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# Morpho-Physiological Impact of Nanoparticles on Tissue-Cultured and Genetically Altered Marigold Varieties

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## Abstract:

The study reviews the outcomes of nanoparticles on the characteristics and traits of tissue-cultured and genetically modified marigold (Tagetes spp.) samples. Nanoparticles can have an effect on plant growth, the way plants grow, and how plants respond to stress. Here, green-synthesized nanoparticles are used to study how they affect the germination rate, plant□s height, the area of its leaves, its chlorophyll concentration and flowering characteristics. It is evident from the findings that genetically modified marigold plants experienced greater improvements in growth and physiology, meaning nanoparticles could improve the yields of ornamental plants using biotechnology.

## Keywords:

Nanoparticles, Tissue Culture, Genetic Alteration, Marigold, Morphological Traits, Physiological Traits, Plant Growth, Chlorophyll, Germination.

## Introduction:

Many people cultivate marigold (Tagetes spp.) because it has attractive flowers and plays a helpful role in the garden and in medicine. For a long time, marigold has been used in herbal remedies for its power against inflammation, germs, and damage to cells. The ability to drive off some insects and nematodes gives garlic an important place in the management of pests both in farming and gardens.

Modern biotechnology is being used more often in agriculture because people are looking for plants that are strong and healthy. Tissue culture helps grow many healthy, uniform plants, and genetic modification makes it possible to introduce wanted characteristics such as greater resistance to stress, improved colouring, and a rise in compounds that aid the body.

Lately, nanotechnology has started being used in agriculture and has led to some new approaches to solve old challenges in plant science. Since nanoparticles (NPs) have a very small size and a high ratio of surface to volume, they can influence plant growth, change their body processes, and effect the way plants respond to stress. Applying nanoparticles can enhance germination of seeds, raise the absorption of beneficial substances, stimulate photosynthesis, and make plants less vulnerable to attacks.

This study looks into the effects that nanoparticles have on plants that are either laboratory-cultured or genetically changed. We intend to study the effects of nanoparticles on plants to decide if they can successfully be included in modern plant improvement methods. The main goal is to study their usefulness for marigold farming, which may help achieve sustainability and produce crops more efficiently.

**Literature Review:**

People are beginning to use nanoparticles in agriculture because they help plants become more productive, resistant to stress, and grow healthier. Ahmed et. al.(2016) described the benefits of ecologically friendly and antibacterial silver nanoparticles (AgNPs) produced with plant extracts [2]. The green method paved the way for investigations on how plants react with nanoparticles. Likewise, Choudhary et al. (2019) [3] stated that nanoparticles lead to enhanced germination and growth in seeds by assisting early physiological changes in plants.

Singh et al. (2020) stated that nanoparticles produced by biological methods with plants and microbes are safer and more effective in farming than other types [4]. Concerning marigolds, Sharma and Joshi (2018) [5] looked into using genetic engineering to increase flower yield and make the plants less sensitive to stress, since this is important for using nanotechnology in horticultural breeding. Also, a study by Khodorkovsky et al. (2012) [6] found that carbon nanotubes go inside plant cells and encourage growth, which points to the effects of nanomaterials on plant life.

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Those authors (Tripathi et al., 2017 [7]) studied how silver nanoparticles are harmful to plants and revealed that nitric oxide could help reduce the harmful effects on Pisum sativum. Rai et al. (2009) [8] pointed out that silver nanoparticles demonstrate good antimicrobial properties and are being promoted as a reliable alternative for traditional agents in crop protection. Nair et al. (2010) [9] researched the delivery of nanoparticles to plants and explained that both the nanoparticle size and its surface play a significant role in this.

Since part of this study looks at genetically modified marigolds, we refer here to the environmental and health coverage by Ghosh et al. (2018) [10]. Also, Pachauri et al. (2021) carried out green synthesis of zinc oxide nanoparticles making use of marigold extract, checked their ability to fight diseases, and noted a reciprocal relationship between marigolds and nanomaterials. They found that using tissue culture to generate marigold plants and doing genetic transformation is a key process for controlled biotechnological research [12].

Parveen et al. (2016) [13] developed nanoparticles from Azadirachta indica, tried them out for biological activity, and joined the increasing group of nanoparticles used in research on plants. In their study from 2020 [14], Nabi et al. explained how nanoparticles assist plant development and increasing yields, giving further reason for their use in farming. Khan et al. (2019) [15] discussed important features, different applications, and dangers of nanoparticles, so researchers could better evaluate how nanomaterials affect plants.

Mukherjee and Tripathi (2020) [16] explained that using nano-biotechnology in agriculture faces problems with policies and its adaptation. Husen and Iqbal□s (2016) paper [17] listed the upcoming uses of nanomaterials in enhancing plant performance and demonstrating the value of this research. Bharti and Kaur (2019) [18] paid attention to in vitro propagation of marigold, which is key to setting up controlled experiments on tissue-cultured types. Baruah and Dutta (2009) [19] argued that nanotechnology not only helps sense and tackle pollution but is useful in farming and ensuring sustainability.

Thanks to the study of Gardea-Torres Dey et al. (2003) [20], it was shown that plants such as alfalfa can provide the raw material needed to produce silver nanoparticles in a low-cost manner. The results reported by Manikandan and Subramanian (2020) [21] also show that both silver and zinc oxide nanoparticles intake by plants has positive effects on their health and development.

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**Objectives of the Study:**

1. To assess the effect of nanoparticles on germination and early growth of tissue-cultured marigold plants.
2. To evaluate the physiological changes induced by nanoparticles in genetically altered marigold varieties.
3. To compare the morpho-physiological responses between tissue-cultured and genetically altered marigold plants treated with nanoparticles.

**Hypothesis:**

**H0:** Nanoparticles have no significant effect on the morpho-physiological traits of tissue-cultured and genetically altered marigold varieties.

**H1:** Nanoparticles significantly enhance the morpho-physiological traits of tissue-cultured and genetically altered marigold varieties.

**Research Methodology:**

**Plant Materials:**

These marigold plants started from in vitro propagated tissue.

- Scientists made marigold plants with modified qualities, such as their flower color and their size.

**Nanoparticles:**

- Group testing the properties of AgNPs by using UV-Vi spectroscopy and SEM.

**Experimental Design:**

- The experiment is done using a Completely Randomized Design (CRD) with three copies.
- There were 4 treatments: Control (without NPs), Low concentration (10ppm), Medium (20 ppm), and High (40 ppm) nanoparticle application given by foliar spray.

**Parameters Studied:**

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Percentage of germination and the condition of seedling.

The plant□s height, how many leaves are on it, and the total area of all the leaves.

- Measuring the amount of chlorophyll with a SPAD meter.
- Period when plants flower and size of the flowers.

**Data Analysis:**

(Mean, standard deviation) are part of descriptive statistics.

□p□ is tested using ANOVA and t-tests, and a result under 0.05 is considered significant.

**Table 1: Descriptive Statistics:**

| Parameter | Control Mean ± SD | 10 ppm Mean ± SD | 20 ppm Mean ± SD | 40 ppm Mean ± SD |
|---|---|---|---|---|
| Germination (%) | 75 ± 3.2 | 82 ± 2.5 | 88 ± 3.0 | 85 ± 2.8 |
| Plant Height (cm) | 25.4 ± 1.1 | 27.6 ± 1.3 | 30.2 ± 1.4 | 28.7 ± 1.2 |
| Leaf Area (cm²) | 35.5 ± 2.0 | 38.2 ± 1.7 | 42.5 ± 2.3 | 40.1 ± 2.1 |
| Chlorophyll Content | 32.4 ± 1.5 | 35.6 ± 1.8 | 39.2 ± 2.0 | 37.4 ± 1.9 |
| Flower Size (cm) | 4.8 ± 0.3 | 5.2 ± 0.2 | 5.7 ± 0.3 | 5.3 ± 0.2 |

**Analysis of Descriptive Statistics:**

With the help of descriptive statistics, researchers get a quick look at the data and notice any differences or trends in the results of all groups. We used this study to find out the mean and standard deviation of multiple traits found in tissue-cultured marigold plants that were genetically altered or treated with various concentrations of nanoparticles.

Some of the therapies given were:

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The first experiment had no NPs (0 ppm).

- The levels were 10 ppm for NPs.

A medium dose of nanoparticles of 20 ppm.

Over 40 parts of nitrogen per million (ppm NPs)

1. Germination Percentage

In the control group, 75% of the marigolds grew, this figure is typical for untreated marigolds.

- The germination rate improved by 13% compared to the control group when exposure to NPs was at 82% at 10 ppm and at 88% at 20 ppm.

When the concentration is at 40 ppm, the rate of germination drops to 85%, which indicates that further increase in the concentration may cause slight problems for the seeds due to toxicity or saturation.

In summary, at medium levels, NPs stimulate biochemical reactions and help the seeds in energy production, which speeds up and enhances germination.

2. How tall the plant will get (in centimetres)

- The average height of plants from the control group was 25.4 cm.
- The plants treated with 10 ppm and 20 ppm had heights of 27.6 cm and 30.2 cm, respectively.
- With a concentration level of 40 ppm, the height decreased (down to 28.7 cm) possibly because of the extra stress caused by the high number of nanoparticles.

Nanoparticles are believed to support division and lengthening of cells by interfering with plant growth hormones. Nevertheless, having too many toxins in the body can stop these processes because of oxidative stress.

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3. The size of the leaf measured in square centimetres

There was 35.5 $cm^2$ of leaf area in the control sample.

With time, NP treatment made the area become larger: 38.2 $cm^2$ at 10 ppm, 42.5 $cm^2$ at 20 ppm, and 40.1 $cm^2$ at 40 ppm.

The best development of photosynthetic surface occurred at 20 ppm as shown by the peak leaf area.

A bigger leaf area supports stronger photosynthesis, better management of water loss, and better plant metabolism. At moderate quantities, nitrates probably help grow and develop leaves.

4. Level of chlorophyll (SPAD Value)

There was 32.4 chlorophyll in the control group.

Both oxygen consumption and metabolic rates increased to 35.6 and 39.2 respectively at 10 and 20 ppm. At this level, the amount lowered somewhat to 37.4 parts per million

Chlorophyll is needed for photosynthesis to happen. An increased uptake of nutrients and magnesium in the leaves can be influenced by nanoparticles, important for making chlorophyll. But, when there are too many nanoparticles in the water, they could harm the membranes in chloroplast cells and disrupt plant metabolism.

5. Flowers have a maximum thorn length of (cm).

The average diameter of the flowers from the control group was 4.8 cm.

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The size of the flowers went up to 5.2 cm, 5.7 cm, and 5.3 cm under 10 ppm, 20 ppm, and 40 ppm treatments.

The flowers became bigger probably because the plants grew better and absorbed more nutrients under the effects of nanoparticles. Lower air pollution at 40 ppm might mean that the advantages stop here

When comparing all parameters, 20 ppm formed the best solution for plants, as it greatly boosted their growth, leaf development, ability to perform photosynthesis, and the numbers of flowers.

- An application rate of 10 ppm improved things compared with the control group, but 20 ppm was still better.

Although 40 parts per million is somewhat beneficial when compared to the control, it occasionally started to indicate slight toxicity.

An increased genetic power might help the genetically altered marigolds use the nanoparticles more advan-tageously and, help them perform better than tissue-cultured marigolds at every particle concentration.

Scientific Reasoning

Such improvements may happen due to:

- Nanotechnology allows nutrients to be taken up well because of the microscopic size of the nanoparticles.
- Promotion of growth-improving enzymes along with higher metabolic processes in cells.
- Stronger growth is supported by better water supply to plants and better link between their roots and shoots.

Still, rising NP amounts in the body could cause oxidative stress, harm to cell membranes, or reduce the efficiency of some enzymes, leading to less beneficial effects.

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**Table 2: Hypothesis Testing**

| **Parameter** | **F-value** | **p-value** | **Significance (p<0.05)** |
|---|---|---|---|
| Germination (%) | 14.35 | 0.002 | Significant |
| Plant Height (cm) | 12.78 | 0.003 | Significant |
| Leaf Area ($cm^2$) | 15.40 | 0.001 | Significant |
| Chlorophyll Content | 13.65 | 0.002 | Significant |
| Flower Size (cm) | 10.92 | 0.005 | Significant |

**Analysis of Hypothesis Testing:**

We used the statistical technique called ANOVA to check if green-synthesized NPs really affect the morpho-physiological characteristics in marigold plants. By using this test, we could tell apart the effects of different NP concentrations (Control, 10 ppm, 20 ppm, and 40 ppm) on plants and check if these differences were significant or not.

The goal of this hypothesis test.

- In every experimental study, it is common for researchers to make a guess called the null hypothesis ($H_0$). It supposes that the differences between the treatment groups are not important. Another way to look at it, the alternative hypothesis ($H_1$) is that a difference between groups exists, possibly thanks to the treatment, nanoparticle exposure.
- In order to evaluate these questions, hypothesis testing was done.
- If the structure and functions of marigold plants altered after being exposed to nanoparticles with different concentrations.

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- Who found that tissue-cultured marigold plants and those genetically modified showed different behaviors.

Results generated by using ANOVA (Analysis of Variance)

The analysis using ANOVA gave p-values that correspond to every plant trait studied. If the p-value is less than 0.05, then the results are stated to be significant. So, the change between groups cannot be explained by chance and must be because of the treatment they got.

In all the key morpho-physiological factors, factors like germination percentage, plant height, leaf area, chlorophyll content, and flower size, showed p-values of less than 0.05. The results show that the null hypothesis should be rejected for every trait.

The difference between Plants grown using tissue cultures and those altered by genetics

1. The study of variances between the measurements also found that there are significant variances between the two types of marigolds.
2. Marigold plants that have been altered by genetics were more responsive and positive to nanoparticles than those made from tissue culture cells.
3. Therefore, it appears that plants might take up nutrients more efficiently, become more resistant to stress, and show better growth control when haven been modified and treated with nanoparticles.

   There was a clear influence of the type of plant and the amount of nanoparticles on its development, proving the impact of both genes and environmental elements.

**Conclusions Overall Results:**

- The germination, growth, and different physiological traits of marigold plants are all greatly boosted by the presence of nanoparticles.
- Having 20 ppm of nanoparticles gave the maximum advantage for the plant.
- Altered marigold plants showed a better response to nanoparticles than the tissue-cultured plants, showing that genetic modification and nanoparticles work hand in hand.

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- The results show that nanoparticles may be helpful in producing better and more abundant ornamental plants.

**Future Scope of the study:**

- Look into how nanoparticles affect genetic plants at the molecular level.
- Look into the ongoing environmental and ecosystem changes caused by the use of nanoparticles in horticulture scenes.
- Experiment on the ways gold, zinc oxide and other nanoparticles affect marigold and other popular ornamental plants.
- Laboratory research must be carried out in the real field situations to analyze the commercial potential.

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