



BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES USING *PINUS ROXBURGHII* NEEDLE EXTRACT: PHYSICOCHEMICAL PROFILING AND INSECTICIDAL EFFICACY AGAINST *TRIBOLIUM CASTANEUM* (HERBST)

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ABSTRACT

Persistent infestations of the red flour beetle (*Tribolium castaneum*) present an ongoing challenge in post-harvest grain storage, where heavy reliance on synthetic pesticides continues to foster chemical resistance and leave undesirable residues. This study reports a green, sustainable route for synthesizing silver nanoparticles (AgNPs) mediated by methanolic needle extract of the Himalayan pine *Pinus roxburghii* Sarg. Quantitative colorimetric profiling confirmed substantial reserves of total phenolic content (38.3 mg GAE/g dry extract) and total flavonoid content (2.5 mg RE/g dry extract), along with strong free radical scavenging potential (IC₅₀=48.89 µg/mL). GC-MS analysis detected major secondary metabolites—including melezitose (10.2%), glucopyranose derivatives (10.2%), and octadecenoic acid esters (4.95%)—which serve as natural reducing and capping agents. Surface plasmon resonance (SPR) centered at 450 nm in UV-Vis spectroscopy confirmed successful bioreduction of Ag⁺ to stable Ag⁰. Contact-toxicity bioassays against adult *T. castaneum* revealed marked concentration- and time-dependent insecticidal responses. The synthesized AgNPs achieved 70% mortality at 200 mg exposure after 72 h, clearly exceeding the 50% mortality recorded for the crude methanolic extract under identical conditions. These results show that combining *P. roxburghii* phytochemicals with nanosilver architectures provides an eco-friendly, potent bio-insecticide suitable for integrated pest management programs in stored agricultural commodities.

Keyword: *Pinus roxburghii*, Silver Nanoparticles, Green Synthesis, *Tribolium castaneum*, Biopesticide, Stored-Grain Protection.

Introduction

Post-harvest grain loss caused by coleopteran pests remains a major bottleneck in maintaining global food supply chains. The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is an infamous secondary pest of stored cereals, grain flours, and milled products. Conventional management relies heavily on synthetic fumigants (such as phosphine and methyl bromide) and synthetic contact insecticides. However, indiscriminate long-term application has triggered resistance, posed chronic mammalian health hazards, and contaminated storage facilities with toxic chemical residues. Consequently, the search for plant-derived, biodegradable, and targeted biopesticides has gained significant momentum.

Conifers of the family Pinaceae, particularly *Pinus roxburghii* Sarg. (Chir pine), are distributed across the subtropical and temperate valleys of the northwestern Himalayas. The foliage of *P. roxburghii* produces a vast array of secondary metabolites, including mono- and sesquiterpenoids, polyphenols, flavonoids, and organic acids, which evolved as chemical defenses against herbivores and microflora. While crude botanical extracts provide measurable contact and repellent activity, their field use is frequently hampered by volatility, chemical instability, and low bio-persistence.

Green nanotechnology offers a clean, sustainable platform to overcome these limitations. By employing plant extracts as primary electron donors and capping agents, aqueous silver ions can be converted into stable zero-valent silver nanoparticles (AgNPs) without toxic synthetic reducing surfactants like sodium borohydride or hydrazine. Biogenic AgNPs possess exceptionally high surface-area-to-volume ratios and unique physicochemical properties that facilitate cuticular abrasion, membrane disruption, and internal oxidative stress in insects. This study examines the phytochemical composition of *P. roxburghii* needle extracts, validates the synthesis and physicochemical traits of biogenic AgNPs, and assesses their contact toxicity against adult *T. castaneum*.

2. Materials and Methods

2.1 Botanical Material and Reagents

Fresh needles of *Pinus roxburghii* Sarg. were collected from Himachal Pradesh, India. Silver nitrate (AgNO_3), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, rutin, ascorbic acid, aluminum chloride (AlCl_3), and analytical grade methanol were purchased from standard chemical suppliers. Double distilled water was used throughout the synthesis and characterization steps.

2.2 Preparation of Methanolic Extract

Collected needles were thoroughly rinsed with tap water followed by distilled water to remove particulate debris. The plant material was shade-dried at ambient temperature for approximately three weeks and ground into a uniform powder using an electrical grinder. Powdered needle biomass (100 g) was

macerated with 100 mL methanol in an Erlenmeyer flask and incubated on an orbital shaker at 37° C. The slurry was filtered through Whatman No. 1 filter paper, and the resulting crude extract was refrigerated at 4° C for further analysis.

2.3 Qualitative Phytochemical Profiling

Qualitative screenings were performed on the methanolic extract to detect major secondary metabolite classes using standard protocols:

- Carbohydrates & Reducing Sugars: Molisch's test and Fehling's test.
- Alkaloids: Wagner's, Mayer's, and Dragendorff's reagent tests.
- Phenolics, Flavonoids, & Tannins: Ferric chloride (FeCl₃) test and alkaline lead acetate test.
- Proteins & Resins: Ninhydrin test and water precipitation turbidity test.

2.4 Quantitative Phytochemical Assays

2.4.1 Total Phenolic Content (TPC)

TPC was determined via the Folin-Ciocalteu colorimetric method. Gallic acid was used as the reference standard. Sample absorbance was read spectrophotometrically at 765 nm, and phenolic concentration was computed from the linear calibration curve:

$$y = 0.0232x + 0.6889 \quad (R^2 = 0.9907)$$

Values were expressed as milligrams of Gallic Acid Equivalents per gram of dry extract (mg GAE/g dry weight).

2.4.2 Total Flavonoid Content (TFC)

TFC was quantified using the aluminum chloride (AlCl₃) colorimetric assay with rutin as the reference standard. The absorbance was recorded and evaluated using the standard rutin regression equation:

$$y = 0.013x + 0.549 \quad (R^2 = 0.999)$$

Data were calculated as milligrams of Rutin Equivalents per gram of dry extract (mg RE/g dry weight).

2.4.3 DPPH Free Radical Scavenging Assay

The antioxidant potential of *P. roxburghii* extract was evaluated by mixing 1.0 mL of extract at graded concentrations (10 to 120 µg/mL) with 1.0 mL of methanolic DPPH solution (0.1 mM). After a

30-minute incubation in the dark, absorbance was recorded at 517 nm against an ascorbic acid reference. Radical scavenging activity (%RSA) was calculated as follows:

$$\%RSA = \left[\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right] \times 100$$

The inhibitory concentration required to scavenge 50% of radicals (IC_{50}) was calculated via linear regression.

2.5 GC-MS Characterization of Bioactive Phytoconstituents

Volatile constituents were separated and identified on a Trace 1300 Gas Chromatograph coupled to a TSQ 8000 Triple Quadrupole Mass Spectrometer equipped with a TG-5MS capillary column (30 m × 0.25 mm × 0.25 μm). Ultra-pure helium served as the carrier gas at a constant velocity of 1.0 mL/min. Oven temperature was held at 60° C for 2 min, increased at 3° C/min to 250° C, and held isothermally for 5 min. Mass spectra were recorded, and compounds were identified by matching mass fragmentation patterns with the National Institute of Standards and Technology (NIST) spectral library.

2.6 Green Synthesis and Physicochemical Characterization of AgNPs

For nanosilver synthesis, 1.0 mL of the methanolic needle extract was mixed with 100 mL of aqueous silver nitrate solution (1 mM) and incubated at room temperature in the dark. The bio-reduction of silver ions ($Ag^+ \rightarrow Ag^0$) was tracked by visual color change and confirmed via:

- UV-Visible Spectroscopy: Scanned across 200–800 nm to detect the characteristic Surface Plasmon Resonance (SPR) peak.
- FTIR Spectroscopy: Air-dried samples were analyzed over 4000–400 cm^{-1} at a resolution of 4 cm^{-1} to assess surface-capping biomolecules.
- XRD Crystallinity: Measured on a Bruker D8 Advance diffractometer using Cu K α radiation ($\lambda = 1.5406$ Å) operated at 40 kV and 30 mA across $2\theta = 5^\circ$ – 40° .

2.7 Insecticidal Contact Bioassays

The insecticidal toxicity of both crude needle extract and synthesized AgNPs was tested against laboratory-reared adult *Tribolium castaneum* using filter paper contact bioassays. Whatman No. 1 filter discs placed inside Petri dishes were uniformly coated with four concentrations (50, 100, 150, and 200 mg). Solvent-treated discs served as negative controls. Ten unsexed, healthy adult beetles were introduced into each dish, and all assays were conducted in triplicate ($N = 30$ per treatment). Mortality was recorded at 24, 42, and 72 h post-exposure. Beetles showing no movement upon gentle tactile stimulation with a fine camel-hair brush were scored as dead. Percent mortality was calculated using standard methods:

$$\text{Mortality (\%)} = \left(\frac{\text{Number of dead insects}}{\text{Total number of exposed insects}} \right) \times 100$$

3. Results and Discussion

3.1 Phytochemical Screening and Secondary Metabolite Abundance

Qualitative testing of the methanolic needle extract of *Pinus roxburghii* revealed an abundance of bioactive constituents, including polyphenols, flavonoids, tannins, alkaloids, reducing sugars, and resins. In contrast, coumarins, quinones, and mucilages tested negative.



Fig 1: Qualitative phytochemical screening assay of *Pinus roxburghii* needle extract

Table 1: Qualitative Phytochemical Screening of Methanolic *Pinus roxburghii* Needle Extract

Sr. no.	Name of test	Test applied / Reagent Used	Inference / Result
1.	Carbohydrates	Molisch's test	++
		Barfoed test	-
2.	Alkaloids test	Wagner's test	+
		Mayer's test	+
		Dragendroff's test	-
3.	Cardic glycosides	Keller Killani test	+
4.	Phenols	Ferric chloride test	++
5.	Flavonoids	Lead acetate test	+
6.	Tannins	Ferric chloride test	+
7.	Triterpinoids	Salkowski test	++

8.	Resin	Turbidity test	++
9.	Coumarin	Sodium hydroxide test	-
10.	Quinone	Sulphuric acid test	-
11.	Gum and mucilages	Alcohol test	-
12.	Saponin	Foam test	+
13.	Protein	Ninhydrin test	+

3.2 Phenolic content, flavonoid content and antioxidant activity

The gallic-acid calibration curve showed strong linearity ($R^2 = 0.9907$), and the extract was calculated to contain 38.3 mg GAE/g dry weight of total phenolics. The rutin calibration curve was also highly linear ($R^2 = 0.999$); the thesis reports a total flavonoid value of 2.5 mg/g dry weight. Because the assay used rutin, this value is presented here without converting it to another equivalent system. In the DPPH assay, the extract showed a concentration-dependent increase in radical scavenging activity: 32.87% at 10 $\mu\text{g/mL}$, 37.77% at 20 $\mu\text{g/mL}$, 41.55% at 40 $\mu\text{g/mL}$, 51.75% at 60 $\mu\text{g/mL}$, 60.12% at 80 $\mu\text{g/mL}$, 72.29% at 100 $\mu\text{g/mL}$ and 76.10% at 120 $\mu\text{g/mL}$. At 120 $\mu\text{g/mL}$, the corresponding value for ascorbic acid was 93.75%. The reported IC_{50} of the extract was 48.89 $\mu\text{g/mL}$.

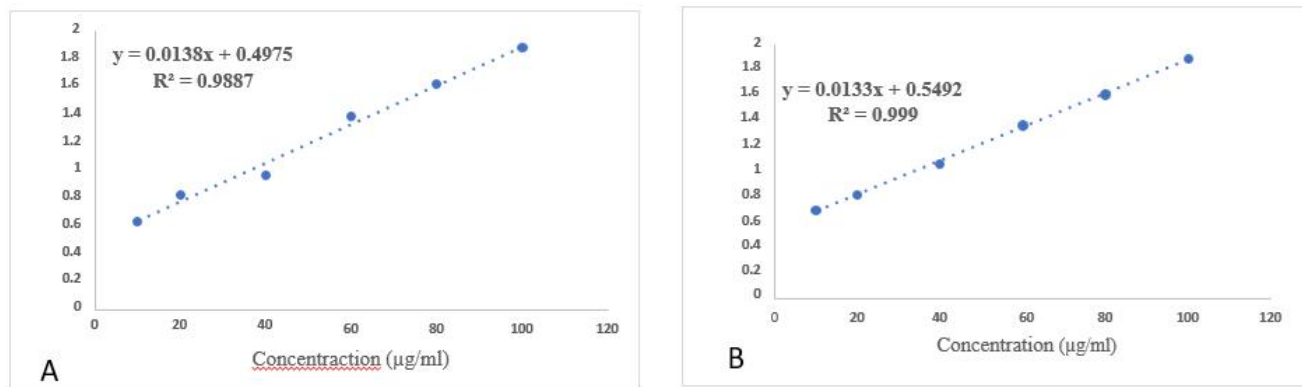


Figure 2: Quantitative phytochemical profile of *Pinus roxburghii* extract: (A) Gallic acid standard curve and (B) Rutin standard calibration plot

The DPPH radical scavenging assay demonstrated consistent, concentration-dependent antioxidant capacity. Free-radical quenching climbed from 32.87% at 10 $\mu\text{g/mL}$ to 76.10% at 120 $\mu\text{g/mL}$, yielding an IC_{50} value of 48.89 $\mu\text{g/mL}$. Under identical conditions, standard ascorbic acid achieved 93.75% inhibition at 120 $\mu\text{g/mL}$. This antioxidant activity indicates an abundance of hydrogen- and electron-donating moieties capable of driving the reduction of ionic silver (Ag^+) to zero-valent metallic nanoparticles (Ag^0).

Table 2: Concentration-Dependent DPPH Free Radical Scavenging Activity of *P. roxburghii* Extract and Ascorbic Acid

Conc. (µg/mL)	Ascorbic acid (%)	Plant Extract Scavenging (%)
10	52.51	32.87
20	58.82	37.77
40	68.83	41.55
60	72.37	51.75
80	78.38	60.12
100	85.99	72.29
120	93.75	76.1
Calculated IC50		48.89 µg/mL

3.3 GC-MS Volatile Metabolite Profiling

GC-MS analysis resolved 27 distinct peaks from the methanolic needle extract, spanning saccharide derivatives, long-chain fatty acids, esters, and terpenoids. Major identified compounds included melezitose (10.2% , RT 19.61 min), glucopyranose derivatives (10.2% , RT 19.61 min), trans-13-octadecenoic acid methyl ester (4.95%, RT 25.72 min), octasiloxane (4.99%, RT 24.04 min), and ledol (0.36% , RT 18.19 min). These polyhydroxy and carboxylic compounds are essential for both metal reduction and colloidal capping to prevent particle aggregation.

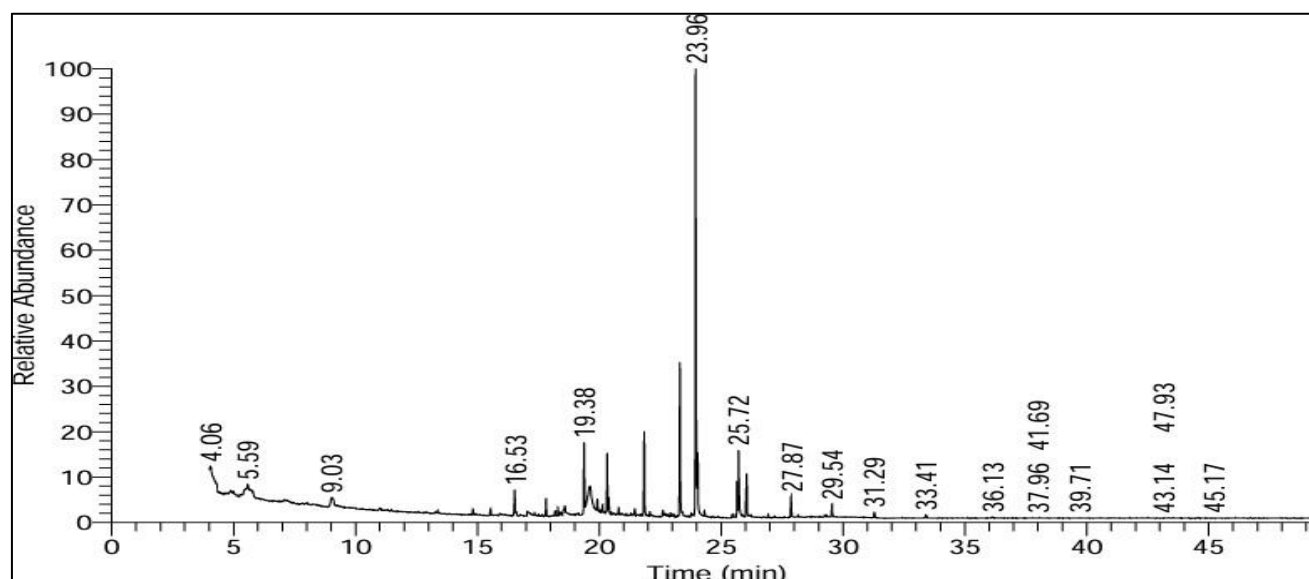


Fig2: Total Ion Chromatogram from GC-MS analysis of *Pinus roxburghii* methanolic needle extract

Table 3: Predominant Phytochemical Compounds Identified in *P. roxburghii* needle extract via GC-MS

S. no.	RT(min)	Compound Name	Area %	Molecular Formula	Molecular Weight (g/mol)
1	4.07	2,2-Dimethoxybutane	0.81	C ₆ H ₁₄ O ₂	118
2	4.07	2-[2-[2-(2-Methoxyethoxy)ethoxy]ethoxy]ethyl acetate	0.81	C ₁₁ H ₂₂ O ₆	250
3	4.07	Ethanol, 2-(1-methylethoxy)-,acetate	0.81	C ₇ H ₁₄ O ₃	146
4	4.07	Ethanol, 1-[2-[2-(1-methylethoxy)ethoxy]ethoxy]-	0.81	C ₉ H ₂₀ O ₄	192
5	4.07	Monoethylmalonate monoamide	0.81	C ₅ H ₉ NO ₃	131
6	4.31	3-Methoxy-hexane-1,6-diol	1.08	C ₇ H ₁₆ O ₃	148
7	4.31	Tetradecanoic acid, 2-hydroxy-	1.08	C ₁₄ H ₂₈ O ₃	244
8	4.31	Decanoic acid, 2-hydroxy-	1.08	C ₁₀ H ₂₀ O ₃	188
9	4.31	3-Hydroxydecanoic acid	1.08	C ₁₀ H ₂₀ O ₃	188
10	4.31	Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester	1.08	C ₁₂ H ₂₄ O ₃	216
11	4.91	4,6-Decadiyn-3-ol, 3-isopropyl-9-(methoxyethoxymethoxy)-	0.81	C ₁₇ H ₂₈ O ₄	296
12	5.59	cis-Bicyclo[4.2.0]octa-3,7-diene (Xylene isomers)	3.64	C ₈ H ₁₀	106
13	9.03	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	2.31	C ₂₆ H ₅₄	366
14	15.54	Caryophyllene /Isocaryophyllene	0.44	C ₁₅ H ₂₄	204

3.4 Spectroscopic and Structural Confirmation of AgNPs

Mixing the transparent needle extract with aqueous silver nitrate solution induced a distinct transition to a deep brownish-yellow shade. UV-Vis spectrophotometry displayed a prominent Surface Plasmon Resonance (SPR) absorption band centered at 450 nm, consistent with the localized surface resonance of spherical nanosilver.

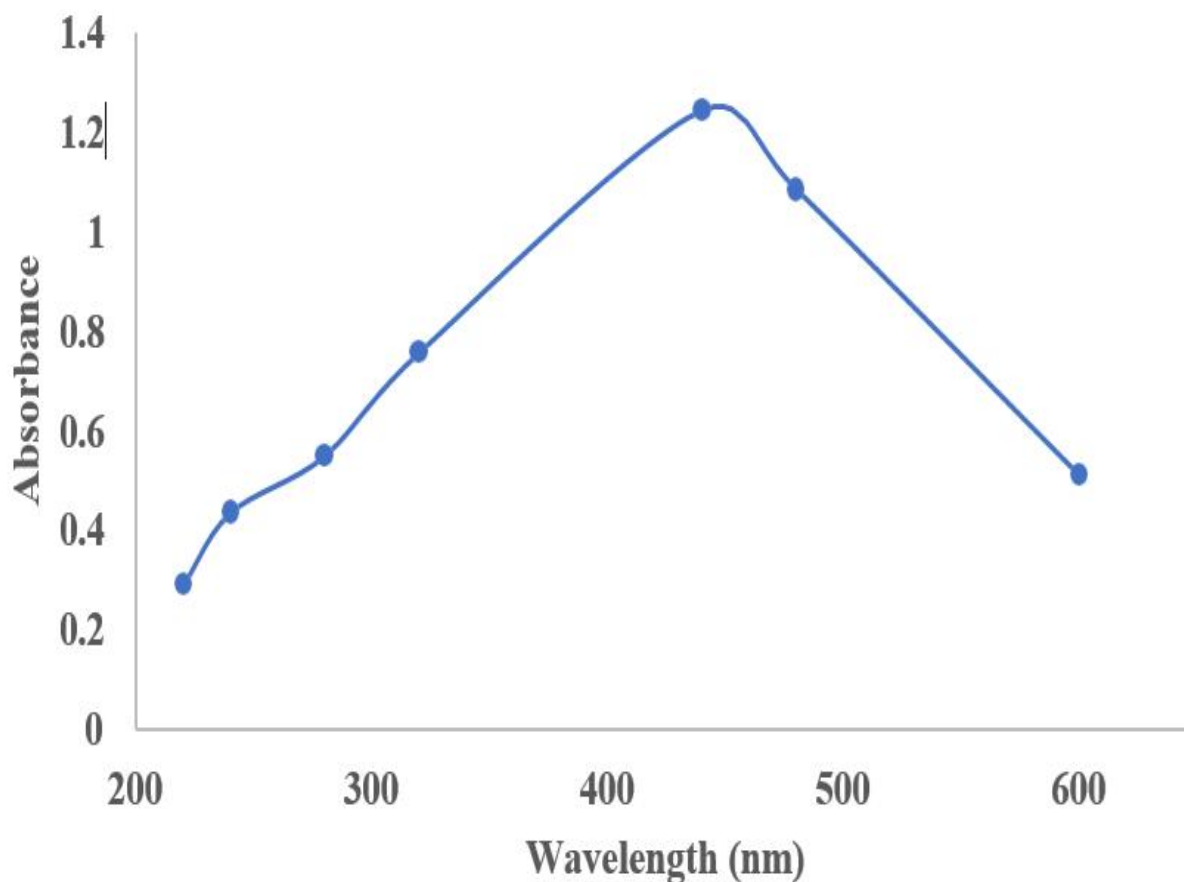


Fig 3: UV-Visible absorption spectrum of biosynthesized silver nanoparticles

FTIR analysis confirmed the involvement of functional organic groups in stabilizing the nanostructures. The broad absorption band at 3324 cm^{-1} corresponds to hydrogen-bonded OH stretches in polyphenols and NH stretches in amines. Aliphatic C-H symmetric and asymmetric stretching modes appeared at 2929 cm^{-1} and 2857 cm^{-1} . Distinct peaks near $1634\text{--}1643\text{ cm}^{-1}$ represent carbonyl (C=O) stretching and aromatic skeletal vibrations, while signals at $1045\text{--}1085\text{ cm}^{-1}$ indicate C-O-C ether and alcoholic vibrations. These spectral shifts show that oxidized phytoconstituents remain electrostatically bound to the nanosilver core, providing steric capping that prevents particle coalescing.

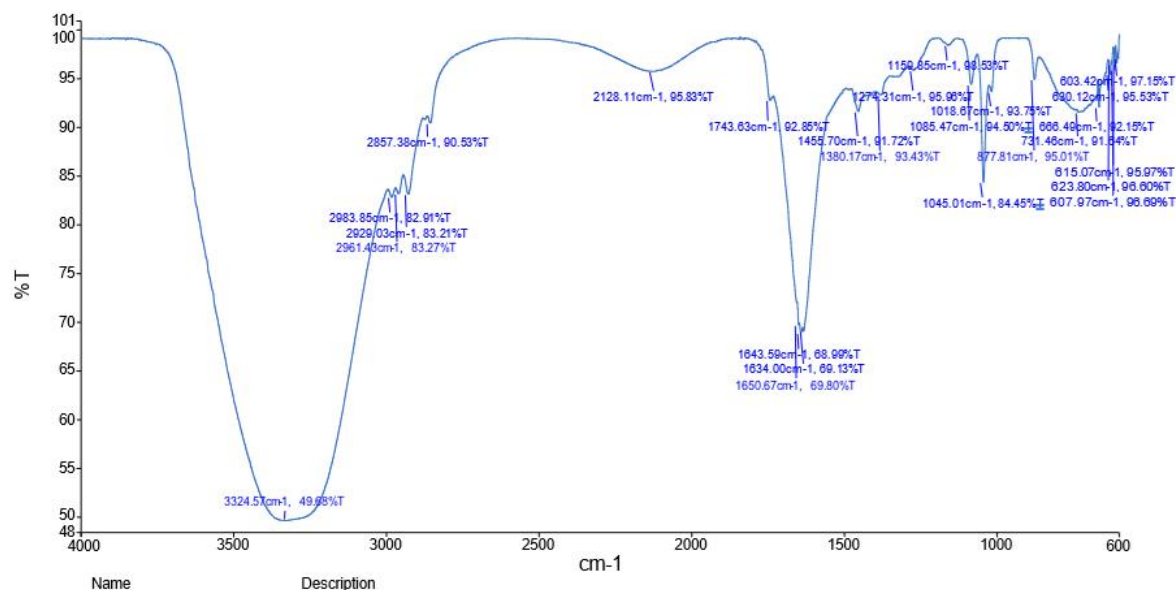


Fig 4: FTIR transmission spectrum of silver nanoparticles synthesized via *P.roxburghii* needle extract

SEM images showed predominantly spherical particles with some clustering and a slightly rough surface. The thesis describes the particles as relatively uniformly distributed but does not provide a robust particle-size distribution from image analysis; therefore, no numerical size range is assigned in this article. XRD displayed sharp reflections around 2θ values of approximately 38° , 44° , 64° and 77° , corresponding to the (111), (200), (220) and (311) planes of face-centered cubic silver. Together, the UV–visible, FTIR, SEM and XRD results provide complementary evidence for formation of crystalline Ag-NPs associated with plant-derived surface chemistry.

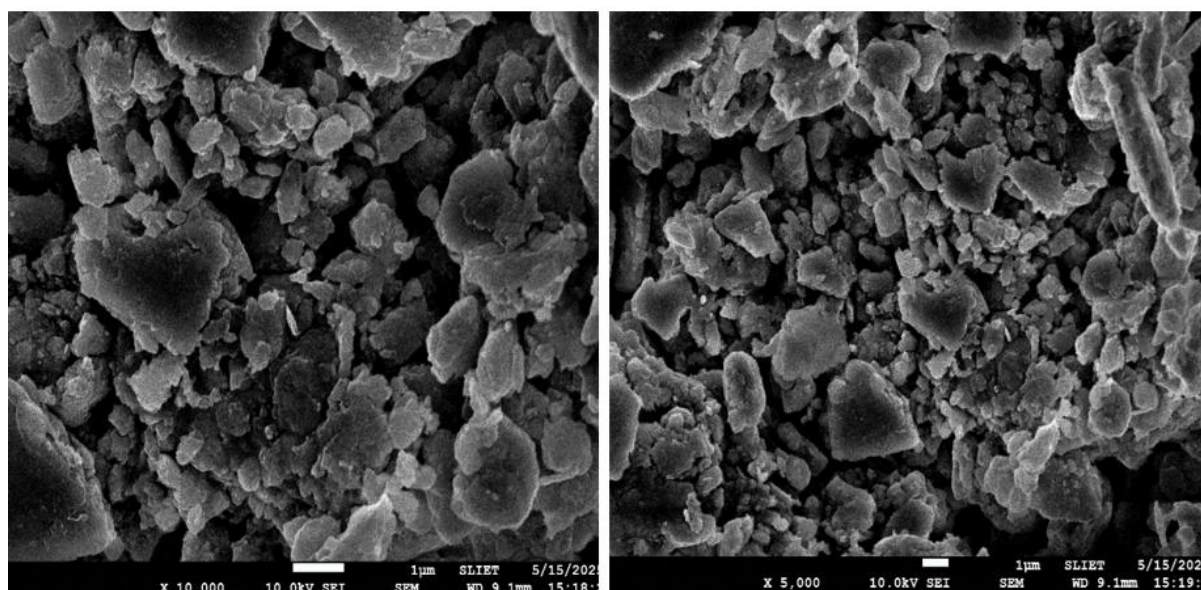


Fig 5: SEM micrographs of the synthesized Ag-NPs at two magnifications

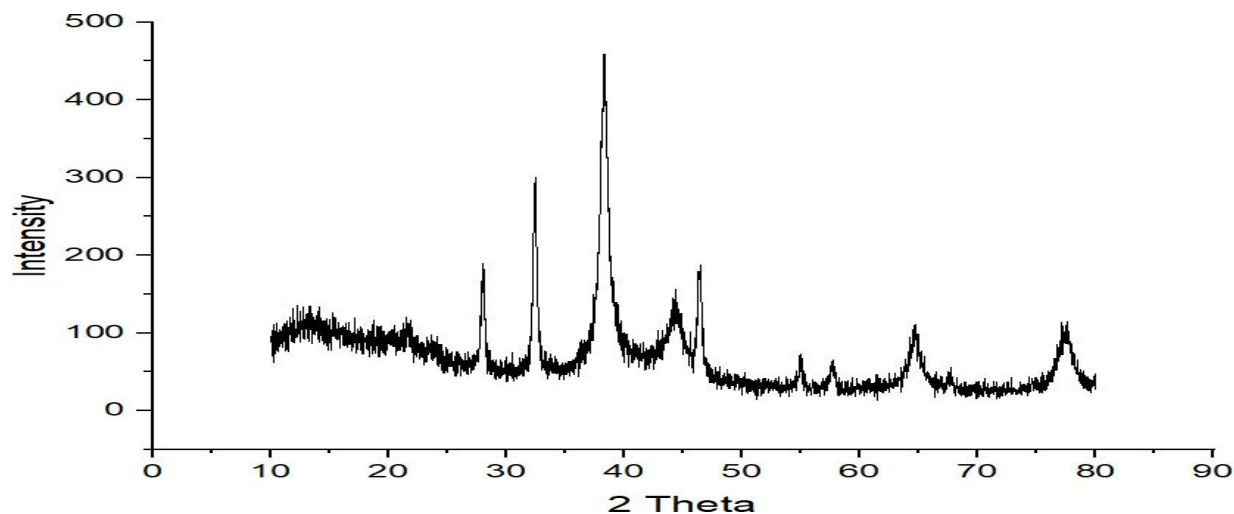


Fig 6: X-ray diffraction pattern of green-synthesized silver nanoparticles

3.5 Insecticidal activity

The insect bioassay showed clear concentration- and exposure-time-related increases in mortality for both treatments. With the crude extract, mortality at 24 h increased from 0% at 50 mg to 30% at 200 mg; at 42 h it ranged from 20% to 40%, and at 72 h from 30% to 50%. The Ag-NP treatment produced 10–30% mortality at 24 h, 20–60% at 42 h and 30–70% at 72 h across the same concentration range. At 200 mg after 72 h, the Ag-NP treatment produced 70% mortality compared with 50% for the crude extract. The average mortality values across the three observation times at 50, 100, 150 and 200 mg were 20%, 33%, 40% and 53% for Ag-NPs, respectively, while the corresponding values for the crude extract were 16.67%, 26.67%, 36.67% and 40%.



Fig 7: Figure Contact toxicity bioassay against adult *Tribolium castaneum*: (A) Stock insect cultures; (B) Petri dish setup before incubation; (C) Petri dish response after incubation illustrating pest

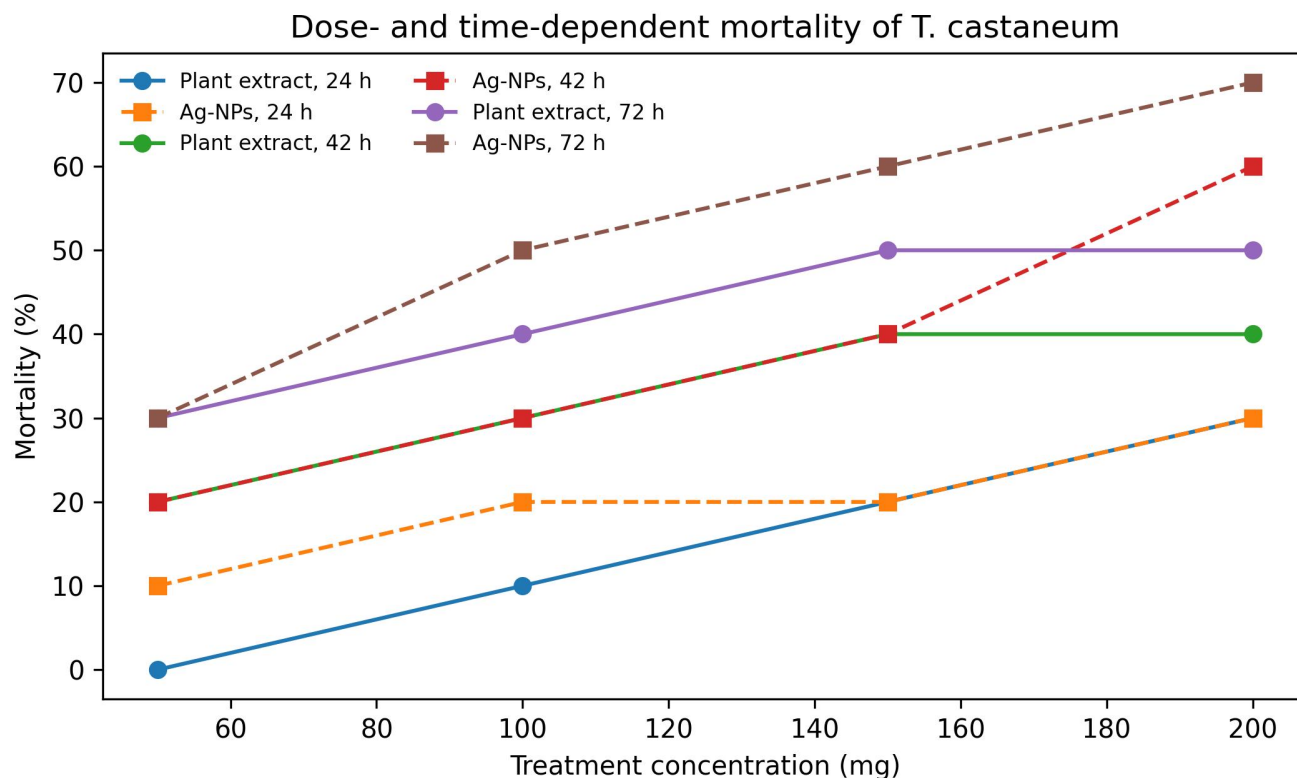


Fig 8: Mortality of *T. castaneum* in response to crude *P. roxburghii* extract and Ag-NPs across concentration and exposure time, replotted from thesis data

Table 4: Contact toxicity of crude *Pinus roxburghii* extract and synthesized AgNPs against adult *Tribolium castaneum*

Extract	Sample Weight (mg)	Exposure/Duration n hours	Number of Deaths	Mortality Rate (%)	Mean duration Mortality (%)
Crude Extract	50	24	0	0%	16.67
		42	2	20%	
		72	3	30%	
	100	24	1	10%	26.67
		42	3	30%	
		72	4	40%	
	150	24	2	20%	36.67
		42	4	40%	
		72	5	50%	
	200	24	3	30%	40.00
		42	4	40%	
		72	5	50%	

Synthesized AgNPs	50	24	1	50	20.00
		42	2		
		72	3		
	100	24	2	20	33.33
		42	3	30	
		72	5	50	
	150	24	2	20	40.00
		42	4	40	
		72	6	60	
	200	24	3	30	53.33
		42	6	60	
		72	7	70	

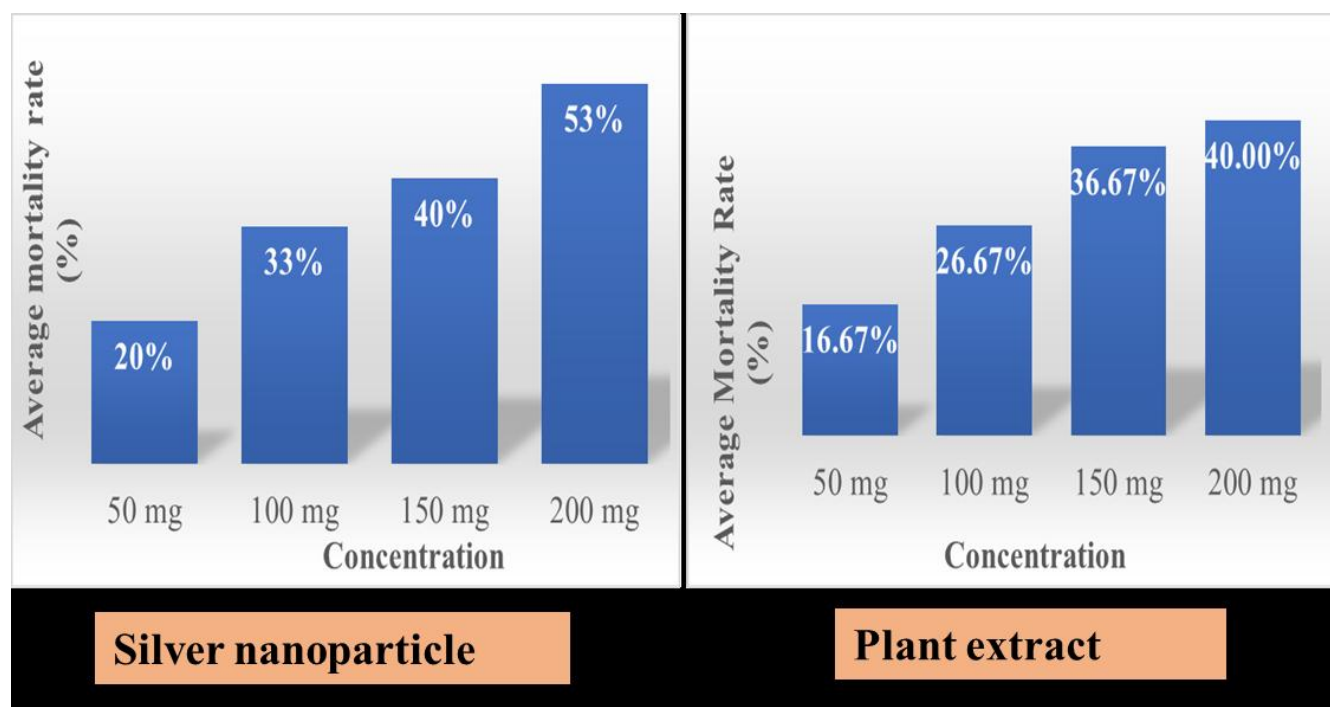


Fig 9: Comparison of average mortality rates for the crude extract and Ag-NP formulation

4. Discussion

The phytochemical evaluation of *Pinus roxburghii* needle extract confirmed rich concentrations of phenolics, flavonoids, tannins, and terpenoids, corroborating Kanchan et al. (2020) and Mehmood et al. (2024), who established the prominence of these secondary metabolites in Himalayan conifer defenses. The presence of essential flavonols like quercetin and kaempferol aligns with findings by Naeem et al. (2010), while volatile terpenoids (such as caryophyllene and ledol) match the conifer essential oil profiles documented by Chaudhary et al. (2014). Quantitatively, the extract yielded a total phenolic content of 38.3 mg GAE/g (3.83 µg/mL) and a total flavonoid content of 0.025 mg GAE/g DW (0.25 µg/mL), alongside strong radical-scavenging activity ($IC_{50} = 48.89 \mu\text{g/mL}$). Although Thapa et al. (2018) reported higher phenolic and flavonoid yields from bark than needles, the current needle extract provided ample electron-donating power, supporting observations by Sharma et al. (2016) regarding pine-derived antioxidant capacity for bio-reduction.

Optical and structural characterization verified that the synthesized silver nanoparticles (AgNPs) exhibited an intense Surface Plasmon Resonance (SPR) peak at 450 nm, consistent with the typical 420–450 nm absorption range reported for biogenic nanosilver by Sridhara et al. (2013) and Chowdhary et al. (2014). FTIR spectral bands at 3324 cm^{-1} (–OH/–NH), 2929 cm^{-1} (C–H), and 1634 cm^{-1} (C=O) confirmed that plant phenolics and proteins acted as natural capping agents, closely matching waste-mediated nanoparticle spectra reported by Kadam et al. (2020). XRD diffractograms displayed sharp Bragg reflections at $2\theta \approx 38^\circ, 44^\circ, 64^\circ$, and 77° , indexing to the face-centered cubic (111), (200), (220), and (311) planes of metallic silver; these values align with standard JCPDS file no. 04-0783 and confirm crystalline purity without secondary phases, as similarly observed by Oda et al. (2019), Das et al. (2020), Aygün et al. (2021), and Sarwer et al. (2022). In contact bioassays against *Tribolium castaneum*, the synthesized AgNPs achieved 70% mortality at 200 mg after 72 h compared to only 50% for the crude extract, confirming that nanoscale bioformulations enhance cuticular disruption and significantly improve pest-control efficacy over crude plant extracts.

5. Conclusion

This study demonstrates a single-step, green synthesis of stable silver nanoparticles mediated by needle extracts of the conifer *Pinus roxburghii*. Rich reserves of phenolics, flavonoids, and reducing sugars serve as natural reducing agents while providing organic capping that prevents particle agglomeration. Physicochemical characterization confirmed successful nanostructure formation with an SPR absorption peak at 450 nm. In contact toxicity assays against the primary grain pest *Tribolium castaneum*, the synthesized AgNPs achieved up to 70% mortality, consistently outperforming the raw botanical extract. These findings show that *P. roxburghii*-mediated AgNPs provide a viable, eco-friendly alternative to synthetic pesticides for stored-product protection.

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