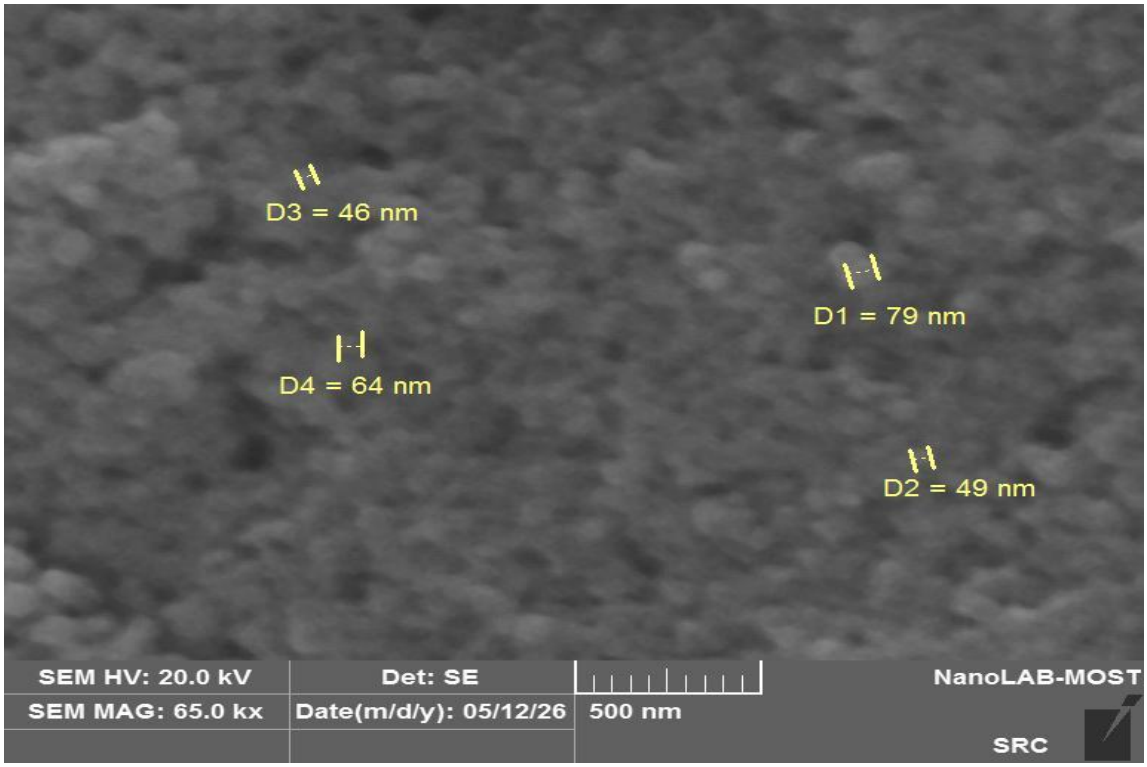


Fig. 5: SEM of AgNPs

3.2. Effect of AgNPs concentration on germination percentage

No significant effect of AgNPs concentration on overall germination percentage. Mean of seeds germination across all species were (88.74, 84.25, 87. 50 and 85.44% at control 0.0, 0.1, 0.5 and 1.0 mg/ml respectively). All differences were below 9.03, this indicates no significant effect. Although individual species showed visible trend (e.g. chickpea germination decreased with increasing AgNPs concentration; common bean increased at 1.0mg/ml). Generally, the effect of concentration is not significant, this suggests that genetic and physiological differences among legume species are primary determinants of germination under these conditions, exceeding the influence of AgNPs.

However, Zhang *et al*. (2026) highlights that the interaction of AgNPs with seed germination shows different responses when a plant of the same species is treated with different concentrations of these particles. Mung bean displayed positive response at low concentrations (0.1, 0.5 mg/ml), This agree with the finding of Ghodake *et al*. (2026) who reported an enhancement seed germination in *V. radiataat* treatment with AgNPs at low concentrations. Fenugreek exhibited same patterns to mung bean. High concentration inhibition is similar with Ghodake *et al*. (2026), showed that an enhancement of fenugreek germination at low concentration of AgNPs, while higher concentrations could be inhibitory. Pea showed an unexpected10% reduction at 0.1mg/ml, which may be an experimental irregularity or because pea is relatively tolerant to AgNPs within 0.5-1.0 mg/ml but may be sensitive at very specific low concentrations. While Rahman *et al*. (2023) observed that low concentration of AgNPs significantly increased pea germination to 100% while high concentration act as toxici effect. Common bean was the only species that demonstrated significant stimulation at the highest concentration. This unique response may be related to species-specific tolerance mechanism. Debnath *et al*. (2020) and Almutairi and Alharbi (2015) demonstrated that variable responses of common bean to AgNPs, some studies showing increasing, others showing inhibition depending on concentration and the characteristics of NPs. Chickpea was more sensitive species, inhibition at all concentrations.

The interaction between plant species and AgNPs concentration demonstrate that response of seed germination to AgNPs showed differences among the tested plant species. As illustrated in Table 1: *V. radiata, T. foemum-graecum and P. sativumdis* played consistently high germination percentages at four concentrations of AgNPs, with range values: 93.84 to 100.00%. Especially, *V. radiata and T. foemum-graecum* reached 100% germination at 0.1 and 0.5 mg/ml, although *P. sativum* reached 100% at 0.0, 0.5 and 1.0mg/ml. This shows that three species were tolerant to the AgNPs concentrations.

In comparision, *P. vulgaris* recorded significantly lower germination percentages, lying between 68.75% and 81.25% at 0.0 (control) and 0.5mg/ml respectively, whereas *C. arietinum* reported the lowest value56.25 at 0.5mg/ml, then rise to 62.50% at 0.1 and 1.0mg/ml. This lower germination of *C. arietinum* indicates higher sensitivity to the AgNPs treatment than other species.

Plant species	**Concentrations(mg/ml)**				**Mean of seed germination**
	C_1	**C_2**	**C_3**	**C_4**	
Vigna radiata	95.84	100.00	100.00	95.84	97.92
Trigonella foenum-graecum	97.84	100.00	100.00	93.84	97.92
Pisum sativum	100.00	90.00	100.00	100.00	97.50
Phaseolus vulgaris	68.75	68.75	81.25	75.00	73.44
Cicer arietinum	81.25	62.50	56.25	62.50	65.63
Mean of concentration	88.74	84.25	87.50	85.44	86.48
LSD (α = 0.05) **Plant species(P) = 10.10 AgNPs concentrations(C) = 9.03** **P × C = 20.20**					

Table 1. Effect of AgNPs on seed germination percentage

Conclusion

The target aim of this research to detect the effect of green synthesized AgNPs on germination seed percentage of important legumes: Mung Bean, Fenugreek Chickpea, Bean and Pea as important food sources. *Silybum marianum* aqueous leaf extract used in green synthesis of AgNPs. This approach of synthesis consider as sustainable ecofriendly method without using hazard and toxic materials. Nanoparticles have exhibited different properties and have been studied by UV–Vis, FTIR, AFM, SEM analyses. No significant effect of AgNPs concentration on overall germination percentage. Wherease individual species showed visible trend e.g. chickpea germination decreased with increasing AgNPs concentration, common bean increased at 1.0mg/ml. Generally, the effect of concentration is not significant, Mung bean and Fenugreek displayed positive response at low concentrations (0.1, 0.5 mg/ml), Common bean was the only species that demonstrated significant stimulation at the highest concentration. Chickpea was more sensitive species, inhibition at all concentrations.

REFRENCES

1. Abada, E., Mashraqi, A., Modafer, Y., Al Abboud, M. A. and El-Shabasy, A. (2024). Review green synthesis of silver nanoparticles by using plant extracts and their antimicrobial activity. *Saudi Journal of Biological Sciences*, 31(1), 103877. https://doi.org/10.1016/j.sjbs.2023.103877
2. Akintelu, S. A., Bo, Y., and Folorunso, A. S. (2020). A review on synthesis, optimization, mechanism, characterization, and antibacterial application of silver nanoparticles synthesized from plants. Hindawi, *Journal of Chemistry*, 2020, Article ID 3189043. https://doi.org/10.1155/2020/3189043
3. Arnoldi, A., Boschin, G., Zanoni, C., and Lammi, C. (2015). The health benefits of sweet lupin seed flours and isolated proteins. *Journal of Functional Foods*, 18, 550-563. https://doi.org/10.1016/j.jff.2015.08.012
4. Alatar, A.A., Qahtan, A.A. and Faisal, M. (2024). Eco-friendly synthesis of silver nanoparticles: unraveling their influence on callus proliferation, secondary metabolite production, and antioxidant activities in *Ruta chalepensis* L. *Plant Cell Tiss Organ Cult* **157**, Article number 55 (2024). https://doi.org/10.1007/s11240-024-02778-6
5. Chavan, S., and Badhe, P. (2023) . Milk thistle (*Silybum marianum*): Review on its morphology, chemistry & pharmacological activity. *Journal of Emerging Technologies and Innovative Research (JETIR)*,**10**(5), p:299–300. https://www.jetir.org/view?paper=JETIR2305C44
6. Debnath, B.; Sarkar, S. and Das, R. (2020). Effects of saponin capped triangular silver nanocrystals on the germination of *Pisum sativum*, *Cicer arietinum*, *Vigna radiata* seeds & their subsequent growth study. IET Nanobiotechnology. 2020, Vol. 14 Iss. 1, pp. 25-32. doi: 10.1049/iet-nbt.2019.0161
7. Dhir, R., Chauhan, S., Subham, P., Kumar, S., Sharma, P., Shidiki, A., and Kumar, G. (2024). Plant-mediated synthesis of silver nanoparticles: Unlocking their pharmacological potential—a comprehensive review. *Frontiers in Bioengineering and Biotechnology*, 11, 1324805. https://doi.org/10.3389/fbioe.2023.1324805

8. Didinger, C., and Thompson, H. J. (2021). Defining nutritional and functional niches of legumes: A call for clarity to distinguish a future role for pulses in the Dietary Guidelines for Americans. *Nutrients*, 13(4), 1100. https://www.mdpi.com/2072-6643/13/4/1100

9. Gepts, P., Beavis, W. D., Brummer, E. C., Shoemaker, R. C., Stalker, H. T., Weeden, N. F., and Young, N. D. (2005). Legumes as a model plant family: Genomics for food and feed—Report of the Cross-Legume Advances through Genomics Conference. *Plant Physiology*, 137(4), 1228-1235.https://doi.org/10.1104/pp.105.060871

10. Ghodake1, G. S.; Kim, M.; Sung, J. S.; Chandrasekaran, K.; Patil, S.; Mehta, V.; Syed, A.; Bahkali, A. H. and Kim, D.-Y. (2026). Greener synthesis of silver nanoparticles from Zingiber officinale rhizome extract for multidrug-resistant pathogen control, seed vigor enhancement, and fish embryo toxicity assessment. Frontiers in Cellular and Infection Microbiology. Volume 16 - 2026 DOI 10.3389/fcimb.2026.1798925

11. Halawani, E. M. (2017). Rapid Biosynthesis Method and Characterization of Silver Nanoparticles Using *Zizyphus spina christi* Leaf Extract and Their Antibacterial Efficacy in Therapeutic Application. *Journal of Biomaterials and Nanobiotechnology*, 2017 (8): 22-35 DOI: 10.4236/jbnb.2017.81002

12. Husen, A. (Ed.). (2025). *Plant Response to Silver Nanoparticles: Plant Growth, Development, Production, and Protection*. Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-97-7352-7

13. Ijaz, M., Zafar, M., and Iqbal, T. (2021). Green synthesis of silver nanoparticles by using various extracts: A review. *Inorganic and Nano-Metal Chemistry*, 51(5), 744–755. https://doi.org/10.1080/24701556.2020.1808680

14. Iraqi, O.; Aouji, M.; Oubihi, A.; Imtara, H.; Noman, O. M.; Mothana, R. A. Tarayrah , M.; Taboz, Y. and Habsaoui, A. **(2025).** Exploring the chemical composition, antioxidant, and antibacterial properties of milk thistle (*Silybum marianum* L.).*Frontiers in Sustainable Food Systems*.**9**, 1551584.https://doi.org/10.3389/fsufs.2025.1551584

15. Khan, I., Saeed, K., and Khan, I. (2017). Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*, 12(7), 908-931doi:10.1016/j.arabjc.2017.05.011

16. Khan, M. S.; Zaka, M.; Abbasi, B. H.; Latifur, R.; Shah, A. (2016). Seed germination and biochemical profile of *Silybum marianum* expose monometallic and bimetallic alloy nanoparticles. IET Nanotechnology. 2016 Dec;10(6):359-366.doi: 10.1049/iet-nbt.2015.0050.

17. Messina, M. J. (1999). Legumes and soybeans: Overview of their nutritional profiles and health effects. *The American Journal of Clinical Nutrition*, 70(3): 439S-450S.https://doi.org/10.1093/ajcn/70.3.439s

18. Mohammadinejad, R., Pourseyedi, S., Baghizadeh, A., Ranjbar, S., and Mansoori, G. A. (2013). Synthesis of silver nanoparticles using *Silybum marianum* seed extract. *Int. J. Nanosci. Nanotechnol*., Vol. 9, No. 4, Dec. 2013, pp. 221-226 https://www.ijnnonline.net/article_4170_f5a0b733305496d21494ff3f9697b687.pdf

19. Nasirvand, S.; Zakaria, R. A. and Ebrahimi, H. A. (2023). Green Synthesis of Silver Nanoparticles Using *Silybum Marianum* (L.) and Portulaca Oleracea (L.) Extracts and Their Antibacterial and Antifungal Activities. Heliyon, Jul. 2023, 18 Pages http://dx.doi.org/10.2139/ssrn.4515486

20. Rahman, M. S., Chakraborty, A., Kibria, A., and Hossain, M. J. (2023). Effects of silver nanoparticles on seed germination and growth performance of pea (*Pisum sativum*). *Plant Nano Biology*, Vol 5, August 2023, 100042.https://doi.org/10.1016/j.plana.2023.100042

21. Rochfort, S., and Panozzo, J. (2007). Phytochemicals for health, the role of pulses. *Journal of Agricultural and Food Chemistry*, 55(20), 7981- 7994. https://doi.org/10.1021/jf071704w

22. Salama, H. M. H. (2012). Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). *International Research Journal of Biotechnology*, 3(10), 190-197.

23. SAS Institute Inc. Statistical Analysis System (SAS) User's Guide: Statistics, Version 9.1. Cary, North Carolina, USA: SAS Institute Inc. (2012).

24. Sivalingam, A.M., Pandian, A., Rengarajan, S.; Ramasubbu, R.; Parasuraman, G.; Sugumar, V.; Dutta, R. and Devaraj,n. (2023). Extraction, biosynthesis, and characterization of silver nanoparticles for its enhanced applications of antibacterial activity using the *Silybum marianum* Linn. plant. *Biomass Conv. Bioref.* **14**, 30227–30238 (2024). https://doi.org/10.1007/s13399-023-04907-1

25. Shah, M., Nawaz, S., Jan, H., Uddin, N., Ali, A., Anjum, S., Giglioli-Guivarc'h, N., Hano, C. and Abbasi, B. H. (2020). Synthesis of bio-mediated silver nanoparticles from *Silybum marianum* and their biological and clinical activities. *Materials Science and Engineering C: Materials for Biological Applications*, 112, 2020,110889. https://doi.org/10.1016/j.msec.2020.110889

26. Shareef, A. A. Farhan, F. J, and Alriyahee, F. A.A. (2024). Green Synthesis of Silver Nanoparticles Using Aqueous Extract of Typha domingensis Pers. Pollen (qurraid) and Evaluate its Antibacterial Activity. *Baghdad Science Journal,*vol 21(1)P:28-40 https://doi.org/10.21123/bsj.2023.7624

27. Singhal, G.; Bhavesh,R.; Kasariya, K.; Sharma, A. R. and Singh, R. P. (2011). Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. Journal of Nanoparticle Researc. Vol 13, (2011) p: 2981-2988 https://doi.org/10.1007/s11051-010-0193-y

28. Valková,V., Ďúranová, H.; Bilčíková, J. and Habán, M. (2020). Milk thistle (*Silybum marianum*): A valuable medicinal plant with several therapeutic purposes. *Journal of Microbiology, Biotechnology and Food Sciences*,9(4),836–843. https://doi.org/10.15414/jmbfs.2020.9.4.836-843

29. Vanlalveni, C., Lallianrawna, S., Biswas, A., Selvaraj, M., Changmai, B., and Rokhum, S. L. (2021). Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: A review of recent literature. *RSC.Advances*,11(5):2804–2837. https://doi.org/10.1039/D0RA09941D

30. Yousaf, H., Mehmood, A., Ahmad, K. S., and Raffi, M. (2020). Green synthesis of silver nanoparticles and their applications as an alternative antibacterial and antioxidant agents. *Materials Science and Engineering: C*, 112, July 2020, 110901. https://doi.org/10.1016/j.msec.2020.110901

31. Zainab M. Almutairi, Z. M. and Alharbi, A. (2015). Effect of Silver Nanoparticles on Seed Germination of Crop Plants. *Journal of Advances in Agriculture*, 4(1), 280-285 https://doi.org/10.24297/jaa.v4i1.4295

32. Zuhrotun, A., Oktaviani, D. J., and Hasanah, A. N. (2023). Biosynthesis of gold and silver nanoparticles using phytochemical compounds. *Molecules*, 28(7),3240. https://doi.org/10.3390/molecules28073240