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Effect of Silver, Iron, and Sulphur Nanoparticles on Stress Physiology and Plant *(Vigna radiata)* Nutrition

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Abstract*— Phytotoxicity assessment plays a crucial role in understanding the potential environmental impact of nanoparticles. This study aimed to screen the phytotoxicity of silver nanoparticles (AgNPs), iron nanoparticles (FeNPs), and sulphur nanoparticles (SNPs). Vigna radiata (Mung bean) was germinated with two concentration gradients of nanoparticles (500mg/L and 1000mg/L). The extent of phytotoxicity was assessed to understand the effect of AgNPs, FeNPs, and SNPs on overall plant growth and nutrition. The nutritive values of mung bean seeds were determined in terms of total starch, soluble sugar, and amylase. Preliminary analysis of physiological and biochemical parameters revealed evidence of phytotoxicity in the plants. AgNPs exhibited the highest phytotoxicity at both concentrations (500mg/L and 1000mg/L), followed by FeNPs and then SNPs. Interestingly, the application of 500mg/L of SNPs led to the attainment of maximum growth and yield. The study highlighted the significance of sulphur and iron nanoparticles as vital nutrients for enhancing the productivity of Mung beans. However, it is noteworthy that preliminary results of AgNPs, SNPs, and FeNPs had a suppressive effect on plant growth and showed phytotoxicity, particularly at higher concentrations.

***Index Terms*— Nanoparticles, Phytotoxicity, Stress Physiology**

I. INTRODUCTION

Mung bean *(Vigna radiata)* is a top legume crop grown under warm tropical climates. Mung bean requires less water and is cultivated in arid zones worldwide. The crop is highly enriched with nutrients and phytochemicals (Dahiya et al., 2015). The growth, production, yield, and protein bioavailability of mung bean has decreased due to contaminated agricultural soils with the accumulation of heavy metals (Mao et al., 2018).

The increasing utilization of nanoparticles, possibly through their incorporation in fertilizers, may inadvertently introduce them into the soil and environment. These nanoparticles possess unique properties that facilitate interactions with living systems in the soil and surroundings, potentially functioning as catalysts and posing risks to plant life. Nevertheless, when subjected to various dilution levels, nanoparticles exhibit substantial variations in both physiological and biological activities. Nanoparticles with different compositions, sizes, concentrations, and physical and chemical properties have been reported to influence the growth and development of various plant species with both positive and negative effects (Chang et al., 2010). As nanoparticles are generally useful for plants in both physiological and biological aspects. Consequently, there is significant concern regarding the potential environmental impacts of nanoparticles. Most studies showed the nanoparticles could produce toxic effects above a certain concentration (Rico et al., 2011). In this study, we evaluate phytotoxicities developed in increasing concentrations of nanoparticles on Mung beans *(Vigna radiata)*.

II. OBJECTIVE

The objective of this study was to comprehensively assess the phytotoxic effects arising from the exposure of Mung bean *(Vigna radiata)* progressively at different concentrations of nanoparticles. We aim to investigate the impact of these nanoparticles on crucial aspects of plant physical growth factors and nutrition. By conducting this research, we seek to gain a better understanding of how nanoparticles at different concentrations influence the overall health and development of the Mung bean plant.

III. MATERIALS AND METHODS

Plant exposure to nanoparticles

Mung bean seeds were chosen as the plant sample, obtained from the local market in Patna, Bihar. The seeds were identified in the Department of Botany, Patna University. These seeds were weighed, washed in distilled water (DW) and immersed in solutions containing nanoparticles (FeNPs, AgNPs, and SNPs) at concentrations of 1000mg/L and 500mg/L for duration of 6 hours. Some seeds were also soaked in distilled water, served as the control.

Plant growth measurements

The Nanoparticle's exposed seeds were then incubated, and various growth parameters were recorded, including the rate of germination, the day of emergence of the first leaf, and the vigour index of the seedlings. After 21 days of growth, the plants from both the control group and the Nanoparticle's exposed groups were carefully extracted.

Measurements were taken for plant height, shoot length,

and root length of the seedlings using a ruler. The fresh weight and dry weight of the seedlings were determined using a digital balance. These evaluations were conducted to examine the effects of nanoparticles on the growth and development of Mung bean seedlings.

Total Chlorophyll assay

Total chlorophyll, along with its constituent's chlorophyll a and chlorophyll b, were quantified according to the methodology described by Arnon in 1949. For this analysis, 2.5 grams of freshly harvested leaves were homogenized with 10 ml of 80% acetone. The resulting mixture was then subjected to filtration and centrifugation at a speed of 5000 – 10000rpm for a duration of 5 minutes. Subsequently, the supernatant was adjusted to a final volume of 20 ml using 80% acetone.

To ascertain the chlorophyll content, the absorbance of the prepared solution was measured at two specific wavelengths, namely, 645nm and 663nm, with respect to a solvent (acetone) blank. Utilizing the acquired absorbance values, the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll were determined via appropriate equations, enabling a comprehensive assessment of the photosynthetic pigments in the samples under investigation.

Total chlorophyll = [20.2 (A645) + 8.02(A663)] V/1000 x W (mg g-1 fresh weight)

Chlorophyll a : 12.7(A663) – 2.69(A645)

Chlorophyll b : 22.9(A645) – 4.68(A663)

Estimation of Starch

Starch estimation was conducted following the method of McCready et al. (1950). The residual mass obtained after extracting total soluble sugars from the plant material was mixed with 5.0 ml of distilled water, and then 6.5 ml of 52% perchloric acid was added. After stirring the mixture, it was centrifuged for 20 minutes at 2000 rpm. The supernatant was collected, and this process was repeated three times. The supernatants from each step were pooled and brought to a total volume of 100.0 ml with distilled water. The mixture was then filtered using Whatman filter paper (No.42). A 1.0 ml sample of this filtrate was used for starch analysis, following the same procedure as that of total soluble sugars. The quantity of starch was calculated in terms of glucose equivalent, and a conversion factor of 0.9 was used to obtain the starch value. The amount of starch was expressed as mg/g fresh weight of tissue.

Estimation of Total Soluble Sugar

Total soluble sugars were estimated using the phenol-sulphuric acid reagent method (Dubois et al., 1956). Fresh normal plant material (500 mg) was homogenized with 10.0 ml of 80% ethanol. After centrifugation at 2000 rpm for 20 minutes, the supernatants were collected. To each 1.0 ml of alcoholic extract, 1.0 ml of 5% phenol was added and mixed, followed by the rapid addition of 5.0 ml of 96% sulphuric acid. The tubes were gently agitated during this process and then allowed to stand in a water bath at 26-30°C for 20 minutes. The optical density (OD) of the characteristic yellow orange colour developed was measured at 490 nm using a spectrophotometer, after setting the transmission at 1000mg/L against the blank. A standard curve was prepared using known concentrations of glucose. The quantity of total sugar was expressed as mg/g fresh weight of tissue.

Estimation of Amylase Activity

Amylase activity was assessed by employing the 3, 5-dinitrosalicylic acid (DNSA) colorimetric procedure of Bernfeld (1955) to measure the production of maltose and other reducing sugars from amylopectin. For this analysis, 200 mg of fresh plant material was crushed in 4.0 ml of 0.02 M phosphate buffer (pH 6.9), and the resulting homogenate was centrifuged at 2500 rpm for 20 minutes. The supernatant obtained from the centrifugation was used for enzyme activity determination. The reaction mixture comprised 1.0 ml of the enzyme extract and 1.0 ml of the substrate solution (1.0 gm soluble starch dissolved in 100 ml of 0.02 M phosphate buffer, pH 6.9 containing 0.0067 M NaCl). This reaction mixture was incubated at 30°C for 45 minutes, and the reaction was halted by adding 1.0 ml of DNSA reagent. The tubes were then placed in a boiling water bath for 15 minutes, followed by immediate cooling under tap water. After cooling, 20 ml of distilled water was added to the mixture, leading to the development of a yellow colour due to unhydrolyzed starch. The optical density of this mixture was measured at 560 nm against a zero-min. blank. The amylase activity was expressed as mg starch hydrolysed per hour per gram fresh weight of tissue.

IV. RESULTS AND DISCUSSIONS

Plant growth measurements

Plant growth and development are affected by biotic and abiotic stresses. They can either increase or decrease seed germination, root formation, biomass output, biological as well as organic inactivity.

In this study, we observed and recorded the plant height of various nanoparticle-treated samples (Table 1). The control group had a plant height of 13 cm. In contrast, plants exposed to AgNPs at 1000mg/L concentration showed the smallest height of 12.5 cm. Among them, plants treated with SNPs exhibited the highest plant height, measuring 14.5 cm, followed by FeNPs at 13.8 cm. At the 500mg/L concentration, all nanoparticle-treated plants displayed a 30% increase in plant height. Notably, the results indicated that AgNPs induced the highest stress compared to FeNPs, SNPs, and the control. The presence of sulphur nanoparticles significantly influenced plant height, with the tallest plants observed in SNPs-treated samples and the shortest in AgNPs-treated ones. All levels of sulphur nanoparticles resulted in a considerable enhancement in plant height.

Although, previous investigations by Stampoulis et al. (2009) have reported on the toxicity of other nanoparticles,

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such as copper nanoparticles, toward mung bean *(Vigna radiata*), resulting in a notable reduction in their seedling growth rate.

The morphological findings indicate that silver nanoparticles (NPs) exert higher stress on plants compared to iron, sulphur, and the control group. At the same concentration of silver nanoparticles, the plant growth is impeded due to nanoparticle accumulation, while sulphur induces relatively lower stress than iron. The adverse impact of NPs on plant growth can be attributed to direct contact with the roots, leading to particle aggregation on the root surfaces, potentially hindering nutrient flow and thus inhibiting growth at higher NP concentrations.

Estimation of Total Chlorophyll

Chlorophyll is an essential class of primary compounds crucial for capturing sunlight and facilitating photosynthesis, which is vital for plant growth. Our study investigated the response of samples to AgNPs, FeNPs, and SNPs at concentrations of 1000mg/L and 500mg/L, and their effects on chlorophyll content were examined. Results revealed that FeNPs had a greater impact on chlorophyll content reduction compared to SNPs, followed by AgNPs. We obtained similar results, indicating that iron stress induced a reduction in Chl. a (by 3.71 mg/g) and Chl. b (by 2.43 mg/g). Notably, this reduction was more pronounced in Chl. a compared to Chl. b.

These outcomes suggest that the impact of magnetic nanoparticles on chlorophyll content is concentration-dependent, and that the presence of iron in particular may lead to significant reductions in both Chl. a and Chl. b levels, with a relatively higher impact on Chl. a.

Table 1 Plant *(Vigna radiata*) growth measurements

Physical Parameter	Control	Treated at 1000 mg/L concentration			Treated at 500mg/L concentration		
		FeNPs	AgNPs	SNPs	FeNPs	AgNPs	SNPs
% of germination	1000%	70%	50%	80%	90%	70%	100%
Seedling vigour index	1300	966	625	1160	1332	931	1520
Day of emergence of first leaf	3rd	5th	4th	6th	4th	3rd	5th
No. of leaves	6	7	5	7	7	6	8
Colour of leaf	Dark green	Light green	Dark green	Light green	Light green	Dark green	Light green
Plant height (cm)	13	13.8	12.5	14.5	14.8	13.3	15.2
Shoot length (cm)	6.5	9.5	9	10	10	9.8	10.2
Root length (cm)	6.5	4.3	3.5	4.5	4.8	3.5	5
Plant fresh weight (mg)	35	24	19	31	27	21	33
Plant dry weight (mg)	18	11	8	15	13	11	18

Table 2 Total Chlorophyll in Nanoparticles treated plant.

	At 1000 mg/L concentration				At 500 mg/L concentration			
	AgNPs	FeNPs	SNPs	Control	AgNPs	FeNPs	SNPs	Control
Absorbance at 645 nm	0.50	0.35	0.34	0.673	0.58	0.45	0.39	0.598
Absorbance at 663 nm	0.54	0.33	0.55	0.598	0.60	0.43	0.54	0.508
Chlorophyll a	6.858	3.155	6.073	14.96	6.06	4.525	5.8	12.67
Chlorophyll b	8.9228	6.471	5.212	13.67	10.48	8.29	6.42	9.35
Total chlorophyll content	14.43	7.07	11.28	28.63	16.528	12.53	12.53	22.02

Previous research has also demonstrated that FeNPs can lead to lower chlorophyll content, resulting in visible chlorotic symptoms and significantly reduced plant yield (Mimmo et al., 2014).

Based on the findings reported by Racuciu et al. (2007), it was observed that the chlorophyll content of maize plants exhibited an increase at low concentrations (10-50 µl/L) of

magnetic nanoparticles, while higher concentrations of these nanoparticles led to inhibition of chlorophyll production.

Estimation of Total Starch

Starch is a complex carbohydrate and the primary energy storage molecule in plants. During seed germination, starch serves as a reserve of energy that can be used by the emerging seedling until it can produce its own energy through photosynthesis (Smith and Zeeman, 2020).

The observed increase in sugar content is mainly attributed to the hydrolysis of starch, a process facilitated by enzymes with hydrolytic activity (Kaplan and Guy, 2004). Additionally, carbohydrates can function as signaling molecules (Hanson et al., 2009) and play a role in the plant's adaptive mechanisms to cope with stress (Ramel et al., 2009). Analysis of Total Soluble Starch (TSS) from Figure 1 reveals a synthesis of stress in plants treated with AgNPs, FeNPs, and SNPs at both concentrations. The stress induced by silver

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nanoparticles (1.715 mg/ml) was found to be the lowest compared to iron nanoparticles (2.019 mg/ml), followed by sulphur nanoparticles (1.81 mg/ml).

A study by Wang et al. (2022) investigated the effect of iron nanoparticles on starch accumulation in rice plants. They found that iron nanoparticles at low concentrations enhanced starch accumulation, but higher concentrations caused a reduction in starch content, indicating a concentration dependent response. The disturbances in starch metabolism can have significant implications, leading to reduced plant growth and affecting overall development (Corbesier et al., 1998).

Estimation of Total Soluble Sugar

Sugars, such as glucose and sucrose, are the end products of amylase digestion of starch. Once starch is broken down, sugars become readily available to the growing seedling as a quick and easily accessible source of energy (Roitsch and González, 2004).

The data obtained in this study (Figure 2) indicated that treatment of seedlings with silver, iron, and sulphur resulted in an increase in total soluble sugars (TSS) content with increasing concentrations, specifically at 1000mg/L. In the case of AgNPs, the TSS content of seedlings notably increased at 1000mg/L, with an absorbance of 2.89 nm.

However, at high concentrations (1000mg/L and 500mg/L), silver caused a reduction in TSS content (by 0.352 mg/ml), indicating stress caused by AgNPs. SNPs and FeNPs induced comparatively less stress than AgNPs.

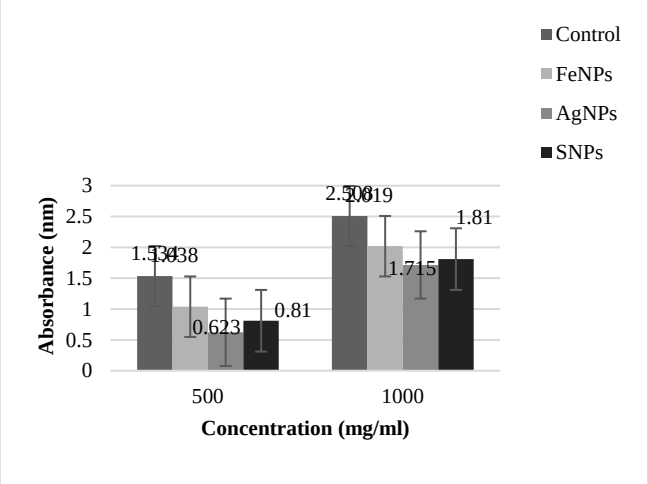

Figure 1 Total Soluble Starch in Nanoparticles treated plants.

Research by Siddiqui et al. (2018) demonstrated that exposure to silver nanoparticles in cucumber plants led to a significant reduction in total soluble sugar content. This suggests that silver nanoparticles may induce stress responses that affect sugar metabolism.

The reduced dose of AgNPs may serve as a catalyst for improving the biochemical metabolism of plants. These results align with the findings of Khane et al. (2022), who reported that the application of silver at different doses leads to an increase in soluble sugar levels.

Moreover, our findings are in the agreement with Aldoust and Isoda (2013), who emphasized the significance of iron nanoparticle treatment for enhancing the photosynthetic potential of plants.

Estimation of Total Amylase Activity

Amylase is an enzyme found in various organisms, including plants like mung bean seeds. Its main function is to break down complex starch into simpler sugars during the process of seed germination (Anderson et al., 1992). The study shows that amylase content was significantly influenced by the treatment of mung bean seeds with nanoparticles. Specifically, the highest amylase content was observed in mung bean seeds treated with silver nanoparticles (AgNPs) at a concentration of 1000mg/L, with an absorbance of 2.667 nm.

Mung bean seeds treated with iron nanoparticles (FeNPs) and sulphur nanoparticles (SNPs) exhibited a lower amylase content of 1.345 nm and 0.798 nm respectively. Interestingly, the study indicates that a reduced dose of silver nanoparticles (AgNPs) i.e., 500 mg/L could potentially serve as a catalyst to enhance biochemical metabolism and growth of seedlings in plants.

Research by Singh et al. (2015) examined the impact of sulphur nanoparticles on plant growth and metabolism in mustard plants. They observed that sulphur nanoparticles promoted growth and increased enzymatic activities, including amylase, which could be linked to improved starch breakdown during germination.

Moreover, a study by Tripathi et al. (2017) compared the effects of various nanoparticles on wheat seedlings. They reported that silver nanoparticles induced more significant oxidative stress and alterations in enzymatic activities, including amylase, compared to other nanoparticles like titanium dioxide and zinc oxide nanoparticles. This suggests

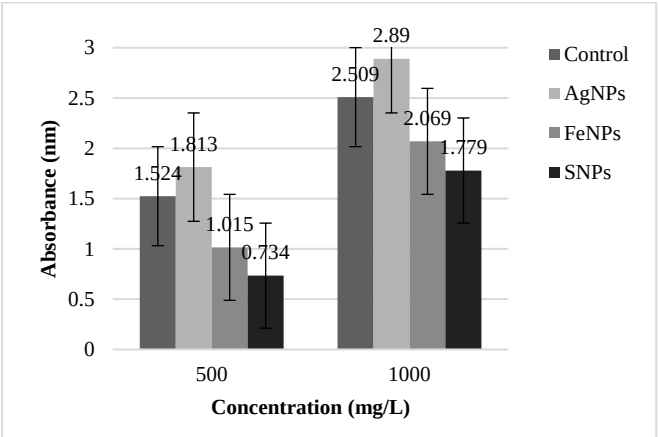

Figure 2 Total Soluble Sugar in Nanoparticles treated plants.

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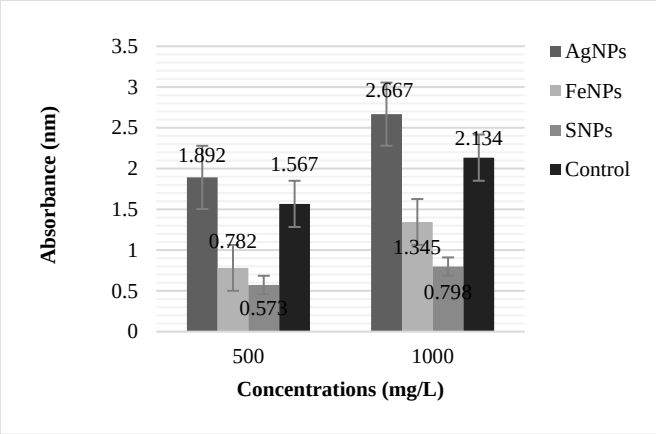

Figure 3 Total Amylase Activity in Nanoparticles treated plants.

that lower concentrations of AgNPs might have positive effects on seed germination and amylase activity.

V. CONCLUSION

In this study, we investigated the toxicity of different nanoparticles (S, Fe, and Ag) at concentrations of 1000 mg/L and 500 mg/L, along with a control group. Among these, silver nanoparticles exhibited the highest toxicity, followed by iron and sulphur nanoparticles. Our findings suggest that the release and accumulation of nanoparticles in the cultivation media significantly contributed to the observed phytotoxicity. However, we also observed that silver, sulphur, and iron nanoparticles had a remarkable positive impact on enhancing the growth and yield of mung beans. At higher concentrations, AgNPs, SNPs, and FeNPs displayed suppressive effects on plant growth and showed phytotoxicity. Interestingly, the application of 500 mg/L of SNPs resulted in achieving maximum growth and yield, indicating that at low concentration sulphur and iron nanoparticles play essential roles as nutrients for enhancing the production and productivity of mung beans. These findings suggest the potential use of nanoparticles to improve crop growth and yield, with careful consideration of appropriate concentrations to avoid phytotoxic effects.

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