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Colletotrichum Diseases of Forest Nurseries and their Biological Management in vitro using Rhizoplane Mycoflora of Grasses

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Abstract: ***Microbes are the promising source for the biological management of many plant pathogenic organisms. Biocontrol agents are the viable alternative to chemical fungicides and also reduces developing fungicidal resistance in pathogens. Grasses and their roots are liable sources for microbial activity. The aim of the study was to isolate and identify major saprophytic rhizoplane mycoflora of selected perennial grass species Alloteropsis cimicina, Ischeamum indicum, Opplismenus compositus, Panicum repens and Perotis indica. Fungal species were isolated from rhizoplane regions by agar plate method in different seasons. Total 55 fungal isolates belongs to six genera, nine species of fungi and two were non-sporulating fungal isolates. The fungal isolates were identified and tested for their hypersensitive activity in sensitive plant species. Thirty six isolates out of 55, exhibited in vitro antagonism to phytopathogenic fungal species – Colletotrichum gleoesporioides. The fungus C. gleoesporioides is the major foliar pathogen cause anthracnose, leaf spot and blight are the major disease symptoms observed in nurseries. Among fungi tested Trichoderma harzianum was found to be more effective antagonistic activity against the pathogen in vitro.***
Key words: Perrenial grasses, Rhizoplane fungi, Colletotrichum gloeosporioides, Biological control, In vitro

I. INTRODUCTION

Forest nurseries established with the aim of providing healthy plant stocks is hindered by a number of factors. Diseases pose a major threat of which fungi forms a major pathogen for the successful production of seedlings and their by hindering planting programmes [1], [2]. Rots, Wilts, damping off and various foliar diseases are the major disease symptoms found in seedlings. Foliar diseases in plants are caused by a number of fungal pathogens among which *Colletotrichum* sp. are the major ones. *Colletotrichum gloeosporioides* Penz. causes anthracnose, leaf spot and leaf blight diseases on a wide variety of plants. *Colletotrichum gloeosporioides* is a common pathogenic fungi found more abundantly distributed in tropical and subtropical regions of the world than in temperate regions. A number of fungicides are available and indiscriminate usage resulting in environmental pollution has led in search of an alternative and eco-friendly methods.

Plant-microbe interactions especially between roots and microbes provide a wide array of opportunities to explore the complexities in association as well as their interaction in the growth and development. Rhizosphere and Rhizoplane regions supports a large number of microorganisms. The role of soil microbes is very complex, they help in nutrient cycling, provide nutrients to plants and stimulate plant growth [3], [4], [5].

Grasses form an important component of ecosystem which keeps rejuvenating in every growing season. They produce fibrous roots which homes abundance of diverse microbes. Rhizosphere and Rhizoplane regions supported populations of fungal communities of anamorphic ascomycetes, teleomorphic ascomycetes, zygomycetes and certain non-sporulating fungi [6]. A great diversity of rhizosphere and rhizoplane microorganisms have been described and also in many cases been used as bio-controlling agents. The present work has been carried out to study the antagonistic activity of rhizoplane fungi against *C. gloeosporioides* for their effectiveness *in vitro*.

II. MATERIALS AND METHODS

A. Isolation of pathogenic fungi

Disease survey was carried out in Central nurseries of Kerala located at Chettikulam, Kannavam, Kulathupuzha and Nilambur. Infected seedling samples were collected and were brought to the laboratory. The samples were washed thoroughly, blotted and were inoculated on antibiotic amended PDA medium. The associated fungal species were isolated and identified by referring standard manuals [7], [8], [9], [10], [11].

B. Isolation Of Rhizoplane Fungi

Rhizoplane fungi were isolated from roots of grass species *Alloteropsis cimicina, Ischeamum indicum, Opplismenus compositus, Panicum repens* and *Perotis indica*. Root samples were washed thoroughly in slow running tap water, blotted and were fragmented into 1 cm long segments. Root segments were inoculated on antibiotic amended PDA medium. Fungal colonies were isolated and identified by referring to standard manuals as described earlier.

C. Dual Inoculation Of Colletotrichum Gloeosporioides And Potential Antagonist On Pda

Dual inoculation of pathogen and an antagonist was set up. A 5 mm disc of pathogen with similar size of each potential antagonist was taken from the growing edge of five day-old pure culture using a cork borer. The control plates were inoculated for pathogen and antagonists separately. Three replications per treatment were set up for each pathogen and antagonist combinations. Inoculated petri-dishes were incubated at room temperature. Daily growth measurements of fungal colonies were taken for 7 days. The percentage inhibition of radial growth of pathogen was calculated using formula [12].

$$\text{Percentage of Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 – Test organism in Control
R_2 – Test organism in Dual culture

D. Statistical Analysis

Antagonistic ability of fungal isolates were statistically analysed and compared by Duncan's Multiple Range Test (DMRT) using SPSS (ver. 21) software developed by IBM Corporation.

III.RESULTS AND DISCUSSION

Disease survey conducted in the Central Nurseries of Kerala resulted in the observation of seedling diseases, leaf spots and blights. A number of fungal species were isolated and among the isolates Colletotrichum gloeosporioides (Fig 1 & 2) was found to be a major pathogen producing symptoms appeared as irregular spot, light to dark brown in colour surrounded by necrotic margin (Table 1) (Fig 3 - 8). Rhizoplane fungi isolated from grasses - Alloteropsis cimicina, Ischeamum indicum, Opplismenus compositus, Panicum repens and Perotis indica resulted a total of 55 fungal isolates belongs to six genera, nine species of fungi and two were non-sporulating fungal isolates. The fungal isolates were identified and tested for their hypersensitive activity in sensitive plant species (Chilli, Tomato and Tobacco). Thirty six isolates out of 55, exhibited in vitro antagonism to phytopathogenic fungal species – Colletotrichum gleoesporioides. The fungal organisms namely Gliocladium, Chaetomium and Trichoderma have been known for their antagonistic activity [13], [14], [15]. The result showed the inhibition percentage of Trichoderma harzianum (Fig 3) to be highest (73%-78%). Other tested isolates were seen to be showing moderate antagonistic activity against the pathogen (Table 2) (Fig 9). Trichoderma sp. have been known for their antagonism interaction and various species of Trichoderma namely T. koningii, T. harzianum and T. viride, respectively have been studied for their antagonistic activity in-vitro [16] [17]. Colletotrichum sp. are known to cause various foliar diseases and their management with natural agents have been practised [18], [19], [20], [21], [22]. Biological agents against forest nursery diseases are being practised to some extent. [23] reported that Trichoderma viride, T. harzianum and Pseudomonas fluorescens were effective against damping-off pathogens Rhizoctonia solani and Cylindrocladium quinqueseptatum.

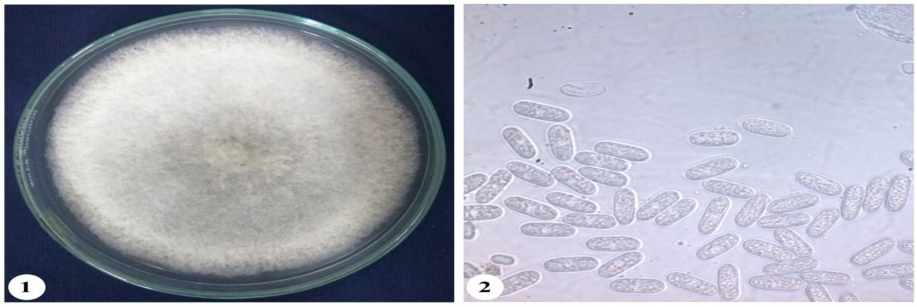

Fig 1 & 2. 1. Pure culture of Colletotrichum gloeosporioides & 2. Conidia of C. gloeosporioides

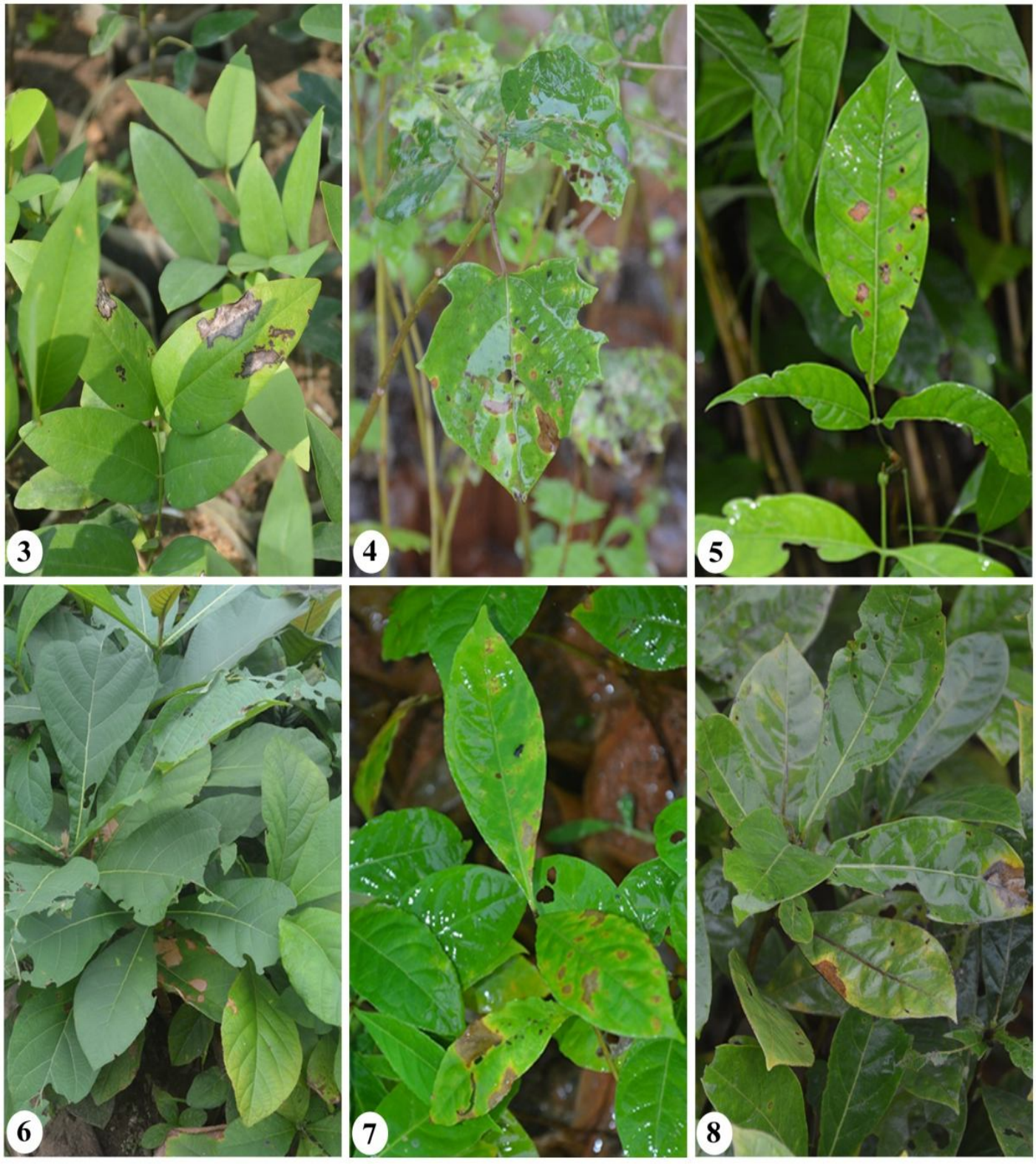

Fig 3-8. Leaf spot blight diseases of Colletotrichum gloeosporioides of 3. Cassia fistula, 4. Gmelina arborea, 5. Swetinia macrophylla, 6. Tectona grandis, 7. Terminalia bellirica and 8. T. arjuna.

Table 1. Colletotrichum gloeosporioides and associated fungi isolated from various seedlings from Central Nurseries of Kerala

Sl. No.	Tree species	Fungal diseases	Fungal organisms	Central nurseries
1	Acacia auriculiformis	Leaf spot / blight	Cladosporium Cladosporioides Colletotrichum gloeosporioides* Curvularia lunata Myrothecium roridum Pestalotiopsis sp.	KPZ
2	Acacia mangium	Leaf spot / blight	Cladosporium cladosporioides Colletotrichum gloeosporioides* Curvularia lunata Myrothecium roridum Pestalotiopsis sp.	KPZ
3	Aegle marmelos	Leaf spot	Alternaria alternata Colletotrichum gloeosporioides* Phoma sp.	CKM
4	Cassia fistula	Leaf spot	Colletotrichum gloeosporioides* Curvularia lunata Pestalotiopsis sp.	NBR
5	Gmelina arborea	Leaf spot	Colletotrichum gloeosporioides* Curvularia lunata	KNM, NBR, KPZ
6	Pongamia pinnata	Leaf spot	Bipolaris sp. Colletotrichum gloeosporioides*	KNM, NBR
7	Saraca asoka	Leaf spot	Colletotrichum gloeosporioides*	CKM
8	Swetinia macrophylla	Leaf spot / blight	Alternaria alternata Cladosporium cladosporioide s Colletotrichum gloeosporioides* Curvularia lunata Pestalotiopsis sp.	KNM, CKM
9	Tectona grandis	Leaf spot / blight	Alternaria alternata Cladosporium cladosporioide Colletotrichum gloeosporioides* Curvularia lunata Pestalotiopsis sp.	KNM, NBR, CKM
10	Terminalia arjuna	Leaf spot	Cladosporium cladosporioide s Colletotrichum gloeosporioides*	CKM
11	Terminalia bellirica	Leaf spot	Colletotrichum gloeosporioides* Pestalotiopsis sp.	KNM, KPZ
12	Thespesia populinea	Leaf spot	Colletotrichum gloeosporioides* Fusarium oxysporum	KNM

*Dominating Fungus associated with naturally infected plant materials
CKM- Chettikulam, KNM- Kannavam, KPZ- Kulathupuzha, NBR- Nilambur

Table2. Antagonistic activity of fungal species from rhizoplane regions of grasses against Colletotrichum gloeosporioides in vitro

Sl. No.	Fungal organisms	Antagonistic activity of fungal species (%)[1]				
		A. c.	I. i.	O. c.	P. r.	P. i.
1	Aspergillus flavus	0.00±0.00[2] a[3]	0.00±0.00 a	0.00±0.00 a	33.45±0.33 e	0.00±0.00 a
2	Aspergillus niger	0.00±0.00 a	30.67±0.56 d	0.00±0.00 a	35.77±0.24 f	39.67±0.34 f
3	Curvularia lunata	26.67±0.45 b	0.00±0.00 a	16.73±0.08 b	0.00±0.00 a	0.00±0.00 a
4	Fusarium oxysporum	0.00±0.00 a	21.43±0.11 c	40.48±0.75 g	21.43±0.14 b	42.67±0.61 g
5	Fusarium sp.	0.00±0.00 a	0.00±0.00 a	26.91±0.08 c	23.81±0.35 c	31.43±0.54 c
6	Penicillium chrysogenum	0.00±0.00 a	32.36±0.77 e	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
7	Penicillium sp.	29.43±0.74 cd	31.42±1.44 de	38.76±0.38 f	40.06±1.72 g	37.16±1.08 e
8	Phomopsis sp.	30.33±0.73 d	0.00±0.00 a	30.38±1.59 e	40.44±1.23 g	35.76±0.87 d
9	Trichoderma harzianum	73.30±1.35 e	75.78±0.85 f	75.77±0.77 h	77.87±1.30 h	74.76±1.05 h
10	NSF-1	30.13±1.09 d	19.05±1.06 b	28.75±0.40 d	29.76±0.91 d	25.68±1.10 b
11	NSF-2	28.57±1.05 c	19.05±0.63 b	27.75±0.39 cd	0.00±0.00 a	0.00±0.00 a

[1]Data is an average of three replicates, [2] Standard deviation and [3]Experiment was conducted in a factorial design. Means carrying same letters in a row are not significantly different (DMRT, $P_{0.05}$)

A. c. - Alloteropsis cimicina, I. i.- Ischaemum indicum, O. c.- Opplismenus compositus, P. r.- Panicum repens,
P. i. - Perotis indica

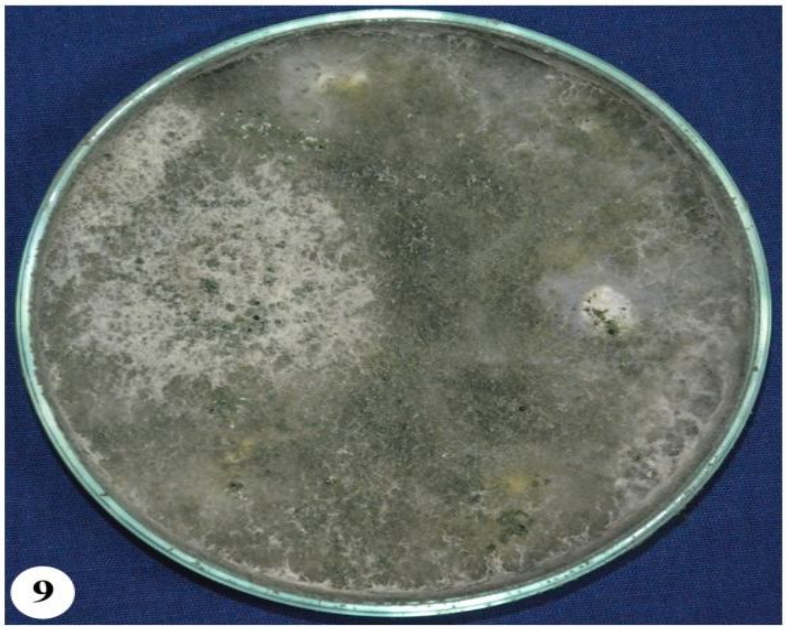

Fig 9. Antagonistic activity of Trichoderma harzianum against Colletotrichum gloeosporioides

IV. CONCLUSION

Shallow fibrous roots of grasses harbours a variety of microbes which are important for plants growth and development and can be possibly applied as an alternative for the management of plant diseases. The desirable demand of plant stock has led to the over dependence on chemicals for time bound supply of materials but the negative effects that they imply has not been stressed so far. The alternative strategy for environmental friendly applications now a days need more serious attention for sustainable management of natural resources. Natural biocontrol agents give an insight into the eco-friendly management of diseases thereby helping in growth and development. Among various natural agents, the fungus Trichoderma harzianum proves their antagonistic potential for their effective application as an alternative to the chemical control against a wide set of fungal plant pathogens [24]. Thus, the potentialities of such agents can also applied not only in agricultural field but in forestry sector also.

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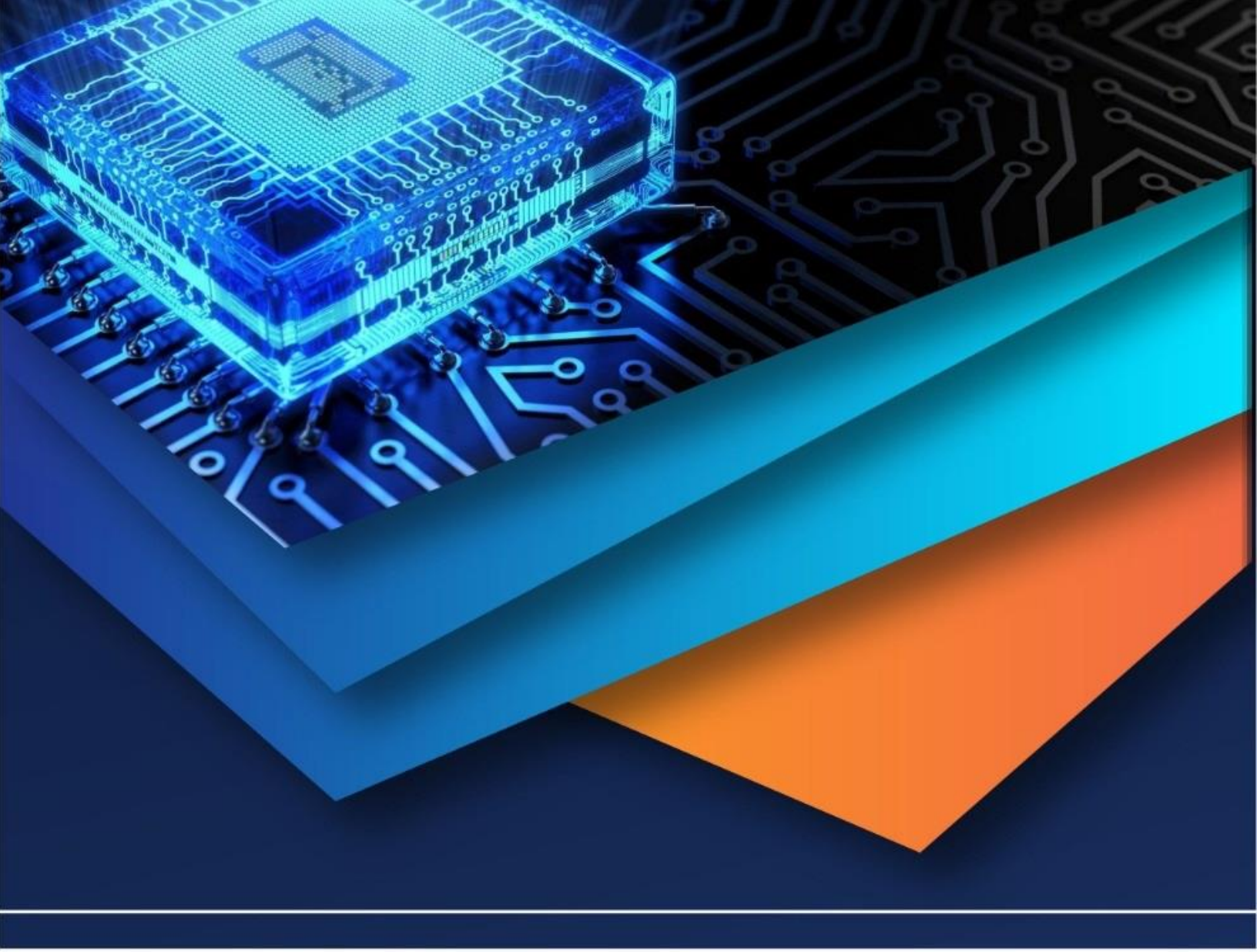

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