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Evaluation of Plant Growth Promotion by Rhizobium-Inoculated Biofertilizer in Leguminous and Non-Leguminous Crops

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Abstract: Rhizobium species are widely recognized for their role in symbiotic nitrogen fixation in leguminous crops. However, their potential to enhance growth in non-leguminous crops remains underexplored. This study evaluates the plant growth-promoting efficiency of Rhizobium-inoculated biofertilizer on Vigna radiata (Green gram) and Zea mays (Maize) under controlled conditions. Biofertilizer formulations containing Rhizobium sp. were applied at sowing, and their effects on germination, shoot and root length, fresh and dry biomass, and chlorophyll content were assessed after 30 days. Results revealed a significant improvement ($p < 0.05$) in all growth parameters of Vigna radiata, while non-leguminous maize showed moderate growth enhancement. ANOVA indicated significant differences between treatments, with Rhizobium-inoculated biofertilizer outperforming control and chemical fertilizers. These findings highlight the scope of Rhizobium biofertilizers as sustainable growth promoters for leguminous crops and as potential growth enhancers for non-legumes.
Keywords: Rhizobium, Biofertilizer, Leguminous crops, Non-leguminous crops, Plant growth promotion, Nitrogen fixation.

I. INTRODUCTION

Nitrogen (N) is one of the most essential macronutrients for plant growth and development, as it plays a vital role in chlorophyll synthesis, protein formation, and enzymatic activities. Modern agricultural systems heavily rely on chemical nitrogen fertilizers to ensure high crop yields. However, excessive and continuous application of chemical fertilizers has led to severe ecological problems, including soil acidification, groundwater contamination, and greenhouse gas emissions (Choudhury & Kennedy, 2004; Bhattacharyya & Jha, 2012). These environmental concerns have shifted global attention towards sustainable and eco-friendly alternatives such as biofertilizers. Biofertilizers are formulations containing live microorganisms that enhance nutrient availability to plants through natural processes such as nitrogen fixation, phosphorus solubilization, and production of plant growth-promoting substances (Vessey, 2003). Among these, Rhizobium, a genus of symbiotic nitrogen-fixing bacteria, has been widely used in leguminous crops. Rhizobium forms root nodules in legumes where it fixes atmospheric nitrogen (N_2) into ammonia (NH_3), which is assimilated by the plant, significantly reducing the need for chemical nitrogen fertilizers (Sulieman & Tran, 2015). Legume-Rhizobium symbiosis is considered one of the most efficient and sustainable nitrogen-fixing systems in nature (Oldroyd et al., 2011). Recent studies indicate that the role of Rhizobium may not be limited to legumes alone. Certain strains exhibit plant growth-promoting (PGP) activities even in non-leguminous crops through mechanisms such as phytohormone (auxins, cytokinins, gibberellins) production, siderophore secretion, phosphate solubilization, and biological control of soil-borne pathogens (Yanni et al., 2016; Sharma et al., 2013). This broadened perspective suggests the potential application of Rhizobium-based biofertilizers beyond traditional legumes, opening new avenues for integrated nutrient management in sustainable agriculture.

Despite these promising attributes, there remains a paucity of systematic studies comparing the growth-promoting effects of Rhizobium inoculation on both leguminous and non-leguminous crops under controlled conditions. Evaluating these effects can provide critical insights into optimizing biofertilizer formulations for diverse cropping systems, particularly in resource-constrained regions where reducing chemical fertilizer dependency is imperative.

The present study aims to evaluate the influence of Rhizobium-inoculated biofertilizers on growth parameters of Vigna radiata (Green gram) as a representative leguminous crop and Zea mays (Maize) as a representative non-leguminous crop. The specific objectives include:

1) Assessing the effect of Rhizobium inoculation on germination, shoot and root growth, biomass, and chlorophyll content.
2) Comparing the growth responses between leguminous and non-leguminous crops.
3) Analyzing statistical significance using ANOVA and Tukey’s HSD test.

By addressing these objectives, this research seeks to provide empirical evidence for the broader utility of Rhizobium-based biofertilizers in sustainable agriculture systems.

II. MATERIALS AND METHODS

A. *Experimental Site and Design*

The experiment was conducted under controlled conditions at MIET, Meerut, maintained at a temperature of 28 ± 2°C, relative humidity of 65–70%, and a photoperiod of 12 h light/12 h dark. A Completely Randomized Design (CRD) was employed with three treatments and five replications for each crop.

B. Test Crops

Two crops were selected:

- Leguminous crop: *Vigna radiata* (Green gram)
- Non-leguminous crop: *Zea mays* (Maize)

Certified seeds of both crops were procured from . Seeds were surface sterilized with 0.1% $HgCl_2$ for 2 min and thoroughly washed with sterile distilled water prior to sowing (Somasegaran & Hoben, 2012).

C. *Soil Preparation and Potting*

Loamy soil was air-dried, sieved (2 mm), and sterilized by autoclaving at 121°C for 30 min on two consecutive days. Soil physico-chemical properties were as follows: pH 7.2, organic carbon 0.8%, available N 180 kg ha^{-1}, P 12.6 kg ha^{-1}, and K 140 kg ha^{-1}, analyzed as per standard methods (Jackson, 1973). Plastic pots (25 cm diameter) were filled with 5 kg soil for each treatment.

D. *Biofertilizer Preparation*

Rhizobium culture (*Rhizobium* sp.) was isolated from nodules of *Vigna radiata* following standard procedures (Vincent, 1970). The isolate was mass-multiplied in Yeast Extract Mannitol Broth (YEMB) at 28°C for 5–7 days until reaching 10^8 CFU mL^{-1} (Somasegaran & Hoben,2012).

Carrier-based formulation: Sterilized peat soil was used as a carrier. The broth culture was mixed with the carrier and allowed to cure for 24 h to maintain a viable population of 10^8 CFU g^{-1} (Reddy et al., 2002). For seed inoculation, a jaggery solution (10%) was used as an adhesive.

E. *Treatments*

Three treatments were tested:

- T_1 (Control): No fertilizer
- T_2 (Chemical fertilizer): NPK applied at recommended dose (40:20:20 kg ha^{-1} for legumes, 80:40:40 kg ha^{-1} for maize)
- T_3 (Rhizobium inoculation): Seeds coated with ***Rhizobium***-inoculated biofertilizer @ 10 g kg^{-1} seed.

F. *Crop Sowing and Management*

Seeds were sown at a depth of 2 cm in pots and irrigated with sterile distilled water as needed to maintain field capacity. No pesticides or additional nutrients were applied to avoid interference with treatment effects.

G. *Growth Parameters Recorded*

Observations were recorded 30 days after sowing (DAS) on the following parameters:

- Germination percentage (%)
- Shoot length (cm)
- Root length (cm)
- Fresh weight (g) and dry weight (g): Dry weight recorded after oven drying at 65°C for 48 h
- Chlorophyll content (mg g^{-1} FW): Estimated using Arnon's method (Arnon, 1949) by extracting pigments in 80% acetone and measuring absorbance at 645 and 663 nm using a UV-Vis spectrophotometer.

H. Statistical Analysis

All data were subjected to one-way ANOVA to test for significant differences among treatments at $p < 0.05$, followed by Tukey's HSD test for mean separation. Analysis was performed using SPSS software version 25 (IBM Corp., Armonk, NY, USA).

III. RESULTS AND DISCUSSION

A. Germination Percentage

The germination percentage of both **Vigna radiata** (green gram) and **Zea mays** (maize) was not significantly influenced by Rhizobium inoculation ($p > 0.05$). All treatments exhibited germination rates above 90%, indicating that Rhizobium inoculation does not adversely affect seed germination. Similar observations were reported by Yanni et al. (2016), who found that Rhizobium does not alter initial germination but enhances post-germination growth.

B. Effect on Growth Parameters

The application of Rhizobium-inoculated biofertilizer significantly ($p < 0.05$) improved shoot length, root length, biomass, and chlorophyll content in **Vigna radiata** compared to the control. In **Zea mays**, growth enhancement was moderate but statistically significant compared to the untreated control (Table 1).

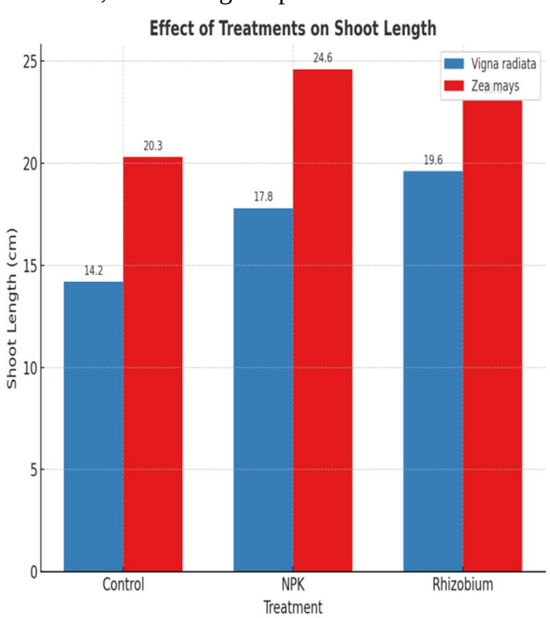
Table 1. Effect of Treatments on Growth Parameters of Vigna radiata and Zea mays (Mean ± SD, n = 5)

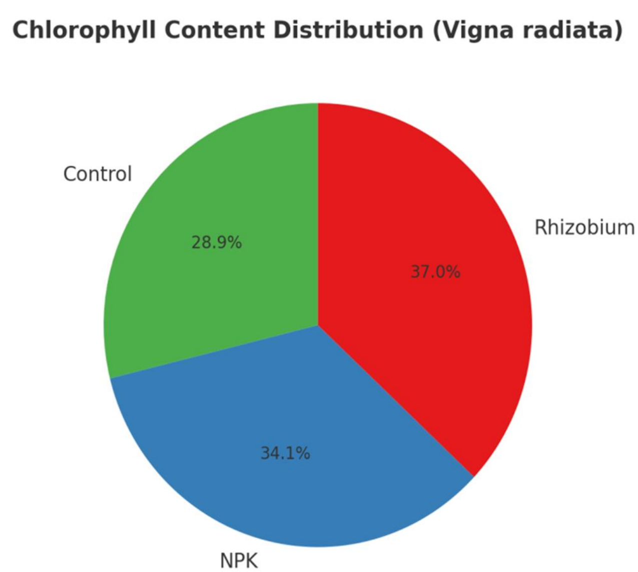
Crop	Treatment	Shoot Length (cm)	Root Length (cm)	Fresh Weight (g)	Dry Weight (g)	Chlorophyll (mg g^{-1} FW)
Vigna radiata	Control	14.2 ± 0.8^b	6.3 ± 0.4^b	3.1 ± 0.2^b	1.12 ± 0.1^b	1.42 ± 0.06^b
	NPK	17.8 ± 1.0^a	7.8 ± 0.6^a	3.9 ± 0.3^a	1.34 ± 0.1^a	1.68 ± 0.07^a
	Rhizobium	19.6 ± 1.2^a	8.4 ± 0.5^a	4.5 ± 0.4^a	1.56 ± 0.2^a	1.82 ± 0.08^a
Zea mays	Control	20.3 ± 1.1^b	9.4 ± 0.6^b	4.2 ± 0.3^b	1.48 ± 0.1^b	1.38 ± 0.05^b
	NPK	24.6 ± 1.3^a	11.2 ± 0.8^a	5.1 ± 0.4^a	1.74 ± 0.1^a	1.62 ± 0.06^a
	Rhizobium	23.1 ± 1.2^a	10.8 ± 0.7^a	4.8 ± 0.3^a	1.68 ± 0.2^a	1.58 ± 0.07^a

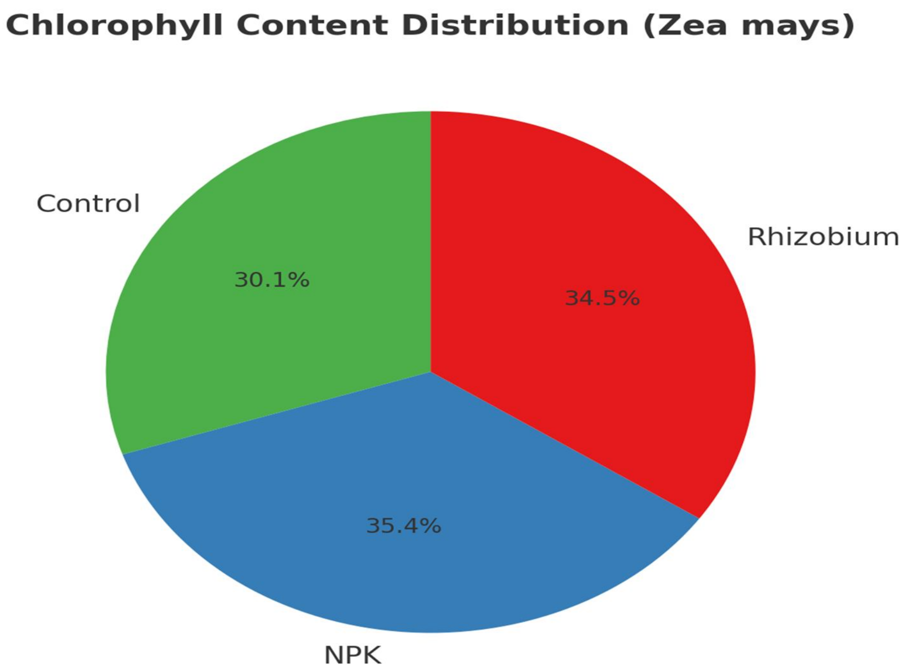
Means within a column followed by different superscripts differ significantly at $p < 0.05$ (Tukey's HSD).

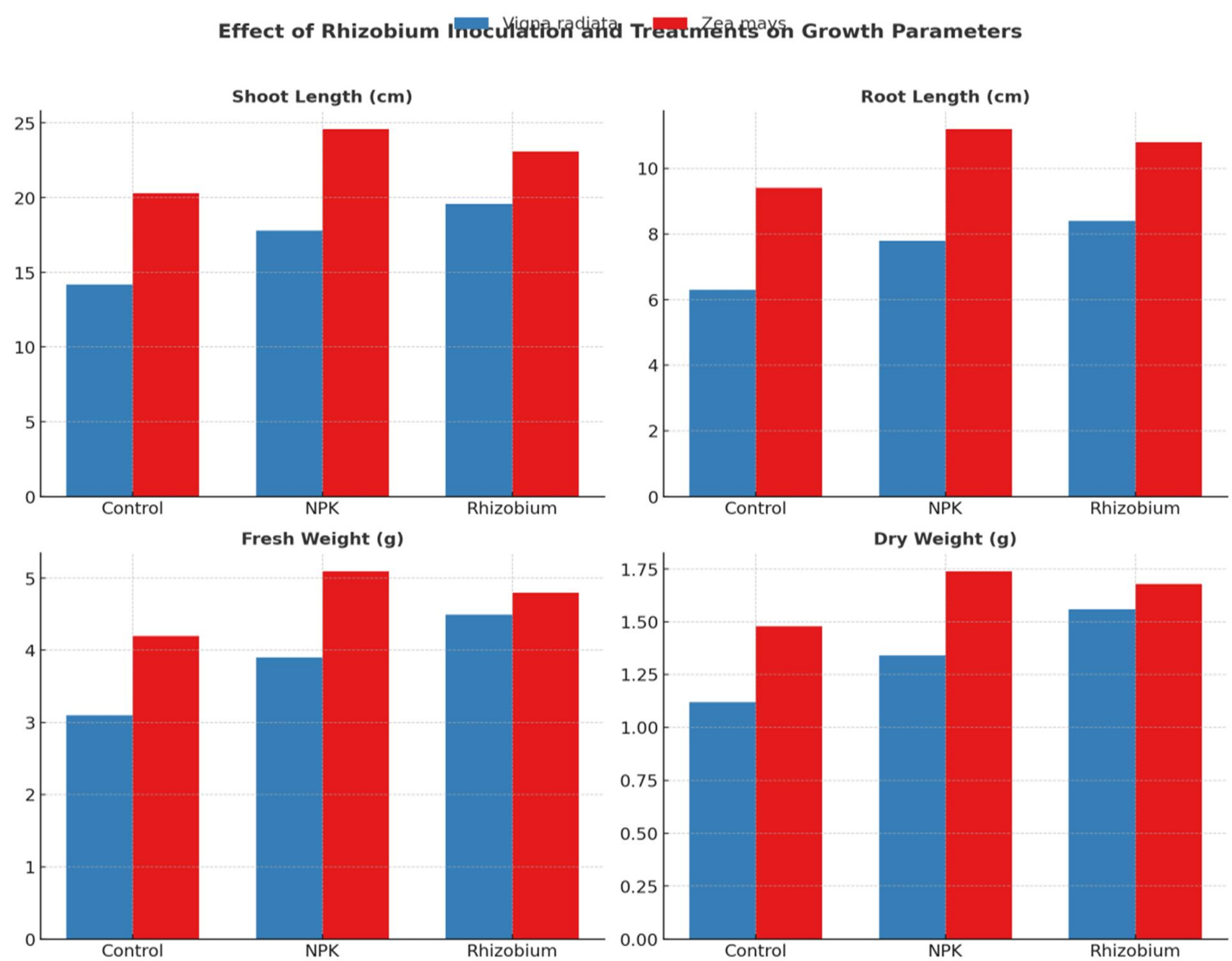
C. ANOVA Results

Statistical analysis revealed significant differences among treatments for all parameters except germination ($p > 0.05$). For shoot length, F-value = 24.68 ($p < 0.001$), indicating a strong effect of treatments. Similar trends were noted for root length, biomass, and chlorophyll content, confirming the positive role of Rhizobium inoculation.






Chlorophyll Content Distribution (Zea mays)
Control
Rhizobium
NPK
30.1%
34.5%
35.4%


Vigna radiata
Zea mays
Effect of Rhizobium Inoculation and Treatments on Growth Parameters
Shoot Length (cm)
Root Length (cm)
Fresh Weight (g)
Dry Weight (g)
Control
NPK
Rhizobium

D. Discussion

The improvement in growth parameters in Vigna radiata due to Rhizobium inoculation is primarily attributed to biological nitrogen fixation, which enhances nitrogen availability for plant metabolic processes (Sulieman & Tran, 2015). Rhizobium-legume symbiosis provides the host with significant nitrogen input, leading to enhanced chlorophyll synthesis and photosynthetic activity (Oldroyd et al., 2011). This explains the higher chlorophyll content in Vigna radiata inoculated with Rhizobium compared to the control.

In Zea mays, a non-leguminous crop, moderate growth promotion was observed. This may be due to plant growth-promoting traits (PGPR effects) exhibited by Rhizobium, including indole-3-acetic acid (IAA) production, siderophore release, and phosphate solubilization (Bhattacharyya & Jha, 2012; Sharma et al., 2013). Yanni et al. (2016) reported similar findings, where Rhizobium strains improved maize growth through phytohormonal effects and enhanced nutrient uptake despite the absence of nodulation.

Interestingly, the performance of Rhizobium inoculation in non-legumes was comparable to chemical fertilizers in certain parameters such as root length and chlorophyll content, suggesting that Rhizobium-based biofertilizers could partially substitute chemical fertilizers in sustainable cropping systems. However, the degree of growth enhancement in maize was less pronounced than in Vigna radiata, reinforcing the specificity of Rhizobium-legume interaction for nitrogen fixation (Vincent, 1970).

Overall, these results underscore the dual role of Rhizobium as a symbiotic nitrogen fixer in legumes and a PGPR in non-legumes, aligning with previous research advocating the broader use of Rhizobium-based biofertilizers in integrated nutrient management systems (Vessey, 2003; Yanni et al., 2016).

IV. CONCLUSION

A. Conclusion

The present study demonstrated that Rhizobium inoculation significantly enhanced plant growth parameters in the leguminous crop (*Vigna radiata*) compared to control and was comparable to chemical fertilizer treatment. Improvements were observed in shoot and root length, biomass accumulation, and chlorophyll content, reflecting the beneficial role of symbiotic nitrogen fixation in legumes.

In the non-leguminous crop (*Zea mays*), Rhizobium inoculation resulted in moderate but statistically significant improvements in growth attributes over the control, which can be attributed to plant growth-promoting traits such as phytohormone production, siderophore secretion, and phosphate solubilization. These findings confirm the dual role of Rhizobium as both a symbiotic nitrogen fixer in legumes and a plant growth-promoting rhizobacterium (PGPR) in non-legumes.

Overall, Rhizobium-based biofertilizers offer a sustainable alternative to chemical fertilizers, reducing dependency on synthetic inputs and contributing to environmentally friendly crop production systems.

B. Recommendations

1) Field-Level Validation: Large-scale field trials are recommended to confirm greenhouse results and evaluate Rhizobium efficacy under varying soil and climatic conditions.
2) Multi-Strain Formulations: Explore consortia of Rhizobium with other beneficial microbes (e.g., phosphate-solubilizing bacteria, mycorrhizae) for synergistic effects on both leguminous and non-leguminous crops.
3) Economic Analysis: Conduct cost-benefit analysis comparing Rhizobium inoculation with conventional fertilizers to assess economic feasibility for farmers.
4) Long-Term Soil Impact Studies: Investigate the effect of repeated Rhizobium application on soil health, microbial diversity, and nutrient cycling.
5) Policy Support: Government and extension agencies should promote biofertilizer adoption through subsidies, farmer training programs, and awareness campaigns to reduce reliance on chemical fertilizers.

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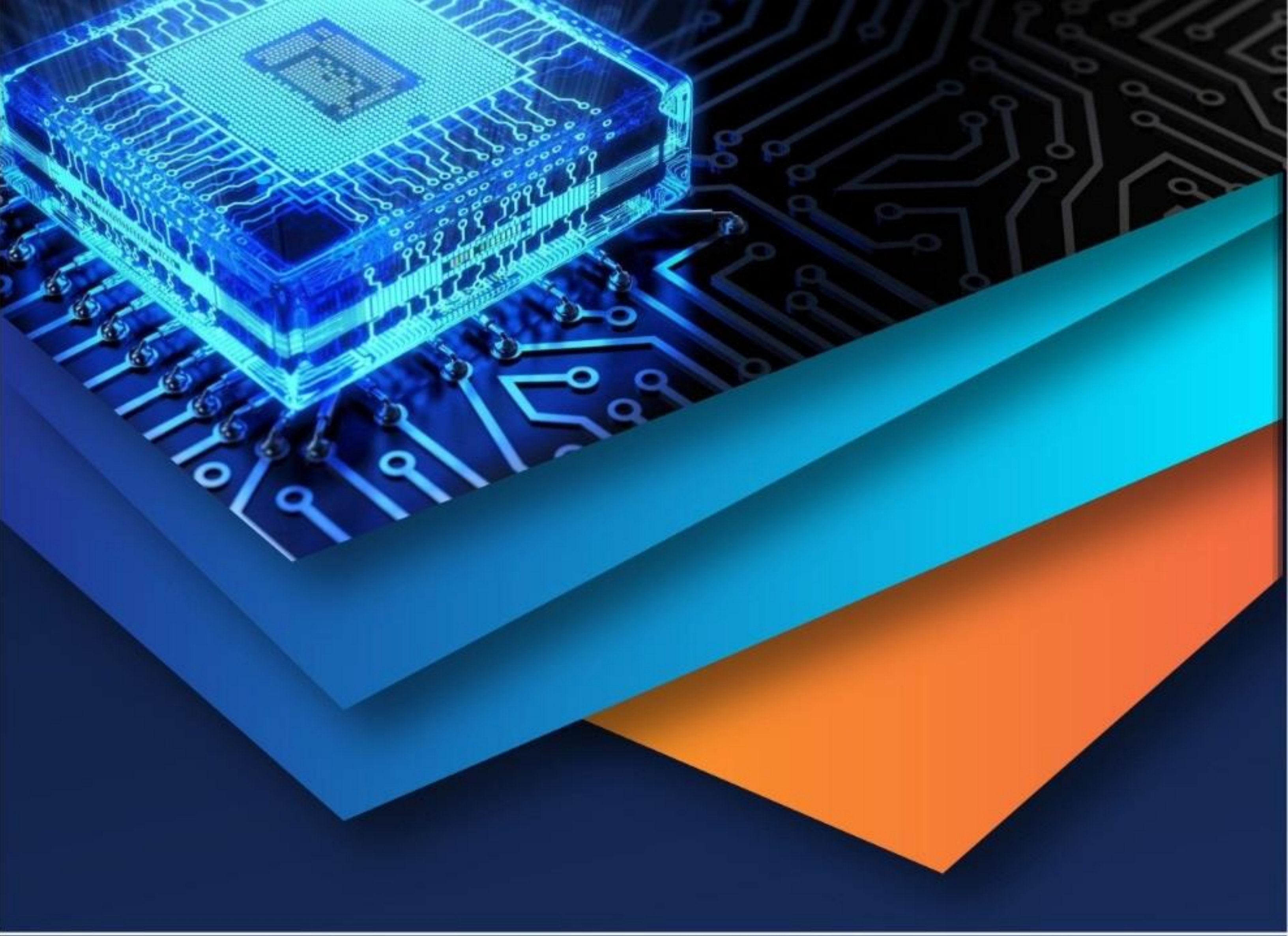

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