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Pollen Morphological Studies of some Common Garden Plants of Apocynaceae Family

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Abstract: ***Every man is a taxonomist from the cradle to the grave since he is surrounded by plants and trees, therefore they can be considered as the primary companion in the biotic community. The importance of plants has been realized and well documented since ancient period. Based on different aspects, they are classified and concluded into various families. The scientific study of spores and pollen grains, both living and fossils make up the subject "Palynology". Study of the morphology of pollens are highly significant as it can be useful in establishing relationship amongst various taxa, resolving disputed taxonomical problem, building phylogenetic tree, tracing ancestry of a particular clade. Besides, this discipline gives a sound knowledge on pollination ecology, constructing phylogenetic classification as well as in palynotaxonomic studies. Apocynaceae family has been considerably studied with respect to their pollen morphological characters. The family Apocynaceae Palynologically depicts eurypalynous nature with variation in apertural characters. They represent simple porate aperture to compound colporate one. This palynological variation is helpful in taxa identification at the generic level.***
Keywords: Apocynaceae, Taxonomy, Palynology, Eurypalynous

I. INTRODUCTION

A pollen grain consists of two wall layers namely the outer sculptured, ornamented exine and the inner intine. Pollen grain characters are genetically labeled and display unique traits in wall ornamentation, aperture and reticulation. Pollen grains are produced in profuse number per anther to ensure successful pollination followed by germination that eventually led to seed production. This feature makes it a potential tool for taxonomic study as it overcomes the major drawback of other features namely, seed, fruit, flower, etc. which are produced in limited number, a necessary handicap with these parameters. Study of the morphology of pollens are highly significant as it can be useful in establishing relationship amongst various taxa, resolving disputed taxonomical problem, building phylogenetic tree, tracing ancestry of a particular clade. Besides, this discipline gives a sound knowledge on pollination ecology, constructing phylogenetic classification as well as in palynotaxonomic studies.

In case of angiosperms, pollen grains are produced from the sporogenous tissue of the anther of flower present. Morphologically pollen grains of angiosperms exhibit highest level of variation that can be utilized in plant taxonomy. They display unique traits of apertural variation from the simplest inaperturate type to the highly advanced echinate form. Pollen characters are permanent and are guided by the genetic makeup of the parent plant, hence providing us with very convincing data which can be utilized in building a systematic classification of angiosperms.

Apocynaceae or dogbane family is one of the relevant families of angiosperms which contain almost 5100 species in 5 subfamilies. In India the family is represented by 24 genera and 52 species. The members are widely used for their economically important nature; some of them provide crude drugs like cardiac glycosides, reserpine, vinblastin, vincristine, etc. from members like Rauvolfia serpentina, Catharanthus roseus. They are also widely used as ornamental plants like Nerium, Vinca, Allamanda, Mandevilla, Plumeria, Thevetia, etc.

Apocynaceae family has been considerably studied with respect to their pollen morphological characters. The family Apocynaceae Palynologically depicts eurypalynous nature with variation in apertural characters. They represent simple porate aperture to compound colporate one.

This palynological variation is helpful in taxa identification at the generic level. However, this data is yet to be applied for building a pollen key that would be useful for re-categorizing the existing taxonomic position of genera under family Apocynaceae. The present study has been conducted on genera under the Apocynaceae family namely; Tabernaemontana, Allamanda, Catharanthus, Calotropis, Nerium, Plumeria and Vallaris.

Pollen grains were collected from freshly opened buds of flower preferably of same age to obtain uniformity in result, just before the anthesis stage. Grains are collected and subjected to acetolysis and acetcarmine staining procedure in order to analyze pollen morphology, pollen viability and pollen germination.

II. MATERIALS AND METHOD

The plant materials were collected from the campus of Nirmala college for women, Coimbatore. Matured flower buds of selected plants from Apocynaceae family were taken for the pollen study.

A. Acetolysis (Erdtmann, 1969)

The pollen grains were first treated with 70% ethyl alcohol and centrifuged for 5 minutes at 1700 rpm. The supernatant is decanted and the pollen grains are treated with acetolysis mixture. The acetolysis mixture used for processing pollen grains contains glacial acetic acid and sulphuric acid in the ratio 9:1. The tubes were immersed in boiling water bath for 3 - 5 min with continuous stirring. The samples were allowed to cool and centrifuged at 1700rpm for 3 minutes and the supernatant was decanted. Distilled water is added to the sample and centrifugation is repeated for 3 times. After decanting the pollen grains were stained with acetocarmine. For Light Microscopic observation, the acetolysed pollen grains were washed with distilled water and add two drops of 0.01 percentage diluted acetocarmine for 2 minutes

B. Pollen Viability Studies – Acetocarmine Technique (Stanley and Linskens, 1974)

Pollen viability was done by acetocarmine staining using dissecting forceps, scalpel and needle, anthers of various species were opened and transfer the pollen dust on to a microscopic cavity slide containing a drop of sucrose solution (Approximate 10 gram of sucrose is mixed with 100 ml of distilled water to make the solution for germination). Mature anthers were crushed and pollen grains mixed thoroughly with the aceto-carmine stain. Cover slips were placed on to different slides for each species. The slides were then observed under light microscope. For each plant species flowers were collected from at least 3 different plants. For each plant, seven slides were prepared. For each slide ten randomly selected fields were observed under the 10 X objective lens. To determine pollen fertility, darkly stained pollen grains were recorded as fertile and viable, and unstained or very lightly stained ones were considered as sterile or non-viable. Pollen fertility was calculated by dividing the number of viable pollen grains by the total number of grains counted in the field of view and averaging them for all plants in that species. Pollen viability was expressedas percentage pollen fertility in each plant species.

III. RESULTS AND DISCUSSION

A. Pollen Morphological Studies.

Results of pollen morphological studies are given in Table 1, figure 2 & 3. Pollens of the taxa studied are mostly psilate. The pollen of Nerium oleander (plate 4) showed pororate apertures. The members like Allamanda cathartica (plate 1), Calotropis gigantea (plate 2), Catharanthus roseus (plate 3), Plumeria rubra (plate 5), Vallaris glabra (plate 7) and Tabernaemontana divaricata (plate 6) exhibit colporate type of pollen aperture. That means all pollens except the pollen of Nerium oleander shows colporate pollen. Pollen shape was sub spheroidal in the case of Allamanda cathartica, Nerium oleander and Tabernaemontana divaricata. The members like Catharanthus roseus, Vallaris glabra and Plumeria rubra showed spheroid pollen. The number of apertures was three in all the selected members

Table: 1. Pollen morphology studies of the selected plant species of Apocynaceae family

Scientific name	Type of pollen	Type of aperture	Number of Aperture
Allamanda cathartica L.	Sub spheroidal	Colporate	3
Calotropis gigantea (L.) W. T. Aiton	Spheroidal	Colporate	3
Catharanthus roseus (L.) G. Don	Spheroidal	Colporate	3
Nerium oleander L.	Sub spheroidal	Pororate	3
Plumeria rubra L.	Spheroidal	Colporate	3
Tabernaemontana divaricata R. Br. ex Roem. & Schultz	Sub spheroidal	Colporate	3
Vallaris glabra (L.) Kuntze	Spheroidal	Colporate	3

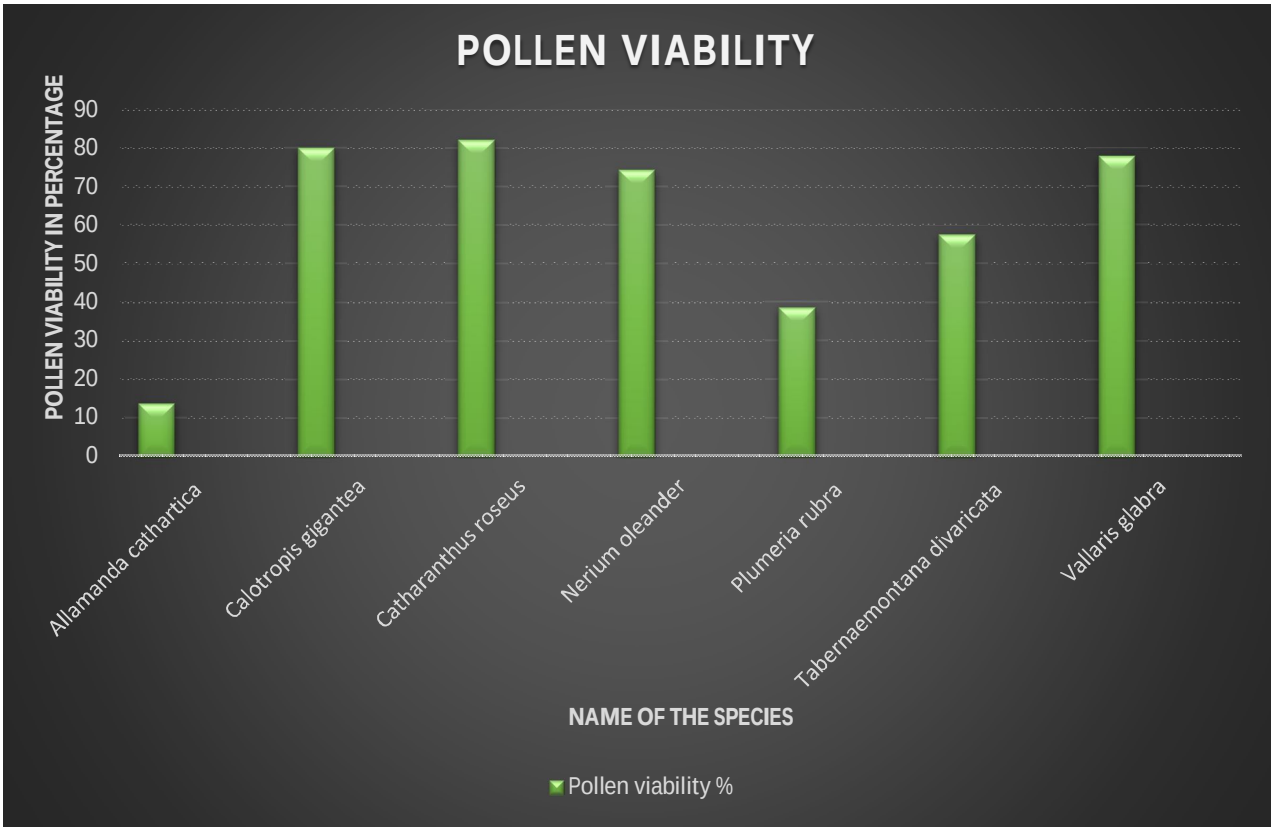
B. Pollen Viability Studies

Results of pollen viability studies are given in Table 2, figure 4. Of the seven taxa studied, the maximum viability showed in Catharanthus roseus (82.14%) followed by Calotropis gigantea (80%) and Vallaris glabra (77.77). The minimum viability resulted in Allamanda cathartica (13.63%). The viable/fertile pollen stains pink to deep red with aceto-carmine, while non-viable/sterile (mostly shriveled) pollen does not take any stain and thus remains almost white and transparent.

Table: 2. Pollen viability studies of the selected plant species of Apocynaceae family

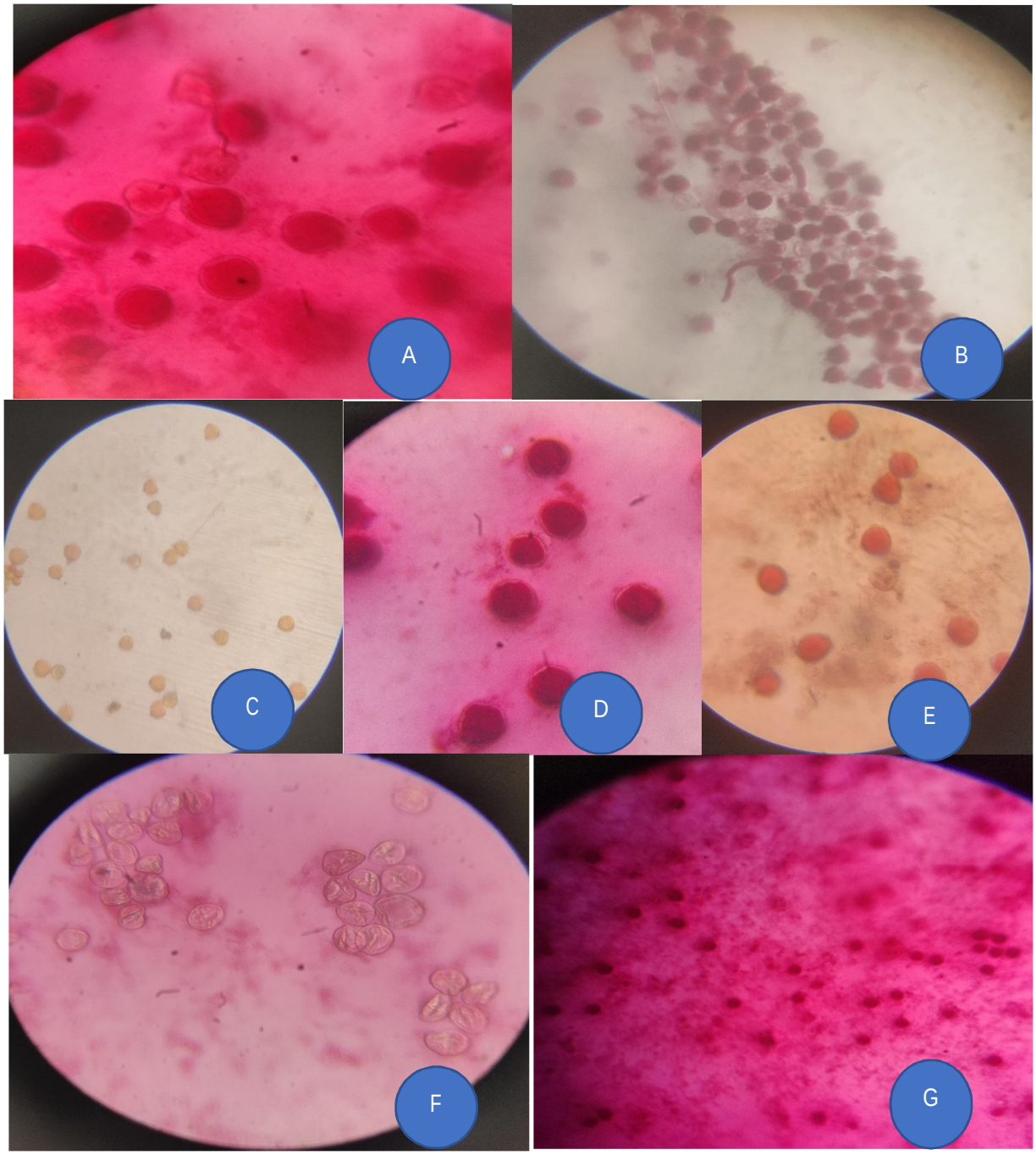
Name of the species	Total no. of pollen grains	Non-viable pollen grain	Viable pollen grains	Pollen viability %
Allamanda cathartica L.	22	19	3	13.63
Calotropis gigantea (L.) W. T. Aiton	15	3	12	80.00
Catharanthus roseus (L.) G. Don	84	15	69	82.14
Nerium oleander L.	39	10	29	74.36
Plumeria rubra L.	52	32	20	38.46
Tabernaemontana divaricata R. Br. ex Roem. & Schultz	66	28	38	57.58
Vallaris glabra (L.) Kuntze	54	12	42	77.77

Figure 4 - Pollen viability of selected plant species of Apocynaceae family



C. Pollen Morphology of Selected plant Species of Apocynaceae

D. Pollen Viability of Selected plant Species of Apocynaceae



(A) Allamanda; (B) Calotropis; (C) Catharanthus; (D) Nerium; (E) Plumeria; (F) Tabernaemontana; (G) Vallaris

IV. CONCLUSION

In this study, the plants like *Catharanthus roseus*, *Plumeria rubra*, *Vallaris glabra* and *Tabernaemontana divaricata* showed tricolporate type of aperture. *Allamanda cathartica*, *Calotropis gigantea* and *Nerium oleander* showed tripororate type of aperture. All the plants were equal number of aperture and showed psilate-type of perforation. Exine ornamentations formed of excrescences are primitive and those formed of depressions are advanced.

Mainly pollen morphological changes within Apocynaceae are in the context of apertural area reduction from colpate to porate. In term of identification, Apocynaceae remains rather eurypalynous. Some species can be distinguished on the basis of aperture, shape and size.

Pollen Viability Studies showed that the maximum viability observed in *Catharanthus roseus* followed by *Vallaris glabra*. The minimum viability resulted in *Allamanda cathartica*. The viable pollen stains pink to deep red with aceto-carmine, while sterile (mostly shrivelled) pollen does not take any stain and thus remains almost white and transparent. From this study we conclude that *Catharanthus roseus*, *Calotropis gigantea* and *Vallaris glabra* showed colporate type of aperture and more pollen viability. These are primitive characters as per the earlier literature. *Allamanda cathartica* and *Plumeria rubra* showed more advanced features like porate type of aperture and less pollen viability.

Pollen morphology is one of the significant tools in solving some taxonomic problems on the family, generic or specific level and has become part of the. collaborative and multidisciplinary approach in plant evolution and systematic. On the other hand, pollen fertility and viability data is a valuable tool for the taxonomists in attempting to distinguish speckled hybrids from the parent plants and is also useful to determine the degree of satiability and fertility

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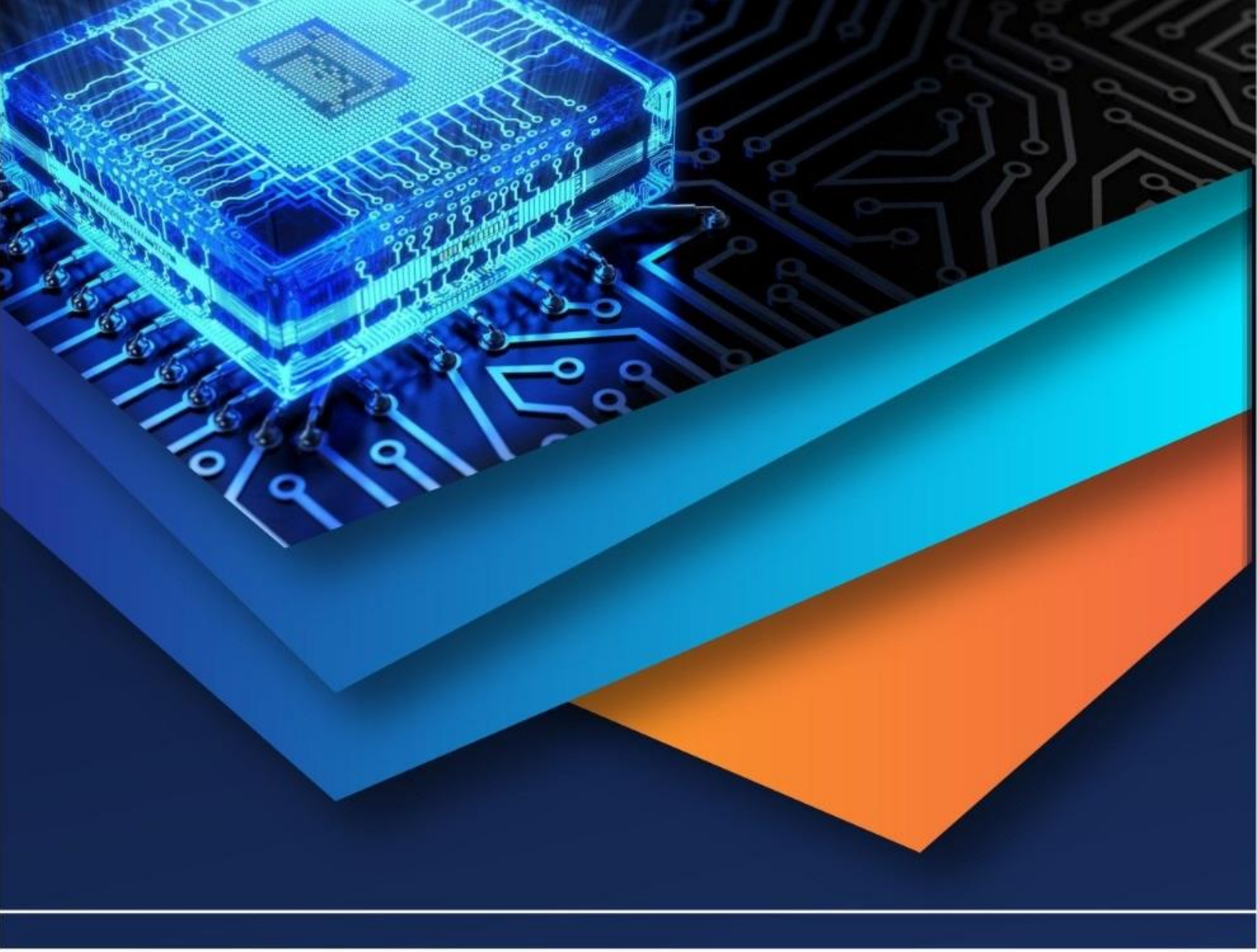

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