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**EFFECTS OF IRON AND COPPER OXIDE NANOPARTICLES ON CALLUS
INDUCTION AND *IN VITRO* REGENERATION OF POTATO (*SOLANUM
TUBEROSUM* L., CV. TELMAN)**

*Potato is widely used as a model species for plant tissue culture, micropropagation, and genetic improvement studies due to its responsiveness to in vitro manipulation and its economic relevance. Iron (Fe) and copper (Cu) are essential micronutrients involved in fundamental physiological and biochemical processes in plants. The application of metal oxide nanoparticles has recently emerged as a promising strategy to improve nutrient bioavailability and morphogenic responses in plant tissue culture. This study investigated the effects of iron oxide (Fe₃O₄) and copper oxide (CuO) nanoparticles on callus induction and in vitro regeneration of potato (*Solanum tuberosum* L., cv. Telman). Explants cultured on Murashige and Skoog (MS) medium supplemented with optimal concentrations of Fe and Cu nanoparticles exhibited significantly enhanced callus formation, shoot regeneration, and rooting compared to the control. In contrast, higher nanoparticle concentrations negatively affected morphogenesis, likely due to nanoparticle-induced oxidative stress. These results demonstrate the potential of iron and copper oxide nanoparticles as effective modulators of potato tissue culture when applied at appropriate concentrations. Results suggest that nanoparticles not only influence early morphogenic responses but also have lasting effects on plantlet*

establishment, which is critical for successful transfer from in vitro to ex vitro conditions. Controlled application of Fe₃O₄ and CuO nanoparticles therefore represents a promising strategy for improving tissue culture efficiency in potato. Overall, the data indicate that the effects of nanoparticles on potato tissue culture are dose-dependent, with low to moderate concentrations enhancing callus induction, shoot regeneration, and root development, while higher concentrations impose inhibitory effects due to oxidative stress. Plantlets regenerated under optimal nanoparticle treatments exhibited enhanced rooting performance and higher survival rates during acclimatization. However, elevated concentrations of Cu-NPs resulted in a pronounced decrease in regeneration efficiency. This decline suggests potential copper-induced toxicity, likely associated with the disruption of cellular redox homeostasis and excessive ROS accumulation.

***Keywords:** Solanum tuberosum, iron oxide nanoparticles, copper oxide nanoparticles, callus induction, in vitro regeneration, nanobiotechnology*

Introduction. Potato (*Solanum tuberosum* L.) is a globally significant food crop, ranking among the most widely cultivated and consumed vegetables due to its high nutritional value, adaptability, and economic importance. In Azerbaijan, potato plays a central role in both the national diet and agricultural economy, with one of the highest per capita consumption rates in Central Asia. The tuber is a key ingredient in traditional dishes, including soups, stews, and various local recipes, highlighting its cultural as well as nutritional relevance. Despite its importance, potato production in Azerbaijan has historically faced challenges, including limited access to high-quality, disease-free seed material and vulnerability to biotic and abiotic stresses, such as viral infections, soil-borne pathogens, and drought [3, 4, 10].

Until recently, most certified seed potatoes were imported, restricting local production and increasing dependence on external sources. In response, the joint initiatives of the Food and Agriculture Organization (FAO) and Azerbaijan have strengthened national capacity in potato production through enhanced laboratory facilities for virus diagnostics, in vitro tissue culture, and the establishment of farmer-based seed multiplication schemes [1, 2]. In this context, certified disease-free tubers of potato (cv. Telman) were procured from the Scientific Research Institute of Vegetable Growing, a public legal entity, ensuring the highest phytosanitary standards for subsequent experimental studies. The availability of high-quality, pathogen-free planting material is critical not only for field cultivation but also for controlled experimental research, as it eliminates variability caused by latent infections and ensures reproducibility in tissue culture and genetic studies.

Potato is widely used as a model species for plant tissue culture, micropropagation, and genetic improvement studies due to its responsiveness to *in vitro* manipulation and its economic relevance. Efficient callus induction and plant regeneration are essential for micropropagation, somaclonal variation studies, and genetic transformation. However, regeneration capacity is highly genotype-dependent, and several cultivars, including cv. Telman, exhibit limited morphogenic responses under conventional in vitro conditions. Optimization of tissue culture protocols is therefore necessary to improve regeneration efficiency, enhance propagation rates, and facilitate advanced genetic and biotechnological research [8, 9].

Micronutrients such as iron (Fe) and copper (Cu) are essential for plant growth and development. They act as cofactors in key enzymatic reactions involved in photosynthesis, respiration, electron transport, and antioxidant defense mechanisms. In conventional tissue culture systems, supplementation of these elements in media does not always guarantee efficient cellular uptake, limiting their physiological effectiveness. Nanoparticle-based delivery systems have emerged as a promising strategy due to their high surface area, enhanced chemical reactivity, and improved bioavailability, offering a novel approach to

micronutrient supplementation *in vitro* [5, 6, 7]. Despite increasing research on nanoparticle-mediated nutrient delivery, data regarding their effects on potato callus induction and morphogenesis remain scarce, and systematic studies are required to elucidate their potential benefits.

The present study aims to evaluate the influence of Fe₃O₄ and CuO nanoparticles on callus formation and *in vitro* regeneration of potato cv. Telman. By using certified disease-free tubers sourced from a recognized public legal entity, this study ensures the reliability and reproducibility of experimental results while addressing both biotechnological and nutritional aspects of plant tissue culture. The findings are expected to provide insights into optimized micropropagation protocols and the application of nanotechnology in enhancing potato growth and morphogenesis, contributing to both scientific knowledge and practical improvements in crop production.

Materials and Methods.

Plant Material and Explant Preparation

Certified disease-free tubers of potato (*Solanum tuberosum* L., cv. Telman) were procured from the Scientific Research Institute of Vegetable Growing, a public legal entity, ensuring the highest phytosanitary standards for subsequent experimental use. Tubers were sprouted under controlled conditions (22–25°C, darkness). Young shoots were excised, and leaf and internode segments (0.5–1.0 cm) were used as explants.

Surface Sterilization

Explants were washed under running tap water and surface-sterilized with 70% (v/v) ethanol for 30 s, followed by immersion in 2.5% (v/v) sodium hypochlorite solution containing a few drops of Tween-20 for 10–12 min. Explants were rinsed three to four times with sterile distilled water under aseptic conditions.

Culture Media and Growth Conditions

Murashige and Skoog (MS) basal medium supplemented with 3% (w/v) sucrose and solidified with 0.7% (w/v) agar was used. The pH was adjusted to 5.8 before autoclaving at 121 °C for 20 min. Cultures were maintained at 24 ± 2 °C under a 16 h photoperiod with a light intensity of 40–50 μmol m⁻² s⁻¹.

Preparation and Application of Nanoparticles

Iron oxide (Fe₃O₄) and copper oxide (CuO) nanoparticles with an average particle size of 20–40 nm were used. Stock suspensions were prepared in sterile distilled water and sonicated for 30 min to minimize aggregation. Nanoparticles were incorporated into the culture medium at the following concentrations:

Fe-NPs: 0, 25, 50, and 100 mg L⁻¹

Cu-NPs: 0, 5, 10, and 25 mg L⁻¹

Media without nanoparticles served as controls.

Callus Induction

Explants were cultured on MS medium supplemented with 2.0 mg L⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg L⁻¹ 6-benzylaminopurine (BAP), in combination with the respective nanoparticle treatments. Cultures were maintained in darkness for the first 10 days. Callus induction frequency (%) and callus fresh weight (g) were recorded after four weeks.

Regeneration and Rooting

Actively growing calli were transferred to regeneration medium consisting of MS supplemented with 2.0 mg L⁻¹ BAP and 0.2 mg L⁻¹ α-naphthaleneacetic acid (NAA). Regeneration frequency, number of shoots per callus, and shoot length were recorded after six weeks. Rooting was induced on half-strength MS medium supplemented with 0.5 mg L⁻¹ indole-3-butyric acid (IBA). Rooted plantlets were acclimatized under greenhouse conditions.

Statistical Analysis

Experiments were arranged in a completely randomized design with three independent replicates per treatment. Data were analyzed using one-way analysis of variance (ANOVA), and mean comparisons were performed at $p \leq 0.05$.

Results and Discussion.

Effect of Nanoparticles on Callus Induction

Both iron (Fe_3O_4) and copper oxide (CuO) nanoparticles had a significant impact on callus induction in potato cv. Telman. The highest frequency of callus formation and greatest fresh weight were recorded at 50 mg l^{-1} Fe-NPs and 10 mg l^{-1} Cu-NPs, respectively. These results significantly exceeded those of the control treatment, suggesting that optimal concentrations of nanoparticles can stimulate cellular proliferation and tissue differentiation *in vitro*.

The stimulatory effects observed at these lower concentrations are likely associated with improved micronutrient availability and activation of iron- and copper-dependent enzymatic pathways, which are crucial for DNA synthesis, respiration, and overall cellular metabolism. In contrast, callus formation was markedly reduced at higher nanoparticle concentrations, presumably due to the overproduction of reactive oxygen species (ROS) and resulting oxidative stress, which can damage cellular structures and inhibit morphogenesis [2, 7]. These observations are consistent with previous reports indicating that while micronutrients are essential for growth, excessive accumulation can exert cytotoxic effects.

Effect on Shoot Regeneration. Shoot regeneration of potato cv. Telman was significantly enhanced under optimal nanoparticle treatments. Fe-NPs at 50 mg l^{-1} promoted both shoot number and shoot length, likely through improved chlorophyll biosynthesis and enhanced photosynthetic efficiency. Similarly, low concentrations of Cu-NPs stimulated shoot regeneration, possibly by facilitating electron transport processes and activating antioxidant enzymes, which mitigate oxidative stress and support cellular differentiation.

However, elevated concentrations of Cu-NPs resulted in a pronounced decrease in regeneration efficiency. This decline suggests potential copper-induced toxicity, likely associated with the disruption of cellular redox homeostasis and excessive ROS accumulation. These findings align with prior studies emphasizing the narrow optimal range for copper nutrition in plant tissue culture and the delicate balance required to support morphogenesis without inducing cytotoxic effects [3].

Rooting and Acclimatization. Plantlets regenerated under optimal nanoparticle treatments exhibited enhanced rooting performance and higher survival rates during acclimatization. Improved root development is likely linked to increased metabolic activity and a balanced oxidative state in nanoparticle-treated tissues. These results suggest that nanoparticles not only influence early morphogenic responses but also have lasting effects on plantlet establishment, which is critical for successful transfer from *in vitro* to *ex vitro* conditions.

Overall, the data indicate that the effects of nanoparticles on potato tissue culture are dose-dependent, with low to moderate concentrations enhancing callus induction, shoot regeneration, and root development, while higher concentrations impose inhibitory effects due to oxidative stress. These results underscore the potential of Fe_3O_4 and CuO nanoparticles as tools to optimize *in vitro* propagation protocols for potato and possibly other economically important crops.

Conclusion. This study demonstrates that iron oxide (Fe_3O_4) and copper oxide (CuO) nanoparticles significantly influence callus induction, shoot regeneration, and rooting of potato (*Solanum tuberosum* L., cv. Telman) *in vitro*. At optimized concentrations, both nanoparticles enhance morphogenic responses, likely by improving micronutrient availability, supporting enzymatic activity, and maintaining oxidative balance. In contrast, excessive concentrations lead to reduced callus formation and regeneration, likely due to oxidative stress and nanoparticle-induced cytotoxicity.

Controlled application of Fe₃O₄ and CuO nanoparticles therefore represents a promising strategy for improving tissue culture efficiency in potato, offering potential benefits for micropropagation, genetic studies, and biotechnological applications. Further research is warranted to elucidate the underlying molecular mechanisms and to optimize nanoparticle use across different potato genotypes.

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ВПЛИВ НАНОЧАСТИНОК ОКСИДІВ ЗАЛІЗА ТА МІДІ НА ІНДУКЦІЮ КАЛЮСУ ТА РЕГЕНЕРАЦІЮ КАРТОПЛІ (*SOLANUM TUBEROSUM* L., СОПТ ТЕЛЬМАН) IN VITRO

Картопля широко використовується як модельний вид для досліджень культури рослинних тканин, мікророзмноження та генетичного вдосконалення завдяки своїй чутливості до маніпуляцій in vitro та економічній значущості. Залізо (Fe) та мідь (Cu) є важливими мікроелементами, що беруть участь у фундаментальних фізіологічних та біохімічних процесах у рослинах. Застосування наночастинок оксиду металу нещодавно виявилось перспективною стратегією для покращення біодоступності поживних речовин та морфогенних реакцій у культурі рослинних тканин. У цьому дослідженні вивчали вплив наночастинок оксиду заліза (Fe₃O₄) та оксиду міді (CuO) на індукцію калюсу та регенерацію in vitro картоплі

(*Solanum tuberosum* L., сорт Тельман). Експлантати, культивовані на середовищі Мурашіге та Скуга (MS), доповненому оптимальними концентраціями наночастинок Fe та Si, демонстрували значно покращене формування калюсу, регенерацію пагонів та вкорінення порівняно з контролем. Навпаки, вищі концентрації наночастинок негативно вплинули на морфогенез, ймовірно, через окислювальний стрес, індукований наночастинками. Ці результати демонструють потенціал наночастинок оксиду заліза та міді як ефективних модуляторів культури тканин картоплі при застосуванні у відповідних концентраціях. Результати свідчать про те, що наночастинки не лише впливають на ранні морфогенетичні реакції, але й мають тривалий вплив на приживлення рослин, що є критично важливим для успішного перенесення з умов *in vitro* до умов *ex vitro*.

Таким чином, контрольоване застосування наночастинок Fe₃O₄ та SiO є перспективною стратегією для підвищення ефективності культури тканин картоплі. Загалом, дані свідчать про те, що вплив наночастинок на культуру тканин картоплі залежить від дози, причому низькі та помірні концентрації посилюють індукцію калюсу, регенерацію пагонів та розвиток коренів, тоді як вищі концентрації чинять інгібуючий ефект через окислювальний стрес. Рослини, регенеровані за оптимальної обробки наночастинками, демонстрували покращену продуктивність вкорінення та вищі показники виживання під час акліматизації. Однак, підвищені концентрації наночастинок Si призвели до значного зниження ефективності регенерації. Це зниження свідчить про потенційну токсичність, індуковану міддю, ймовірно, пов'язану з порушенням клітинного окисно-відновного гомеостазу та надмірним накопиченням активних форм кисню (ROS).

Ключові слова: *Solanum tuberosum* L., наночастинки оксиду заліза, наночастинки оксиду міді, індукція калюсу, регенерація *in vitro*, нанобіотехнологія

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